

**ANIMAL
ACOUSTIC STARTLE
RESPONSE SYSTEM**



Single Subject Startle Chamber



Multi-Subject Chamber

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INTRODUCTION

The startle response is used in many branches of research involving behavioral testing. Coulbourn Instruments has developed a system that enables the researcher to standardize this procedure. The data collected with this system can be repeatedly reproduced with complete reliability.

The Animal Acoustic Startle Response System from Coulbourn Instruments is simple to use and user friendly. The system consists of one or more acoustically isolated test chambers, startle platforms, animal holders, system controller, computer, PCI interface card and software.

The software allows for easy setup and operation. It is a fully configurable Windows® based software package for creating protocol and experimental design that does not require the user to learn complex programming languages.

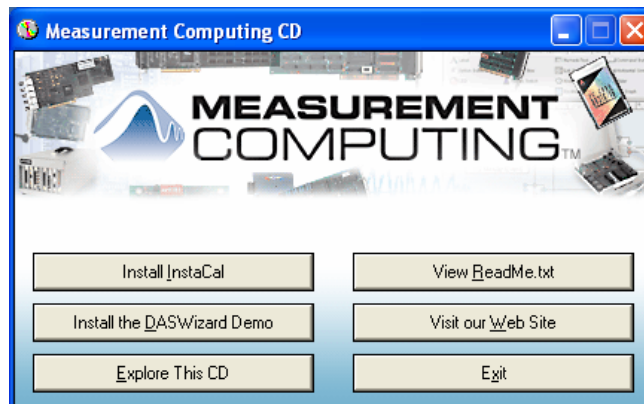
Every effort has been made to provide a system that is both easy to setup and to use. This manual will guide you through the installation of the interface card and software, the hardware setup, calibration procedures, creating protocols, running experiments and data review/analysis. Please take the time to read this manual.

SYSTEM REQUIREMENTS

The minimum recommended computer configuration is a PC-compatible desktop or laptop computer with Pentium III or faster processor, 256 MB RAM, 40 MB hard disk, CD Drive, Windows 2000® or Windows XP®. The desktop computer must have one available PCI interface slot and the laptop must have one available PCMCIA interface slot.

INSTALLING THE PCI INTERFACE CARD (Desktop Computer)

If you are using a laptop computer skip this section and proceed to the next section titled, “INSTALLATION OF THE PCMCIA CARD (Laptop Computer)”. Two Compact Discs were included with your system, “WSTART4” and “InstaCal”, included with the PCI-3 Kit. “InstaCal” must be installed first. DO NOT INSTALL THE PCI INTERFACE CARD IN THE COMPUTER. Before beginning the installation, exit all Windows programs. Insert the “InstaCal” CD into the CD drive of the computer. The program should start automatically but if it does not, select Run from the Programs menu and choose the setup.exe file from the CD drive. Select the OK button and the monitor will display the following screen.



1. Select “Install InstaCal” button. A window will appear instructing you to exit all windows programs. Select Next>.
2. Another window will appear specifying the Install directory, C:\MCC. Select Next>.
3. The installation procedure will specify that icons will be added to the Program Folder, “Measurement Computing”. Select Next>.
4. The installation will begin. Once the installation is completed, a screen will appear offering you the opportunity to view the “Read Me” file for “InstaCal”. It is not necessary that you read this file.
5. Shut down the computer leaving the “InstaCal” Compact Disc in the CD Drive.
6. Now, reboot the computer. After the computer has fully rebooted, shut the computer down and install the PCI-INT32 Interface Card (PCI-3) in any available PCI slot in the computer.
7. Once the PCI-3 Card is installed, reboot the computer.
8. Windows should indicate that new hardware has been located. If not, select “Add/Remove Hardware” from Control Panel.
9. Choose the “Install the software automatically (Recommended)” option and select Next>.
10. Once the PCI-INT32 installation is completed, a new window will appear. Select the Finish button to close the Installation Wizard.



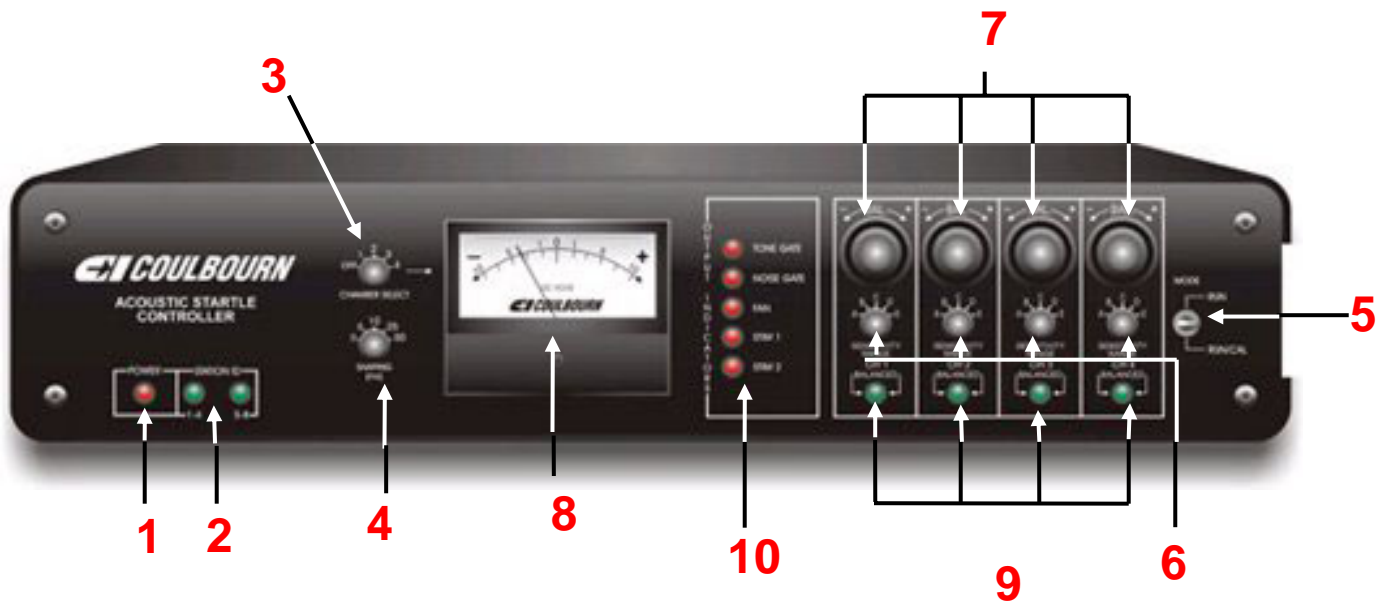
11. Now run “InstaCal” from the Program group Measurement Computing.
12. The PCI-INT32 (PCI-3) Interface Card should be detected. Select OK
13. Exit the “InstaCal” program and remove the Compact Disc from the CD drive.

INSTALLING THE PCMCIA INTERFACE CARD (Laptop Computer)

INSTALLING THE STARTLE SOFTWARE (WSTART4)

1. Place the Wstart4 Compact Disc in the CD drive.
2. Select Start, and then Run.
3. In the Run window, select Browse, and choose the CD drive (e.g., CD Drive [D:])
4. Choose the Setup.exe file and then select OK button to begin the installation.
5. The Startle Software will self install. During the initial installation, you may be requested to confirm that you wish to keep files that already exist on your computer, rather than overwrite them with files from the installation CD. It is recommended that you choose to keep the files that already exist on your computer.

A10-21 CONTROLLER – FRONT PANEL CONTROLS



1 – POWER INDICATOR: On/Off indicator for A10-21 Startle Controller

2 – STATION ID: The Acoustic Startle System can run up to eight chambers (channels) simultaneously. Each A10-21 supports up to four chambers (channels). The **STATION ID** indicator identifies the chambers (channels) interfaced to the A10-21.

3 – CHANNEL SELECT: The **Channel Select** switch routes the signal of the selected channel (1 through 4) to the meter. Used during the zero balancing procedure, the **Balance Control** switch (Refer to No. 7 below) of the selected channel is then adjusted until the meter is centered at 0 Volts. During a session, the switch should be set to the “OFF” position.

4 – SHAPING SWITCH: Sets the Rise duration of the Tone. Options are 0 (no shaping), 5, 10, 25, and 50 milliseconds. Selecting 0 may result in an audible click when Tone is presented. Rise time must be less than the duration of the Tone for the Tone to reach full amplitude.

5 – MODE SWITCH: When zero balancing or calibrating the platforms, the mode switch **MUST** be in the “Run/Cal” (down) position. In the “Run/Cal” position, the Transducer Couplers of all four chambers (channels) are DC coupled. During a session, the **Mode** switch can be set in the “Run/Cal” (down) position or the “Run” (up) position. In the “Run/Cal” position, the response data will include the sum of the total weight on the platform (including any response) less the weight that was present on the platform during the zero balancing procedure. With the **Mode** switch in the

“Run” (up) position, the couplers of all four chambers (channels) are AC coupled. In this position, only the response will be a part of the data.

6 – SENSITIVITY RANGE: There are four **Sensitivity** switches, one for each chamber (channel). There are five sensitivity or gain” ranges. It is recommended that a gain of 1000 (Position “E”) be used for startle response protocols.

- A = 100
- B = 200
- C = 266
- D = 500
- E = 1000

7 – BALANCE CONTROL: The A10-21 has four **Balance Control** switches, one for each chamber (channel). These are used to zero balance the platform of each chamber (channel). The **Mode** switch (Refer to No. 5 below) must be in the “Run/Cal’ position during the zero balancing procedure. Select the chamber (channel) using the **Channel Select** switch (Refer to No. 3 above) and then adjust the **Balance Control** until the meter displays zero volts. This may be done with no weight on the platform or with the animal holder in place. The advantage of the latter procedure is that the weight of the platform will be “subtracted” from the response waveform and only the subject’s weight will offset the signal from “0.” Once the platform is balanced, the **Balance** indicator LED (Refer to No. 9 below) for that channel will be “ON”. It is recommended that the Lock on the **Balance Control** be used once the transducer is zero balanced.

8 – METER: Displays output voltage of the selected chamber (channel) during zero balancing procedure. (Refer to the Nos. 3, 5, and 7 above.)

9 – BALANCED (Indicator): The A10-21 has four Balanced LED indicators, one for each chamber (channel) that are “ON” when the platform has been zero balanced (Refer to No. 7 above).

10 – OUTPUT INDICATORS: There are five visual indicators that correspond to each of the four stimuli (Tone, Noise, Stim 1, and Stim 2) and the fan in the chamber(s). Stim 1 and Stim 2 for each chamber (channel) are available on the rear of the A10-21 Acoustic Startle Controller). During a session, the output corresponding indicator(s) is “ON” during the stimulus presentation. Stim 1 and Stim 2 are outputs that can be used to present other stimuli such as air puff, shock, and visual stimuli. The indicators correspond to the following stimulus options in the Acoustic Startle Software:

<u>Software Stimulus Select</u>	<u>Stimulus Type</u>
1	Tone
2	White Noise
3	Stim 1 (Rear Panel of A10-21)
4	Stim 2 (Rear Panel of A10-21)
5	Stim 1 and Stim 2 simultaneously

INSTALLING THE HARDWARE

The Startle Response Platforms (E45-XX) are placed inside the chamber and connected to the cable that extends from the corner of the chambers. In the E10-24, there are four of these cables for inserting up to four Response Platforms.

There are two auxiliary outputs on the back of the controller labeled “Stim 1” and “Stim 2” associated with each station. These outputs are used for controlling -24 Volt devices such as shock, solenoid for air puffs, or other devices that might be used in addition to or in place of tone and/or white noise for startle response protocols.

Single Subject Acoustic Startle Chamber (E10-21)

With the Single Subject Chamber, connect the audio cable from audio output of the first channel on labeled “Speaker” (rear panel of the controller) to the audio input on the back of the chamber. Connect the transducer cable from the first channel, labeled “Chamber”, (rear panel of the controller) to the transducer connector on back of the chamber. The AC power cord is attached to the back of the chamber and an AC outlet.

Continue to make these same connections for every chamber being used. You must use the outputs from the controller in sequential order. That is, channel one must be used first, then channel two, then channel three, and finally channel four. Once the connections are made it is not necessary that you run subjects at all four stations in sequential order.

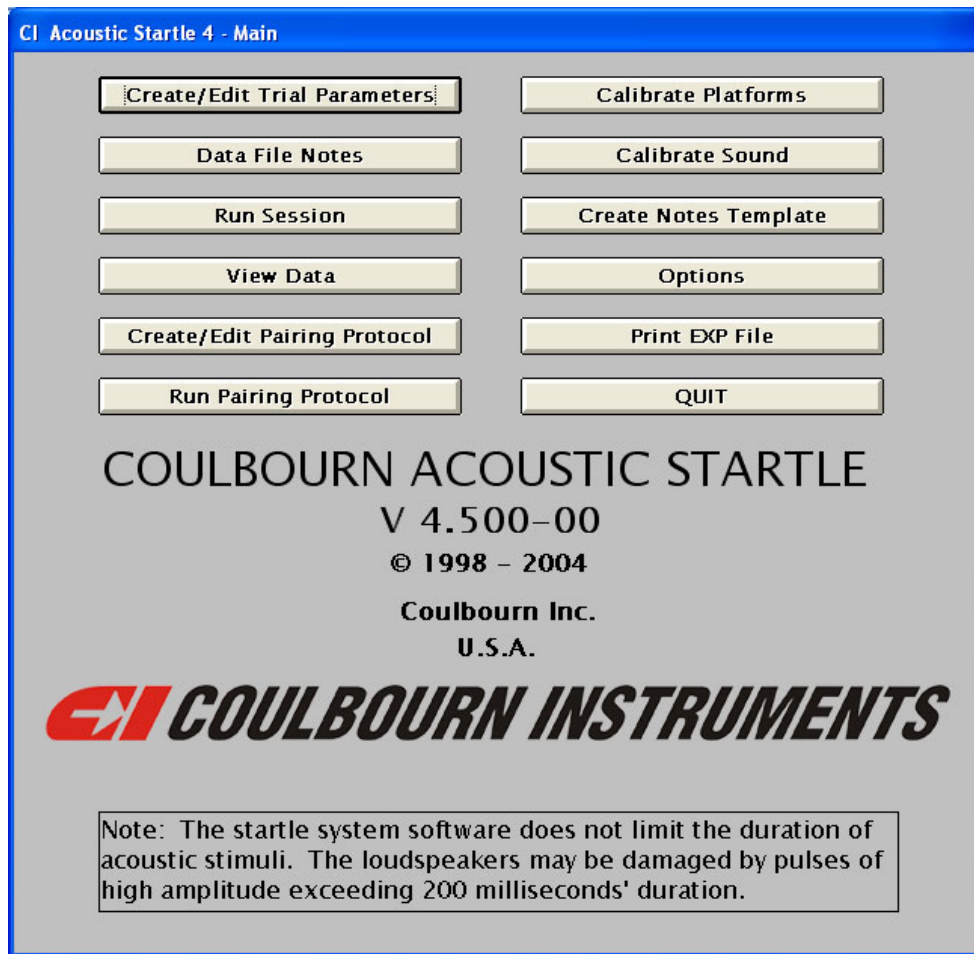
Four Subject Acoustic Startle Chamber (E10-24)

Refer to Appendix A of this manual for the hardware connections when using a Four Subject Acoustic Startle Chamber (E10-24).

STARTING THE WSTART4 SOFTWARE

Select Programs and choose Wstart4 program group. There is one item included in this group. Click on the Wstart4 icon to start the program. If the PCI-3 Card has been installed properly, a window will appear when the program begins; a window will appear indicating the PCI card has been detected. Select "Proceed". You may wish to place a check in the, "Don't show this information again" box. This will eliminate this window when you start the Startle program in the future.

The main menu is displayed when the Wstart4 software is started.



Create Edit Trial Parameters This portion of the program is used to specify the nature of stimuli to be presented on each trial and to arrange their temporal aspects. An experiment file is created trial by trial and then stored on disk for future retrieval. These files may also be edited in this section of the program.

Data File Notes Use this option to enter up to ten (10) lines of text into the data file for the session. Another option enables the entry of fixed headers for each line.

Run Session This section of the program runs a session. The user specifies the needed input files and the root name to be used for the data files. The program runs the session according to the specifications in these files and collects and stores data in disk files.

View Data An analog data review capability is included in the software; it is accessed via this routine. .

Create/Edit Pairing Protocol Used to create paired presentations of stimuli in order to implement protocols such as fear-potentiated startle. This option is used to create and edit such pairing protocols.

Run Pairing Protocol This option is used to run the protocols described in the previous section

Calibrate Platforms The transducers in each of the startle platforms require calibration. The routine accessed by this option will perform these calibrations.

Calibrate Sound It is necessary to calibrate the transducers to be used for the presentation of the auditory stimuli. These routines will allow the user to adjust the frequency, amplitude, and duration of tone presentations and the amplitude and duration of white noise bursts so that the calibrations may be accomplished. Values required for stimulus amplitude are determined by this part of the program.

Create Notes Template To avoid the need to enter fixed text repetitively, this option may be used to enter the unchanging headers for each of the text lines to be included in the data files.

Options Specify parameters that will apply to all experiments such as adaptation period, ventilation fan duration, rise/fall duration of tone, etc. These parameters may be changed at any time.

Print Exp File A feature for providing a hard copy of experiment files that have been created and stored.

Quit Selection of this option causes an exit from the Acoustic Startle System software.

OPTIONS

When you choose Options from the main menu, the Options window will be displayed. Select Options to set or modify the following parameters. The options you select will remain constant for every session unless the parameters are changed.

CI Acoustic Startle 4 - Options

ADAPTATION PERIOD (sec)
 YES NO

POST EXP ADAPTATION (sec)

SAVE RESPONSE WAVEFORMS
 YES NO

PLATFORM CONFIGURATION
 1 TO 4 PLATFORMS 5 TO 8 PLATFORMS

SHAPING SWITCH SETTING
 0 5 10 25 50

VENTILATION FAN
 ALWAYS ON INTERMITTENT NOT IN USE

PER CENT OF ITI
Minimum
Maximum

INTERFACE BOARD
BOARD TYPE
BOARD NUMBER

Cancel OK

Adaptation Period: The first option deals with the adaptation period – the period of time between the start of the experiment and the start of the first trial. If no adaptation period is to be used, click the NO option, and the duration entry window will be disabled. If an adaptation period is to be included, click the YES option and enter the duration of the adaptation period (in seconds) in the data entry box.

Post Exp Adaptation: This determines the period of time between the last trial and the termination of the session. If you wish the session to terminate after the last trial, enter 0 (zero). Otherwise, enter a value in seconds, for the duration of the Post Experiment Adaptation period.

Save Response Waveforms: This option deals with whether or not response waveforms will be saved for later viewing. Summary data files, that include the trial number, trial type, peak response, and latency to peak response, are always recorded. You may also wish to record, for later viewing, the response waveform of each subject for every trial. To View Data after the experiment, click “YES”. Note that the View Data option requires the response waveforms.

Platform Configuration: If your system consists of one A10-21 Acoustic Startle Controller then the “1 TO 4 PLATFORMS” option must be chosen. If you are running more than four subjects simultaneously and your system consists of two A10-21 Acoustic Startle Controllers then the “4 TO 8 PLATFORMS” must be selected. In either case, it is not necessary that you run all four or all eight subjects in a session but it is necessary to identify the number of stations that could be run based on the number of controllers that are connected.

Shaping Switch Setting: Select the value (0, 5, 10, 25, or 50) that is set on the A10-21 Acoustic Startle Controllers Shaping Switch. This is the value, in milliseconds, of the Rise and Fall times when tone is presented. This does not apply to the noise stimulus. If tone is not being used, the setting chosen here is irrelevant. It is important to note that if change this setting on the Controller, you must change the value here to match the setting on the Controller. It is also important that the duration of the tone pulse must be equal to or greater than the Rise/Fall time set on the Shaping Switch. If the duration of the tone is less than the value set on the Shaping Switch, the amplitude of the tone stimulus will not reach the full output.

Ventilation Fan: The final option deals with the use of the ventilation fan in the Startle Chamber(s). The prime consideration when the fan is used is that neither its onset nor offset serve as a cue with regard to the upcoming trial. If the fan is “ALWAYS ON” or “NOT IN USE”, this problem is avoided.

If, however, the fan is used intermittently, especially in the case of invariant inter-trial intervals (ITI), care must be taken to avoid a predictable relationship between the fan activity and the startle stimuli. For intermittent fan use, the fan is turned on following stimulus presentation (i.e., at the onset of the ITI). The per cent of the inter-trial interval for which the fan remains on is determined by the entries in the PER CENT OF ITI data entry area. For each trial, a random value between these set values will be chosen for the fan to be turned off. In this way, the fan is turned off and on randomly with respect to the stimulus presentation even in cases in which the ITI is fixed.

Interface Board: No entries are required here. The Interface Board must be installed prior to running the Startle Software. The board types are PCI if using a desktop computer or PCMCIA if using a laptop computer.

Prior to exiting this window, click the OK button to save the options.

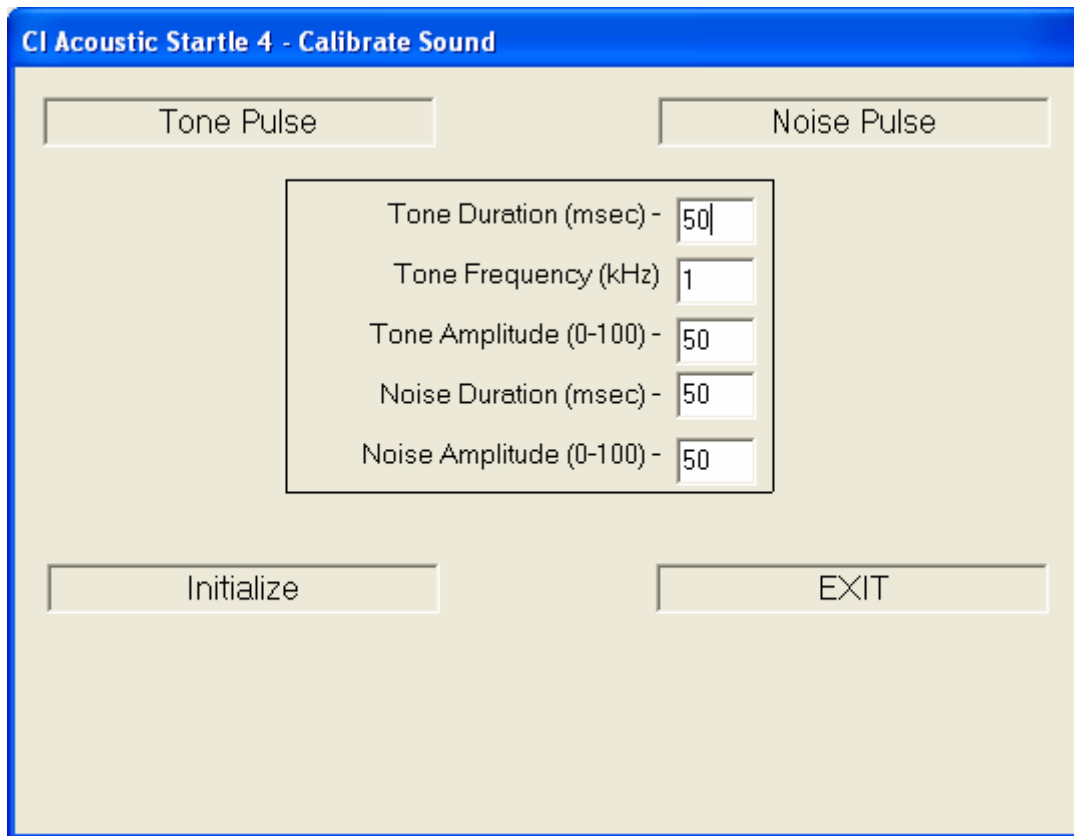
CALIBRATE SOUND

The Calibrate Sound option provides a fast and easy method for presenting tone or noise stimuli for the purpose of calibrating the amplitude. This is necessary to determine the Sound Pressure Levels (SPL) that will be presented based on the amplitude value entries used in the software. When recording SPL, it is important to understand the measurement and the parameters that will affect the measurement. For example, moving the recording transducer as little as one inch can greatly affect the SPL measurement. Extraneous noise and vibrations will also affect the SPL. It is important that the environment be as conducive as possible to recording SPL during both the calibration and the experimental sessions.

SPL is a logarithmic function. The voltages that control the amplitude are linear functions. Therefore, it will require smaller increases in the amplitude values entered in the software to increase the SPL by one decibel (1 dB) at lower amplitudes while larger increases will be necessary at higher amplitudes for a 1 dB increase.

With this switch in the high range, amplitudes from approximately 75 dB to 125 dB can be generated.

As you calibrate the audio signals, make note of the duration, attenuator and frequency multiplier settings. These values will be required when stimulus specifications are entered into experiment files. With this switch in the high range, amplitudes from approximately 75 dB to 125 dB can be generated.



Hardware Considerations with Auditory Stimuli

On the rear panel of the A10-21 Controller, there is a gain switch labeled "High Gain" and "Low Gain". The position of this switch is not changed during a session. Therefore it is important that this switch be set to the same position for calibration noise and all of the frequencies that will be used in an experimental session. The amplitudes of both tone and noise will be affected by the position of this switch. The same

software entries for amplitude will produce very different results when this switch is moved from high gain to low gain or vice versa. Although your results will vary, noise amplitudes from approximately 65 dB to 110 dB can be reached with this switch set in the low range.

Each chamber has a safety switch. This switch is located on the left side of the doorframe of the single subject chamber and on the front of the lid frame on the four subject acoustic chamber. This switch is to protect against unintentional presentations of a loud pulse while the chamber door is open. If it is not possible to place the recording transducer in the chamber with the door closed in the single subject acoustic chamber or the lid closed in the four channel acoustic chamber while calibrating the sound, it will be necessary to press this switch when generating a stimulus. Take precautions to protect yourself from potential hearing damage when generating loud stimuli.

If you wish to take readings in more than one chamber, the audio output on the rear panel of the A10-21 Controller from each preceding channel output must be connected to a startle chamber. That is, if you are using startle chamber 3, the audio outputs of channels 1 and 2 as well as channel 3 must all be connected to a startle chamber. If using the four subject acoustic chamber, you must use the channel 1 output.

Calibrate Tone

When calibrating tones, it is recommended to work with one frequency at a time. For example, if you plan on using frequencies of 1000, 2500, and 5000 Hz, calibrate the amplitude settings for the 1000 Hz tone first, then the 2500 Hz, and then the 5000 Hz tone.

To calibrate the tone you will need to enter the duration, frequency in kilohertz (KHz), and amplitude. Be sure you enter the frequency in KHz and not Hertz. For example, if you wish to generate a frequency of 3,500 Hz (3.5 KHz), the correct entry is 3.5. Once the tone duration and frequency are entered, enter a value for the amplitude. Valid amplitude entries are 0 to 100 in 0.01 increments. With the mouse cursor positioned over "Noise Pulse", left click the mouse. Make sure the switch on the single subject startle chamber doorframe (lid frame of the four channel acoustic chamber) is pressed or the door is closed. It may be necessary to enter a few other amplitude settings before finding the range you are seeking.

Once you have determined all of the values needed to produce the amplitudes at a specific frequency, enter the next frequency, in KHz, and begin the calibration process again. Once the calibration values for all of the frequencies you wish to use have been determined, you can proceed with calibrating noise.

Calibrate Noise

Unlike tone, there is no frequency entry required for noise calibration and therefore there is only one set of calibration values that will need to be generated. Enter the duration and amplitude and be sure the gain switch on the rear of A10-21 Controller is in the desired position. Again, the switch on the left side of the doorframe on the single subject acoustic chamber or the front of the lid frame on the four subject chamber must be depressed. Place the mouse cursor on the "Noise Pulse" in the window and left click the mouse. Continue this process until all the values for all of the amplitudes you intend to use have been determined.\

CALIBRATE PLATFORMS

When this program is selected, a screen entitled *Platform Calibration (Step 1)* will be displayed. The total number of platforms selected in the *Options* menu (i.e., PLATFORM CONFIGURATION) will be enabled. Indicate which platforms are to be calibrated by clicking the appropriate check boxes. Click *Proceed* when platform selection is complete.

CI Acoustic Startle 4 - Platform Calibration (Step 1)

Number of Stations: 4

Station	Calibrate?	Station	Calibrate?
1	<input checked="" type="checkbox"/> Yes	5	<input type="checkbox"/> No
2	<input checked="" type="checkbox"/> Yes	6	<input type="checkbox"/> No
3	<input type="checkbox"/> No	7	<input type="checkbox"/> No
4	<input type="checkbox"/> No	8	<input type="checkbox"/> No

Proceed Cancel

If 5 to 8 platforms are in use, all platform numbers will be accessible. Click on the boxes next to the station numbers of the platforms that will be calibrated. A mark will be placed in the box and the word “No” will be replaced by the word “Yes”, indicating this platform will be calibrated.

Note: If a calibration file already exists and platforms have been added or replaced, check only the boxes for the added or exchanged platforms. There is no need to recalibrate those for which calibration data remain current.

Click *Proceed* when platform selection is complete.

The next screen, entitled *Platform Calibration (Step 2)* allows the user to enter the values for the calibration weights that will be used during platform calibration. At least two (2) weights are required, and one (1) of these may be “0.” Enter the values in ascending order. Consideration must be given to the range of the platform as well as whether or not the animal holders will be placed on the platform(s) during calibration. In no case should the total weight of any one calibration weight being used exceed the range of the platform.

CI Acoustic Startle 4 - Platform Calibration (Step 2)

CALIBRATION WEIGHTS

Weight Number	Weight in Grams	Weight Number	Weight in Grams
1	<input type="text"/>	6	<input type="text"/>
2	<input type="text"/>	7	<input type="text"/>
3	<input type="text"/>	8	<input type="text"/>
4	<input type="text"/>	9	<input type="text"/>
5	<input type="text"/>	10	<input type="text"/>

Proceed Cancel

When this process is completed, click *Proceed*.

Before proceeding there are hardware settings that need to be addressed. It is important that certain parameters on the A10-21 Controller(s) are set appropriately and noted.

1. During platform calibration, the Mode switch must be set to Run/Cal. In this position, the couplers for each station are DC coupled, allowing the platforms to be calibrated with a static weight. During a session, you may wish to set the Mode switch to Run (AC Coupled). This will be discussed later in the Running a Session section of this manual.
2. The Sensitivity/Range Switches for each channel must be set to the same position. It is typically recommended that Position E (gain of 1000) be used. This is especially true if the sessions will be run with the Mode Switch in the Run position. However, if you intend to run sessions with the Mode switch in the Run / Cal position then you may want to use range D (gain of 500) or even C (gain of 266). Refer to the A10-21 CONTROLLER – FRONT PANEL CONTROLS section of this manual for a complete listing of the gains.
3. When calibrating more than one platform, complete the calibration process with each weight at one station before proceeding to the next station. Set the Channel Select switch of the A10-21 Controller to Station 1. At this point the platform can be “zero balanced” by turning the balance control until the meter reads zero or near zero. When using 0 grams as the initial calibration weight, the meter will be helpful.
4. If you plan to run sessions with the Mode switch in the Run / Cal mode, you should zero balance the platform with the animal holder sitting on the platform. This will effectively subtract the weight of the platform from the response.

Once all of the settings have been addressed on the A10-21 Controller, select Proceed to advance to Step 3 of the Platform Calibration Process.

CI Acoustic Startle 4 - Platform Calibration (Step 3)

		STATION							
WEIGHT	1	2	3	4	5	6	7	8	
0	X	X							
50	X	X							
100	X	X							
200	X	X							
400	X	X							
SLOPE	0.01000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000	
INTCPT	0.08000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	
ZERO	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	
		Fit Lines		OK		Cancel			

The weights that were entered during Step 2 of the Calibration Process will be displayed in this window. If you have chosen to use zero weight as the first value, make sure that the meter is at or near zero and then click on the “X” in first row of Station 1. The voltage value that is recorded will be displayed. If you want to make any adjustments to the zero balance (assuming you have entered 0 grams as the first value), adjust the balance control of channel 1 and click in this same box again.

Place the first weight on the platform or in the animal holder if calibrating with the animal holder and using the mouse cursor, click in the appropriate box. Continue this process until all weights for station 1 have been used. Proceed to the next station. Once all of the stations have been calibrated, select the Fit Lines.

If at any time you wish to start over, you can double click on the red, Zero bar below the appropriate station to return the values to zero and begin again.

Once all of the platforms have been calibrated and the lines and slopes have been calculated, select OK to end the Platform Calibration program.

CREATE NOTES TEMPLATE

Choosing this option will display a screen with ten numbered data entry areas. Each of these corresponds to one of the text lines that may be included in the data file. If a constant type of text is to be added to all data files, the template may be used to enter the constant text. If, for example, the first line will always contain the date, the first template line might contain the text Date: ; this will serve as a prompt for date entry when the Data Notes option is used. Each line may be annotated in this way; some or all of the text lines may be left blank.

CI Acoustic Startle 4 - Make Template

1 Date:

2 Eperimenter ID:

3

4

5

6

7

8

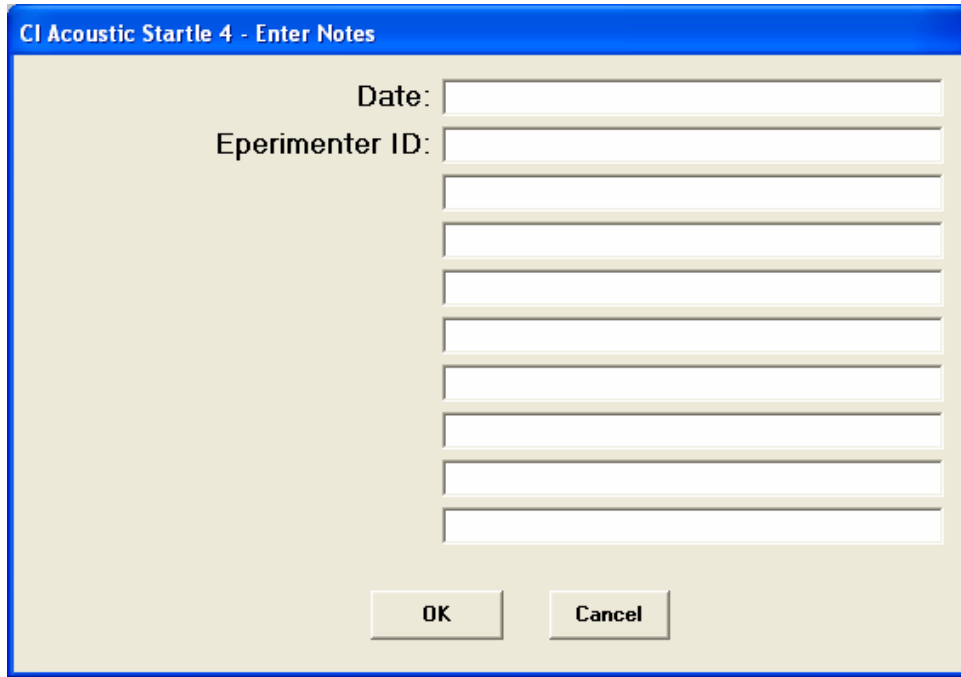
9

10

OK Cancel

DATA FILE NOTES

Choose Data File Notes to enter up to 10 lines of text data into the data file. If the Notes Template has been created, these entries will appear to the left of the line entries in this window. Any data entered in this window will be included in the data file when a session is run. Be sure to update these entries or delete the information before starting a new session.

The image shows a software dialog box titled "CI Acoustic Startle 4 - Enter Notes". The dialog has a blue title bar. Inside, there are two labels on the left: "Date:" and "Experimenter ID:". To the right of "Date:" is a single-line text input field. To the right of "Experimenter ID:" is a single-line text input field. Below these are ten stacked, empty text input fields, each with a thin border and a light gray background. At the bottom of the dialog are two buttons: "OK" and "Cancel".

When the text is written to the data file, both the label and the text entered via this option will be written on the same line. If, for example, Date: was written as the label for the first line and Experimenter ID: was used for the second line, these labels will appear to the left of the first data entry line in the Data File Notes entry screen. If 3/20/98 is entered as the first notes line and CI was entered on the second line, the first two lines of the data file will read as follows:

*Date: 3/20/98

*Experimenter ID: CI

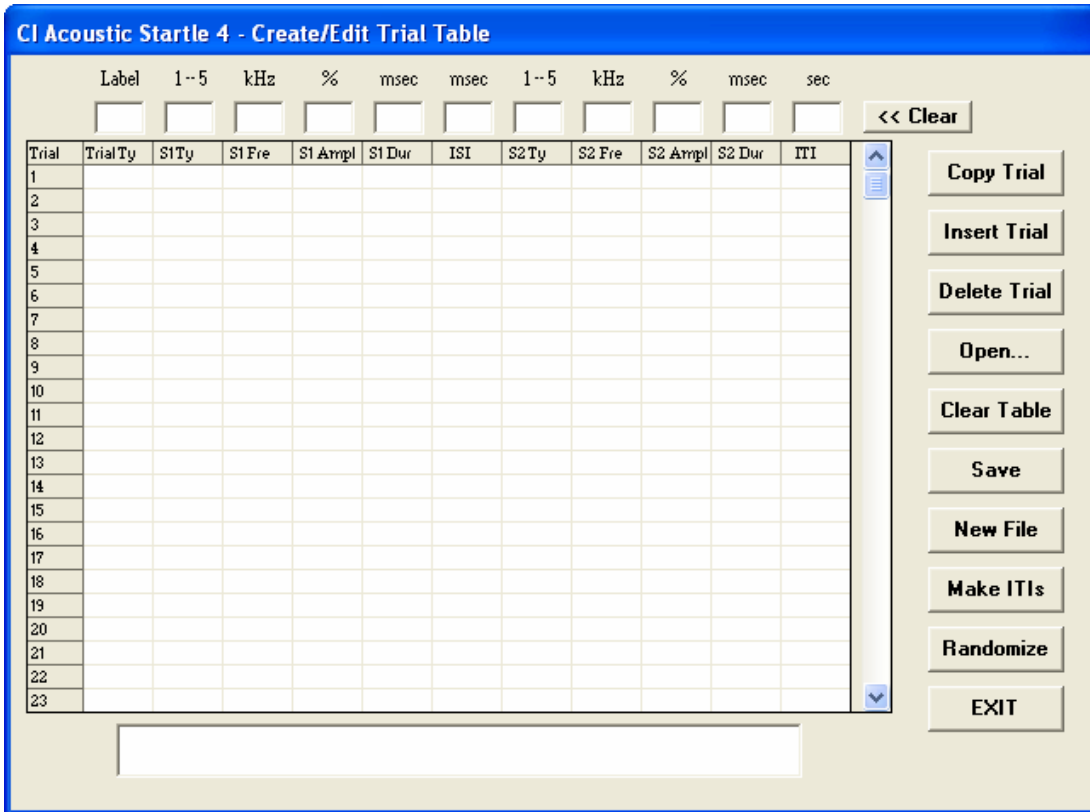
Note that each of the first ten lines of the data file will begin with an asterisk to make identification of the text header lines easier.

Click on OK to save the file notes.

CREATE / EDIT TRIAL PARAMETERS

The first option in the main menu, Create/Edit Trial Parameters, is used to create, edit, and store experiment files. When this option is selected, the program will display a window containing a central grid, an upper text-entry row, and 11 control buttons.

The values for each trial are entered in the text boxes at the top of the window. Each of the boxes corresponds to the label just below it in the grid. The meanings of these labels are as follows:



Note: The stimulus type (S1Ty and S2Ty) is identified using numbers 1 though 5. The stimulus is specified as follows:

Number Entry	Stimulus Type
1.	Tone
2.	White Noise
3.	Stim 1 (-28 Volt Output on Rear Panel of A10-21 Controller)
4.	Stim 2 (-28 Volt Output on Rear Panel of A10-21 Controller)
5.	Stim 1 and Stim 2 simultaneously)

Trial Ty (trial type) - A number used only as an identifier so that different types of trials may be separated for analysis.

S1 Ty (stimulus type) - This value determines if the first stimulus (prepulse) will be tone, white noise, one of the auxiliary outputs, for controlling other stimuli such as shock, or both of the auxiliary outputs. This entry can be left blank if a prepulse stimulus is not being presented.

S1 Fre (stimulus frequency) - If the first stimulus is tone, enter the frequency in KHz. If pre-pulse stimulus is noise, any combination of the auxiliary stimuli, or not used, this can be left blank.

S1 Ampl (stimulus amplitude) - Enter the value from 0.00 to 100.00 to be used to determine the amplitude of the first stimulus. If stimulus “3”, “4”, or “5” is selected, or the prepulse is not used, this can be left blank.

S1 Dur (stimulus duration) - The duration of the first stimulus, in milliseconds (msec), is entered here. The maximum allowable duration is 200 msec; any entry longer than this will be converted to 200 msec. If the prepulse stimulus is not used, leave this entry blank.

ISI (inter-stimulus interval) - This value represents the duration, in milliseconds, of the interval separating the offset of the first stimulus (pre-pulse) to the onset of startle (or second) stimulus. No entry is required if prepulse is not being used.

S2 Ty (stimulus type) - This value determines if the second stimulus (startle) will be tone “1”, white noise “2”, one of the auxiliary outputs, “3” or “4” for controlling other stimuli such as shock, or both of the auxiliary outputs “5”. An S2 stimulus is always required. If you wish to run a trial without any stimulus, use S2 Ty “3” or “4” whichever is not being used.

S2 Fre (stimulus frequency) - If second stimulus is tone, the frequency in KHz must be entered. If startle stimulus is noise or a stimulus controlled by one of the auxiliary outputs this may be left blank.

S2 Ampl (stimulus amplitude) - Enter the value from 0.00 to 100.00 to be used to determine the amplitude of the second stimulus. If stimulus “3”, “4”, or “5” is selected, or the prepulse is not used, this can be left blank.

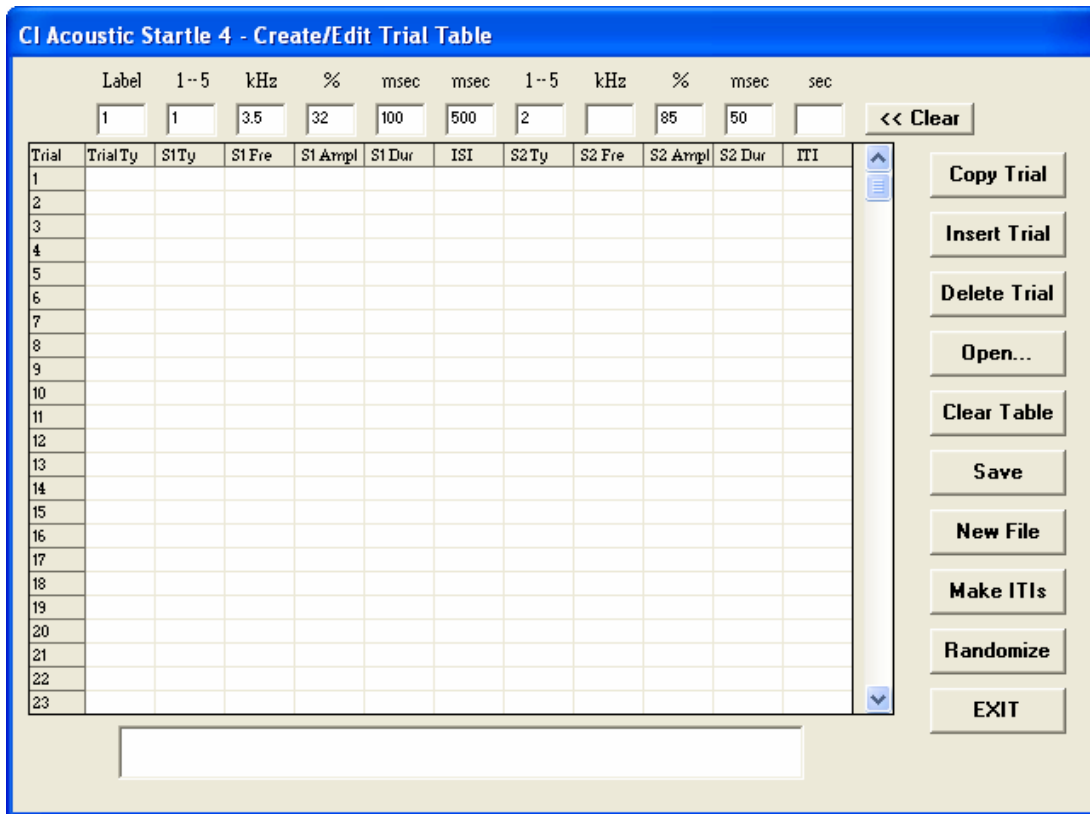
S2 Dur (stimulus duration) - This specifies the duration, in milliseconds, of the second stimulus. The maximum allowable duration is 200 msec; any entry longer than this will be converted to 200 msec.

ITI (inter-trial interval) Enter the duration, in seconds, of the interval separating the end of trial n to the start of trial n+1. It is not necessary to enter ITI values if you wish to use random ITI.

As an exercise, we will create an experiment consisting of three trial types. Trial 1 will be a pre-pulse trial consisting of a tone of 100 msec duration, a frequency of 1.5 KHz, an amplitude of 32%, followed by a white noise 50 msec in duration, an amplitude of 85%, with a 500 msec interval in between the tone and the white noise stimulus. You can use the Tab key or place the mouse cursor in the desired parameter box to advance from column to column to make each entry.

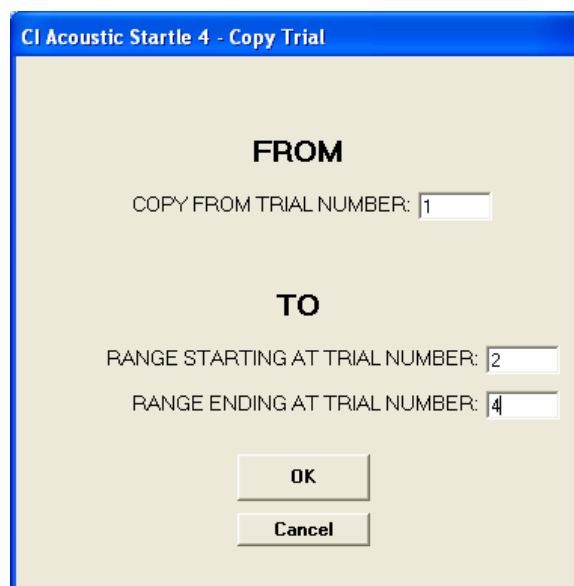
In our example, we will create an experiment in which the three trial types will be presented in blocks of four trials each. Trial Type 1 will be presented on trials 1 through 4, Trial Type 2 on trials 5 through 8 and Trial Type 3 on trials 9 through 12.

With the values for Trial Type 1 showing in the entry row, click on any box in the first row of the grid. This will copy the values from the entry row to the grid; these then become the values for Trial 1.



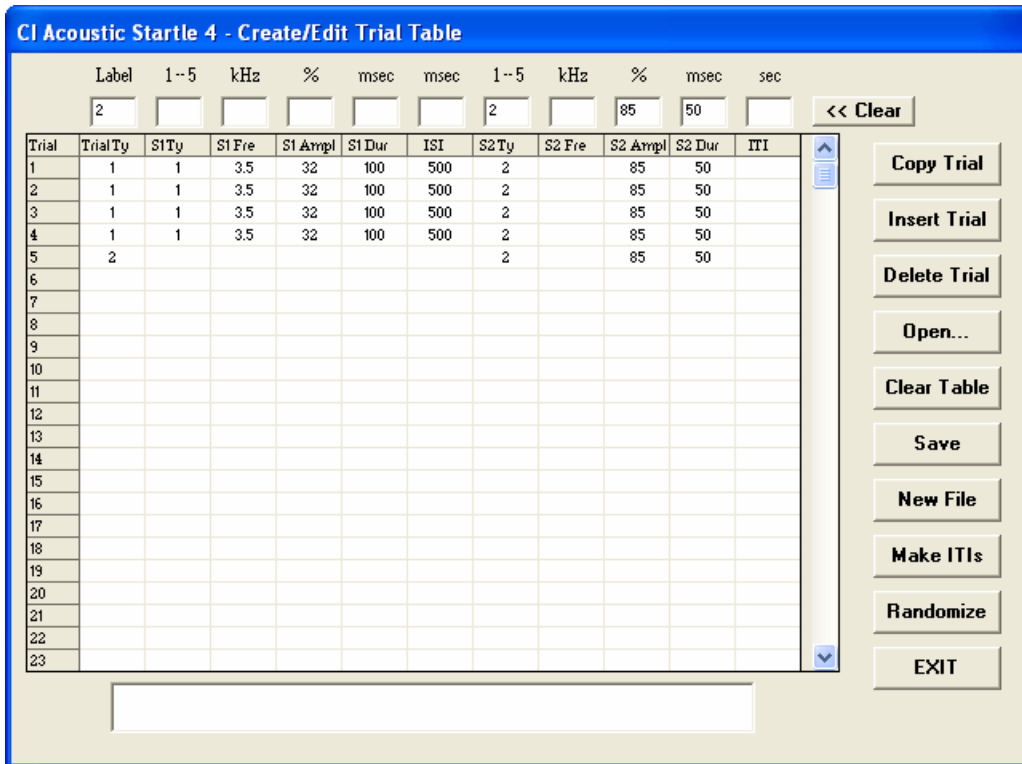
Two methods are available for creating trials 2 through 4. Clicking on each of the appropriate rows will replicate Trial 1 since the values are still showing in the entry row. Trials (data in the grid) may be copied by using the Copy Trial function.

When the Copy Trial function is clicked, a window will open with entry areas for the trial to be copied from and the range of trials to which the data are to be copied. In our present example, we would enter “1” as the trial to be copied from, “2” as the start of the range and “4” as the end of the range.



Trial Type 2 will contain only noise as the Startle Stimulus and we will use the same values for this stimulus as in Trial 1.

Begin by clicking on the << Clear button to clear the entry row. Then enter the values for stimulus 2; since there is no stimulus 1, all entries referring to this stimulus, as well as the ISI, may be left blank or specified as 0.



Either method described above may be used to copy these data into trials 5 through 8.

Finally, Trial Type 3 will consist of two noise presentations. The first stimulus will be a noise at 40% amplitude. The stimulus will be 75 msec long and will be separated from the second stimulus by 250 msec. The second stimulus will be the same noise used in Trials 1 and 2.

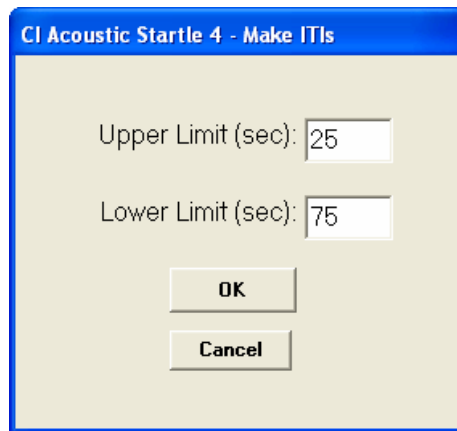
Without clearing the entry row, change the first entry to 3 and make the following entries in columns 2 through 6

2 0 30 75 250

Now use these values to create trials 9 through 12.

The final step is to create the intertrial interval (ITI) duration for each trial. This could have been done by entering the desired values in the ITI column in the same manner as the other parameters were entered. Or we can have the program automatically generate and enter or simply enter the ITI for each trial

Select the Make ITIs button to set the inter-trial intervals for ALL trials in the grid. Click on this button and enter the upper and lower limits for the intervals. If all ITIs are to be the same, use the same value as the upper and lower limits. If the upper and lower limits are different, the program will select random values within the bounds specified and fill in the ITI column of the grid. Any entry may be changed manually via the data entry row. The minimum ITI value for any trial is 12 seconds.



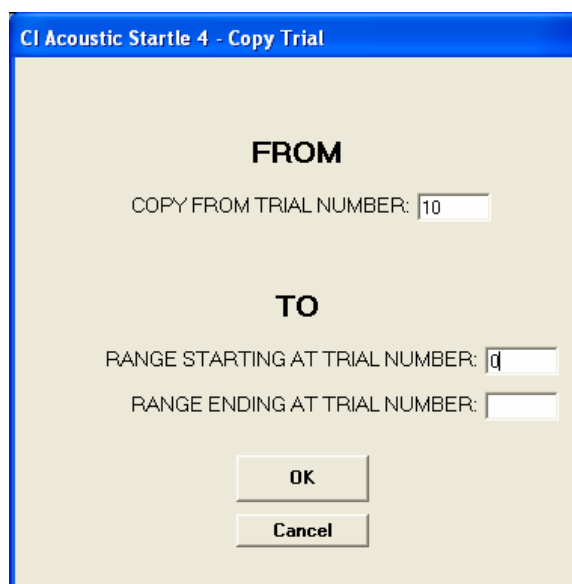
This completes the table for the experiment and it may be saved as a disk file by selecting New File. Do not include an extension. The program will add the extension, "exp", for purposes of identification.

Click on the Clear Table button. The program will ask for verification that the table is to be cleared; click on OK. The remainder of this exercise will consist of making modifications to the first experiment file.

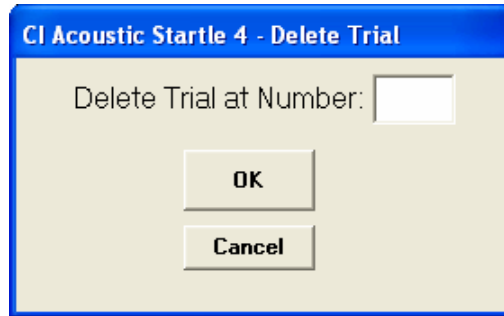
To begin, open the file by clicking on the Open button and selecting the experiment file. If we wanted to add a trial of Type 3 between the current Trials 2 and 3, we first need to make space for the additional trial. Click on the Insert Trial button, and enter '3' as the location for the new trial.



The grid is changed so that a blank trial now appears at Trial 3. To complete the task, we will use another feature of the Copy Trial option. Click on Copy Trial and indicate that the data from Trial 10 should be copied to Trial 0 (specify only that '0' is the starting range). The data from Trial 10 are copied into the data entry row. We may now click on the blank row of the grid to insert the data into Trial 3. This method is easier to use when a particular trial type is to be added in many places in an already existing experiment file.



The Delete Trial button is used to delete a trial from the grid. Click on the button and indicate which trial is to be deleted.



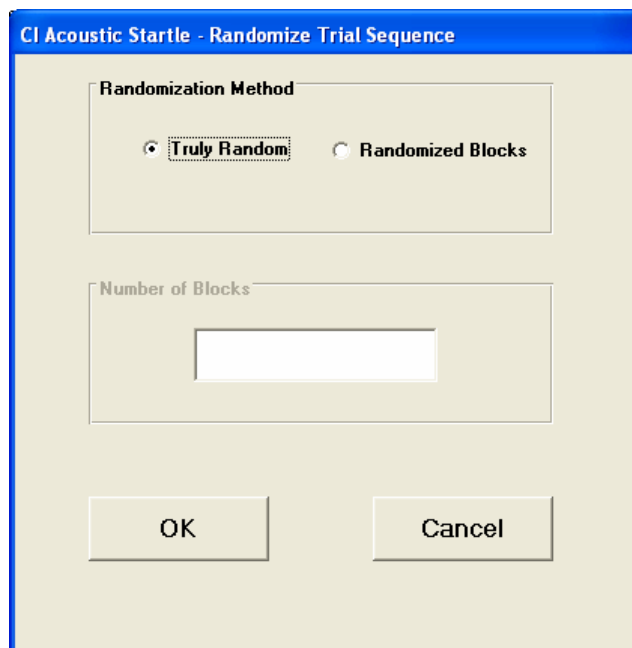
When only the ITI needs to be changed, it is easiest to first copy the trial data into the data entry row, make the change to the ITI, and then copy the data back to the trial row in the grid.

The exercise in creating an experiment was used to introduce you to the various features and tools that are available to you when creating protocols. Another feature available for creating experimental protocols is the Randomize option. This option offers two features. You can use this feature randomize all of the trials that have been entered or to create a random block.

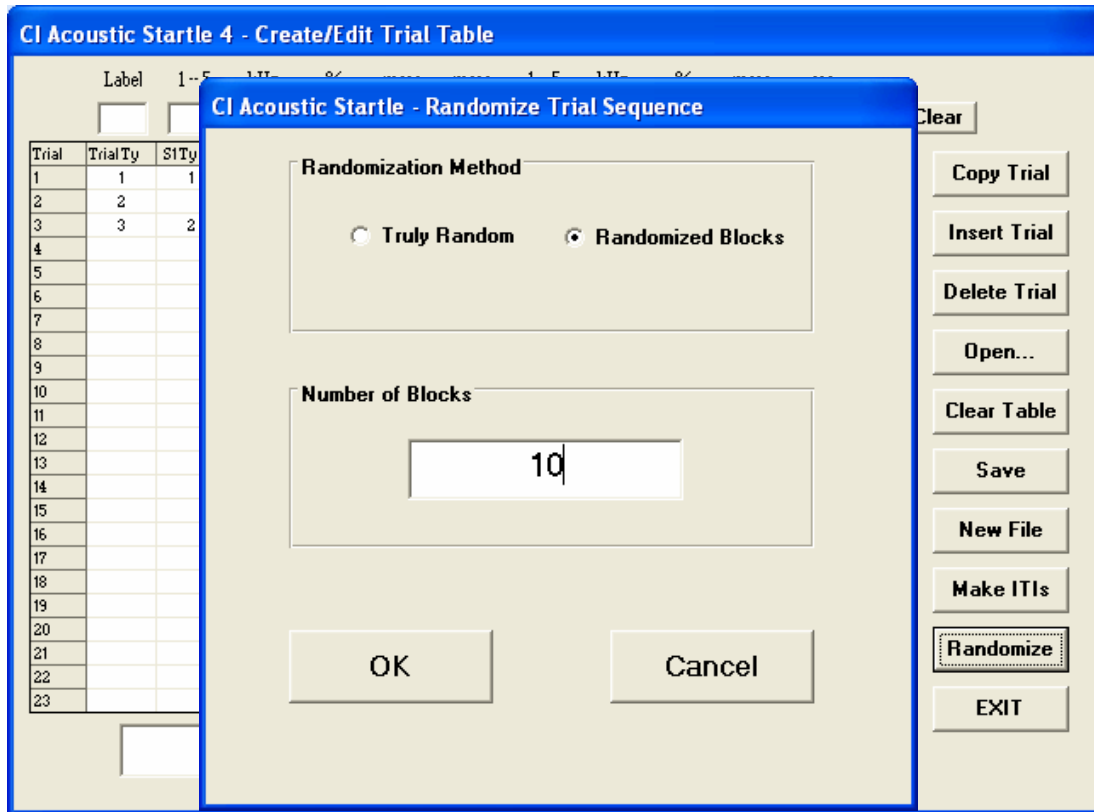
Suppose you have four different trial types that you wish to use but not all of the trials will be used an equal number of times. If there are 75 trials in your session, it can be quite time consuming to enter the first trial, make the changes then enter the second trial, make changes and enter the third trial, etc. We will create an experiment consisting of four trial types such that trial type 1 will be presented 24 times, trial type 2 will be used 20 times, trial type 3 will be presented 15 times and trial type 4 will be used 16 times.

Enter the parameters for trial type one, place it into trial one and using the copy method, copy trial one into trials 2 through 24. Now select the clear button, create trial type 2 and enter this into trial 25. Now copy trial 25 into trials 26 through 44 for a total of 20 more trials. Follow the same method for trial type 3, entering it into trial type 45 and copying it into trial 46 through 59 and finally, create and enter trial type 4 into trial 60 and then copy it to trials 61 through 75.

Now select the Randomize button and choose the Truly Random option and then select the OK button. All 75 trials will be randomized by the program. If you wish to make any changes you can use the Randomize feature again or move trials as described earlier by inserting trials and deleting trials.



The Randomize feature can also be used to create protocols using a Random Block design where an equal number of each trial type will be presented. In this case, you need to simply create and enter one of each trial type into the table, select Randomize, and choose the Randomize Blocks option. For example, suppose there are three trial types and you wish to present each trial 10 times (for a total of 30 trials) such that each trial type will be presented once, randomly, before any trial is presented a second time. Enter one of each trial type into the first three rows. If every presentation of a trial type is to include the same ITI duration then enter the ITI. If you wish to randomize the ITI do not enter the ITI. Once each trial type has been entered, choose Randomize and enter 10 in the Number of Blocks entry box in this window and the program will generate a random block design.

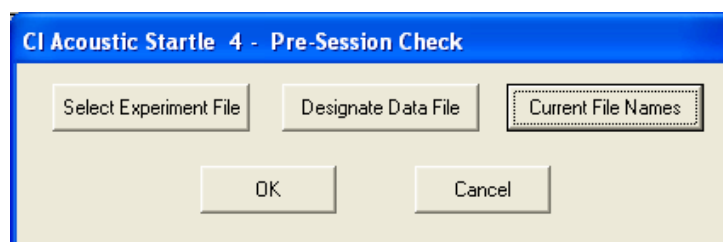


If you did not enter the ITI, select Make ITIs to generate the ITI for each trial.

If you wish to make this protocol completely random then choose the Randomization option again and select Truly Random.

RUNNING A SESSION

Clicking on the Run Session button displays a window in which the user specifies the files to be used during the session. These include the experiment file containing the trial by trial specifications and the root name for the data files to be created.



Click on the Select Experiment File button to see a list of the experiment files that are available. When a file is selected, its contents will be displayed in a grid for verification. Click on OK to complete file selection.

Click on Designate Data File to see a list of the data files which can be located. The file name display is presented in two windows. The left window shows the numeric data files (*.DAT extensions) and the right window shows the raw analog data files (*.A2D extensions). The contents of these files will be discussed below. Enter the root name only. Do not include an extension in the text box and then click on OK. The program will not allow the use of a duplicate file name for either file type.

To review the selections, click on the Current File Names button. If any selections are incorrect, use the appropriate button to modify the selection. When all selections are correct, click on OK in the pre-session window.

The experiment monitor window contains an area for the display of the trial by trial data as they are collected, and buttons to control the start and abort functions. When the BEGIN button is clicked, the experiment begins with the adaptation period if one has been scheduled. An on-screen counter will display the time remaining in the adaptation period. (If the ventilation fan has not been specified as NOT IN USE, it will be activated for the duration of the adaptation period.) Also displayed on the screen will be areas for the plotting of the response waveforms from each of the active platforms as well as display numeric data for the most recent trial for each active platform.

Following the adaptation period, the first ITI will begin. The fan will be controlled according to the settings specified in the Options screen. At the end of the ITI, the first set of stimuli will be presented and data will be collected from each active platform.

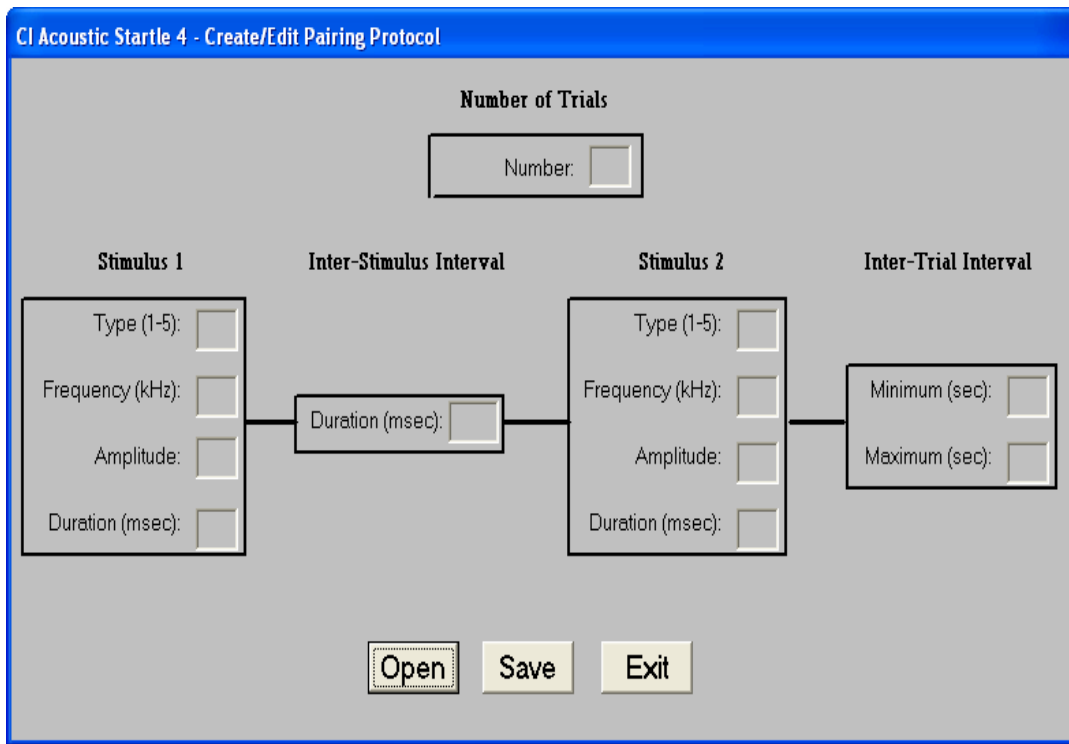
Data are collected for 200 msec following the onset of S2; waveforms are digitized at 1 KHz. For each platform, the data displayed on the screen will be the peak response (in grams) and the latency (in msec) to the peak response. These data will also be written to the data file. If the waveforms are saved, the 200 samples for each platform will be written to the analog data file. Complete descriptions of the data files are given on the following page.

Also presented on the screen is the number of trials remaining in the session, the number of trials completed and the time to the next trial. When all trials have been completed, DONE will be displayed in place of the Next Trial counter.

When the experiment is complete, click on EXIT to return to the main menu. An experiment may be aborted by using Ctrl-Alt F1 (hold down the Ctrl and Alt keys and press the F1 key). The experiment will be terminated immediately during an ITI or at the start of the next ITI when the ABORT button is clicked. Any data collected up to that point will be preserved. Note that ABORT causes an end of program execution and a return to the Windows desktop.

CREATING PAIRING PROTOCOLS

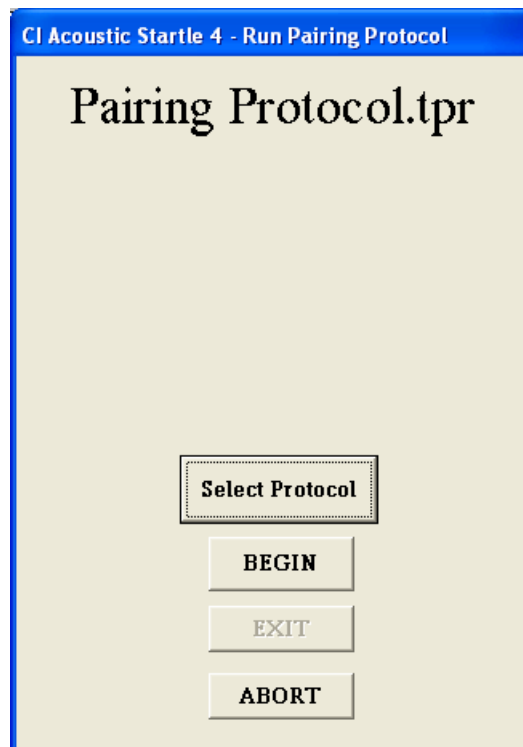
Click on the Create/Edit Pairing Protocols option to use this facility of the software. The screen which is displayed has entry points for the number of trials, S1 type (tone, noise, or user-defined types), inter-stimulus interval, S2 type (as above for S1), and inter-trial interval. (Note that the stimulus frequency and/or amplitude entries will be disabled when these parameters do not apply.)



When all entries are complete, use the Save button to write the specifications to a file. Enter only the root portion of the file name, as the program will append the extension “.tpr ” which identifies it as a pairing protocol file.

RUNNING PAIRING PROTOCOLS

The Run Pairing Protocol option is used to run the trials. Choose this option and then click on the Select Protocol button. Select the desired protocol from the displayed list and then click on the BEGIN button. The trials will be presented as scheduled with on-screen displays of the number of scheduled trials and the number of trials completed. When all trials have been presented, click on the EXIT button to return to the main menu.



DATA FILES

Numeric data are written to a file with a *.DAT extension; the root name of the file is the one provided by the user in the pre-run screen. A file with *.A2D as its extension will also be created if the waveforms are saved. Descriptions of these files follow.

The *.DAT files are text files that begin with ten lines, each of which begins with an asterisk. These are the text entries described above. If no text entries are made, ten lines consisting solely of the initial asterisks will be written.

The next line is a label: NUMBER OF PLATFORMS that is followed by a line which contains a single entry – either 4 or 8.

The next line is a label: CALIBRATION CONSTANTS that is followed by either 4 or 8 lines (depending upon the number of platforms) each of which contains a slope and intercept for a platform.

The next two lines are labels: SORTED BY TRIALS

PLATFORM, TRIAL NUMBER, TRIAL TYPE, PEAK, LATENCY that are followed by the trial-by-trial data. In this first data section, the data are presented based upon trial sequence. If there are four platforms, the first line will be for Platform 1, the second for Platform 2, the third for Platform 3, and the fourth for Platform 4. These four lines will contain the data for the first trial. Following these lines, there will be four lines for each trial. In the eight-platform case, there will be eight lines of data per trial. This sequence will be repeated until data for all trials have been written.

The next two lines are labels: SORTED BY PLATFORM

PLATFORM, TRIAL NUMBER, TRIAL TYPE, PEAK, LATENCY that are followed by the data for Platform 1 across trials, for Platform 2 across trials, and so on.

Here is an example of a partial data file for a session that included four subjects.

```
FILE NAME: Test.dat
*
*
*
*
*
*
*
*
*
*
NUMBER OF PLATFORMS
4
CALIBRATION CONSTANTS
.02321, .01133
.02112, .01333
.02435, .01317
.02237, .01245
SORTED BY TRIAL
TRIAL NUMBER, TRIAL TYPE, PEAK, LATENCY
1, 2, 145, 57
1, 2, 144, 62
1, 2, 135, 74
1, 2, 133, 68
2, 4, 161, 72
2, 4, 133, 65
```

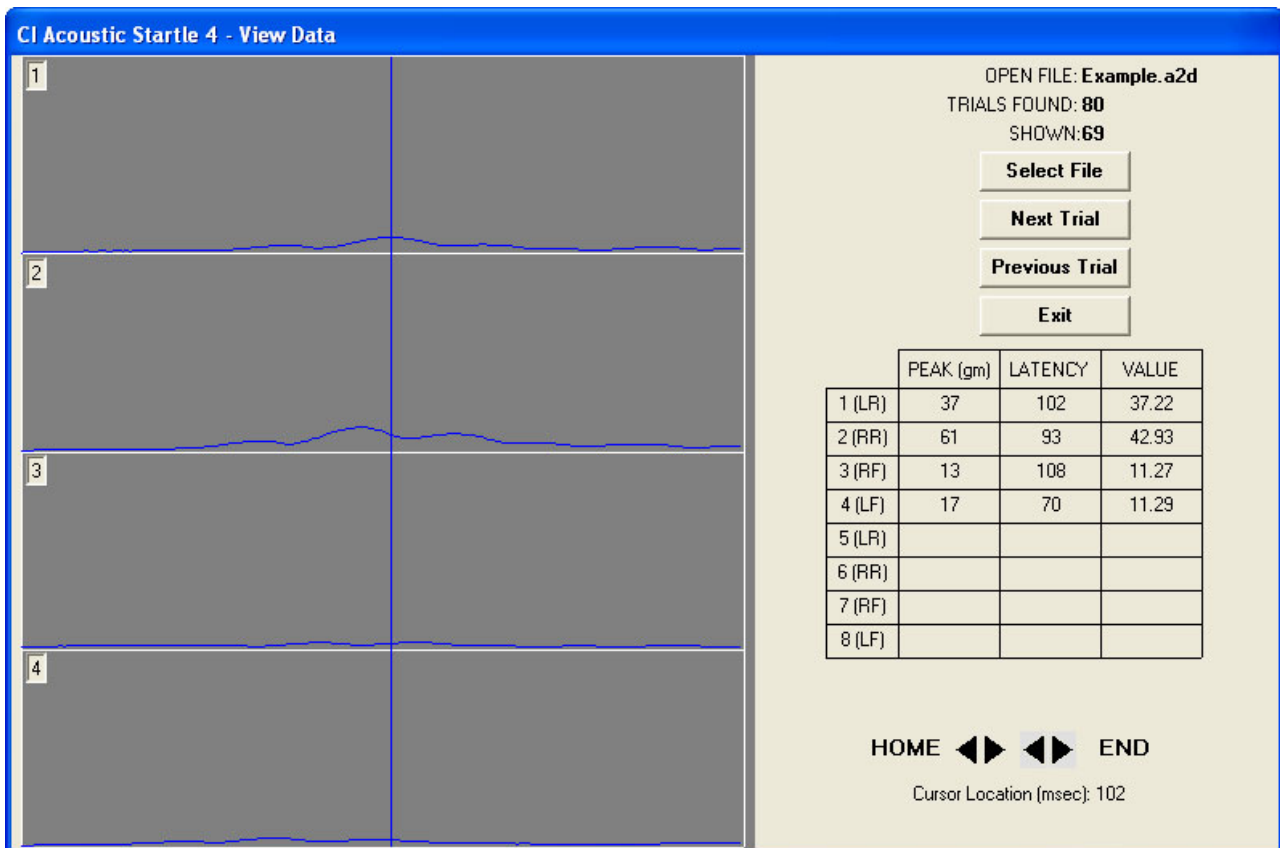
2,	4,	146,	81
2,	4,	112,	77
3,	1,	122,	125
3,	1,	122,	146
3,	1,	120,	131
3,	1,	127,	122

The A2D files begin with the number of platforms and the calibration values and then contain 200 samples per trial per platform. Inactive platforms are represented by 200 entries of 0. In order to keep the data file structure constant, each trial will contribute 800 samples when four platforms are in use and of 1600 samples in eight-platform systems.

VIEW DATA

The View Data option is used to review analog data files. The window displayed includes a Select File button. Click here to open a window showing the available A2D files. Select a file by clicking its name. The screen will show the name of the selected file and the number of trials in the file. Click the Next Trial button and the peak and latency data will be displayed. The current trial number will be shown in the SHOWN text box.

Use the Next Trial and Previous Trial buttons to move through the data file.



The displayed matrix shows the numeric data for the trial. For this reason, the DAT file corresponding to the A2D file being examined must be available to the program. Use the arrow keys next to HOME to move a cursor (a blue vertical line) through the displayed data one sample (1 msec) at a time. Use the arrow keys next to END to move the cursor 10 samples (10 msec) at a time. Notice as you move through the trial, the numbers in the Value column change, reflecting the data point, in grams, at the location of the cursor for

each subject. The time of the trial is also displayed just below the arrow keys and updated as the cursor is moved through the waveform. Double click on HOME or END to move the cursor to the beginning and end of the data respectively.

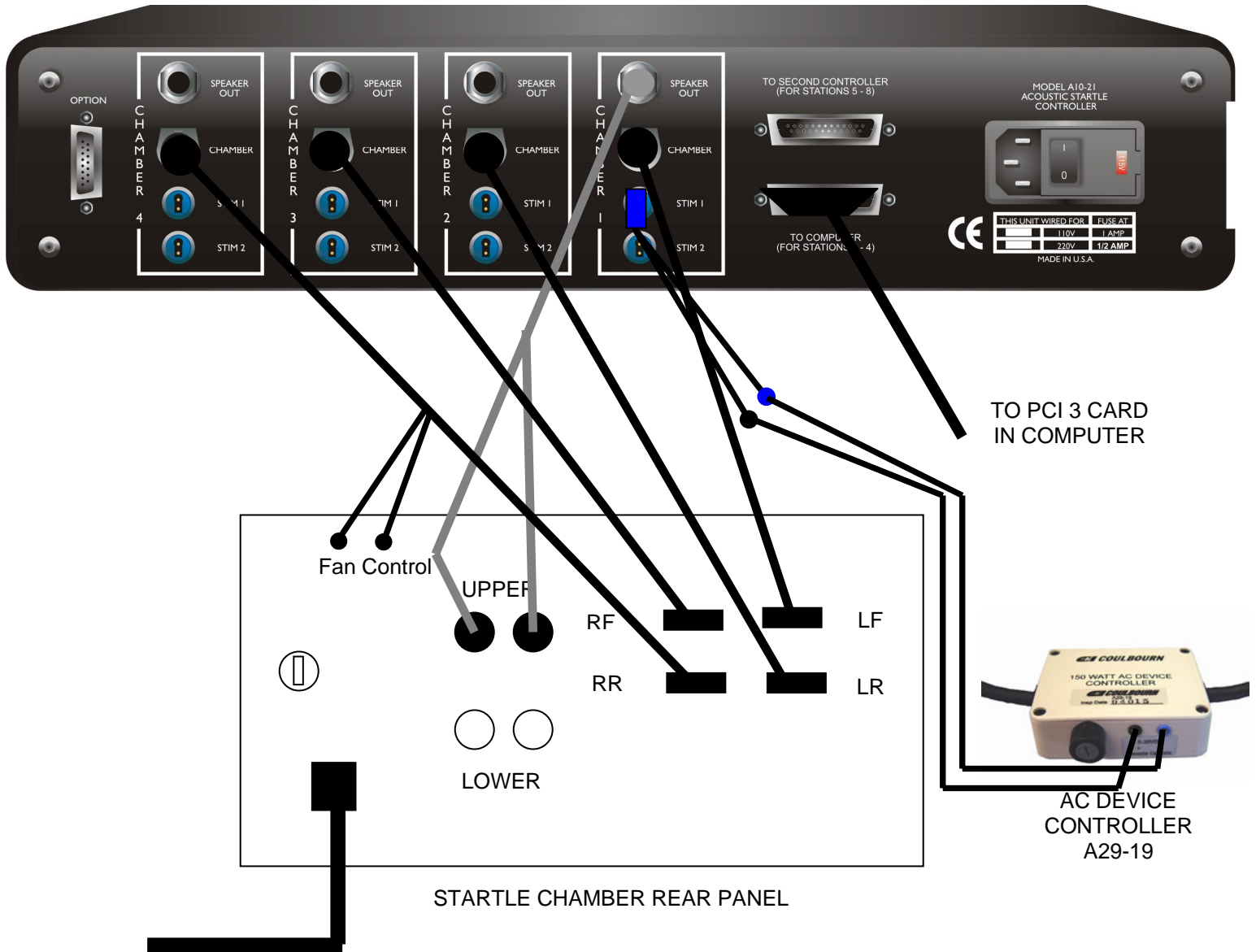
Click on the Exit button to return to the main menu.

SPECIFICATIONS

- Number of subjects: 1 to 8
- Audio source frequency range: 10 Hz to 99 kHz
- Audio amplitude range: 0 to 125 dB SPL
- Trial protocol: Intertrial Interval (ITI), random or fixed range from 10 to 65 seconds
- Pre-pulse and Pulse durations: 1 millisecond (msec) to 200 msec
- Startle transducer platform calibration: static load, computer automated
- Platform weight range: 20 gm to 2.2 Kg (higher ranges are available)

APPENDIX A

CONNECTIONS FOR A10-21 ACOUSTIC STARTLE CONTROLLER TO E10-24 FOUR SUBJECT ACOUSTIC STARTLE CHAMBER



MAIN POWER
TO WALL SOCKET

NOTES:

1. Speaker Output MUST be connected to Channel 1 of A10-21 Acoustic Startle Controller.
2. Platform connections between A10-21 Acoustic Startle Controller and E10-24 Acoustic Chamber can be made in any sequence. Be sure to note the location of the startle platform position in the chamber (i.e., RF, RR, LF, LR) and the Chamber Position (i.e., 1, 2, 3, or 4) to identify the station number by the location in the chamber.
3. "Stim" outputs on rear of controller are connected as needed to Shocker, Air Puff, Stimulus, Visual, etc. Selecting Stimulus 3 in the Acoustic Startle Software will activate the "Stim 1" outputs of all four chamber outputs simultaneously. Selecting Stimulus 4 in the Software activates "Stim 2" of all four chamber outputs simultaneously. Stimulus 5 in the software activates the "Stim 1" and "Stim 2" chamber outputs simultaneously.
4. The AC Power Cord of the Stimulus Light in the Chamber is plugged into the AC Device Controller and the AC Device Controller is plugged in the Mains 110 VAC or 220 VAC Source. Be sure the blue connector from the short cable is wired to the blue connector of the AC Device Controller.