Episodic ABF analysis v 6.0

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Foreword

The Episodic ABF Analysis tool is a MATLAB application designed for simple batch analysis of episodic electrophysiological recordings. Later the import of certain episodic LabChart (ADInstruments) recordings and events detected in Eventer* was implemented. Episodic ABF Analysis v 6.0 is the first publicly released version of this tool.

The analysis functions include the calculation of the mean, the amplitude, the rise and decay slope, and the area under the curve of regions of interest. Analysis can be performed on individual sweeps or on a calculated average sweep.

The results can then be exported as tsv files or Excel spreadsheets.

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The tool uses the abfload function to import abf files (Forrest Collman (2023). fcollman/abfload (https://github.com/fcollman/abfload), GitHub. Retrieved July 10, 2023.)

Bonn, May 2024

Michael Rabenstein

* Winchester, G., Liu, S., Steele, O.G., Aziz, W. and Penn, A.C. (2020) Eventer. Software for the detection of spontaneous synaptic events measured by electrophysiology or imaging. http://doi.org/10.5281/zenodo.3991676

Licenses

Installation and start

The application is written with MATLAB 2024a. If the code is opened with older versions, it may be necessary to open the code once in the MATLAB App Designer and save it with the older version. To run the compiled app, MATLAB Runtime R2024a (24.1) must be downloaded and installed.

After starting the application, it is possible to import selected files (“File selection”) or all files in a selected directory (“Full folder”, Fig. 1). Alternatively, a previously saved session can be loaded.

Fig. 1: Start-up window with ABF mode (top), Labchart txt mode (middle) or Eventer files mode (bottom) selected.

User interface

The “Raw” tab

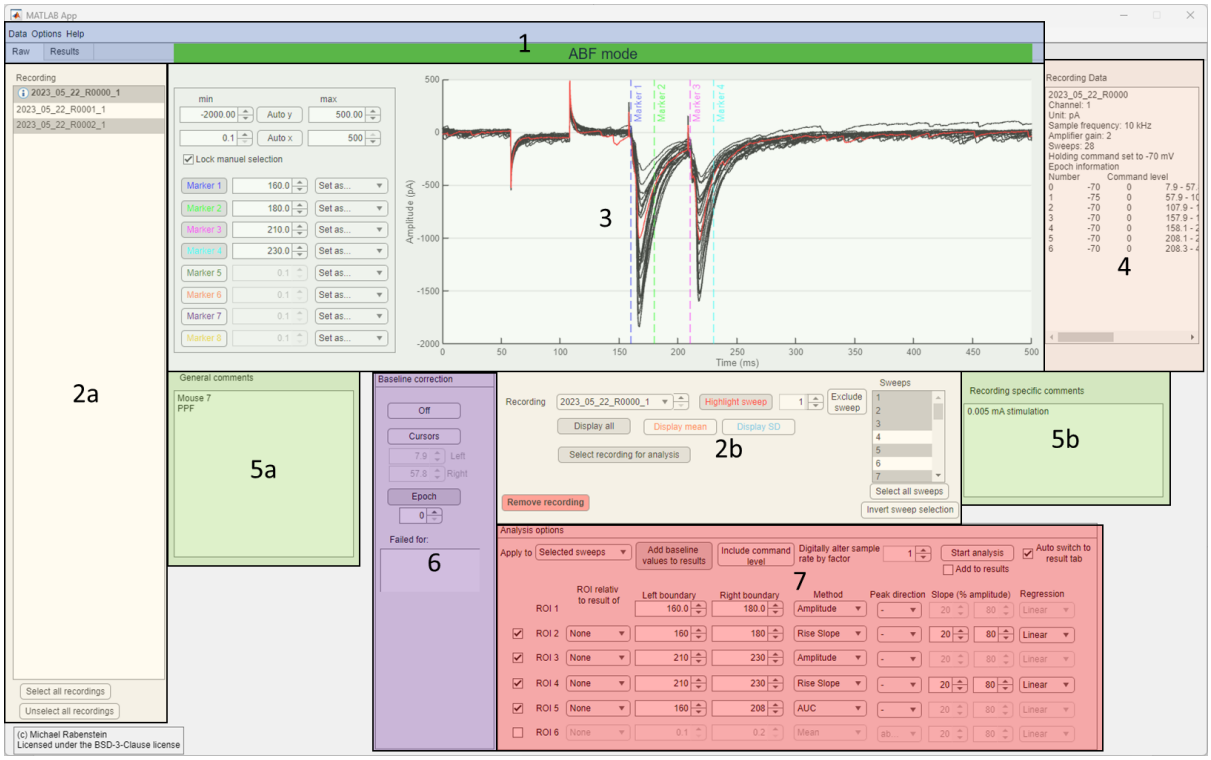

Fig. 2: Structure of the “Raw” tab.

Fig. 2, 1) Options area.
Data: import/save/load functions, switch between ABF, LabChart txt and Eventer files mode, start CSV converter
Options: Set holding potential (ABF mode only), set signal unit (LabChart txt mode only)
Help: Manual (opens in default PDF reader), About (licences), Emergency save (in case the tool runs into an error)
Switch between “Raw” and “Results” tabs
The colored bar indicates the current mode. Green: ABF, orange: LabChart txt, blue: Eventer files.

Fig. 2, 2) Select recordings and sweeps for display and analysis.
In 2a, the imported recordings are displayed. The highlighted one is displayed. The grayed-out recordings are included in the analysis.
In 2b, individual sweeps can be selected or excluded from the analysis. The mean sweep and its standard deviation can be displayed. “Remove recording” removes the selected recording from the session.

Fig. 2, 3) Plot of selected recording and sweeps. Markers can be displayed and their values sent to the ROI spinners in the analysis window.

Fig. 2, 4) General recording information (imported from ABF header or generated during LabChart txt or Eventer files import)

Fig. 2, 5) Comment fields. 5a is made for comments related to all recordings. Comments in 5b are related to the selected recording.

Fig. 2, 6) Baseline correction options

Fig. 2, 7) Analysis options

The “Results” tab

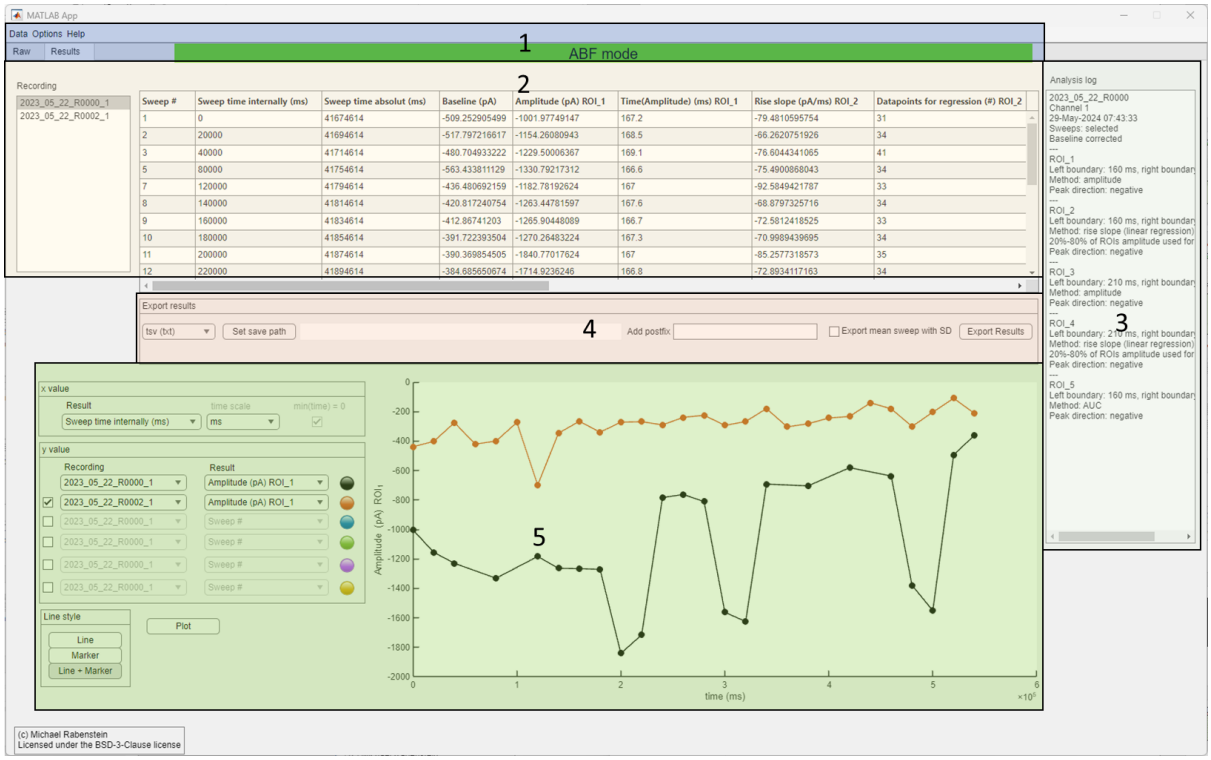

Fig. 3: Structure of the “Results” tab.

Fig. 3, 1) Options area (same as Fig. 2, 1)
Fig. 3, 2) Results. The results of the selected recording will be displayed.
Fig. 3, 3) Log of recording and analysis parameter
Fig. 3, 4) Export path and settings and export button.
Fig. 3, 5) Area for plotting results (only in “ABF mode”)

Import recordings, save or load sessions

General information

The import, save and load functions can be found in the “Data” menu (Fig. 2, 1).
The tool supports the import of ABF2 files and txt files, exported from LabChart.
Either all these files in a folder or selected files can be loaded. Which files are displayed and loaded depends on the activated file mode (“Data” → “Change mode to […]”). Therefore, in ABF mode (default), only ABF files are selected. In Eventer files mode, only full folders can be imported.
Only files created with the same protocol should be loaded in the same session to avoid errors.
After importing files, the “Add files” button becomes available. This allows to add files to the current session. Files with the same name will not be loaded a second time.
If “Replace loaded files” is selected, the already loaded recordings are removed and the new selection is added.
ABF mode only: Since the holding command level cannot be extracted from the ABF header, it can be added manually via “Options” → “Set holding command”. This value will apply to all loaded recordings and will be retained, even if new files are imported.

Compatible ABF files

The tool is designed to open ABF2 files recorded with pClamp 11 using an “Episodic stimulation” protocol. Files without the epoch information in the file’s header will not be loaded. In this case, a message box will inform which files could not be loaded.

Compatible LabChart txt file structure

The tool was designed to load LabChart tab-separated txt files from a specific episodic stimulation experiment. Therefore, the files must have the following structure: A sweep starts with header lines (up to 6 by default, can be changed using the “headerlines” property in the code; stops at first numeric value).
Then pairs of time (s) and value (mV) are listed. The decimal separator is a period (., US format)
The time starts with negative values, because the recording indicates the time of stimulation as 0.
Comments are listed in the third column at their set time, but are not loaded.
The next sweep is indicated by its header.

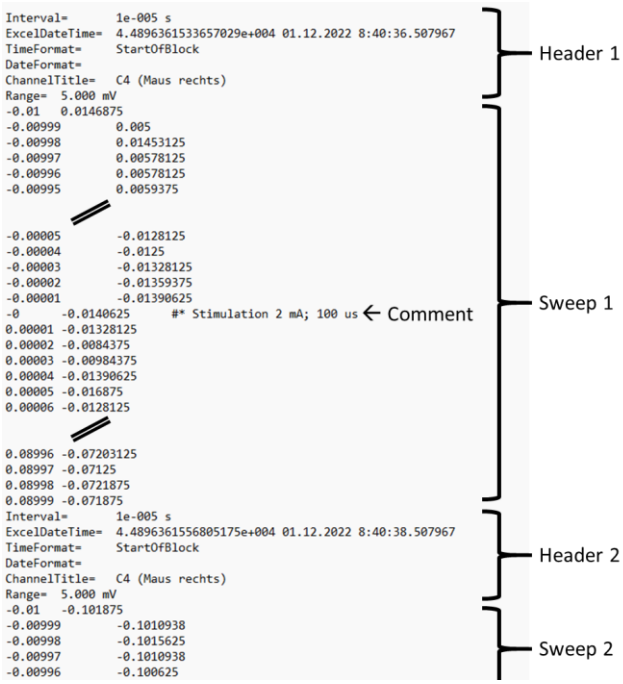

Fig. 4: Structure of compatible LabChart txt files.

The information for the “Recording Data” field is generated based on the recording header and data. The file does not provide episodes to use for baseline correction. Therefore, based on the time point 0, the data will be split into two episodes (pre- and post-stimulation) during the import. If no negative time points are present, the first two datapoints will be used as episode 0 and the rest as episode 1.
The signal unit can be set after startup during the raw import process or later in “Options” -> “Set signal unit” (Fig. 2, 1).

Eventer files

Eventer “ephysIO binary (.phy)” files can be imported. This was implemented with events detected in ABF files using Eventer v1.4.0.
Note that the ABF files created by Eventer are not compatible with this tool.
Eventer exports the detected events and their metadata to different levels of a directory-subdirectory structure (Fig. 5). Therefore, it is only possible to import all recordings in such a

structure. The main directory containing the “filepaths.txt” must be selected as the import directory. The filepaths.txt contains the names of the recordings. The detected events for each recording are stored in subdirectories with “*_YWave*” in the name. The sweeps are stored in “event_data.phy”. The “summary.txt” contains the Eventer detection parameters and is added as general recording information. Since no baseline correction epochs are defined, the first two data points are used as episode 0 and the rest as episode 1.
Note that the recording name is linked to its “*_YWave*” subdirectory via position-based indexing (the first entry in filepaths.txt is linked to the first “*_YWave*” folder). If only some of the recordings are to be imported, their “*_YWave*” subdirectory as well as their entry in “filepaths.txt” must be deleted. Alternatively, unwanted recordings can be removed after import.
Due to the missing information compared to ABF files, the functionality of the tool is limited to the same as in Labchart txt mode.
If Eventer could not detect events in a recording, no “event_data.phy” file is created. Such recordings cannot be imported. A message box lists the concerned recordings.

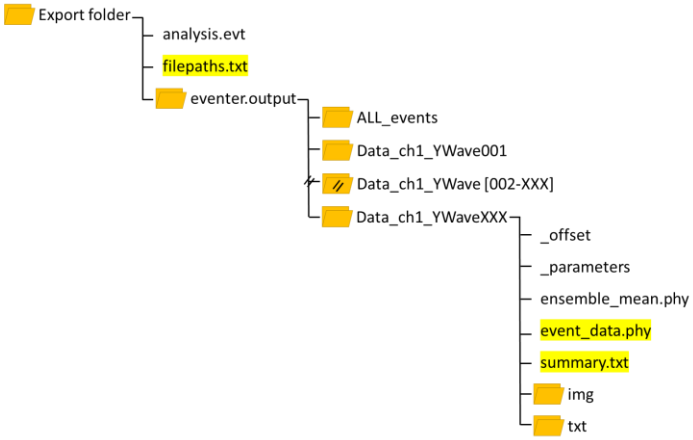

Fig. 5: Eventer export directory-subdirectory structure. The highlighted files will be used during the import.

Import other data types

Other data files can be imported using the LabChart txt path. The provided tool “CSV Converter Episodic ABF analysis” converts csv files into the structure of LabChart txt files. The csv, tsv, txt or tab files to be converted must have the structure as shown in Fig. 6. Several optional header lines (1), optional column names (2), time (x) value in the first column (3) and column-wise arranged sweep data (y) (4). Allowed delimiters are tab, comma, semicolon, space, or bar. Important: Numeric-only headers are not allowed.

Header 1 Header 2 ①				
Time ②	Sweep_1	Sweep_2	Sweep_3	Sweep_4
0.1	0.216100618	0.940844547	0.022354788	0.224536211
0.2	0.174482922	0.606398174	0.80738725	0.561303538
0.3	0.803200368	0.248213892	0.367950786	0.737185145
0.4	0.061350289	0.93910526	0.654947598	0.357182698
0.5	0.479780805	0.289520424	0.680124835	0.99495774
0.6 ③	0.529687534	0.879382856 ④	0.768713823	0.533538191
0.7	0.962626335	0.729738516	0.312568291	0.498704983
0.8	0.486729645	0.354453042	0.477582813	0.832353888
0.9	0.011638026	0.200178744	0.08776743	0.016108688
1	0.605968343	0.897392438	0.15691284	0.883806211

Fig. 6: Structure of files to be converted.

After starting the converter, an input directory must be selected (Fig. 7). The number of header lines to be transferred to the converted file is set with the “Header lines” spinner. Note that the transfer will stop at the first numeric line, even if a higher number of header lines is specified in the spinner. The “Delimiter”, “Time unit” and “Decimal separator” drop-down menus define how the input files are handled and transformed. The signal units can be converted with the “Convert amplitude to” option (e.g., A to pA). Select either a provided power-of-ten value or enter your own (“user defined”). Entries in the “Postfix” field are added to the filenames.

The conversion is started by pressing the “Convert Files” button. The tool will try to convert each csv, tsv or txt file in the input directory. Converted files will be saved to a new subfolder.
If some files could not be converted, their filenames will be displayed.

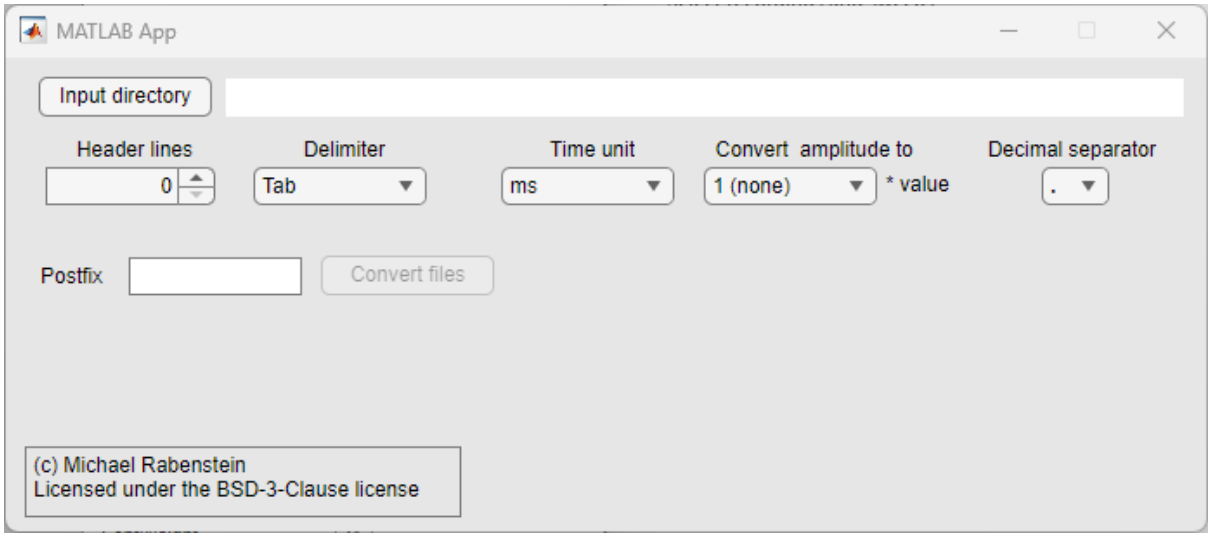

Fig. 7: The “CSV Converter Episodic ABF analysis” tool

Save and load sessions

Sessions can be saved as mat files. This includes the settings on the “Raw” tab, including selected recordings/ sweeps, comments, and analysis options. The results are not saved.
Because the date and time are automatically added to the file name, previously saved sessions will not be overwritten.

When a session is loaded, it overwrites the currently active session and changes the file mode accordingly.

Select recordings and sweeps

Loaded recordings are listed in Fig. 2, 2a. The highlighted one is displayed. The grayed-out recordings are included in the analysis. To include or exclude all recordings, use the buttons at the bottom of Fig. 2, 2a. Individual recordings can be excluded by deselecting the “Select recording for analysis” button (Fig. 2, 2b). Recordings can be removed from the session with the “Remove recording” button.
The selected recording with its selected sweeps is displayed in the plot area (Fig. 2, 3). By default, all sweeps are displayed and one is highlighted in red (buttons “Display all” and “Highlight sweep” are activated). The individual sweeps can be highlighted using the spinner to the right of the “Highlight Sweep” button, by clicking the sweep with the left mouse button in the plot, or by selecting it in the “Sweeps” list box.

Individual sweeps of a recording can be excluded by clicking it with the right or middle mouse button in the plot. Alternatively, the “Exclude sweep” button will exclude the selected sweep.
The included sweeps are greyed-out in the “Sweeps” list box. A single click on a deselected sweep will reinclude it. To include all sweeps or invert the current selection, push the respective buttons under the “Sweeps” list box.

Based on the selected sweeps and the baseline correction settings, a mean sweep and its standard deviation are calculated. To display them, activate the “Display mean” and “Display SD” buttons. The “Show all” and “Highlight sweep” buttons will then be deselected. If necessary, they can afterwards be added to the mean sweep by reactivating the buttons.

Baseline correction

The baseline of the sweeps can be corrected using the options in Fig. 2, 6. In general, the average of a selected range of the selected individual sweeps is subtracted from the sweeps’ data points. The range can be defined either by setting the left and right boundaries (“Cursors”) or by selecting a recording epoch. When an epoch is selected, the left and right boundaries are displayed in the “Cursors” spinners.
The settings will affect the calculation of the mean sweep and its standard deviation.

The baseline correction, together with the state of the “Add baseline values to results” button (in Fig. 2, 7), affects the analysis. If the baseline correction is set to “Cursors” or “Epoch”, the baseline will be subtracted during the analysis (Fig. 8). Activating the “Add baseline values to results” button, will add the calculated baselines to the results table. Since the last calculated baseline value is stored, it is possible to analyze uncorrected data, but add the calculated baseline to the results tables.

Note that the baseline of a mean sweep based on corrected data will always be zero and will therefore be listed as such in the results table. If the individual baseline values of the sweep are

required, the analysis must be repeated but applied to the selected sweeps.
If the baseline correction was enabled, it is noted in the analysis log.

		“Add baseline values to results” button	
		On	Off
Baseline correction	Cursors or Epoch	Analysis performed with baseline corrected data + baseline value(s) added to results table	Analysis performed with baseline corrected data + baseline value(s) not added to results table
	Off	Analysis performed on raw data + baseline value(s) added to results table	Analysis performed on raw data + baseline value(s) not added to results table

Fig. 8: Effects of different “Baseline correction” and “Add baseline values to results” combinations on analysis.

If the baseline correction fails (cursor positions not in recording, selected epoch not present), the affected recordings are listed in the “Failed for:” box. In this case, the uncorrected data will be displayed in the plot and used to calculate the mean sweep.

Plot window and marker

The limits of the y and x axes can be adjusted using the spinners in the left box of (Fig. 2, 3). Pressing the “Auto [y/x]” buttons will set the limits to the maximum values of the recording for the selected axis. If “Lock manual selection” is activated, the setting will be kept when switching recordings. Otherwise the y- and x-axis values are set automatically.

Up to four markers can be displayed and moved along the x-axis. To display them, select the marker buttons. They can be moved using their spinners (up/down arrows or by directly entering values). The position of the markers can be sent to the left and right boundary spinners of the ROI in the analysis options (Fig. 2, 7). Select the target spinner from the markers “Set as…” drop down menu.

MATLAB’s built-in functions can be used to export the plot.

Analysis

General settings and analysis start

The analysis options can be found in (Fig. 2, 7). The first row contains general settings and the “Start analysis” button. The analysis can be applied either to the mean sweep (default) or to the selected, individual sweeps (“Apply to…” drop-down list). The baseline correction values and the command level of the ROI, e.g. for I-V-recordings, can be added to the analysis using the respective buttons. Note that the baseline correction depends on the selection in the “Baseline correction” box, not on the state of the “Add baseline values to results” button.

If the “Include command level” button is active, the command level will be extracted from the ROI and added to the results (only in ABF mode). If the holding potential has not been set (Fig. 2, 1 “Options” → “Set holding command”), it must be set when the button is activated. Note that if the command level cannot be determined (ROI contains epochs with different command levels or the ROI of the mean sweep is based on single sweeps with different command levels), it will be noted in the analysis log and the values will be set to NaN.

It is possible to digitally change the sample rate for the analysis in the range from 0.2 to 5 of the original sample rate. The possible factors are fixed and can be set with the spinner. Values >1 (2, 3, 4, 5) add data points by interpolating the recorded values. Values <1 (0.2, 0.25, 0.333, 0.5) will reduce the sample rate by skipping data points, e.g. a factor of 0.5 removes every second data point. Changing the sample rate does not change the loaded raw data or the calculated mean sweep.
Note that changing the sample rate will slow down the analysis.
Changes to the sample rate are listed in the analysis log.

The “Start analysis” button starts the analysis, based on the settings.
Note that starting another analysis will overwrite the old one with the new results. This can be avoided by enabling the “Add to results” option.
Note that if baseline correction is active but failed, the “Start analysis” button will be disabled.

If the “Auto switch to result tab” option is selected, the result tab will be displayed after the “Start analysis” button is pressed.

The “Add to results” option is available after an analysis has been performed. It allows to analyze different recordings, e.g. with different ROIs, and add the results to the existing results.
Note that if a recording has already been analyzed, the old results will be overwritten by the new ones.
To avoid errors, only the spinner values (left + right bound, “Slope (% of amplitude)”) should be changed when adding results.

Setting the ROIs

Up to six regions of interest can be analyzed at once. To add an ROI, activate it by selecting its check box. The left/ right boundary spinner values (in ms) define the boundaries of the ROI.

The analysis method can be selected from the “Method” drop down menu: “Mean”, “Amplitude”, “Rise Slope”, “Decay Slope” and “AUC” (area under the curve).

If one of the last four is selected, it is possible to define the peak direction. If the peak direction is defined as positive (+) or negative (-), the analysis will treat the ROI accordingly. If “auto” is selected, the algorithm will determine the highest and lowest value after defining the first data point of the ROI as zero. The higher absolute value defines the peak direction for the following analysis. “absolute” does the same, but does not add the sign to the values.

If one of the slope methods is selected, the range the peak amplitude range to be used for the regression, can be defined, with the “Slope (% amplitude)” spinners. The regression type (linear, 1x exponential, 2x exponential) can be selected from the “Regression” drop down menu. If “All” is selected, the regression is performed with all three types and all results are listed.
The difference between rise and decay slope analysis is in the portion of the event, not its direction, that is used for the analysis. The rise slope analysis determines the slope from the baseline to the peak, while the decay slope analysis calculates the slope after the peak.

It is possible to set the ROI boundaries depending on the result of a previous ROI, using the “ROI relative to result of” drop-down menu. If active, the left and right boundaries are not absolute times, but are set relative to the result of the selected ROI. This is most useful when the amplitude method is selected for the former ROI. In this case, the time point of the calculated amplitude is set to zero for the dependent ROI. This allows, for example, to calculate the average of the recording always 20-25 ms after the peak of the event.

If one of the other methods is selected for the former ROI, the left boundary of the former ROI is set to zero for the depending ROI.

The workflow of the analysis

Once the parameters have been set, the analysis can be started. The analysis steps are listed here and the data transformation steps are shown in Fig. 9. as an example for a 20% - 80% rise slope analysis.

1) The raw data will be prepared. If selected, the baseline values are subtracted and the sample rate is digitally changed.
2) The ROI is isolated from the entire data table.
3) The ROI's selected analysis method is executed.
    a. Mean: The mean is calculated from the ROI using MATLAB's mean() function.
    b. Amplitude: The max or min value and its time point of the ROI is determined, depending on the selected peak directions. If "auto" is selected, the higher value is selected and the peak direction is added to the results. If "absolute" is selected, the values sign is removed using the abs() function.
    Note that the amplitude is calculated based on the general baseline settings. If, for example, the amplitude is to be based on the values of the sweep just before the event, either set the baseline correction cursors there or determine this value using the "Mean" analysis and correct the amplitude value afterwards manually.
    c. Rise/ Decay Slope: In the first step, the peak direction is determined or used, based on the settings (described in 3) b.). Then, the first (rise slope) or last (decay slope) data point of the ROI will be to zero ("baseline"). Conversely, the last (rise slope) or first (decay slope) data is selected as the peak of the event ("peak"). Note that the exact location of the peak of the event isn't calculated. This means, for example, that if the right boundary of a rise slope analysis is located in the decay phase, a value lower than the max amplitude will be used in the next step.
    The "peak" value is used to calculate the upper and lower limits of the ROI, based on the "Slope (% amplitude)" settings. For example, if the selected boundaries are 20% and 80%, the cut-off values will be calculated as 20% and 80% of the "peak" value.
    The ROI is further trimmed based on the cut-off values. The left cut occurs at the time of the first value that exceeds the lower cut-off value (rise slope) or falls below the upper cut-off value (decay slope). The right cut occurs at the last time the data point value is less than the upper cut-off value (rise slope) or exceeds the lower cut-off value (decay slope). This means that values in between are ignored in the trimming process, even if they're outside of the cut-off values. An overview of the transformation steps is shown in Fig. 9.
    The selected regression type will be applied to the trimmed data.
    Linear regression uses the polyfit() function and adds the slope and R^2 to the results.
    The exponential regression methods are performed using the fit() function. The tau values and R^2 are added to the results.
    If the slope analysis fails, it be noted in the analysis log. This can e.g. happen if there are not enough data points between the upper and lower boundaries, or if the regression calculated an impossible slope based on the direction of the peak, such as a negative rise slope from a positive peak. This can be caused by additional peaks in the ROI, or the right boundary is in the decay phase.
    Tip: If the peaks of your sweeps do not always occur at the same time, set the ROI

boundaries relative to a ROI in which the amplitude is determined. For rise slopes, set the left boundary to a negative value and the right boundary to 0. For decay slopes, set the left boundary to 0 and the right boundary to a positive value.

d. Area under the curve (AUC): The data set is modified and the AUC is calculated using the trapz() function as follows. First, a linear function is calculated using the first and last ROI data points. This function is used to compute a dynamic baseline. The ROI values are corrected using this baseline. The trapz() function subtracts areas in the positive and negative directions. The selected peak direction suppresses this. For example, if the peak direction is set to positive, all negative values of the corrected ROI will be set to 0, leaving only positive values. If “auto” is selected, the higher value is selected and the peak direction is added to the results. If “absolute” is selected, all values of the corrected ROI are set to positive using the abs() function. This results in the addition of areas in positive and negative directions. An overview of the transformation steps is shown in Fig. 10.
   The absolute AUC and the calculated linear function are added to the result table.

4) The analysis results and log can be found in the “Results” tab.

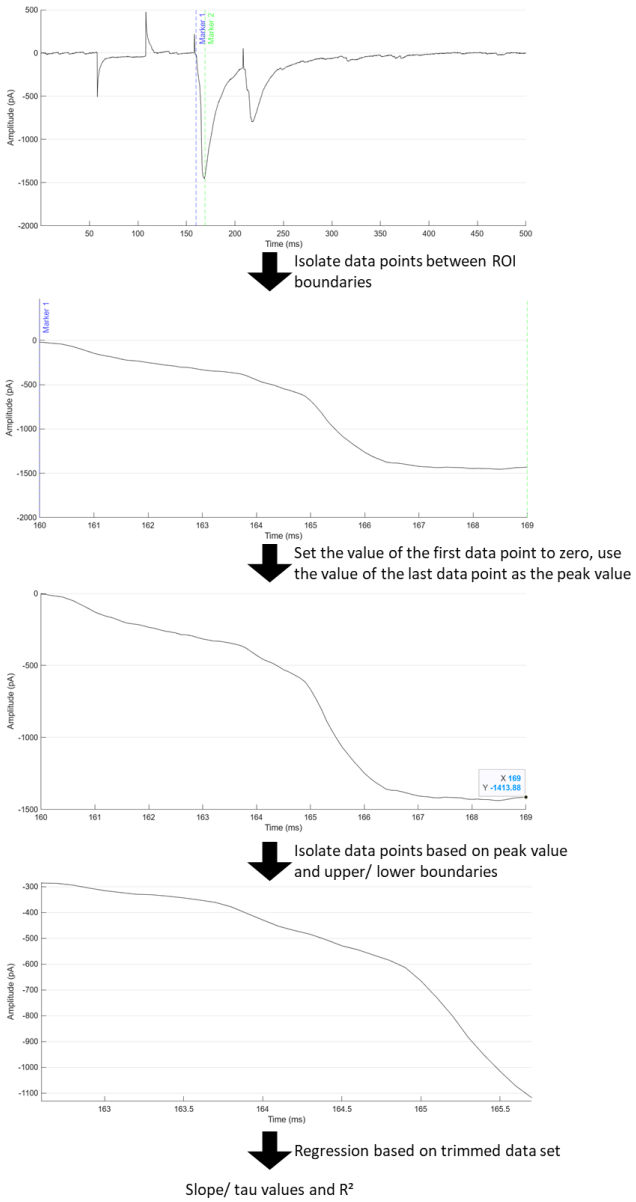

Fig. 9: Data transformation during a rise slope analysis (20% - 80% of amplitude).

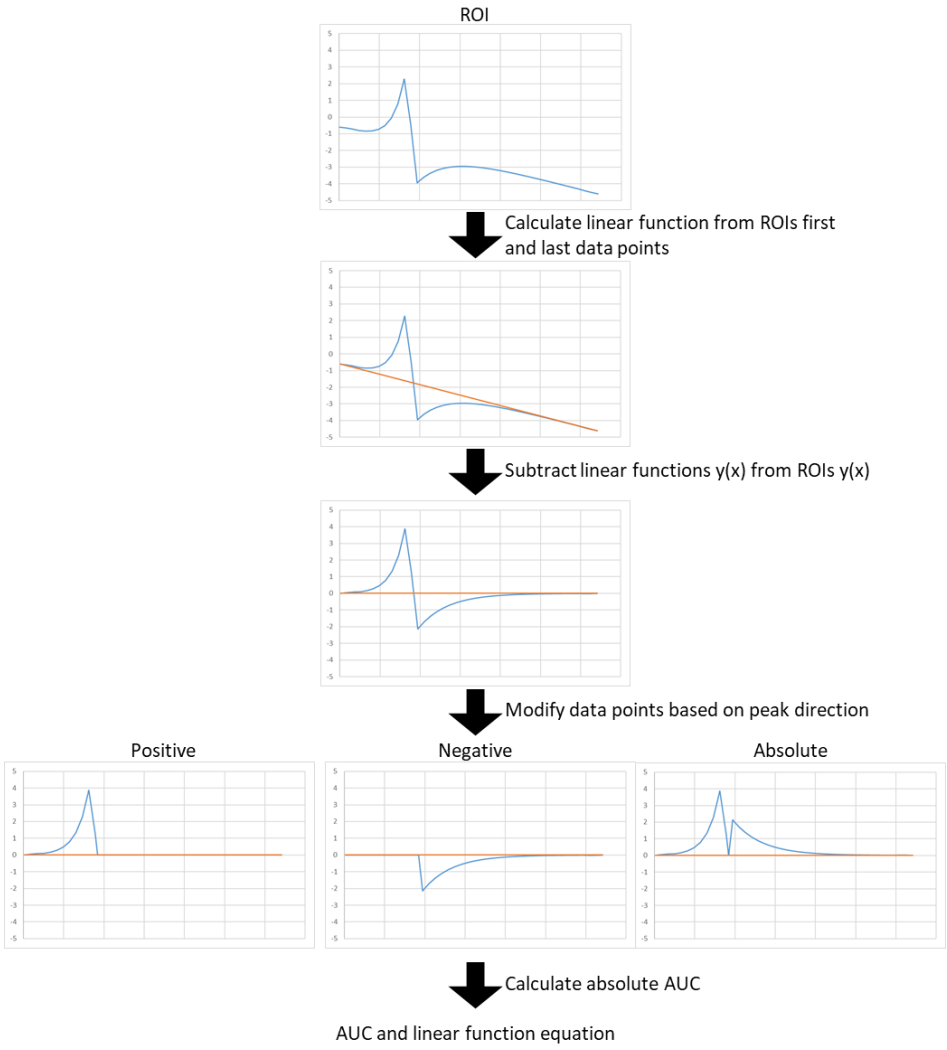

Fig. 10: Data transformation during AUC analysis.

Displaying the results

After analysis, the results can be found in the “Results” tab (Fig. 3).
In the “Recording” box (Fig. 3, 2), the analyzed recordings can be selected. The selected results are displayed in the table and the log in the “Analysis log” box (Fig. 3, 3).
To get an overview, the parameters/ results can be plotted against each other (only in ABF mode; Fig. 3, 5).
Select a result category for the x-axis and a recording result for the y-axis. The selected x-y pairs can be plotted by selecting the “Plot” button.
Note the difference between “Sweep time internally” and “Sweep time absolute”. For the internal sweep time, time 0 marks the start time of the protocol and the start times of the sweeps are calculated from the ABF header variable “sweepStartInPts”. For the absolute sweep time, midnight of the recording day marks time zero. The absolute sweep time is obtained from the ABF header variable “lFileStartTime”.
The absolute start time is useful when a single recording consists of multiple recording files, such as long-term synaptic plasticity recordings. In this case, the recording could consist of two files (before and after stimulation). The absolute time can be used to display the full time course.
The “Line style” box allows you to specify how the data is displayed.
MATLAB’s built-in functions can be used to export the blot.

Exporting the results

The results can be exported as either tab-separated values (tsv) txt files or Excel (xlsx) files (Fig. 3, 4). Exported are the result tables, a general log with the general comments and the excluded recordings, and the analysis log, with the recording specific comments and a list of the excluded sweeps.

The save directory can be selected with the “Set save path” button. If no directory is set before, it will be set during the export process.
The export file and folder names are automatically generated based on the date, time, and recording file name. Therefore, previously exported results won’t be overwritten by a new export command. If desired, a postfix can be added to the filenames (alphanumeric characters only).

If tsv is selected as the export format, a folder containing all exported files will be created in the save directory. The filename extensions indicate their contents. The general log is in the file ending with “_general_log”. The recording specific results tables and analysis logs are in the files ending with “_results” and “_log”.
If all listed results are based on the mean sweeps, it is possible to create an Excel file that lists the results of all recordings in a single table. Important: If the listed results are based on different method settings (function “add to results” active and “Method”, “Peak direction” or “Regression” changed), this may fail. The tool can detect and notify the cases, where the number of columns in the result tables don’t match. If the number still matches, the unified table will be generated, but the results could may be listed under the wrong column names. Changing the left and right boundaries or the “Slope (% amplitude)” parameters won’t cause any problem.

If the results are to be exported as an Excel file, all the data will be saved in a single xlsx file. The results of the different recordings are saved as separate sheets in the Excel file. The first sheet contains the general log. The following sheets are named after the recordings on which the results are based. To avoid duplicate sheet names, the recording names are prefixed with increasing numbers. If the recording name is longer than 30 characters after the prefix is added, only the first 30 characters are used for the sheet name. The first column of the results sheet lists the analysis protocol of the recording. The other columns contain the results table.

If the option is checked, the calculated mean sweeps can also be exported along with their standard deviation. They are saved as separate files in the same format as the other results (file ends with “_mean_sd”). Again, when exported as tsv, every mean sweep with its standard deviation is exported as a separate txt file. When exported as an Excel file, each mean sweep is listed as a separate sheet in the same file.
Note that the exported mean sweep is selected based on the baseline correction settings during the analysis. If it was active, the mean sweep, based on the baseline-corrected individual sweeps, is exported. Otherwise the mean of the uncorrected sweeps is exported.
Digital changes in the sample rate, used during analysis, are not included in the exported mean sweep.

Important: Exporting the results, especially as Excel files, is a slow process. Don’t open the exported files during the export process as this will cause an error. The export is finished, when the “Start analysis” button is enabled again.

Error prevention

Since the tool was designed to analyze specific types of recording and has been tested with a limited amount of data, there may still be errors. Some errors can be avoided by following the instructions below.

- Wait for the tool to finish the current execution before pressing another button.
  - Eventer files in particular can contain thousands of sweeps per recording, which slows the tool down massively. In this case, it is recommended to disable the “Display all” button.
- Do not open the saved or exported files until the process is complete.
- Do not operate the spinners too fast.
- Load only files recorded with the same protocol in the same session.
- If results should be added, change only the spinner values (left + right bound, “Slope (% of amplitude)”)
- Imported recordings must be formatted numerically using the US style (period as decimal separator).

In case an error occurs, it may still be possible to save the session using the “Emergency save” option in the help menu. This way the session can be resumed just before the error. This can be used to determine if the error can be avoided.