

A Guide to ABR Testing with the System 3 RZ6



Updated: 5/24/19

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ABRs - from Set-up to Data Collection

Introduction

This guide is primarily intended for researchers who are new to collecting ABRs using a TDT RZ6/BioSigRZ system. It brings together targeted information from our software and hardware manuals and goes beyond product specific details to answer common questions, including subject or test area considerations that might affect your testing. The information provided is based on our knowledge of the TDT system and years of experience helping our users. It's not intended to favor or dictate any particular experimental protocols, methods, or research goals.

We leave the science up to you, but we're here to support you on your way to successful experiment completion.

Organization of the Guide

This guide begins with a general overview of ABRs; what they are, how they're used, and what type of response signal to expect. It discusses the various aspects of your experiment such as, test subjects, your test area, and the hardware and software you will use. It includes detailed information about important tasks including: running the ABR and viewing and exporting test results, system calibration, and diagnosing noise issues. Several indices are included to provide supplemental information.

ABR Overview

An ABR is a synchronous neural response to an auditory stimulus. ABR testing can be used to estimate hearing sensitivity. The ABR is an early potential, occurring shortly after stimulus onset, and is typically in the 1 – 2.5 microVolt range—so small that it can be masked by the background activity of the brain. Because ABRs are repeatable in response to stimuli, signal averaging can be used to clearly identify the response in recorded data.

A short click or tone stimulus is rapidly presented and response data is collected and averaged to reduce background noise and isolate the signal. Typically, four times as many sweeps averaged together will reduce the background noise by half.

When viewing ABR responses, you can expect to see an averaged waveform trace, consisting of multiple features with at least two distinct peaks. The response shape should be consistent, but can change in response to changes in the auditory stimulus, such as intensity or polarity. After averaging, the response should clearly stand out above the noise floor of the system.

You can expect to see a noise floor falling in the range of 80 – 120 nanovolts, after 512 averages. You can determine the noise floor in your environment by

running an ABR test without the subject present. This simple test is a good way to identify noise issues before you begin collecting data.



Identifying the Threshold

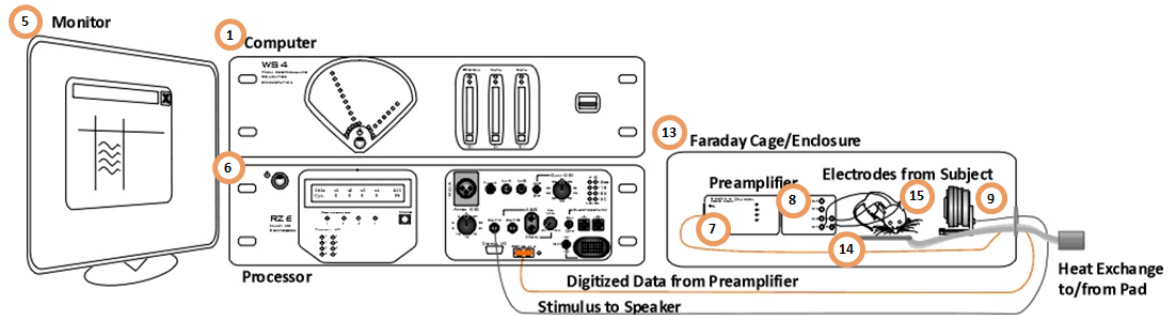
As the level of the stimulus is decreased, the amplitude of the response gets smaller and the latency gets longer—making features less distinct. The lowest intensity at which the response is present is generally considered the hearing threshold for the given set of variables. The determination of response or no response is left up to the researcher. Because noise and artifacts can mask the very small threshold response, quiet recordings are critical to accurate threshold testing.

ABR Recordings

ABRs are a useful and reliable tool for hearing screening. To get the clearest possible responses, you'll need to record using high fidelity equipment in a quiet environment. The subject must be anesthetized and kept warm to prevent movement or stress from affecting the response. Recordings should be performed in a shielded enclosure that has been set-up to minimize noise interferences.

The TDT system for ABR recording is designed to minimize noise at every step, beginning with an optically isolated, battery operated preamplifier that digitizes responses right in the enclosure. The processor (RZ6) brings together stimulus production and data collection on a single device and a single clock for precise timing. The system software for experiment control and data visualization is PC based and is more flexible than clinical systems while still easy to use.

The diagram below shows a typical ABR system. Each part of the system will be discussed in more detail as you read through this guide.



Typical System Using TDT's RZ6 Processor and Medusa Preamplifier

What you'll need:




1. Windows computer (*WS4 shown*)
2. Optibit interface (*installed in computer*)
3. TDT Drivers (*software installed on computer*)
4. BioSigRZ (*software installed on computer*)
5. Computer monitor
6. Multi I/O Processor with optic port (RZ6-A-P1)
7. 4- or 16-channel preamplifier (RA4PA or RA16PA)
8. 4-channel low impedance headstage (RA4LI)
9. Speaker (MF1)
10. Calibration microphone (PCB-378C01)
11. Needle electrode kit (ELE-N)
12. Cable kit (BNC-ABR)
13. Sound attenuating subject enclosure with Faraday cage
14. Heating pad
15. Subject
16. Anesthesia (*not shown*)

The Subject

Planning or setting up your ABR experiment must begin with an understanding of the subject's normal hearing range and factors that might have an effect on its response or recording. ABRs have been measured for many species. In this guide, we'll focus on the three most common species used in ABR experiments and screening protocols: mice, rats, and guinea pigs.

Expected Hearing Range

ABR tests are typically focused on the range in which the species is commonly known to hear well.

Subject		Standard Testing Range	Typical Threshold Range
Mice		4 to 32/48 kHz	25 – 30 dB at 16 kHz
Rats		4 to 24/32 kHz	30 dB at 16 kHz
Guinea Pigs		1 to 18 kHz	30 dB at 8 kHz

Note: The hearing range of laboratory subjects may vary across different strains or colonies and can be affected by age, the environment, and anesthesia.

Age-Related Effects on Hearing

Many lab subjects experience hearing loss due to age. The rate of loss varies across species and strains. This effect has been observed as early as 12 weeks in mice.

Anesthesia Considerations

Two types of anesthesia protocols are typically used for ABR testing. Each has benefits and limitations. Check your IRB approved guidelines for dosages and protocol.

Xylazine(XYL) + Ketamine to start, Ketamine to Boost

This aesthetic protocol uses an injectable combination of muscle relaxer and sedative. Typically, the subject is unconscious in a few minutes, begins to wake ~45 minutes and is completely awake at ~90 minutes after initial sedation. If a subject wakes early or a test runs longer than ~45 minutes, a booster of ketamine can be used to keep the subject anesthetized. Be sure to follow your approved IRB protocol.

Isoflurane (ISO)

This aesthetic protocol uses a fast acting inhalant with a short duration. The subject is typically fully sedated within 4–5 minutes and wakes very quickly when the gas is removed.

Your Test Area

The small size (1 – 2.5 microVolt) of typical ABR response signals makes it especially important to minimize electrical noise in the test area and to ensure all conditions are carefully controlled during testing. Be sure to consider the elements detailed below in setting up your testing area.

Grounding

Use a designated and grounded electrical outlet.

Enclosure Considerations

Many labs are full of cables, metal structures and other items that generate noise as well as electrical wires that serve as antennas for electrical noise. We recommend using a sound attenuating chamber with a built in Faraday cage for ABR testing. It is equally important to use good practices when using the selected enclosure. A mains power cord that extends from outside the enclosure to inside the enclosure undermines its integrity.

To maintain the integrity of the enclosure:

- Keep AC electrical powered (wall/mains powered) devices outside.
- Ensure the preamplifier is completely inside.
- Ensure the preamp charger is unplugged and outside.
- Ensure electrode wires are completely inside.

Speaker Placement

Most enclosures have some amount of acoustical reflection or interference during open field stimulus presentation. The effects will vary depending on the size of the enclosure, the angle at which the speaker is positioned, and the materials used or added for sound dampening. To minimize the effects of distortion or interference, position the stimulus speaker on the same plane as the subject's ear and set at an angle from the sides of the enclosure. See "Appendix A: Sound Chamber Fundamentals" on page 31 for more information on speaker placement.

Vibrations

Vibration from nearby large equipment is another common source of noise. If this is a problem in your lab, the cage and subject can be positioned on an air table or anti-vibration table.

Heating Pads

When the subject is sedated for testing, it cannot generate enough body heat to maintain core temperature. When the subject loses body heat, its hearing system is not as responsive which can artificially raise hearing thresholds. A heating pad is typically used to maintain the subject's temperature. Heating pads that require AC power inside the chamber are not suitable for ABR recordings.

Below are two alternative types of heating pads that can be used for this purpose:

An Isothermal Pad contains a phase change material that changes state near the subject's body temperature. It can be heated in a microwave and can typically hold a constant temperature for an hour or more.

Example: <http://www.braintreesci.com/images%5CDP.pdf>

A Warm Water Recirculator moves warm fluid through a small subject heating pad to keep the subject's body temperatures stable. Because this method uses an external heating element and pump to transfer water to the pad via non-ferrous tubes, the recordings will not be affected.

Examples: <http://www.braintreesci.com/prodinfo.asp?number=HTP-1500> and <http://www.braintreesci.com/prodinfo.asp?number=TP-700>

Important: It is critical that you **place the heat pump outside the Faraday cage** when using this type of pad.

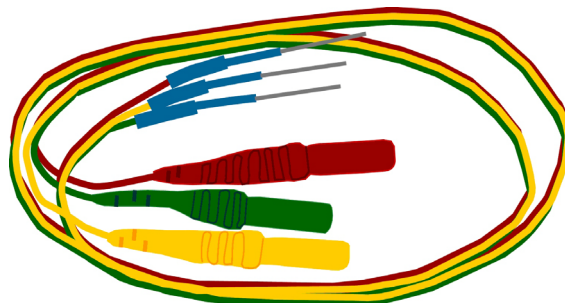
The Test Equipment

The care used in setting up your test area must also be taken when selecting and setting up your test equipment. TDT's ABR system is part of a modular experiment platform. Each module in the system is designed for exceptional signal fidelity, precise timing, low noise, and ease of use.

The sub-sections below discuss the role each part of the system plays in running an ABR experiment and provides information on making the necessary connections.

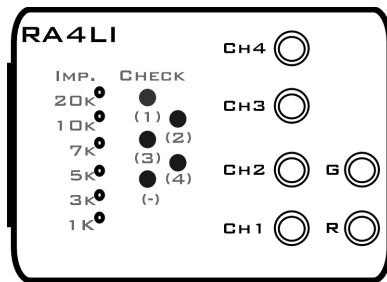
Needle Electrodes

In a research environment with non-human subjects, ABRs are typically recorded using sub-dermal needle electrodes. There are a wide variety of suitable electrodes available. When working with a TDT System, you will need electrodes with 1.5 mm female safety connectors to be used with the RA4LI headstage. TDT recommends disposable, 12 mm long, 27 gauge needle electrodes with 18" color coded lead wires. In general, selecting the shortest available lead wires helps keep the noise floor as low as possible. Some test protocols using small subjects, such as mice, may find that 29 gauge electrodes are more workable. Keep in mind that smaller gauge electrodes are lighter, but are also less robust and more likely to dull quickly.



Low Impedance Headstage

The headstage interfaces the subject and implanted electrodes with the TDT amplifier.

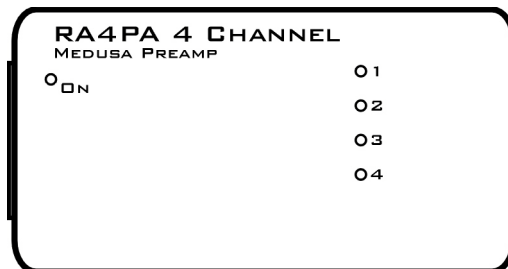


TDT's RA4LI Four-Channel Low Impedance Headstage includes built-in impedance checking and uses 1.5 mm safety connectors which interface with widely available third party needle electrodes.

Gain: **The RA4LI amplifies the incoming signals by 20x.** This gain is later removed from the recording in BioSigRZ software, see page 15.

Impedance test: The built-in impedance tester should be used to check the channel and reference impedance relative to ground after the electrodes are connected to the subject and before data acquisition begins. See page 16 for tips and troubleshooting electrode impedance.

Preamplifier/Digitizer



Biological signals from the RA4LI headstage are input to the RA4PA Medusa PreAmp for further amplification and digitization. The RA4LI and RA4PA connect together directly through a 25-pin connector. The RA4PA transfers digitized data to the RZ6 processor via a fiber optic cable. The fiber optic connection electrically isolates the RA4PA from the RZ6 and ensures that no additional noise is introduced to the biologic signal. This design is intended to be used with the preamplifier running on battery power alone, placed inside the Faraday cage with only the fiber optic cable connecting it to the RZ6.

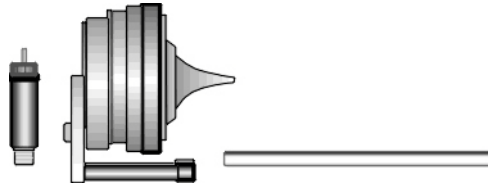
Max Input: ± 4 millivolts max input. **Note:** when you use the RA4PA with the RA4LI, the effective input range is reduced to ± 200 microVolts because of the headstage's 20X gain.

Gain: The RA4PA amplifies the signal by 250x before digitizing and transferring it to the RZ6. The RZ6 automatically accounts for this 250x gain when receiving the data, yielding a **net unity gain**.

Power: The RA4PA is powered by a Lithium-ion battery. It can be charged in 6-8 hours and will power the amplifier for up to 30

hours. The supplied battery charger must **NOT** be connected or inside the enclosure when running experiments. To protect the battery, do not leave the RA4PA on the charger for an extended period of time (1+ week).

Speakers



TDT's MF1 Multi-Field Magnetic Speaker is suitable for testing the hearing range of mice, rats, and guinea pigs. It can be configured for either free- or closed- field use (see "Appendix B: Configuring the MF1 for Closed Field Operation" on page 35). It has a built-in 8-32 threaded hole for use with standard laboratory mounting hardware. Aluminum mount/base fittings are included for easier positioning.

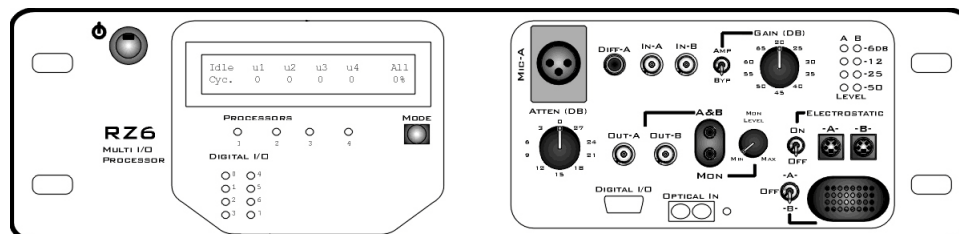
For **Open Field** experiments, place the speaker 10 cm from the subject. Be sure to position it to the side of the subject, in line with the ear, NOT in front of the subject.

Closed Field Setup

Each MF1 comes with a tapered tip for **Closed Field** experiments. The tip mates with the provided 1/8" outside diameter (O.D.) PVC tubing (10 cm included). The MF1 comes with an inline filter (pictured at left) that connects between the RCA cable and speaker input. This filter should always be in place when using the MF1 speakers in closed-field mode. In closed-field mode, the MF1 has undesirably high low frequency output. This filter reduces the speaker output at low frequencies so that the output range can be equalized. When using the tube tip, use the MF1's inline filter and the shortest tube possible. You can cut the 10 cm tube to the desired length. A 3 – 5 cm tube length is typical. The tube should slide snugly into the tube tip. You can push firmly without fear of damaging the tube. If you have trouble inserting the tube, a small amount of silicon spray can be used. It may be easiest to spray silicone lightly on a cloth first then put the tip of the tube on the cloth to apply it.

Warning: other lubricants (WD-40, grease sprays, or similar) should not be used as they can damage the O-rings surrounding the tube tip, or the tubes themselves.

RZ6 Signal Processor



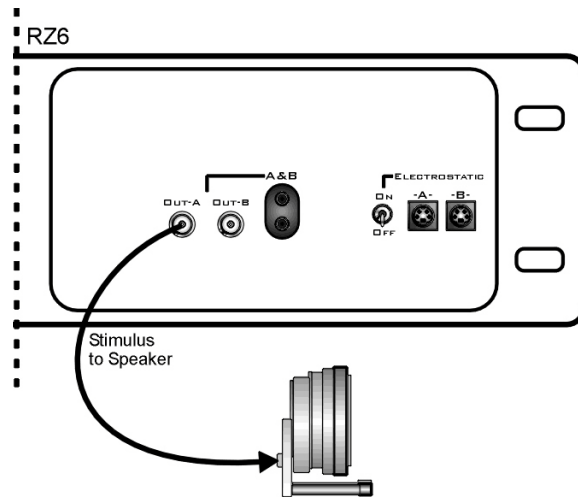
The RZ6-A-P1 Multi I/O Processor provides the processing power for averaging signal inputs and the digital-to-analog converters and amplifiers for producing the stimuli. It also provides the precise timing essential to evoked response experiments.

The power input and optical connection to the PC are located on the back. The input/output support (such as speaker drivers) and attenuation that is incorporated into the device are all accessed on the front panel.

Stimulus Output The RZ6's can generate a stimulus in the frequency range of DC – 88 kHz and is well suited for standard ABR testing.

A built-in stereo power amplifier drives the stimulus signals through TDT's MF1 multi-function speaker via the BNC labeled **Out-A**.

Use the RCA cable and BNC to RCA adapter (included with the MF1 kit) to connect **OUT-A** to the RCA connector on the **speaker**.

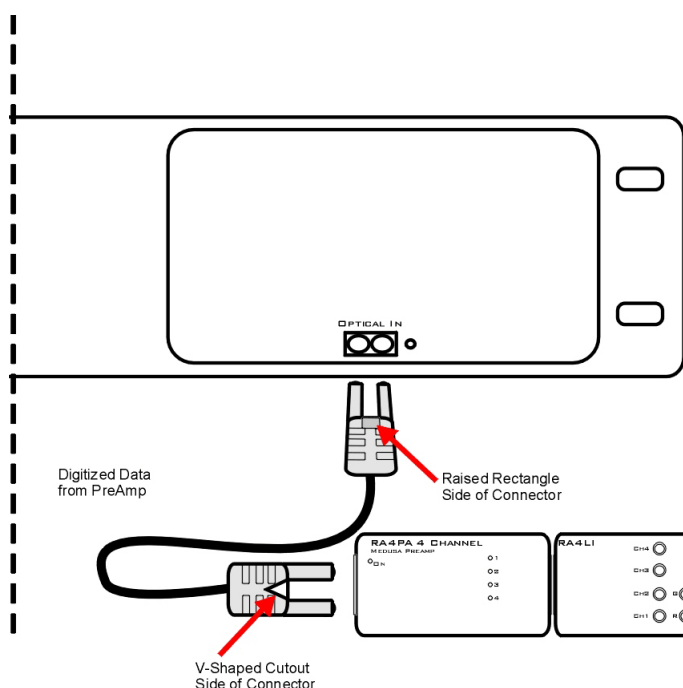


Important!: When using the MF1, the **ON/OFF** switch next to the outputs marked Electrostatic must be in the **OFF** position to reduce noise.

Attenuation Typically, stimulus attenuation is handled via software. The Atten knob on the front panel provides optional manual attenuation applied to the signal before it is output. This can be useful if the sound level is found to be systematically too high across all frequencies during speaker calibration.

Fiber Optic Input Biological signals are digitized on an RA4PA Medusa preamplifier and transferred to the RZ6 via the fiber optic port marked **Optical In**.

Use the provided fiber optic pair (white connectors) to connect the **To Base** port on the preamplifier to the **Optical In** port on the RZ6.



The duplex fiber optic cable has identical one-piece connectors at each end. There is a V-shaped groove on one side of the connector and a raised rectangle on the other. As shown in the image above, plug the connector into the RZ6 port with the raised rectangle side up. Plug the connector into the preamplifier with the V-shaped groove up.

The green LED next to the Optical In port on the RZ6 will illuminate when the preamplifier is connected and both devices are powered on. This indicates that the RA4PA is properly time-locked with the RZ6.

Mic Inputs

The BNC input connector is used during system calibration (see “Calibration” on page 23).

PC and Interface

The RZ6 must be directly connected to a PC, which provides the user interface for the system. The PO5 or PO5e optical interface card is installed in the PC and connected to the RZ6 across fiber optic cables. This adds another layer of noise reduction, separating the processor from the noisy PC environment.

The PC handles:

- Configuring the hardware and software.
- Communicating with the TDT hardware and transferring data across a fiber optic interface.
- Displaying and working with data during and after acquisition.

If you use TDT’s WS4 computer, the optical interface and BioSigRZ software are preinstalled.

If you use your own PC, you’ll need to install the interface card and BioSigRZ software yourself. Step-by-step instructions for installing software and drivers and setting up the processor are available in [System 3 Install Guide](#) that came with your

system. The installation guide also includes information for connecting the WS4 or your PC to the RZ6 Processor, including running a transfer test to ensure communication between them is working correctly.

BioSigRZ Software

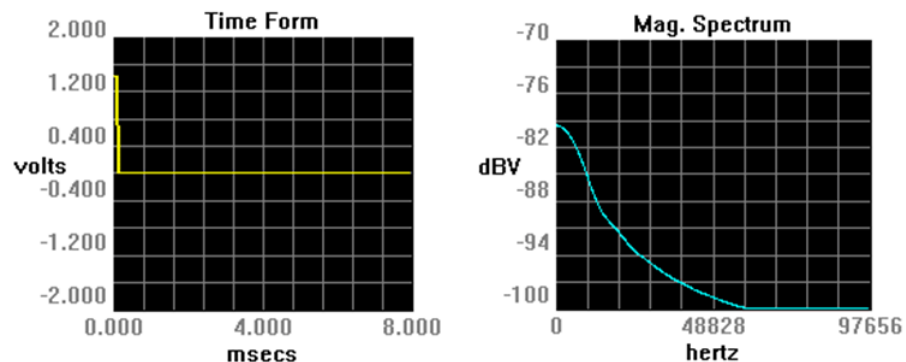
BioSigRZ software is streamlined and optimized for ABR and DPOAE screening. The software provides a simplified interface for selecting a stimulus and configuring how the biological response will be recorded and processed.

Typically, ABR experiments use click and/or tone stimuli.

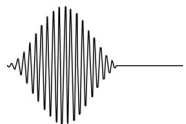
Click ABRs



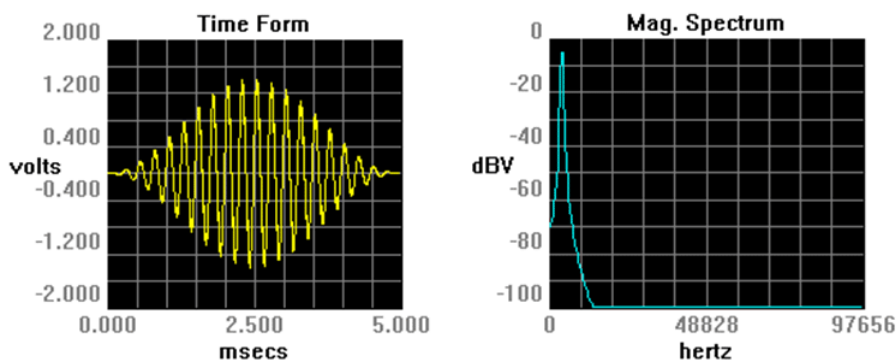
A click (square wave) is a broadband signal that stimulates a larger frequency range with each stimulus presentation. Because a wide range of frequencies are stimulated at once, the response tends to be larger and easier to identify. Screening with click-based ABRs also reduces the number of variables to be tested (no individual frequencies) and thereby significantly reduces the time required to test each subject (typically about 5–10 minutes). Presenting a click stimulus at a few levels can quickly screen for deafness or significant hearing loss. Presenting across more levels can provide basic information about a subject's hearing threshold.



Tone ABRs



Tone pips are typically presented at multiple frequencies to target more specific areas of the subject's hearing range. Tone ABRs take significantly longer to perform (typically up to around 40 minutes) but provide more data.



Out-of-the-Box Experiments

When the software is installed, out-of-the-box experiment files for both types of ABRs are stored at: C:\TDT\BioSigRZ\Configs\ABR. The default values used in the preconfigured experiments are set to values suited to typical experiments. Your protocol or circumstance may differ from these values but these will work as an example or a starting point in most cases.

To open the ABR experiment configuration:

1. Launch **BioSigRZ**.
2. Click the File menu and click **Open Config. File**.
3. Browse to and open one of the following files:

C:\TDT\BioSigRZ\Configs\ABR\Root_ClickAbr.acf

A 0.1 millisecond, single-channel mono-phasic click will be presented at a rate of 21 presentations per second. With each successive presentation, the click phase will alternate between a condensation and rarefaction click to eliminate potential speaker artifacts. Click level will vary from 90 dB SPL to 10 dB SPL in 10 dB decrements. A 10ms response window is acquired from a single acquisition channel on the RA4PA via the RZ6 fiber optic port. A total of 512 responses will be averaged at each level.

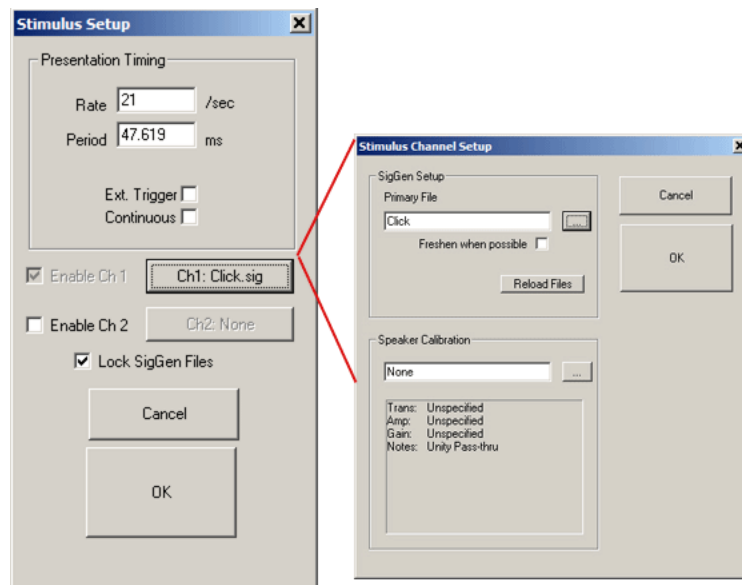
C:\TDT\BioSigRZ\Configs\ABR\Root_ToneAbr.acf

A five millisecond, single-channel cosine-squared gated tone will be presented 21 times per second at the following frequencies: 4 kHz, 8 kHz, 16 kHz, 24 kHz and 32 kHz. For each frequency, the tone level will vary from 90 dB SPL to 20 dB SPL in 10 dB decrements. With each successive presentation, the tone phase will alternate 180 degrees to eliminate potential speaker artifacts. A 10ms response window is acquired from a single acquisition channel on the RA4PA via the RZ6 fiber optic port. A total of 512 responses will be averaged at each frequency and level combination.

The Stimulus Settings

The default stimulus settings represent a typical configuration and should be fine to begin, but you may eventually want to make adjustments.

The stimulus setting dialog boxes can be opened by clicking **Setup|Stimulus**.



The setting notes below provide information about each setting.

Setting

Notes

Presentation Rate:

21/sec (period is 47.619 ms)

Why? This rate minimizes the effects of noise from the 50/60 Hz cycle of mains power – the most common source of noise interference in research labs. If you change the rate, make sure the new rate does not divide evenly into 60 or 50.

How does changing the presentation rate affect ABR recordings? Increasing the rate gives the subject less time to recover between presentations and can decrease the magnitude of the ABR response.

Stimulus Durations:

Stimulus duration can be no more than 95% of the period.

CH1:

Click.sig or TonePip.sig

When the stimulus is defined, either by loading a standard configuration file or by specifying a stimulus file (.sig) in the Stimulus Setup dialog, a complete list of signal parameters and variables are added to the BioSigRZ file. During stimulus presentation, the values of the variables change as a function of the SigGen Index (SGI), a set of variable conditions. This is called the stimulus schedule. You can access this schedule and choose to omit specific SGIs, if desired. See “Appendix C: Modifying the Stimulus” on page 37 for more information on modifying the standard protocol.

Ch1:|Speaker Calibration: Leave blank for now

All speakers have some variation in output across frequencies. When you use a speaker with a known frequency response, you can adjust the stimulus output for these known frequency characteristics. A USB drive with a speaker-specific calibration file is provided with

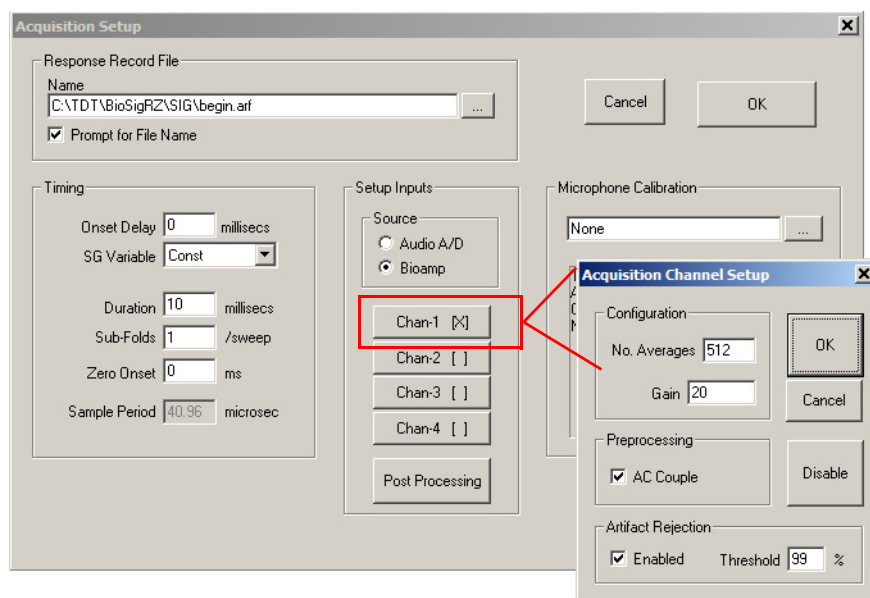
your MF1 speaker. You will need to copy that file to the following directory: C:\TDT\BioSigRZ\TCF. After the file has been copied to this directory you will be able to select it from a list in the Stimulus Setup. You can also make your own .tcf calibration file by following the instructions in Chapter 6 of the [BioSigRZ Manual](#).

The first time you run an ABR, we recommend skipping this step and any other calibration until after you have been able to successfully record an ABR response. If the system has not yet been fully tested, small mistakes in calibration can become more difficult to diagnose. It is important to make sure everything else is working before calibrating and it is a good idea to start with a click stimulus. Responses to click stimuli tend to be larger and easier to acquire.

The Acquisition Settings

The default acquisition settings should be fine to begin, but you may eventually want to make adjustments.

The acquisition settings dialog boxes can be opened by clicking **Setup|Acquisition**.



The setting notes below provide information about each setting.

Setting

Response Record File:

Notes

All the data collected and displayed in the History pane during the ABR test are stored in a response record file (.arf).

By default the file will be saved at:

C:\TDT\BioSigRZ\SIG

You can specify a name in the setup window or select the Prompt for File Name check box to be prompted for a name before each recording session. You will be prompted each time you click Begin.

Duration: 10 ms

Important!: The acquisition duration cannot be more than 95% of the presentation period.

Setup Inputs |

Chan-1 | Source: Bioamp:

The Bioamp selection determines that the signal will be acquired via the RZ6's fiber optic input port. Typically, the RA4PA is used for ABRs, though the RA16PA may also be used as a replacement for the RA4PA without any changes to the software.

Number of Averages: 512

Why? For most experiments, 512 is a good balance between averaging enough responses to get good signal quality and few enough to minimize the time to complete testing. To find the right balance for your experiment, weigh factors such as length of sedation, purpose of testing (quick deafness screening vs threshold testing) and number of subjects to be tested. **The typical range is 256 - 1024 averages.**

Note: Subjects with hearing loss often require more averages than normal hearing subjects.

Gain: 20x (RA4LI)

Why and Where? The RA4LI hardware applies a 20x gain amplification. This must be digitally removed from the acquired signal in software ... **but only once!**

There are two ways to account for the gain and using both—a common mistake—will cause the responses to be dramatically reduced.

To account for the gain, enter **20** in the **Gain** field of the **Chan-1** Acquisition Channel Setup. This is the preferred method.

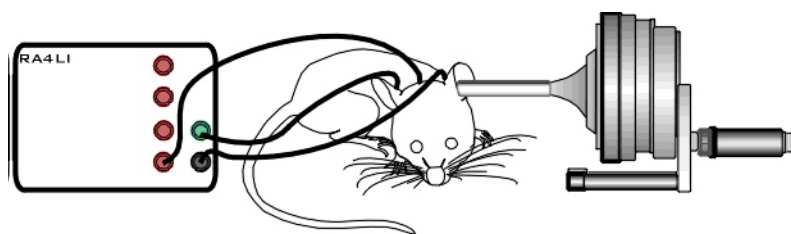
Alternatively, set the Gain field to 1 and select **RA4LI** in the microphone calibration area of the **Acquisition Setup** dialog. However, **do NOT use both methods.**

Running the Experiment

After you feel comfortable with the system, you can begin to collect data. We recommend reading through the entire guide before using a live subject.

Electrode Placement

TDT can provide an electrode kit with suitable needle electrodes for ABR testing. One electrode is required for each channel, plus one for Ground and one for Reference. The electrodes should be inserted just under the skin and connected to the appropriate input on the RA4LI low impedance headstage. Correct placement is essential for acquiring good data.



For this single channel configuration, position electrodes as follows:

Channel 1	Vertex (base of skull)
Reference	Ipsilateral ear (same side as stimulus delivery)
Ground	Contralateral ear (or hind hip or base of tail)

Check Impedance

The RA4LI headstage includes built-in impedance checking. Impedance is a measure of resistance from electrode to ground. High impedance can result in lower response signals or no signal at all. After inserting electrodes, run an impedance check for the subject to verify a good insertion and working electrodes.

To check the impedance level:

- Press the button next to the channel indicator to test channel 1 electrode.
- Press the button next to the (–) indicator to test the reference electrode.

The highest-level LED that is lit indicates the impedance between that electrode and the ground. The impedance of the input channel and the reference (–) must match, ideally at 1 K – 3 K for each.

Note: If all impedance lights are illuminated, it is likely that one of the electrodes is not properly connected. If both the output channel and reference show all impedance lights, it is likely the ground electrode is not making good contact.

Check Sedation

If the subject is not fully sedated throughout testing, subject responses, such as muscle movements or distress, can interfere with ABR recordings. To avoid this, **always check the subject before beginning your experiment and monitor the subject for signs of wakefulness during testing.**

Toe Pinch

For small lab subjects, a toe pinch is the most effective method to check for full sedation. Extend the rear leg and use your fingernails to firmly pinch toe or foot webbing. There should be no recoil. If there is any response, such as leg movement, the subject is not adequately anesthetized.

Note: Startling the subject with a loud clap or loud noise is not a rigorous enough way to check sedation. A subject may not respond to clapping even when only partially sedated. Also, a test subject with hearing loss may not respond regardless of sedation level.

Begin Experiment

BioSigRZ is the user interface for ABR testing. It has three modes of operation: Idle, Running, and Averaging. Before you begin, it is in Idle mode. You can begin stimulus presentation or run the experiment using buttons on the left side of the BioSigRZ software main window. The options change depending on the mode. See below for more information about the operating modes.



To begin stimulus presentation:

1. Click the **Start** button. You may be prompted to specify a response record file where data will be stored.
2. If prompted, type a name in the **File Name** field and click **Open**.

You are now in **Running Mode**.

The stimulus signal is presented using the first SigGen Index (SGI). No data is collected.

To begin data averaging:

1. Click the **Begin** button.
2. A Subject Information window opens. Information entered here is stored with the data in the response record file.
3. Enter any subject information and click **OK**.

You are now in **Averaging Mode**.

The first stimulus is repeatedly presented and the ABR response data is acquired and included in the running average until the desired number of responses has been collected at that level. As averages are completed, the averaged ABR records will be appended to the History plot and the .arf data record file.

Modifying the Stimulus Schedule

BioSigRZ provides several functions that allow you to override the current stimulus schedule and control data averaging in real-time.

Advance

Current SGI is halted and running average is saved. Stimulus presentation advances to the next SGI in the stimulus schedule.

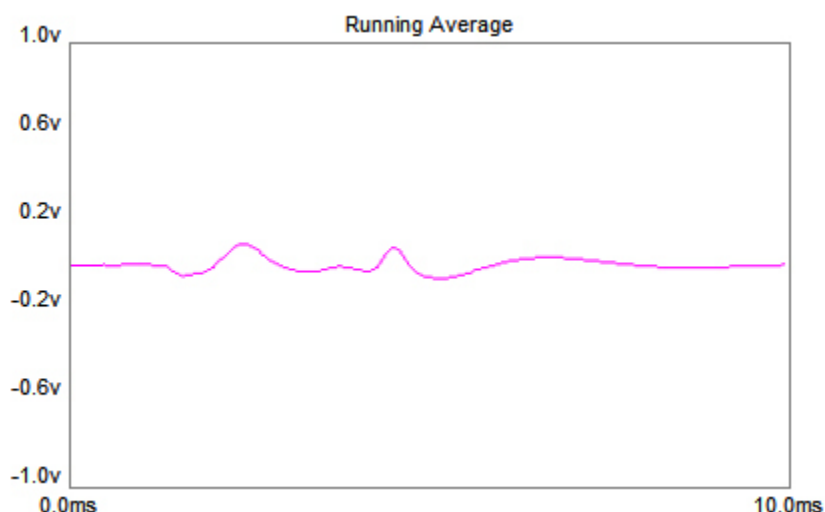
Repeat

Current SGI is halted and running average is saved. Stimulus is presented again at the current SGI.

Skip	Current SGI is halted and running average is discarded. Stimulus presentation is advanced to the next SGI in the stimulus schedule.
Redo	Current SGI is halted and running average is discarded. Stimulus is presented again at the current SGI.

Multi-Purpose Plot

During averaging, the Multi-Purpose Plot may be used to display a variety of signals. The plot is controlled through the use of the Plot Control Toolbar, available from the right-click menu. You can also control scaling using the toolbar. Consider using the options described below.



Running Avg	This view displays the current running average.
EEG Plot	This view lets you see the last incoming EEG waveform. The signal displayed is not averaged, so sudden/temporary anomalies will be visible. If the waveform jumps, for example, it may indicate that the subject is awake. Other anomalies, such as heartbeat artifact, can be seen here as well.
Zooming	Click and drag from left to right over the area of interest to zoom in and change the scale of the time axis. Click and drag from right to left to zoom out.

Termination

In BioSgRZ, control of termination of the signal presentation and acquisition is called boundary control. When a boundary is reached and the SGI stops incrementing, signal generation will continue for the current SGI, but no more response data will be collected.

Note: You can manually terminate data averaging at any time.

To manually terminate data acquisition:

- Click the **End** button.

This returns the system to **Running** mode. You can halt stimulus presentation and return the system to **Idle** mode or run the experiment again.

To halt stimulus presentation:

- Click **Stop**.

To run the experiment again:

1. Click **Begin**.
2. Enter the new subject information.

Data and Export

Viewing data in the BioSigRZ plots and worksheet.

History Plot

In averaging mode, BioSigRZ acquires data and calculates a running average. Data will be acquired until enough records have been gathered to compute the final averaged signal. At that time, the averaged signal will be appended to the end of the History Plot, the SGI will be incremented, a new stimulus signal will be generated according to the current stimulus parameters, and a new average will be computed. This process continues until the termination of the stimulus schedule is reached or until you manually end data averaging. Information about each SGI is displayed to the left of the history plot (pictured below). Right-clicking this data allows you to control what data is shown.



The Worksheet

The worksheet provides you with several basic functions. It allows you to graphically organize acquired data and perform mathematical and visualization manipulations of the acquired data directly in BioSigRZ. The Worksheet is located to the right of the History plot. Waveforms can be copied from the Worksheet and pasted to other applications for report writing or by choosing to generate a report, you may output data for use by other applications.

To place History Plot records into the Worksheet:

1. Select the desired record(s).
2. Click and drag the selected record(s) from the History Plot to the Worksheet.

The selected History Plot record(s) will be copied to the Worksheet.

Export Data

BioSigRZ has many onboard tools for viewing and working with responses. Records may be exported to an ASCII text file (.txt file) or CSV (spreadsheet) file or you can choose to do further analysis in an environment such as Matlab. We can provide support for exporting data to the desired format, but we leave further analysis up to you so that you can do it your way.

To export to ASCII:

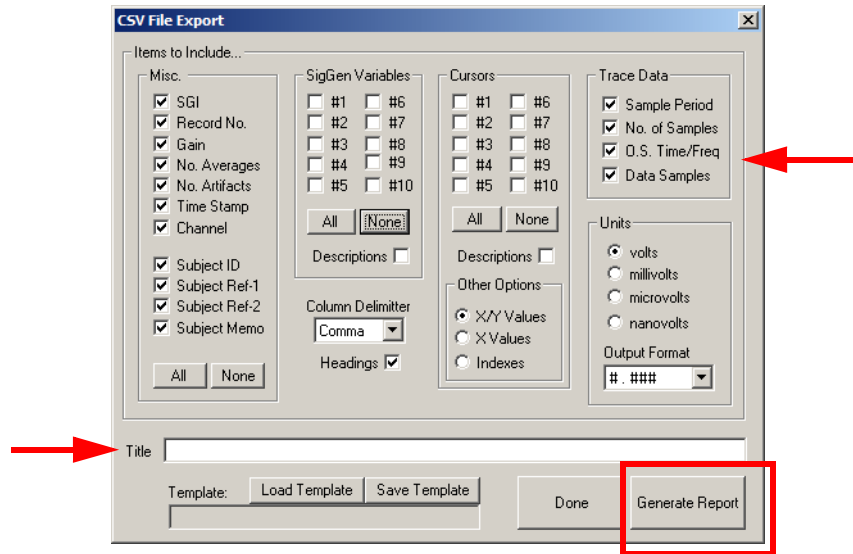
1. Select the desired records in the History plot.

Or to export all records in the History plot, click the label area at the top of the plot to clear all selections. When no records are selected, BioSigRZ will prompt to determine if all records should be exported.
2. On the **Menu** bar, click the **History** menu and click **Export to ASCII** file.
3. You will be prompted to supply the export file name and location where the file will be saved.
4. Click **Open**.

The selected records will be saved to the new file.

To export to CSV:

1. Select the desired records in the History plot.
2. On the **Menu** bar, click the **History** menu and click **Export to CSV** file.



To export the trace data, check the box next to **Data Samples**. If you wish to further customize the export, click the check boxes or radio buttons to toggle on/off the desired options. The settings shown are the typical/recommended export settings.

3. Type a report title in the **Title** field.
4. Click the **Generate Report** button.
5. When prompted, specify a file name and location to store the export file and click the **Save** button.
6. When you are returned to the CSV File Export dialog box, you can change options and generate additional report exports or click **Done** to exit.

See the [BioSigRZ Manual](#) for further information on data viewing tools and export features.

Troubleshooting

Diagnosing Noise Issues

Electrical noise is one of the most common problems users must address. Luckily, most problems can be resolved easily.

Here are a few common sources of noise:

- A preamplifier charger (for the RA4PA/RA16PA) that has been left plugged in.

Always disconnect the preamplifier from mains power and rely on the battery during recordings. No high voltage lines should be used inside the Faraday cage. **Note:** some subject enclosures may include interior lights or fans. These should not be used or connected to power during ABR recordings.

- Ground and reference are placed incorrectly.

See electrode placement on “Electrode Placement” on page 15, for correct placement.

- Electrode impedances do not match.

Channel 1 and Reference impedances should match at 1–3k each.

- Something near the recording area is causing noise?

The nearby area should be as free of high voltage noise sources as possible. Common sources are motors, fans, refrigerators, freezers, lights, and computer monitors.

- The subject enclosure does not have a dedicated ground.

If your noise floor is higher than expected, we recommend tying the cage to a dedicated ground or connecting it to a nearby copper pipe, if possible. Use caution if you use an electrical outlet ground. These are commonly tied to other outlets that could introduce noise.

- An electrode is old or has not been adequately cleaned.

Most electrodes are recommended for one time use. While that is not always practical, electrodes do degrade with use or time. They can become bent or corroded. Try using another electrode. If the noise disappears, the age or cleanliness of the problem electrode may have been the culprit.

- The **preamplifier** (RA4PA/RA16PA) battery is at the end of its charge.

A low battery state may cause poor recording. **NOTE:** impedance lights on the preamplifier may remain lit when the battery is low. Fully charging the RA4PA Medusa PreAmp battery takes 6–8 hours.

If you have checked the list above and have not been able to identify the source of the noise, you can perform further testing to narrow down the possibilities.

A Saline Test

A saline test can be used to check the baseline noise floor during recording.

To perform a saline test:

1. Fill a non-metal beaker with saline.
2. Place the three electrodes 50% submerged and spaced evenly through the saline. A rubber band, positioned around the beaker after the electrodes are positioned inside, may help hold them in place.
3. Put the beaker in the location where the subject would be during ABR recording.
4. Run BioSigRZ and record several SGIs.

The noise floor should be ~80 – 100 nanoVolts after 512 averages. During a saline test, the saline replaces the subject, so if the noise floor is significantly higher than expected, it is likely the noise is environmental.

If the results show that the noise is not environmental, then it may be an issue related to the subject, such as sedation or age issues. To confirm this you will need to run an ABR with a live subject and no stimulus presentation. The easiest way to achieve this is to simply disconnect the speaker. When running this test, the noise floor will typically go up by 20%. If the noise floor increases by more than 20% the problem is likely subject related (sedatives, etc).

Troubleshooting On-The-Fly

If a problem occurs during an experiment, the BioSigRZ Multi-Plot window provides several views useful for on-the-fly troubleshooting. Here are a few common problems that can be identified and resolved quickly so you can resume your experiment.

The EEG view allows you to monitor recordings during testing.

If you see:

You may have:

A heart beat in the signal. Incorrectly placed electrodes.

Random jumps

in signal level.

An animal that is awake or not fully sedated.

You can also view a running average of the ABR responses.

If you see:

You may have:

No ABR response

at any SGI.

A speaker problem. Check the speaker by opening the door during an audible frequency or click SGI to listen for the stimulus.

A hearing impaired animal. If using a tone, switch to a click to increase the odds of getting a response (tests over a broad spectrum of frequencies).

A deaf animal. Try the same test again with a normal hearing animal.

Noisy signal.

A noisy environment. Run a saline test as described above.

Getting Help

If you are unable to diagnose the noise problem, contact TDT for assistance at support@tdt.com or +1.386.462.9622.

When you contact us be prepared to answer questions such as, what frequencies you're testing, what subject and anesthetic you're using, and when the system was last calibrated. We may also ask you for data, screen-shots, or photos of your experimental setup. We may also ask you to participate in a remote desktop session, which requires an Internet connection to your experiment PC running BioSigRZ software.

Calibration

All speakers have some variation in output across frequencies. When you use a speaker with a known frequency response in BioSigRZ, you can adjust the stimulus output for these known frequency characteristics by selecting your speaker from a list in the Stimulus Setup.

Standard calibration files for common speakers and microphones are provided with BioSigRZ and a speaker-specific custom calibration file might also have been provided with your speaker (provided on a USB drive in the speaker kit). If a calibration file is not provided for your specific speaker model or the distance (free-

field experiments) or length of tube (closed-field experiments) is different than those measured for the standard files, you can create a custom file using BioSigRZ's Calibration tools and a calibration microphone.

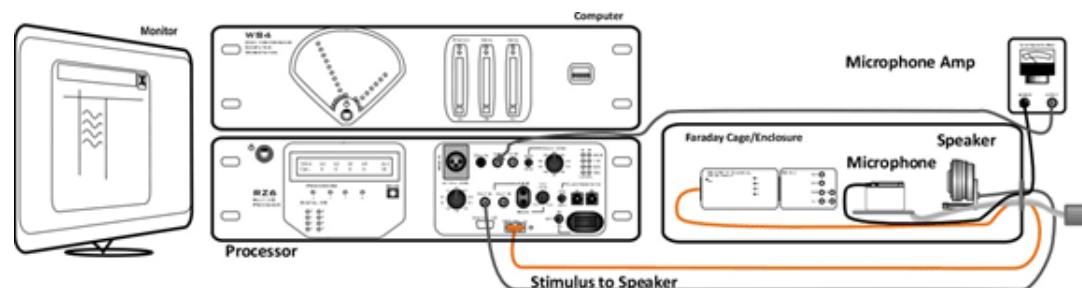
The first time you run an ABR, we recommend skipping calibration until after you have been able to successfully record an ABR response. If the system has not yet been fully tested, using a custom calibration file can make problems more difficult to diagnose.

How Often to Calibrate

After your system is set-up and working well, how often you calibrate is up to you. Some researchers prefer to calibrate every day, others prefer once a week, monthly or longer. If you are using a robust speaker, like the TDT MF1 magnetic speaker, you may choose to calibrate less often. If you are using a more specialized speaker, like the TDT EC1 or ES1 electrostatic speakers, you may choose to calibrate more often. If you are new to collecting ABR data, consider running a calibration check often enough to confirm that you are seeing less than 5 dB variability, then reducing frequency of calibration to something suitable to your protocol and experiment conditions. We do recommend calibrating anytime your setup (layout or hardware) has changed.

Hardware Setup

To calibrate your system you should have everything set-up as you will have it setup for your experiment, with the exception of the subject. During calibration you will place a calibration microphone with a flat response in the band of interest in the place of the subject. The Amp/BYP switch on the front of the RZ6 must be set in the 'Amp' position, and the Gain knob should be set to 40dB to start.



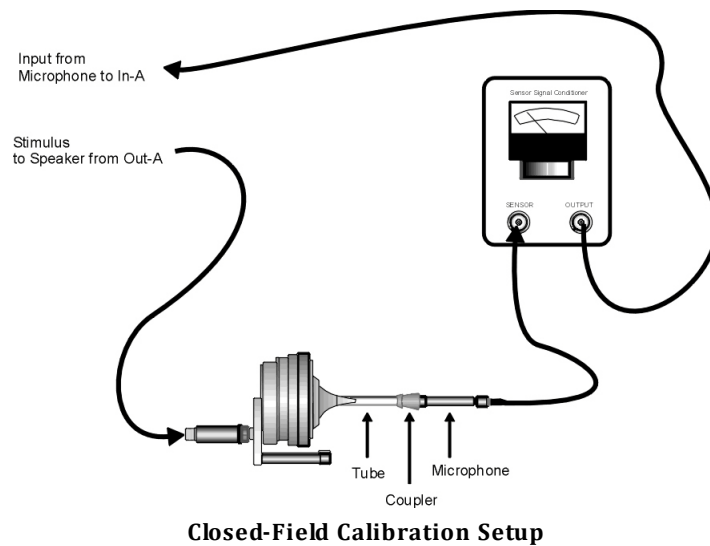
Free-Field Calibration Setup

Important: Use only one input for channel A at a time. Attempting to input signals from multiple sources will produce an erroneous signal.

Important: To ensure accurate calibration, give the microphone 10 minutes to warm-up before testing.

Closed Field

For a closed field calibration you need to interface your Calibration Microphone to your speaker tube using some sort of coupler. Couplers can be made from parts common in most labs (pictured below), such as ear insert adapters, syringes, and putty. All of the connections between the speaker, tube, coupler and microphone need to be airtight. Any gaps, holes or other leaks will cause inaccurate calibrations.



Possible coupler materials:



Eartip



Putty

Tone Calibration

During calibration, BioSigRZ performs a sweep over the frequency range of interest and generates a speaker response curve along with a correction curve that would be required to flatten the response. An FIR filter is calculated from the correction curve and stored in the contents of the calibration file. When you load the calibration file, that filter is loaded onto the RZ6 so that any Tone Pips presented through the RZ6 will be calibrated to have a flat response across all frequencies.

The extension for calibration files is .tcf. All TCF files should be stored at: C:\TDT\BioSigRZ\TCF. For more information on file types and calibration see the [BioSigRZ Manual](#).

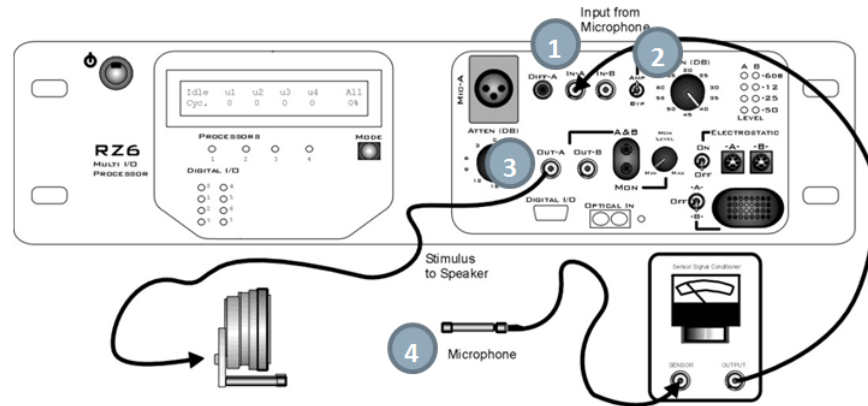
Click Calibration

The RZ6_Click_Calibration.rcx circuit is designed to allow you to calibrate the RZ6 for click stimuli. This file is part of the <https://www.tdt.com/files/examples/BioSigRZExamples.zip> zipped example folder, which can be downloaded from the TDT website. Download and extract the zipped files to C:\TDT\BioSigRZ\RCX.

To use this circuit there are a few steps required for setup:


1. Connect your Calibration Microphone to the In-A port on the RZ6.

2. If your microphone has a sensitivity of less than 50 mV/Pa, ensure your Amp/BYP switch on the front of the RZ6 is set to Amp.
3. Connect your speaker to Out-A or Electrostatic-A (if using Electrostatic-A, be sure the Electrostatic On/Off switch is in the On position).
4. Place your microphone in the same position that your subject's ear would be located.



5. Launch TDT's RpvdsEx software and open **C:\TDT\BioSigRZ\RCX\RZ6_Click_Calibration.rcx**.
6. In the circuit, double-click the ConstF component labeled Microphone Amplification Level and set its value to match the Gain knob on your RZ6. Typically 40–60 dB works well, if your microphone has 1–3 mV/Pa sensitivity. If you are bypassing the amplifier, set the gain in the circuit to 1.
7. In the circuit, modify the ConstF labeled Microphone Calibration Voltage to match the sensitivity of your microphone.

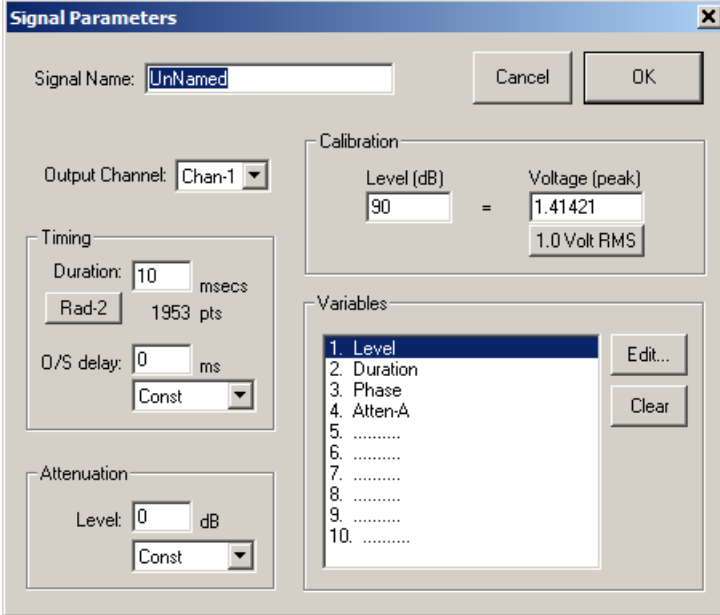
To run the circuit:

8. Click the **Compile, Load, and Run**  button on the tool bar.
9. Start with a low voltage in the **Desired Voltage Amplitude** ConstF and slowly increase this value. Re-compile, load and run (step 8) after you make any modification to the circuit.
10. When the dB SPL level shown in the top right corner of the screen matches your desired level, make a note of the voltage you are using in the ConstF labeled **Desired Voltage Amplitude**.
11. This voltage and the corresponding dB level should be entered in the click signal stimulus configuration file found at: C:\TDT\BioSigRZ\SIG\Click.sig.
 - a. To open the SigGenRZ stimulus design software (included with BioSigRZ), click the Windows **Start** menu, click **All Programs**, click **TDT Sys3**, click **SigGen Solutions**, and then click **SigGenRZ**.

This program can be used to edit stimulus files.

- b. To open the standard click stimulus file, click **File**, click **Open**, browse to **C:\TDT\BioSigRZ\SIG\Click.sig**, then click **Open**.

- c. On the main toolbar, click **Modify**, and then click **Signal**.



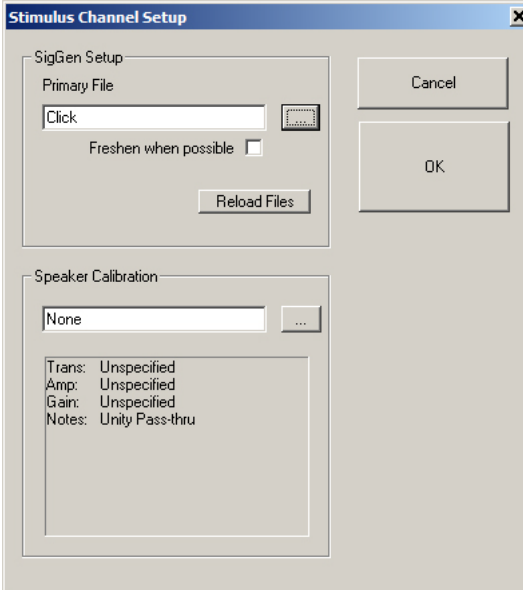
The **Signal Parameters** dialog box contains the following sections:

- Signal Name:** A text field containing "UnNamed".
- Output Channel:** A dropdown menu set to "Chan-1".
- Timing:**
 - Duration:** 10 msec (with a Rad-2 button and 1953 pts).
 - O/S delay:** 0 ms (with a Const dropdown).
- Attenuation:**
 - Level:** 0 dB (with a Const dropdown).
- Calibration:**
 - Level (dB):** 90
 - Voltage (peak):** 1.41421
 - 1.0 Volt RMS** (checkbox)
- Variables:**
 - A list box containing: 1. Level, 2. Duration, 3. Phase, 4. Atten-A, 5., 6., 7., 8., 9., 10.
 - Edit...** and **Clear** buttons.

- d. In the Signal Parameters dialog box, enter the voltage and the corresponding dB level in the Calibration area, and then click **OK**.
12. To save the change, click **File** then **Save**.

This will save the change to the stimulus used by BioSigRZ's standard click configuration.

13. Run the stimulus file in BioSigRZ **without** a calibration (.tcf) file applied to the stimulus. Your stimulus is already calibrated, and adding a .tcf file will cause the calibration to be incorrect.



The **Stimulus Channel Setup** dialog box contains the following sections:

- SigGen Setup:**
 - Primary File:** A text field containing "Click" and a file selection button.
 - Freshen when possible:** An unchecked checkbox.
 - Reload Files** button.
- Speaker Calibration:**
 - A dropdown menu set to "None" and a selection button.
 - Trans:** Unspecified
 - Amp:** Unspecified
 - Gain:** Unspecified
 - Notes:** Unity Pass-thru

See the [System 3 Manual](#) for detailed information on TDT equipment and see the [BioSigRZ Manual](#) for computer requirements and detailed software reference.

Testing Speakers and Microphones

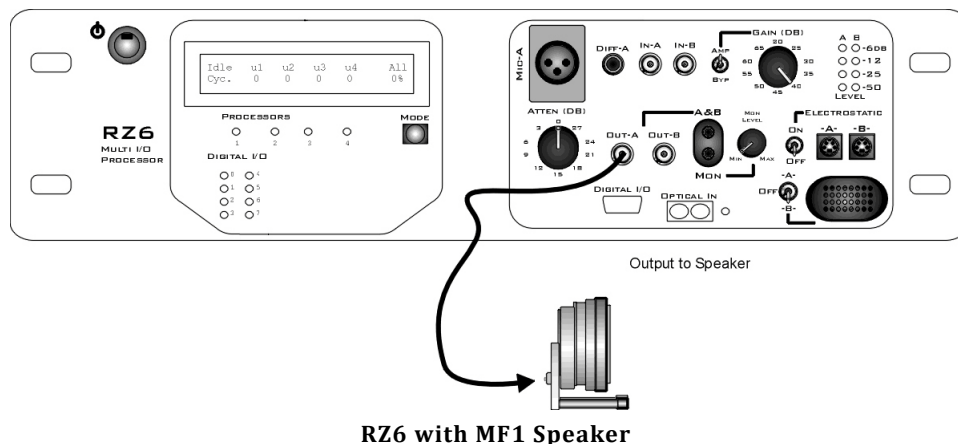
A simple RPvdsEx test circuit can be used to ensure your speakers and microphones are working. The test circuit, SpeakerMicTest.rcx, is part of the [BioSigRZExamples.zip](#) zipped example folder, which can be downloaded from the TDT website.

Speaker Test



If you suspect that your speakers may not be functioning correctly, download and extract the zipped files to C:\TDT\BioSigRZ\RCX\, then double-click the SpeakerMicTest.rcx file to launch RPvdsEx and open the file.

To use this circuit there are a few steps required for setup:

1. Connect your speaker to Out-A.



To test your speaker:

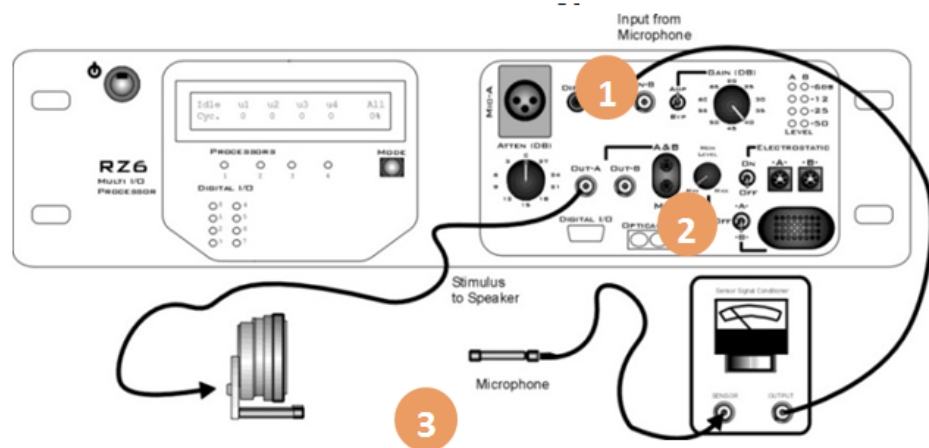
- Click the  **Compile, Load, Run** button on the toolbar. You should be able to hear a tone from the speaker.
- Click the  **Stop** button to stop the test.

Microphone Test

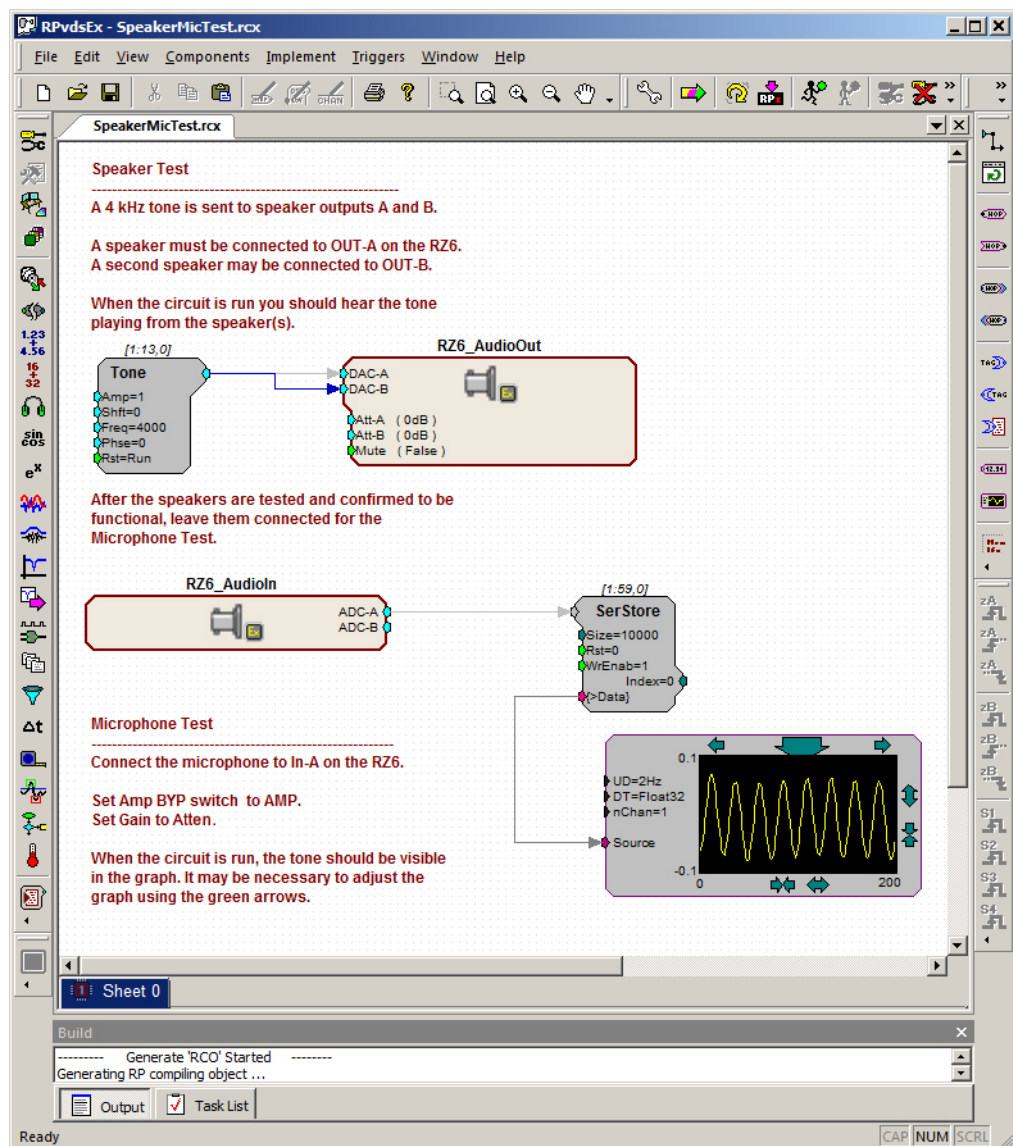
After you've confirmed at least one speaker is working, connect the calibration microphone and run the circuit again. Important: To ensure accurate calibration, give the microphone 10 minutes to warm-up before testing.

To use this circuit there are a few steps required for setup:

1. Connect your Calibration Microphone to the In-A port on the RZ6.
2. If your microphone has a sensitivity of less than 50 mV/Pa, ensure your Amp/BYP switch on the front of the RZ6 is set to Amp.
3. Place your microphone where your subject's ear would be located.



RZ6 Open Field Calibration Setup



You should be able to see a sine wave in the graph. You may need to adjust the scale using the green arrows on the sides of the graph. Click the Stop button again to stop the test.

Appendix A: Sound Chamber Fundamentals

Reflections and interference inside of a sound chamber can distort the stimulus frequency or have an undesirable effect on the stimulus level. This appendix provides supplemental information on these issues and correctly setting-up your sound chamber.

Reflections and Interference

Sound waves are a mechanical force in which pressure is used to transmit energy through a physical medium. Sound waves can be reflected off surfaces. The reflected signal strength is based on the angle of incidence and damping factor of the reflecting surface.

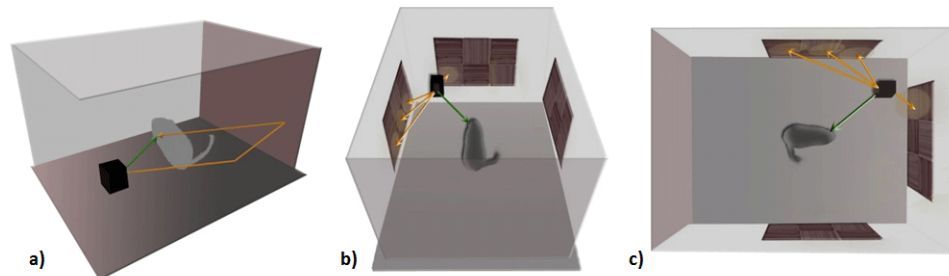


Figure 1 – Sound wave reflection vectors inside a sound chamber.

Constructive & Destructive Interference

The signal strength at a specific point of interest (e.g. subject's ear, calibration microphone location) can increase or decrease depending on the phase delay, frequency, distance of travel and damping factor of the material.

For this reason, the measured dB SPL of a specific frequency may vary greatly depending on the combination of the primary signal and its reflections at the recording site, which may appear as drop outs or spikes in the calibrated frequency response for your speaker.

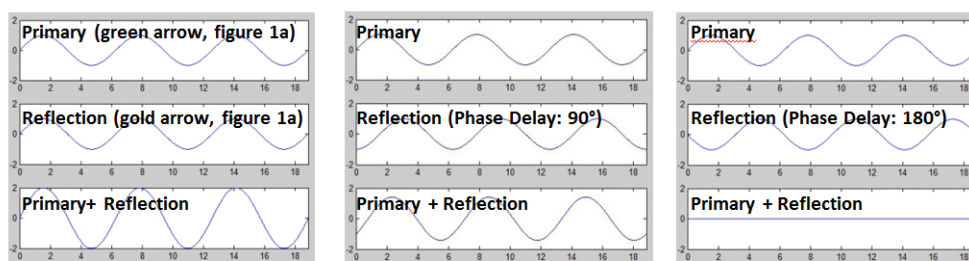


Figure 2 – The summation of signals during constructive interference (left), partial interference (middle), and fully destructive interference (right). The level of interference is based on the frequency specific phase delay, which is based on wavelength and distance of travel for the primary and reflected signals.

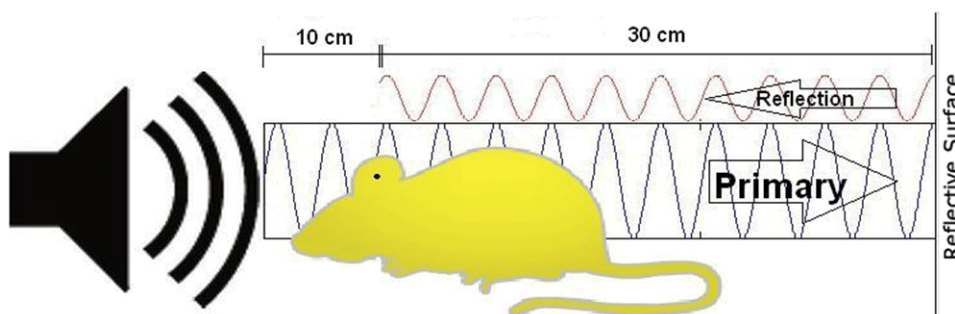


Figure 3 – A standing wave that is present at the subject's ear when the reflection returns.

Constructive and destructive interference is caused by standing waves and their reflections. In the model configuration above (Figure 3), with a speaker–subject distance of 10 cm, a subject–wall distance of 30 cm, and a reflective distance of 70 cm, any stimulus longer than 0.175 ms will generate a temporary standing wave.

The table below further emphasizes how reflections can affect standing waves and tone pips at various frequencies. The Δ value is the relative distance or tolerance between fully constructive and fully destructive interference. This value represents a 180° phase shift over a reflected distance or $\lambda/4$. The speaker–subject spacing and tolerance is very sensitive to change, especially at higher frequencies. If a speaker's position is displaced by a distance of 2.68 mm, for a 32 kHz stimulus, the reflection's phase is shifted 180° and could greatly reduce the perceived intensity.

Parameters

c (m/s)	f (Hz)	λ (mm)	Δ (mm)
–	–	c/f	$\lambda/4$
343.2	4000	85.8	21.45
343.2	8000	42.9	10.725
343.2	16000	21.45	5.3625
343.2	32000	10.725	2.68125

Table 1 – Speaker position offset tolerances with respect to frequency.

Ways to Reduce Reflections and Interference

To reduce reflections and interference:

- Increase the distance of travel for the reflections by changing the angle of incidence. If the angle of incidence is near a corner but not focused directly at a corner, the reflections will bounce off both walls and scatter throughout your chamber rather than bounce off one wall before converging at the center of the chamber.
- Increase the damping factor by covering hard surfaces (metal, tile, wood, plastic, etc.) with soft, spongy, non-reflective, sound absorbing materials (carpet, foam, etc.).

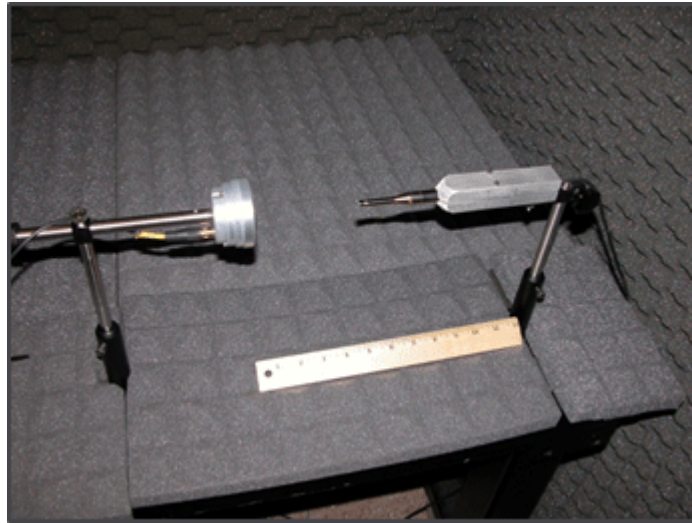


Figure 4 – A sound chamber used for testing and calibrating speakers.

The walls and table top in Figure 4 are covered by 1 inch foam pads with 1 inch tall pyramid projections (total thickness of 2 inches) for increased scattering.

**Important
note:**

The external shell is metallic and tied to earth ground, which doubles as a Faraday cage that will shield against electromagnetic radiation.

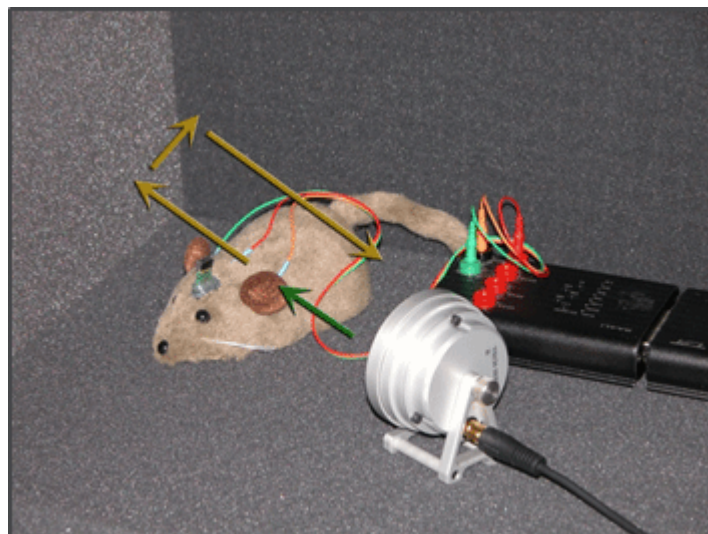


Figure 5 – An example of a good sound chamber.

The floor and walls in Figure 5 are covered in soft, absorbing surfaces (1.75 inch foam padding) with significant damping power. The speaker and subject are aligned slightly off-axis with the wall to maximize scattering.

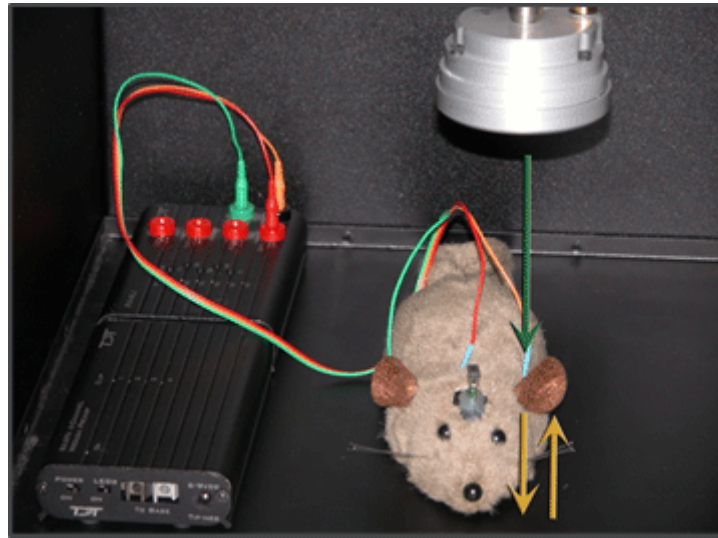


Figure 6 – An example of a bad sound chamber.

The speaker orientation in Figure 6 is an example of poor sound chamber preparation for two reasons. First, the speaker is angled directly at the floor, so the stimulus will be reflected directly back into the subject's ear. Second, the floor is a hard reflective surface that offers little to no damping.

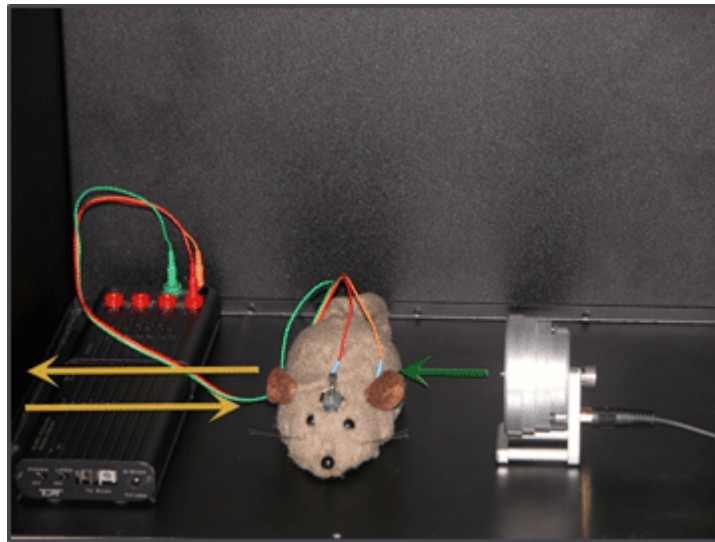
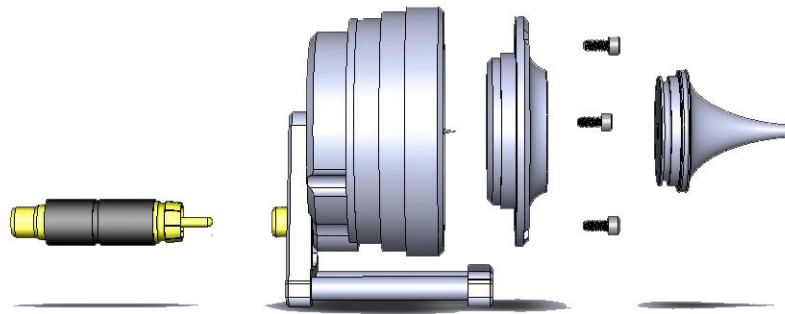


Figure 7 – An example of a bad sound chamber.

Figure 7 is also incorrect because the speaker is aligned orthogonal to the wall, which also provides a direct reflective path back to the subject's ear. In both cases, depending on the exact distance of travel, the reflections will accumulate at the subject's ear to create constructive or destructive interference, which will distort the stimulus and give an undesired sound pressure level.

Appendix B: Configuring the MF1 for Closed Field Operation

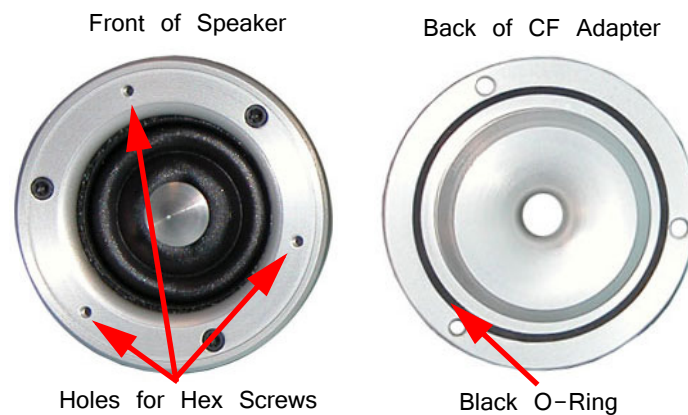


MF1 Closed Field Speaker Assembly Diagram

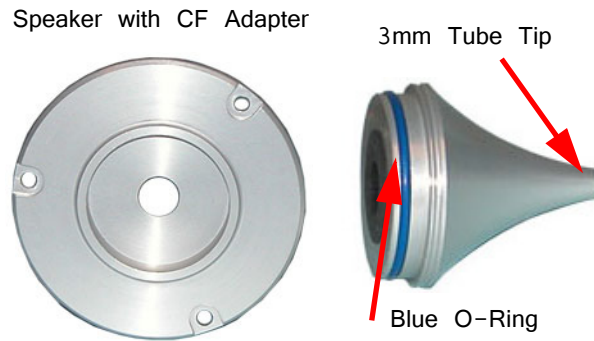
Important: Closed Field Adapter, Tip, and CF Line Filter used only for Closed Field Configuration!

To configuring the MF1 for closed field operation:

1. Ensure Black O-ring is in place on back of CF Adapter, as shown.



2. Attach the CF Adapter to the front of the speaker using three of the provided 1/4 x 4-40 hex screws.



3. Ensure the blue O-ring is in place at the base of the desired tip, as shown.

Insert the tip into the groove on the CF adapter. Ensure the tip is bottomed in its socket. Gently insert the tube into the narrow end of the tip.

4. Attach the CF filter to the provided RCA cable.

CF Line Filter



For Closed Field Configuration Only

5. Attach a BNC to RCA adapter to the BNC amplifier port of your source device.

Back of Speaker
(shown with included stand)



Amplifier Line In

6. Connect the MF1 to the amplifier using the RCA cable (with CF filter attached).

For more information on the MF1 Speaker, see the [System 3 Manual](#).

Appendix C: Modifying the Stimulus

When the stimulus is defined, a complete list of signal parameters and variables are added to the BioSigRZ file. During stimulus presentation, the values of the variables change as a function of the SGI. This is called the stimulus schedule.

You may wish to present a subset of the current stimulus schedule. Both the stimulus schedule and the variable definitions can be modified in BioSigRZ. To do so, display the stimulus schedule (as below) then select the SGIs to be included in the subset for presentation.

To view the current stimulus schedule:

- Select **Modify Schedule** from the **Operations** menu.

Or select **SigGen Variables** from the **Setup** menu.

Or click the **Modify Schedule** button.

The **SigGen Variable Control** dialog box is displayed.

The **SigGen Variable Control** dialog displays the current stimulus schedule. Variable values are shown for each SGI. Select SGIs to include in signal presentation by using standard Windows techniques, such as click, shift-click, and ctrl-click. You can also choose to Do Odd or Do Even, using buttons in the dialog box. When no SGIs are selected, BioSigRZ will present the entire stimulus schedule. Otherwise, BioSigRZ will present only the selected SGIs.

Modifying Variables

The variables can also be modified to meet your needs.

To modify a variable:

- Click the *variable button* for the desired variable, such as [Freq].
- In the *Signal Variable* dialog box, make any changes then click **OK** to confirm and return to the *SigGen Variable Control*.

Different variables may have different editable options. For example, the variable frequency for a tone stimulus may be configured by entering a Value List (shown below). For a click stimulus, the Phase may be configured using an Alternating variable method.

Another commonly modified variable is the Gate Time for tone stimuli. Typical ABR gate times are .2 ms – 2 ms.

Note: Changing the gate time will change the size and shape of the response.

Important! The changes will only be saved in the configuration file, if the **Lock SigGen Files** check box is selected in the **Stimulus Setup** dialog box. If this check box is not selected, any changes to variables or SGI selections for presentation will be lost.

Also, if you select the **Reload Files** button in the Stimulus Channel Setup window, the changes will be overwritten in favor of the values in the .sig file.

The schedule tool was designed for quick edits you may want to make while testing or setting up a new paradigm. For permanent edits to your stimulus, TDT recommends using SigGenRZ (included with BioSigRZ installations) to modify the stimulus files (.sig) directly.

Customizing the Stimulus

SigGenRZ is a signal design software that enables you to create stimuli in a dialog box driven environment. Most importantly, it allows most settings to be used as variables for your experiments. All of the signals used in the BioSigRZ out-of-the-box experiment configurations have been created in SigGenRZ. It is also a helpful tool for modifying aspects of the stimulus that are not editable from within BioSigRZ.

To modify a file directly in the SigGenRZ software you will need to follow these basic steps:

1. Open the stimulus file (.sig) in SigGenRZ.
2. Make changes in the file.
3. Save the file or save the file with a new name.
4. Open the desired BioSigRZ configuration file (.acf).
5. Load or reload the stimulus file.
6. Save the BioSigRZ configuration file.

Opening the File

The stimulus files (.sig) for the BioSigRZ out-of-the-box experiment configuration are stored at:

C:/TDT/BioSigRZ/SIG/

For ABRs, there is one file for click stimuli and one for tone stimuli: **Click.sig** and **TonePip.sig**, respectively.

It is generally a good idea to leave the original files unchanged and in place, but you can still use and modify these files then rename the file.

To open the signal file you wish to modify:

1. Click the **Start** button on the Windows taskbar, point to **Programs**, point to **TDT Sys 3**, and click **SigGenRZ**.
2. Select **Open** from the **File** menu.
3. Click the desired file name.
4. Click **OK**.

To save the file with a new name:

1. Select the signal by clicking anywhere in the signal window.
2. Choose **Save As** from the **File** menu.

3. The **Save As** dialog box will be displayed.
4. Type a new name for the signal and click **OK**.

SigGenRZ builds a signal from mini-signals called segments. The standard ABR stimuli have only one segment. Some settings affect the entire signal while others affect only a segment.

The most common changes to the signal settings include *Timing Duration* and, for click calibration, *Calibration Level* and *Attenuation Level*. These can be found in the **Signal Parameters** dialog box.

To open the Signal Parameters dialog box:

- Click **Signal** from the **Modify** menu.

The most common changes to the segment parameters include: *Gate Type*, *Gate Time* and *Duration*. Parameter settings that define the segment can be modified in the **Edit Signal Segments** window.

To access the Edit Signal Segment window:

- Select **Segment** from the **Modify** menu of the main window.
- or
- Double-click the segment marker in the signal window.

Segment Marker

In the Edit Signal Segment window, you'll find a variety of Windowing/Gating and other parameters.

Gate Type

From the Gate Type box, you can select a variety of gating or windowing functions, including:

- Cos2 (10% – 90%)
- Ramp
- Blackman
- Hanning

For click stimuli, gating is not necessary and the Gate Type should be set to None.

For tone pips, Cos2 is the default setting.

Appendix D: Noise Exposure

ABR testing is a common method used to investigate the link between noise exposure and hearing loss. As a result, some of our customers have asked for more information on using their TDT system for implementing noise exposure in the lab. This appendix includes basic information on noise delivery and our best advice for lab set up. For investigators new to noise exposure experiments, we also recommend consulting other researchers with experience in the field.

Noise exposure testing typically compares the results of hearing measurements before and after exposure. The goal of noise exposure delivery in the lab is to reproduce or simulate the effects, including hearing damage or deafening, of real-world noise exposure.

Key elements of noise exposure:

- High dB noise delivery
- Extended duration (hours) of continuous sound delivery
- Filtered or unfiltered noise (tones can also be used to investigate frequency-specific hearing loss)

Hardware Requirements:

- TDT Signal Processor, such as the RZ6 Auditory Processor
- Amplifier
- Speaker
- Microphone and mic amplifier (for calibration and testing enclosure)
- PC for experiment control

When considering your hardware set-up, the first question to ask is what level sound you will be using. The TDT amplifiers and speakers are not intended for delivering sound greater than 100 dB over long periods, typical of most noise exposure protocols. That type of use will damage the speaker and will almost certainly result in clipping the stimulus. We suggest using an external amplifier and speakers.

External Amplifiers

You might already have an appropriate amplifier in your lab. One of the most common amplifiers we have seen in labs is the Crown D-75 or Crown D-75A. Unfortunately, this is no longer available. If you have one, this is a good choice. If you don't already have a suitable amplifier, we can recommend the CSA 280Z (<http://jblcommercialproducts.com/en-US/products/csa-240z-280z-2120z>) manufactured by JBL (formerly Crown). The CSA 280Z is a two channel amplifier that provides up to 80 watts of power.

Speakers

Because speaker models change frequently, it is difficult for us to recommend a specific speaker. A commonly available three way, 6 inch by 9 inch car speaker is a good choice for delivering sound.

Setting Up Signal Generation in RPvdsEx

All of the tasks performed by your RZ6 are configured via Real-time Processor Visual Design Studio, RPvdsEx, files. BioSigRZ and all other TDT software use RPvdsEx files behind the scenes to control TDT hardware. You will use this same software to deliver noise exposure.

The RPvdsEx is a nuts and bolts tool with virtually limitless flexibility, but it wasn't really designed for running experiments. That means the user interface will look a little different from our applications software. For this purpose, however, it will work just fine and all you will need to do when it is time to start signal presentation is open a file and click run.

Before you run signal presentation, however, you will need to make some basic adjustments to the file.

TDT has three versions of a basic timed sound presentation file:

Filtered Noise	RZ6_TimedNoise_Calibrated.rcx
Unfiltered Noise	RZ6_TimedNoise_Calibrated_NoFilter.rcx
Tone	RZ6_TimedTone_Calibrated.rcx

These files are included in the BioSigRZ Examples folder found at: <http://www.tdt.com/files/examples/BioSigRZExamples.zip>

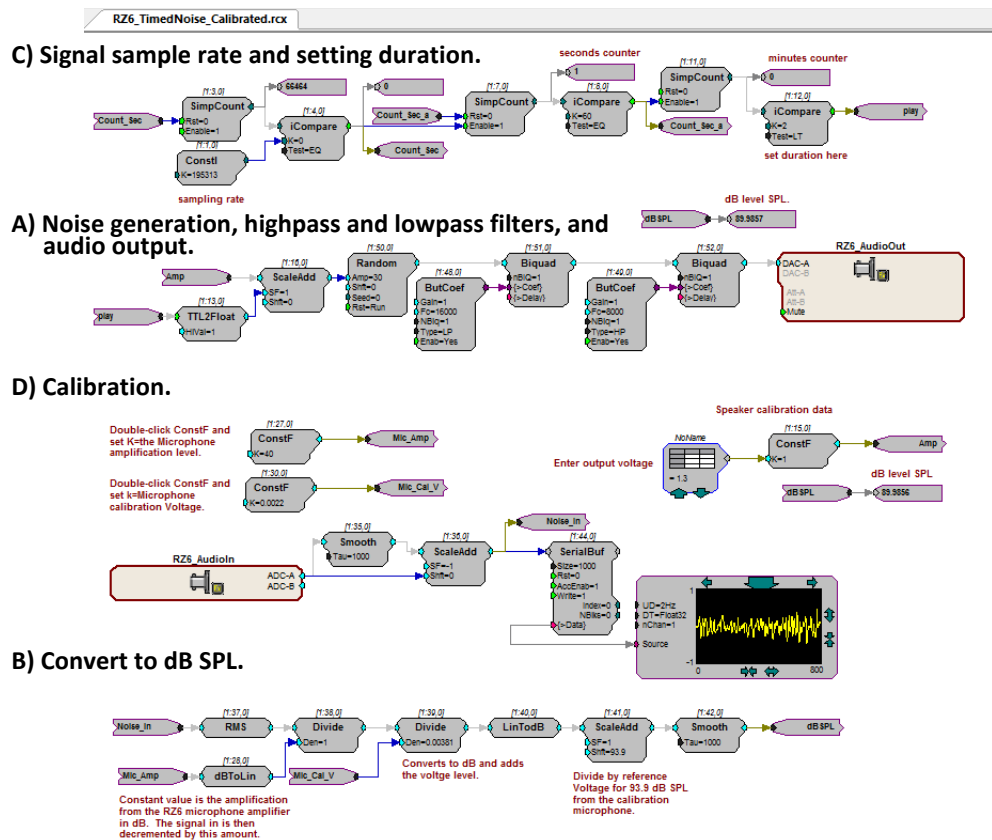
After you've downloaded and extracted the folder, choose the file that is right for you and double-click it to run RPvdsEx and open the file.

Modifying the RPvdsEx File

The processing functions that make up this task appear as components ordered and connected in the processing chain and arranged on a large sheet. You can use the



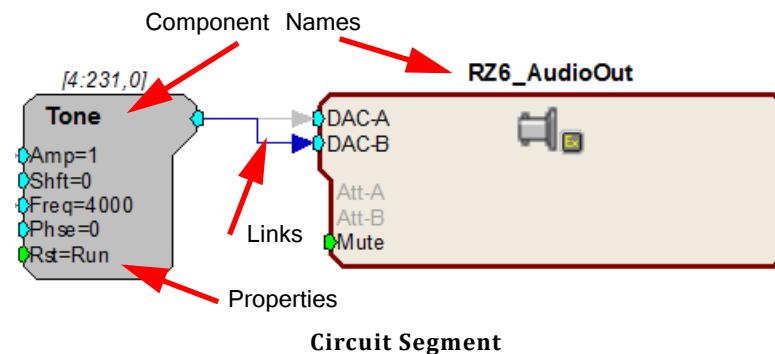
Pan button or the horizontal and vertical scroll bars to move around the sheet. The illustration below shows the circuit and identifies the tasks in each section.



Random Noise Generation Circuit

Working with Circuit Components

This circuit is configured for you, but you will need to change the properties of some of the components.



To modify the properties of any component, double-click it and enter a new value in the properties dialog that is displayed and then click OK.

To create a link, click an output port on a component, then click the input port on the next component in the circuit.

To add a component, double-click the workspace and select a component.

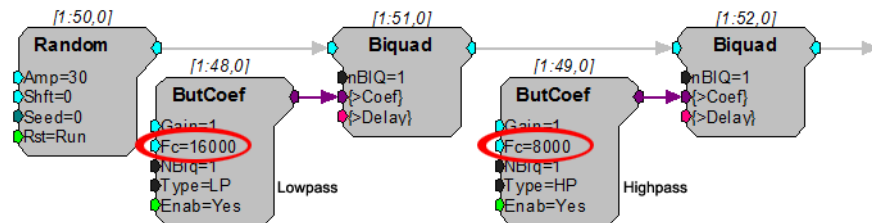
To delete a component or link, click it and press the DELETE key on your keyboard.

For more information on working with circuits, See the *RPvdsEx Manual*.

To modify the file:

1. Open the appropriate RPvdsEx file.
2. If you are using filtered noise, the first step is setting the filter values. If not, you can skip to the next step.

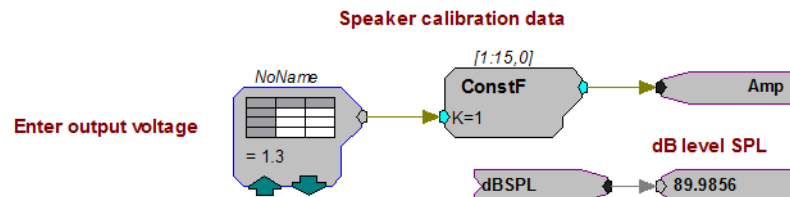
Find **section B**, in the illustration above. By default the circuit uses a lowpass filter with a corner frequency at 16 kHz and a highpass filter with a corner frequency at 8 kHz.



These components generate the filter coefficients used by the Biquad filters. To set the filters, modify the corner frequencies (Fc) in the ButCoef components as needed.

3. The TDT System outputs a voltage. To ensure the output voltage is calculated correctly for the desired dB results, you need to calibrate the system.

Find **section C**, in the illustration above. The voltage is set in the circuit segment pictured below. By default it is set to 1.3 V, which should be a good starting point. For now, leave the default voltage as is.

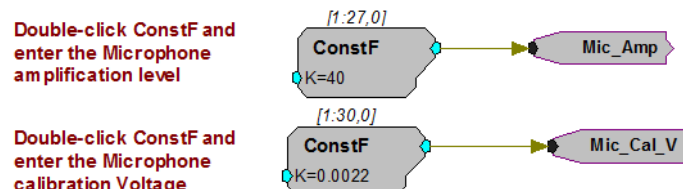


The expected dB SPL is displayed for reference. The actual dB will depend on your complete system, including the external amplifier and speaker.

To begin calibration, place the calibration microphone in the subject enclosure in place of the subject. We recommend the PCB-378C01 PCB microphone and mic amplifier, mentioned on pages 24–26.

4. Enter the microphone sensitivity for your calibration microphone.

Find this circuit segment pictured below.



Double-click the first ConstF component and enter the microphone amplification level indicated by the microphone Gain knob setting on the face of the RZ6. Click OK.


Note: the microphone amplification switch on the RZ6 front panel, must be set to AMP.


Using the microphone sensitivity provided by the manufacturer, determine the calibration microphone voltage (usually listed on spec sheet in millivolts per Pascal). Double-click the second ConstF component and enter the value in volts. Click **OK**.


5. **Section B** of the circuit, calculates the dB SPL. You shouldn't need to make any changes in this segment, but this a good time to save the circuit with a new name.

To run the circuit for calibration:

6. To begin calibration, make sure the microphone is in place and the chamber

is closed, then click the **Compile, load, run**  button on the main toolbar.

7. To stop presenting the sound, click the **Stop**  button.

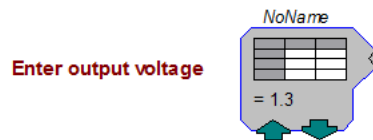
8. To restart, press the **Run**  button.

Both Run and Compile, Load, Run restart the presentation, however, Compile Load, Run also restarts the circuit timer. Run resumes from current value in timer.

To modify the file:

9. Use the level recorded by the microphone to adjust the voltage.

To update to the corrected voltage, locate the DataTable component pictured here.

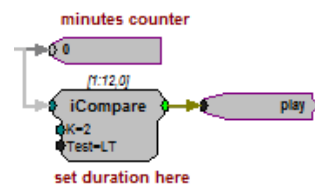


Click the green up or down arrows to adjust the value.

If none of the preset values is suitable, you can double-click the DataTable component to modify the table.

Note: TDT devices are limited to ± 10 V. If the calibration results in a voltage outside this range, your stimulus will be clipped and distorted. If this becomes a problem, you need a more sensitive speaker or a more powerful amplifier.


10. Finally, before beginning signal presentation, set desired duration in minutes.




Double-click the **iCompare** component labeled “set duration here” and enter a value for K equal to the desired number of minutes you want the signal to play. For example, $k = 120$ (2 hours). Click **OK**.

11. Save the changes you've made. Be sure the file is saved using a new name.

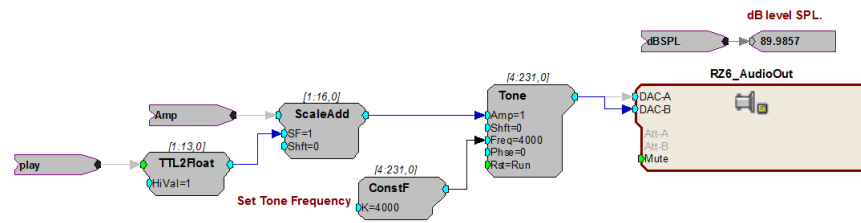
To run the circuit for calibration:

12. To begin, click the **Compile, load, run**  button on the main toolbar.

13. To stop presenting the sound, click the **Stop**  button.

Using Tones

If you are using the timed tone signal file, you will need to set the tone frequency. Find the section of the file pictured below.



To set the tone frequency:

- Double-click the **ConstF** component, set the desired frequency in Hz, and click **OK**. Then run the circuit as explained above.

Preparing a Suitable Enclosure

Noise exposure is performed inside a chamber to protect the researcher's hearing. You will also need an enclosure inside the chamber to limit the movements of the agitated subject and ensure it is positioned near the speaker. If you do not already have a suitable animal enclosure, you can build one from widely available materials. This section provides general recommendations for your chamber and enclosure.

Sound Proof the Chamber

Line the inside of the chamber with acoustic foam to eliminate constructive or destructive interference with the signal. See Appendix A for more information.

Build the Enclosure

Use mesh hardware cloth to create an oval tube or cylinder with a maximum width that matches your selected speaker. Mesh hardware does not interfere with noise moving through the chamber but will resist the subject's attempts to escape the space and be strong enough to support the weight of a speaker placed at the top of the tube.

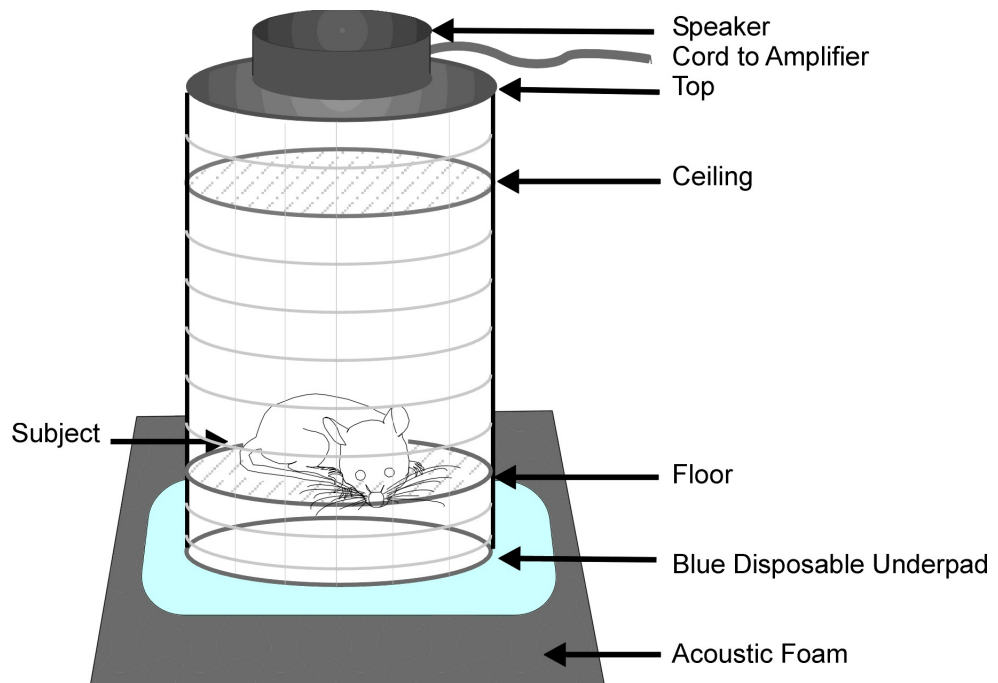
Use mesh hardware cloth to create a raised floor a few inches above the acoustic foam and a lowered ceiling a few inches below the speaker. This will prevent the subject from damaging the acoustic foam or the speaker in any attempt to escape the space.

Protect Foam with Replaceable Underpad

Place a blue disposable underpad on the acoustic foam below the tube to collect droppings and prevent damage to the foam.

Position the Speaker

Position the speaker directly above the tube, facing down toward the subject.



Completed Enclosure with Subject

Ensure Consistent Signal Delivery

After you build the enclosure, verify that SPL at all test levels or frequencies is even across all spaces in the enclosure.

Working with Multiple Subjects

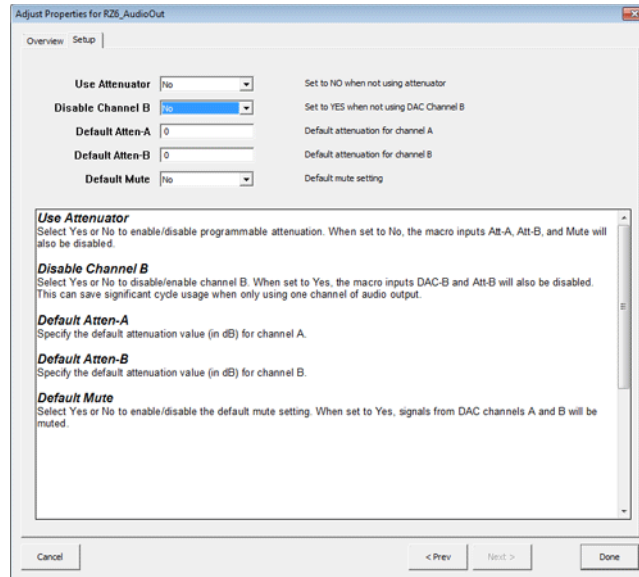
If you need to expose more than one subject at a time, you can use mesh hardware cloth to divide the tube into four equal size enclosures. The smaller space may also help limit the subject's movements and agitation.

For more than four subjects, add a second tube and speaker in the enclosure and add a second channel to the RPvdsEx circuit file.

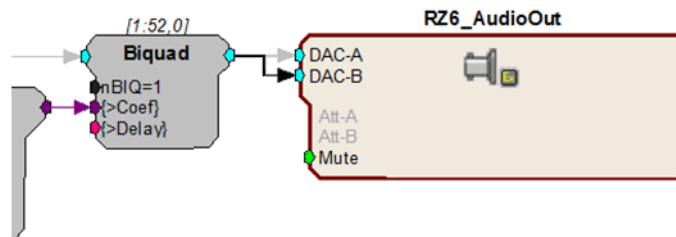
To add a second channel:

1. Open the RPvcsEx file.
2. Find the RZ6_AudioOut macro component pictured below. Double-click the component to display the properties.

- On the Setup tab, change Disable Channel B to **No**, and click **Done**.



- To route the signal to channel B, double-click the teal output port of the Biquad filter that precedes the RZ6_AudioOut then click the teal input port for DAC-B.



- Be sure to save your changes.

Connect the second output of your RZ6 to a second external amplifier and speaker.