Protocol for extracting amplitude and latency data from ABRs collected on Tucker Davis Technologies rig.

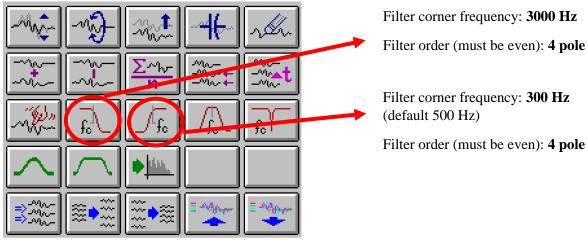
Preparing data for program

If you already know how to export filtered ABRs to CSVs skip to Section titled ABR Waveform Analysis Program

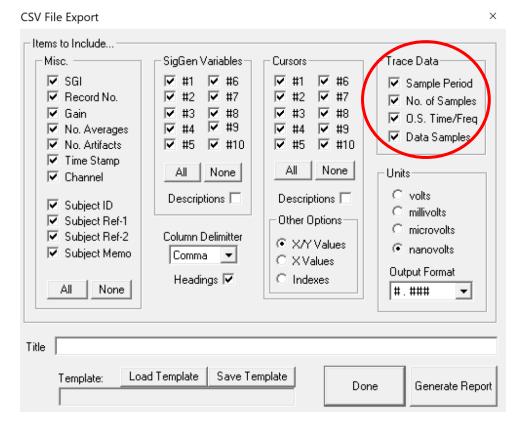
In BioSigRZ:

Open up the access record file (.arf) containing your data.

- Top toolbar > History > Access Record File (navigate to folder where data are stored and select file > Open > Select access record group no. (highlights blue when selected) > Load > Load all
- Select all SGIs (click top trace, hold shift, click bottom trace) and drag over to the worksheet window
- Filter corner frequencies of all traces by clicking the circled buttons below (sample values recommended for mice shown in text boxes).



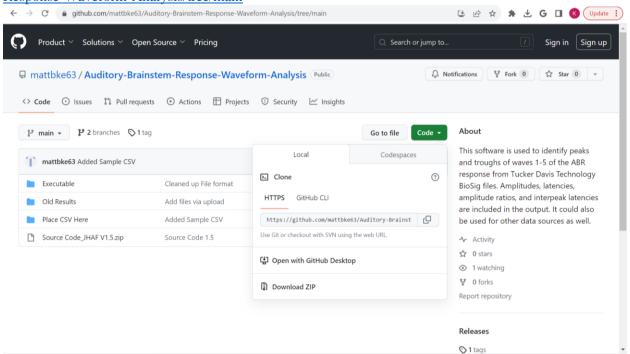
• Top toolbar > Worksheet > Export to CSV > Check the 4 circled boxes under Trace Data indicated in the image below > Generate Report > Save as .csv file > Done



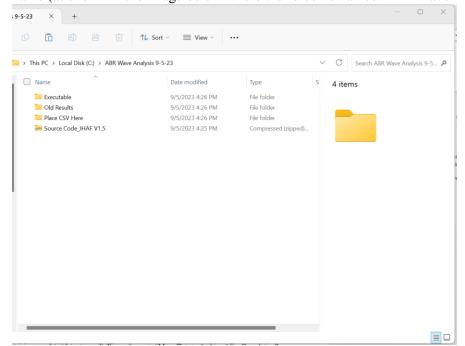
• Repeat these steps for all files you would like to extract amplitudes and latencies. If your dataset includes more than 3 frequencies of tones (with 9 levels) be careful not to exceed the worksheet size limit.

ABR Waveform Analysis Program

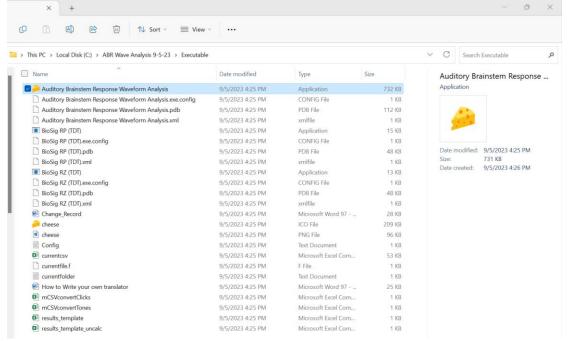
• Go to github and download program: https://github.com/mattbke63/Auditory-Brainstem-Response-Waveform-Analysis/tree/main



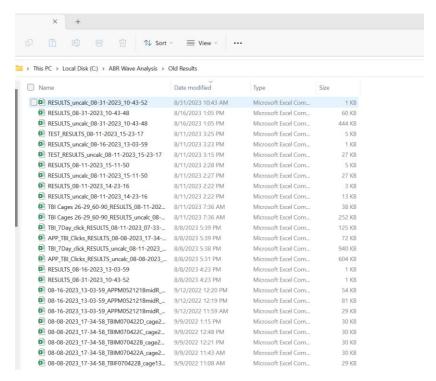
- Select green "Code" button, and Download ZIP
- Once downloaded, unzip and copy the "Auditory-Brainstem-Response-Waveform-Analysis-main" folder and all subfolders in C drive. This root directory can be renamed to a shorter file name (as shown in the image below where the folder is named ABR Wave Analysis 9-5-23).



 The "Executable" folder contains the program as an application called Auditory Brainstem Response Waveform Analysis with a cheese icon (selected in image below)



• The "Old Results" Folder will contain all "RESULTS" files from previously executed runs (date stamped) including calculations generated by the program, and all "RESULTS_uncalc" files containing raw data (date stamped). If you choose to "Move Data to Archive After Completion" then input files will also appear in this folder. If you assign your file a "Saved File Name" this will appear before the word "RESULTS" or "RESULTS_uncalc" in the file name. Upon first download there will only be a file called "New Text Document" which can be deleted. Shown below is an example "Old Results" folder for some runs where the files had a saved name and some where they do not.



• The "Place CSV Here" folder is where you should move all CSVs you wish to analyze after exporting them in the manner described above in BioSigRZ or BioSigRP. There is no limit to the number of files you can analyze, though it is recommended that you try a small subset of data first to verify parameters. The files shown below are named by experiment_cage and subject number_sex_timepoint_ear_frequency/click. If using a custom file output you will need to first build a translator and then indicate this in subfield for "Data Type Accepted" in the application explained more below. Clicks and tones can be analyzed in the same batch so long as the duration of the recording is the same for both.

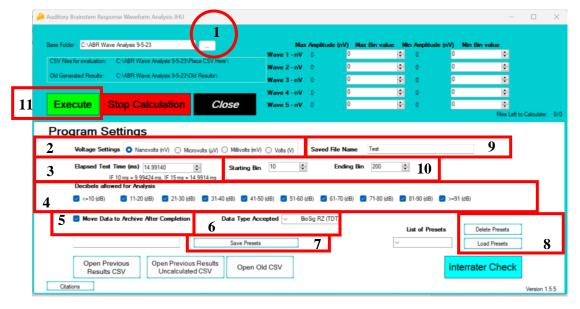
TBI_1A_M_Baseline_R_8_12_16kHz.csv	12/6/2021 10:44 AM	Microsoft Excel Co	82 KB
TBI_1A_M_Baseline_R_24_32kHz.csv	12/6/2021 10:45 AM	Microsoft Excel Co	55 KB
TBI_1A_M_Baseline_R_Click.csv	12/6/2021 10:37 AM	Microsoft Excel Co	31 KB
TBI_1B_M_Baseline_L_8_12_16kHz.csv	12/6/2021 10:46 AM	Microsoft Excel Co	82 KB
TBI_1B_M_Baseline_L_24_32kHz.csv	12/6/2021 10:46 AM	Microsoft Excel Co	55 KB
TBI_1B_M_Baseline_L_Click.csv	12/6/2021 10:38 AM	Microsoft Excel Co	31 KB

Once downloaded, unzipped, and placed in the C drive, the program can be launched from the "Executable" folder by double clicking on the "Auditory Brainstem Response Waveform Analysis" application. Upon the first launch you may get a message like below:



If so, click Run anyway and you will not be prompted with this message again in future launches of the program.

• First set the base folder for the program by clicking the circled (1) ... button in the image of the user interface (pictured below). This folder should be the root folder containing "Executable" "Old Results" and "Place CSV Here" (and a zipped folder containing the source code) folders. The program will auto-populate the "CSV Files for evaluation:" field to the path where the "Place CSV Here" folder is and the Old Generated Results: to the path where the "Old Results" folder is based on this root directory.



- Configure your program settings to match the format of your CSV files. Select the voltage (2) setting in your CSV files (this must be the same for all files being processed together). All units will be converted into nanovolts (nV) in the output so we recommend exporting in TDT to nanovolts and selecting nanovolts here.
- Set the "Elapsed Time" in ms of your files (3). The CSV file will have time in bins (e.g., ranging from 0-365 for a 15 ms recording) which will be used to calculate the exact latency and inter peak latencies. Be sure to get the exact value in ms from BioSigRZ (a 15 ms recording is 14.9994 not 15.0000 ms which is explicitly found for your recording duration by exporting the file in

BioSigRZ as an ascii file). **Important:** Do not analyze files with different durations in ms in the same batch as calculations are based on the total elapsed time and the bin number- you will have incorrect values in your output for incorrectly labeled durations. An error message will pop up in the program if any file has a different number of bins (and therefore a different duration) than the previous file analyzed.

- Select which intensities you would like to analyze (4). These are ranges so if you have an intensity of 85dB that you would like to include in your analysis select the button for 81-90 dB. When starting off, it is recommended that you do not check all boxes to first familiarize yourself with the program/output.
- To remove CSV files from the "Place CSV Here" folder after the execution is complete check the Move Data to Archive After Completion button (5). This will help you to analyze in batches one dataset without redundantly including it in subsequent analyses. You may not want to check this box if you want to do a first pass only looking at suprathreshold intensity (e.g., 90 dB) and then following up a second pass on multiple intensities across the full level series.
- There are 3 built-in templates for data processing, a custom matlab format used by the Lauer lab, a BioSigRP (TDT) for setups running with an RX6 processor, and BioSigRZ for newer setups using and RZ6 processor. Be sure to select the correct Data Type Accepted (6) to ensure the data are read in correctly.
- To streamline future data processing, you can save your settings as a preset (7) (call it something that is obvious to the settings, e.g., 15 ms 90-10dB analysis. To delete a preset you no longer want saved hit delete presets, and to load a preset you've saved click the load presets button (8).
- To assign your execution with a file name, enter it under "Saved File Name" (9) prior to executing the program, otherwise output files will all be named "RESULTS.csv" and "RESULTS_uncalc.csv" with a timestamp.
- The bins for analysis can be limited based on your trace duration (10) with recommended start bin of 10 and end bin of 200 for a 15 ms recording. This will help to avoid any false triggers by the summating potential and noise floor.
- Once you are satisfied with your settings hit "Execute" (11).
- The ABR waveform of the first CSV file will appear (one intensity at a time) plotted as bin number by amplitude (see main graph below). The peaks of waves will always be indicated by orange dots, and the troughs of waves will always be indicated as cyan dots. The X coordinates of each of these can be manually adjusted by clicking the up/down tickers for the Max Amplitude (orange) (1) and Min Amplitude (blue) (2) individually for each of the 5 waves. If you hover over a spot on the waveform, it will tell you the coordinates of that spot and you can type the entry in instead of toggling up or down one digit at a time. While the program uses an algorithm of increasing and then decreasing slopes for maxes and decreasing and then increasing slopes for mins, you MUST spot check every single trace to be sure that it is not incorrectly identifying the mins and maxes. One major issue is grabbing the summating potential instead of wave 1, or misidentifying noise as peaks (especially in lower intensities or later waves). This program is intended to streamline the processing but the algorithm is not **perfect.** As you can see from the plot below, these peaks and troughs are accurately representing the maxes and mins of Waves 1-4 of the ABR response with a questionable identification of wave 5. When you are satisfied with the placement of the Min and Max value for each of the 5 waves (if they are present) you can hit the "Next Graph" (3) button. Traces are read in from the CSV sequentially, so if you selected all intensities for analysis the next lowest intensity will be the next graph. If you only selected 90 dB then the next trace will be for the next subject at 90 dB. To go back to the previous graph (or keep moving backwards) you can hit the "Previous Graph" button

Set all Bins to 0 der C:\ABR Wave Analysis 9-5-23 C:\ABR Wave Analysis 9-5-23\Old Results 3 -2310.524 Stop Calculation Close Decibel Level:90.000000 4000 Set all Bins to 0 ÷ -3794.680 ÷ Stop Calculation Close 0 -686.567 Sub ID: TBIM070422B_29 Decibel Level:80.000000 6000 4000 2000 -2000--4000--6000

which will appear above (3) once you have advanced past the first trace (shown in second picture below labeled (4).

As you can see the 5th wave is not identified correctly by the algorithm. See the corrected wave 5 at 80 dB shown below by modifying the Max Bin value and Min Bin value corresponding to only wave 5.

Bin Number
ate data (Bin number, Amplitude) / Zoom with Scroll Wheel / Reset Zoom with Double Click / Pan Graph with left click and drag (Cursor must be on graph for both c Ve

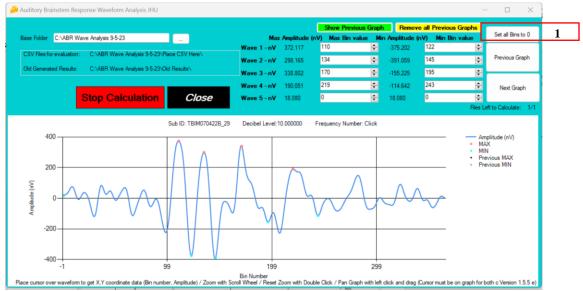


Comparing traces for a subject at lower intensities to higher intensities can help with user identification of individual waves. This can be done with the "Show Previous Graph" button (1) shown in the image above to display 90 dB and 80 dB simultaneously.

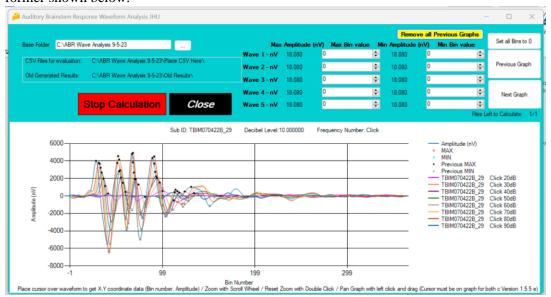


In the above image you can see 90 and 80 dB traces plotted on top of one another. You can confirm the placement of all peaks and troughs of Waves 1-4 and see the questionable identification of wave 5 in the 90 dB trace. The "Previous Graph" and "Next Graph" buttons can be used to easily toggle back and forth between levels to change cursor placements, while the green "Show Previous Graph" button can be used to view all levels for a subject simultaneously.

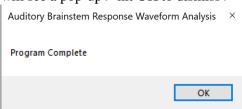
At the lowest intensities (e.g., 10 dB shown below) especially noisy signals may yield false triggers. To quickly reject all peaks defined by the algorithm select (1) "Set all Bins to 0" button.



Once all peaks have been identified at all levels, it is advisable to look at all levels stacked together for internal validation that peaks are being identified consistently. This can be done by clicking the green "Show Previous Graph" button repeatedly. There is no limit to the number of traces that can be shown simultaneously, but it is advisable to either look within one stimulus and subject at all levels or to look across subjects within one stimulus and level. See example of the former shown below.

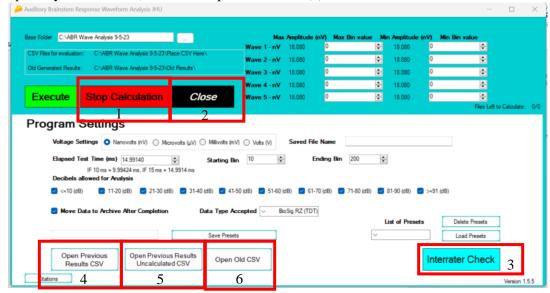


• When you are satisfied with all of your mins and maxes for your batch and you reach the end of all files in the directory you will see a pop-up > hit OK to dismiss >

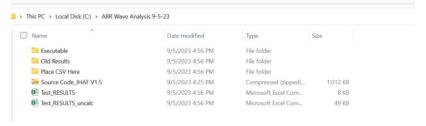


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- If you are part way through your analysis and you need to stop the program to finish analyzing at a later time, you can hit the red "Stop Calculation" button (see (1) below) and it will exit and the program complete pop-up will appear.
- When you are finished using the program you can close in the X in the corner of the user interface or hit the black "Close" button (see (2) below).
- "Interrater Check" button (see (3) below) and select the result file corresponding to the timestamp for your analysis. If it is the file you have most recently completed you will need to navigate to the base directory to find the file, otherwise all older runs will be moved to the "Results Files" folder and will get a time stamp added to the file name. These files can be shared to a second rater who can toggle through each trace to confirm they agree with each of the mins and maxes that were selected but be advised that it will over-write the results file that is loaded in, so save a copy somewhere else if you will calculate an inter-rater reliability statistic. To open the most recent results file you just tested not as an inter-rater check, click the "Open Previous Results CSV" button (4) and to open the merged spreadsheet containing all the raw data that was in each separate CSV in one file click the "Open Previous Results Uncalculated CSV" (5) button. To open any old CSV file, click the "Open Old CSV" (6).

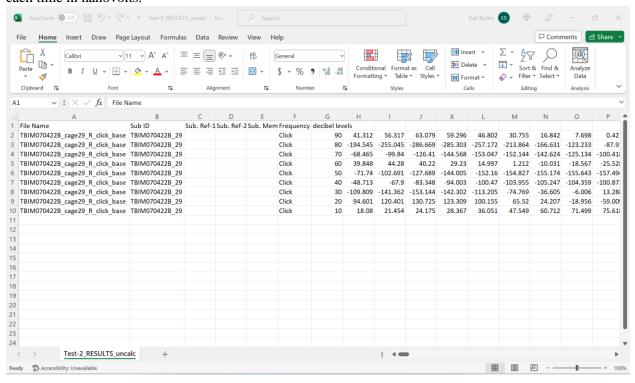


Once you have completed your first run with the Sample data provided, the base directory will be populated with your "RESULTS" and "RESULTS_uncalc" csv files. Because a file name was identified prior to running the data through the program, these files shown in the image below are called "Test_RESULTS.csv" and "Test_RESULTS_uncalc.csv"

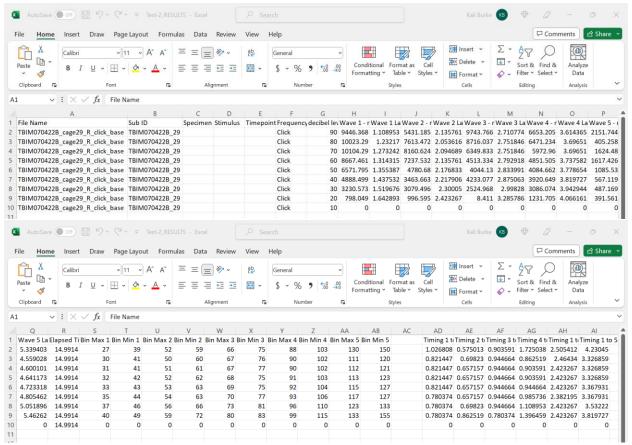


Understanding the output

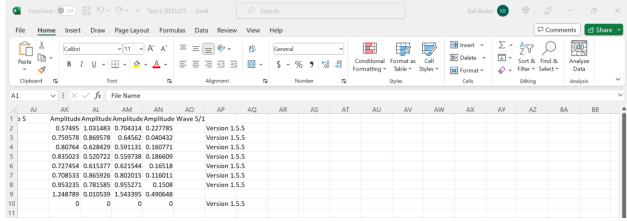
RESULTS_uncalc file = a file that merges all of the CSVs you placed into the "Place CSV Here" folder into one CSV. This is the raw data that is otherwise unchanged. Column A is the file name (e.g., this file was called "TBIM070422B_cage29_R_click_base"", so all cells from that CSV contain that in the "File Name" column. Column B titled "Sub ID" is what was typed into the "Notes" field in BioSigRZ when running the ABR. Columns C, D, and E can be extracted from your file names if you include the stimulus type (e.g., "Click") or time point (e.g., "baseline") but will otherwise not populate if that information isn't in the file name. Column F "Frequency" is populated from the raw data CSV where it specifies the frequency tested. Column G "decibel levels" includes each level of the level series tested for that subject at that frequency that was included in the analysis. Note: if you only analyze one intensity for all subjects, it will only include that level and no other levels from the original raw data in this CSV. Columns H - ... will contain the voltage at each of the X bins present in your data set. In this example the data was sampled 365 times so the data extends from columns H to NI, with the voltage represented at each time in nanovolts.



• Results file = The file containing calculations for Wave 1-5 including latencies of each wave, amplitudes (absolute value of min + absolute value of max, for each wave), as well as interpeak latencies and amplitude ratios. Columns A-G are redundant with the Results_uncalc file. Column H "Wave 1 – nV" is the amplitude in nV of Wave 1. Column I "Wave 1 Latency" is the timing of the max point for Wave 1. This repeats separately for each of the 5 waves in columns J-Q.

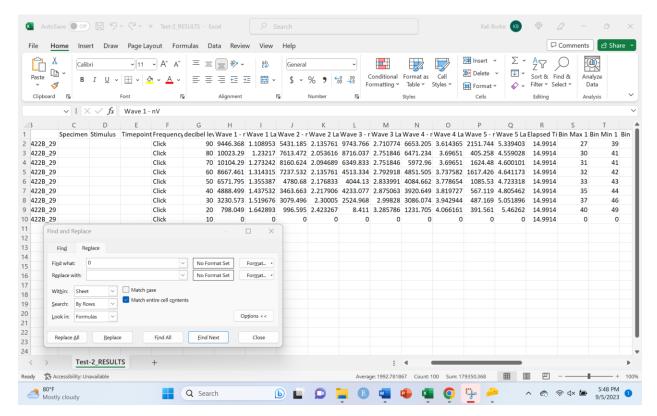


Column R "Elapsed Time" is the exact ms entered into the program and used for the calculations from time bin to ms. Column S "Bin Max 1" is the value selected by the user for the max of wave 1. Column T "Bin Min 1" is the value selected by the user for the min of wave 1. This is repeated for the rest of the waves (II - V) in columns T-AB. Column AD contains the interpeak latency between wave 1 and 2 (calculated as latency wave 2 – latency wave 1). Timing 2 to 3 is the interpeak latency between wave 2 and 3 (calculated as latency wave 3 – latency wave 2). These interpeak latencies continue in columns AF-AI.



Column AK "Amplitude Wave 2/1" is the amplitude ratio between Wave 2:1 (calculated amplitude of Wave 2/Amplitude Wave 1). Column AL "Amplitude Wave 3/1 is the amplitude ratio between wave 3 and wave 1 (calculated amplitude Wave 3/Amplitude Wave 1). This is continued for other amplitude ratios in columns AM-AN.

AN IMPORTANT NOTE IS THAT CELLS CONTAINING VALUES OF EXACTLY 0 NEED TO BE REPLACED WITH EMPTY CELLS PRIOR TO ANALYSIS. This can be done by selecting the columns with data, typing Ctrl + F, clicking replace, checking the box "Match Entire Cell Contents", and filling the find value with "0" and then replace with nothing, and clicking "Replace All". If you skip this step your data will not be accurate because it will include 0 as a numerical value for calculations.



Data are output in columns which would make them easily able to be analyzed using R to examine differences across various factors of interest, to make input-output plots, or to add additional calculations for analysis than those built into this program. Custom code for analyzing data or generating plots in R studio are available upon request.

This ReadMe was written for usage of the software as updated 9/5/23 in version 1.5.5. Please see the Github page for any updates to the program that may occur after this date.