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Auditory brainstem response (ABR) waveform analysis program ☆

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ABSTRACT

Auditory brainstem responses (ABR) are a high-throughput assessment of auditory function. Many studies determine changes to the threshold at frequencies that span the normal hearing range of their test subjects, but fewer studies evaluate changes in waveform morphology. The goal of developing this program was to make a user-friendly semiautomatic peak-detection algorithm to encourage widespread analysis of the amplitudes and latencies of the ABR, which may yield informative details about the integrity of the auditory system with development, aging, genetic manipulations, or damaging conditions. This method incorporates automated peak detection with manual override and inter-rater validation to calculate the amplitude and latency for waves 1–5, as well as interpeak latencies and amplitude ratios between waves. The output includes raw data and calculations in a format compatible with graphical and statistical software.

- The method yields a high-throughput peak-detection algorithm with manual override and inter-rater capabilities to streamline ABR waveform analysis.
- Data output includes amplitudes, latencies, amplitude ratios, and interpeak latencies for generation of input-output curves.
- While complete automation of peak detection with this tool is dependent on good signal-to-noise ratios, relevant amplitude and latency calculations are fully automated, and manual spot-checking is simplified to significantly reduce the time to analyze waveforms.

Specifications table

Subject area:	Neuroscience
More specific subject area:	Automation of data processing for functional assessment of auditory periphery and brainstem
Name of your method:	Auditory Brainstem Response Waveform Analysis Software
Name and reference of original method:	ABRs - Physiological evidence for delayed age-related hearing loss in two long-lived rodent species (P. leucopus and P. californicus) [1]
Resource availability:	https://github.com/mattbke63/Auditory-Brainstem-Response-Waveform-Analysis.git

☆ Related research article: [1] G. Capshaw, S. Vicencio-Jimenez, L.A., Screven, K. Burke, M.M. Weinberg, A.M. Lauer, Physiological evidence for delayed age-related hearing loss in two long-lived rodent species (Peromyscus leucopus and P. californicus), *J. Assoc. Res. Otolaryngol. (2022) 1–15*. 10.1007/s10162–022–00860–4

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Introduction

Recording the auditory brainstem response (ABR) is a useful method to rapidly assess auditory system function in animals. The presentation of transient sound stimuli evokes a robust onset-dependent response in normal hearing individuals that is easily recorded via electrodes placed on the scalp or subdermally. The ABR can be used to quickly identify auditory peripheral and brainstem status using high-intensity stimuli (e.g., 90 dB broadband click), or to evaluate auditory sensitivity across the hearing range in response to stimuli bracketing thresholds. Auditory sensitivity is reported as an ABR threshold, defined as the lowest sound level at which ABR waveforms are distinguishable from background physiological noise (see [2] for a complete guide to conducting ABRs in small mammals and [3] for a description of a thresholding algorithm). In addition to auditory thresholds, ABR wave morphology can be probed to assess the time it takes various processing centers of the auditory brainstem to receive incoming sounds (e.g., latency between waves of the response) and the integrity of the ascending auditory pathway (e.g., using amplitudes of individual ABR waves).

The ABR in mammals includes 4 to 5 easily identifiable waves which are stereotyped in their time course and reflect evoked activity in the auditory nerve and brainstem. Animal studies have been critical for identifying the generators of each ABR wave in the ascending auditory tract, although multiple cell populations in various nuclei contribute to the centrally generated ABR components in mammals (e.g., [4,5]). Wave 1 is generated by the auditory nerve and spiral ganglion neurons. Wave 2 is driven mostly by the globular bushy cells in the cochlear nucleus. Wave 3 is generated partly by spherical cells from the cochlear nucleus and the medial superior olive. Wave 4 is driven by projections from the lateral lemniscus to the inferior colliculus. Wave 5 is not always identifiable in mice [6], whereas in humans wave 5 is robust. In contrast, wave 1 is robust in mice, whereas it can be difficult to distinguish in humans. Numerous studies have been published on biological factors that affect the ABR waveform including the species/strain, age, and sex of the individual [7]. For example, [8] noted a marked sex difference in the amplitudes of waves 1, 3 and 5 in humans in which males have smaller amplitudes than females. The age of the individual also has significant consequences on the ABR waveform (e.g., in mice: [9]; reviewed in humans by [10]) with amplitude reductions in wave 1, and in later waves to a lesser degree, with advancing age [11]. Among laboratory mice, strain differences are present in the amplitudes and latencies of waves 1 and 4, with significant differences in the wave 1:4 amplitude ratio and 1–4 interpeak latency across strains [12]. These biologically based differences are critical to consider when using ABRs diagnostically in humans and other animals.

In addition to biological factors, ABR waveforms can be probed to estimate the effects of various experimental manipulations on the status of various structures in the peripheral and central auditory systems. For example, several studies by Liberman and colleagues have suggested that the suprathreshold amplitude of ABR wave 1 reliably reduces following acoustic overexposure, even in cases where thresholds have returned to pre-exposure sensitivity (mice: [13,14]; guinea pigs: [15], commonly referred to as hidden hearing loss). In addition to long-lasting wave 1 amplitude reductions, preserved wave 5 amplitudes following acoustic trauma reflect an imbalance of peripheral and central gain which has been suggested to be an indicator of tinnitus (e.g., in humans, Wave 1:Wave 5 [16,17]; in mice, [18]). In studies of patients receiving ototoxic drugs such as cisplatin, evaluation of the ABR waveform has been useful in detecting early ototoxicity, where ABR wave 5 showed significant delay prior to the onset of hearing loss shown in conventional audiometry [19]. In cases such as these, ABR waveform analysis is a necessary diagnostic step that could inform treatment intervals or drug choices to preserve the patient's quality of life. The studies highlighted here are not intended to be an exhaustive list of demonstrable effects of noise, ototoxicity, or injury on the ABR waveform but rather to illustrate the need for characterizing not only thresholds but waveform morphology when examining auditory disease.

Here we developed a custom Windows-based software to automate the detection of peaks and troughs of the individual waves of the ABR waveform and output the amplitude and latency values for offline statistical analysis and data visualization. This software provides a user-friendly interface that enables automatic, user-supervised extraction of ABR wave data, including individual wave amplitudes and latencies. In animal research, such characterizations can improve our understanding of the ways in which various damaging exposures (e.g., from noise, physical insult, genetic manipulation, ototoxic drugs, etc.) affect the physiology of the peripheral and central auditory systems. Correlational studies of the structure-function relationships including ABRs and anatomical characterizations in the inner ear and the brainstem will further strengthen our understanding of the mechanisms of auditory system injury. Our goal in developing this software was to ease the burden of manual peak/trough detection, to reduce bias, and encourage researchers and clinicians to quantitatively examine the fine temporal structure of the ABR waveform. Ultimately, this will facilitate the use of rapid, high throughput techniques as a diagnostic measure of more than just hearing sensitivity (e.g., specific deficits associated with age or injury that are correlated with changes to ABR wave morphology).

Method details

Automation

This program was developed to read .CSV output files from Tucker Davis Technologies (TDT) BioSigRZ software with modifications to read in .CSV output files from TDT BioSigRP. Users can generate additional templates to import .CSV output files generated using custom software with the base package of this program. The program reads the file name, subject identification (sub ID), frequency, decibel levels, and raw trace data from the imported .CSV files, and this metadata is preserved in the output results files.

When importing .CSV data to the program, the user must specify input parameters describing their data, including the waveform amplitude unit of measure ('Voltage Setting'), the duration of the recording ('Elapsed Test Time' in milliseconds), and the stimulus levels for analysis ('Decibels allowed for Analysis'). Based on the input recording duration, the program creates a matrix of the number

of sampled events (bin numbers) that are derived from the total duration of the recording. Regardless of input voltage, amplitude is converted to nanovolts (nV) to keep the calculations consistent and output in results files is also in nV.

ABR data typically does not have peaks in the first several data samples, reflecting the latency to the sound-evoked response, therefore the peak detection algorithm does not identify potential ABR wave peaks/troughs until the 10th bin to avoid false triggers in the beginning of the signal. This delay is supported by data that showed there is a 0.9 ms delay between signal onset and arrival of the sound at the tympanic membrane in mice [5], which corresponds to bin 22 based on a 14.9914 ms total duration of the recording and a bin size of 0.04096 ms. While this value of start bin is modifiable by the user, we recommend a value of 10 in order to not miss the identification of the first peak corresponding to wave 1, not identify false triggers before bin 10, and to account for potential latency differences in the peak of wave 1 across stimulus frequency. The program's algorithm leverages this expectation and uses a formula to compare neighboring bins and identify local maxima/minima that indicate the individual ABR waves. As a first pass, the algorithm will examine 5 bins above and below the current bin. If there is no bin within this range with a higher amplitude, it is considered a peak value (i.e., a local maximum).

$$b_n > b_{n-1} \ldots b_n > b_{n\pm5}$$

Where :

b_n = Current bin being evaluated

The algorithm continues evaluating the input data from that peak value point to identify its corresponding trough. A similar formula is applied to detect a local minimum.

$$b_n < b_{n-1} \ldots b_n < b_{n\pm5}$$

Following identification of the bins containing the local maximum and minimum of a putative ABR wave, a semi-arbitrary voltage threshold is applied to ensure that the peak detection algorithm is not being triggered by a poor signal-to-noise ratio. This voltage threshold is used as a minimum amplitude triggering identification of true peaks/troughs of the ABR waves. The minimum voltage amplitude used in this program was derived from multiple ABR recordings using young adult, normal hearing laboratory mice (CBA/CaJ strain) and validated in 80 ABRs collected from the experiments in [20]). The formula for the ABR wave detection trigger is a linear slope used to get a minimum criterion for 250–400 nV between 8 and 42 kHz used is:

$$\text{Starting Trigger Threshold} = \left(-\frac{3}{680} * \text{Stimulus Frequency} + \frac{7400}{17}\right) * \left(\frac{\text{Decibel Value}}{90}\right)$$

The starting trigger threshold for click uses a static starting value of 650 instead of the above formula. If the threshold for the ABR wave detection trigger is not met, the identified peak and its corresponding trough are considered noise and are not labeled as an ABR wave. If the ABR wave detector trigger threshold is met, the identified peak is labeled as a component (peak or trough) of the ABR wave. The program continues in this manner for all 5 ABR waves, each with a labeled peak and trough.

If the signal-to-noise ratio of the input recording data is too high, as in the case of subjects exposed to traumatic brain injury, of advanced age, or a with a history of noise exposure, the ABR wave detection trigger may not yield 5 peaks and troughs. In such cases, the program will then iteratively reevaluate the input data with a slight modification to the ABR wave detection trigger:

$$\text{New Trigger Threshold} = \text{Current Threshold} - (\text{Starting Trigger Threshold} * .05)$$

The new trigger threshold reduces the criteria necessary to identify peak-trough pairs as ABR waves in datasets with excessive noise or low amplitude signal. This process will repeat, reducing the multiplier in the above formula by 5 percent with each iteration until it reaches the lowest set limit that prevents the trigger threshold from reducing to less than half of the original trigger threshold used in the calculation. If the algorithm reaches the point at which it is unable to identify a peak/trough pair that meets the above criteria it will conclude that there is no ABR wave present in the data and assign a latency and amplitude value of 0. This iterative process with a set limit is necessary because we expect the ABR signal to decrease to a level indiscriminable from the noise floor at low stimulus levels (e.g., at and below auditory threshold level for that stimulus).

The last step in the algorithm to find any peaks and valleys is to adjust the last bin for evaluation. There is also the option for the user to input the start and end bins. The input value for the end bin is where the algorithm considers the end of the response and beginning of the noise. While the full ABR should generally occur in the first 5–6 ms for mice, not all mice or experiments are the same (e.g., shorter or longer recording durations), and therefore the algorithm will expand the final bin in intervals of 20 bins and re-run the Starting Trigger Threshold and New Trigger Threshold calculations until it reaches the maximum number of bins. This step in the automated identification of peaks allows the algorithm to favor data towards the beginning of the signal (start bin) rather than potentially generating false positives from the noise at the end of the trace data. The recommended end value should be 75 % of the total bins or approximately bin 200 for a 15 ms recording.

Manual override

A key benefit to the user interface of this program is the ability for an investigator to override the automated peak/trough selection. Each peak and trough can be moved in bin intervals if visual inspection indicates that the automated peak detection is identifying peaks/troughs that are not the ABR response (e.g., if the summating potential is especially large, automated detection may determine it as wave 1), or if the program is skipping peaks/troughs from waves that are especially reduced (e.g., if the amplitude of wave 5 is smaller than surrounding noise). When analyzing ABR data recorded using stimuli presented at multiple levels, the software

will automatically re-scale the waveform data for ease of identifying peaks/troughs. Users can select the option to 'Show Previous Graph' to plot multiple ABR waveforms simultaneously, which will re-scale the y-axis to the highest amplitude waveform and allow for confirmation at suprathreshold levels that the ABR waves are being identified consistently/accurately.

Results output

Upon execution of the program, data analysis is streamlined for the user. The program generates two .CSV files: a "RESULTS" file containing the main output of the peak detection algorithm and a "RESULTS_UNCALC" file that contains the raw data imported and read by the program. The RESULTS.CSV file includes all metadata from the original imported data (e.g., Filename, Subject ID, Stimulus, Frequency, Decibel Level) and calculations of wave amplitudes, latencies, ratios, and interpeak latencies for each of the 5 identified ABR waves. Data are organized by file name, stimulus, and level for each of the dependent variables extracted from the wave identification. This file additionally includes the bin numbers for the peak and trough markers corresponding to all detected waves. ("bin 1″ corresponding to the peak of wave 1, "bin 2″ to the trough of wave 1, … "bin 10″ corresponding to the trough of wave 5). While exported data includes all these variables, users should analyze only the dependent variables which are relevant given their experimental design and predictions.

Inter-rater reliability

We have also incorporated an inter-rater reliability check in the program that allows for multiple users to view, validate, and adjust the peaks/troughs that were detected in a previous session. Users can share the Results.CSV and Results_UNCALC.CSV files with an experienced ABR viewer, who will be able to load these files into the program for point validation using the Inter-rater Check button. This allows for rapid validation of wave identification by multiple viewers to reduce subjective bias and user errors.

Conclusion and future work

The main goal of developing this software was to ease the burden of manual peak detection of ABR waves to enable researchers and clinicians to extract the most information on ABR waveform morphology, including amplitudes and latencies of the individual waves of the response. The program is designed to easily import and analyze ABR data recorded using TDT software and hardware; however, data recorded using other ABR acquisition programs can be easily coded into a template file in order to be analyzed using this program. Results data are output into tables in a .CSV format, including a file containing raw response amplitude data and a file with the amplitudes, latencies, amplitude ratios, and interpeak latencies that are automatically computed by the program's algorithm. Output data are formated and clearly labeled for quick statistical analysis. Additionally, output data files can be shared and validated by experienced raters using the inter-rater function.

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Data availability

The source code for the program is available for download on GitHub. Sample data to test in the program will be available upon request.

CRediT authorship contribution statement

Kali Burke: Conceptualization, Methodology, Software, Writing – original draft, Visualization, Validation, Writing – review & editing. **Matthew Burke:** Software, Visualization, Validation, Writing – original draft, Writing – review & editing. **Amanda M. Lauer:** Supervision, Writing – review & editing.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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