NeuroExpress program for analyzing patch-clamp data

** Introduction to the Windows 10 version **

NeuroExpress is a Windows-based program designed to perform analysis of electrophysiological recordings made in whole-cell **patch clamp experiments** or using sharp electrodes. It has various modules for analyzing different types of data, such as **current step responses** (I-V data), miniature **postsynaptic currents** or **spike arrival times**. The program is a standalone executable (NeuroExpress.exe) and it can run in Windows 7 or higher operating systems. The suggested memory size is 4 GB, but it can likely run with less RAM. If you experience the error message 'Out of memory', please contact the author.

The program should be placed into a **local folder**, which is not designated as Read-only. One example is c:\Program files\NeuroExpress. The program can read **Axon Binary files** (ABF) directly. For minis, acquisition mode should be **gap-free** and the current should be recorded in **channel #0**, holding voltage in channel #1. It can also read ATF, TXT and ASCII files but those need to be in a format that is compatible with the program. Time, voltage and current channels need to be in separate columns.

When opening files, the user has to select the **requested data format** in the combobox. If the file is readable, the program performs the analysis immediately. The content of the file will be displayed in the upper panel(s) and analyzed data will appear in the bottom plot panels. The user can change settings for the analysis (right side panel) or save the calculated parameters into an **Excel** worksheet (press the **'Send'** button). Also, the content (event list) of the plot panels can be exported into Excel. All functions can be accessed through **popup** menus, that are associated with the buttons in the right upper corner of the display panels (**'Func'**).

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** Usage feedback **

The program can send a short data packet to the developer that contains information on the number of successfully/unsuccessfully **opened files** and the **type of analysis** performed by the user. This information is sent via internet when the user exits NeuroExpress. The program collects absolutely **no personal information**, names of opened files or parameters calculated by it. The developer would appreciate if the users contact the author in case of crashes or when experiencing difficulties, so the program can be **further improved, and bugs uncovered**. E-mail: attilaszuc@gmail.com

** Work folder **

The program will create a work folder at c:\Users*username*\AppData\Local\NeuroExpress. The program **configuration file** NeuroExpress.ini is stored here. Also, the program will save the settings and **manual correction data** for each analyzed file when the menu 'Autosave analysis settings' is checked. Such data can be reloaded when the menu 'Load last settings' is checked.

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** User experience, smart features **

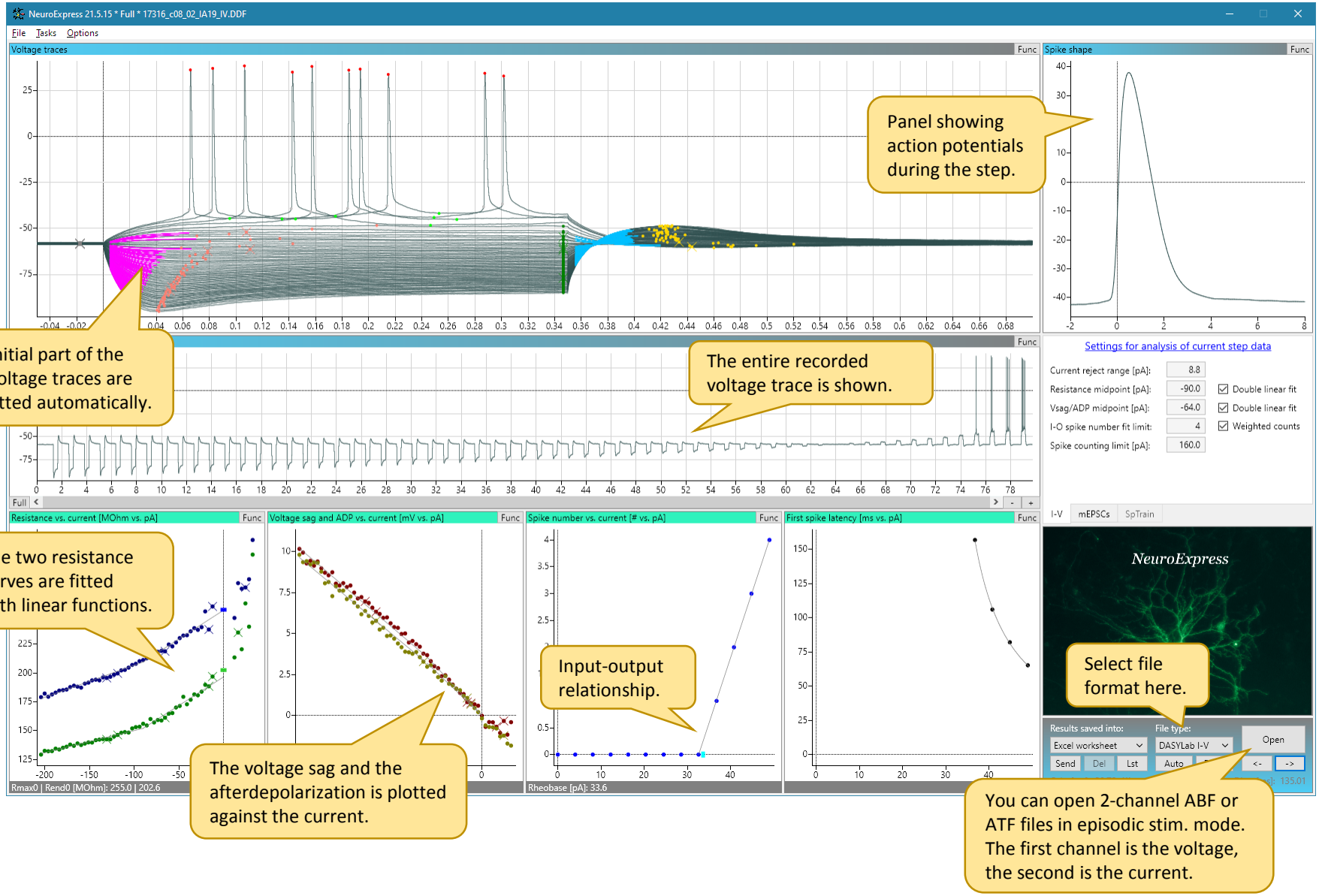
The author tries to make the program user friendly, fast and robust. Calculation time for most tasks remains under 1 s. As an example, current step data from an ABF file containing 50 sweeps each 1 s long and acquired at 20 kHz are loaded and **fully analyzed well within 1 s**. The program will perform all the analysis tasks including spike detection, search for extrema, fitting of voltage traces automatically and all the results can be saved into an Excel worksheet or TXT file. Analysis of mini events is also fast. A current trace of 200 s containing a few hundred events and acquire at 20 kHz is analyzed within 1 s.
Parameters for the analysis can be set manually or using the mouse wheel (incrementing or decrementing, try it). Whenever a parameter is changed the mini analysis is re-ran and the results are shown immediately.
If the mini recordings contain artefacts or test pulses to be excluded, the user can bring up selection cursors and set the range to exclude using the mouse (click and drag the cursor). Such sections can be ignored and the data outside of such regions are used for the statistics. Ignored sections will turn gray.

Analysis of physiological properties

** General instructions **

To perform analysis of voltage traces evoked by current step stimulation the recording must be in a format that is suitable for the program. **ABF and ATF** files containing 2 channel recordings can be analyzed. The acquisition mode of the recording must be **episodic stimulation** for this analysis. Measurement units of the voltage channel must be **mV** and **pA or nA** for the current channel. The first channel contains the voltage response of the neuron while the second channel contains the current that is injected. These are rectangular current steps **starting at a negative level** and incremented in small steps to more depolarizing levels. As an example, a recording containing step responses starting from -200 pA, incremented by +10 pA and ending at +200 pA is OK. The length of each episode can be 0.1 – 4.5 s at 20 kHz sampling rate for the recording. The maximal number of current steps in a recording can be 192. The program will load the ABF of ATF file into the memory and then immediately extracts dozens of physiological parameters from the voltage traces. Voltage deflection data, resistance, time constant, estimated membrane capacitance and other parameters will be displayed as functions of the injected current. These will appear in the bottom plot panels. The content of each panel can be selected by the popup/dropdown menu (use the right mouse button to explore freely). Linear or exponential functions will be used automatically to fit various relationships, e.g. the resistance vs. current plot or the spike number vs. current relationship.

Analysis of physiological properties



Analysis of physiological properties

**** Settings and controls ****

These are parameters for the analysis of current step responses. Plots will be updated when the user enters a new value for a selected parameter. All parameters are accessible via the Settings dialog box.

Current reject range:	No voltage traces are used for resistance calculation within this current range centered at I=0 pA. Estimation of resistance values can be increasingly less accurate when applying current steps with smaller amplitude.
Resistance midpoint:	This is the midpoint (breakpoint) current level that separates the 2 linear parts of the resistance curves.
Vsag/ADP midpoint:	This is the midpoint (breakpoint) current level that separates the 2 linear parts of the voltage sag and afterdepolarization curves.
I-O spike # fit limit:	The I-O curve is considered as linear up to this spike count level.
Spike counting limit:	Spikes are counted and summed up to this current current level to calculate cumulative spike counts.

Analysis of physiological properties

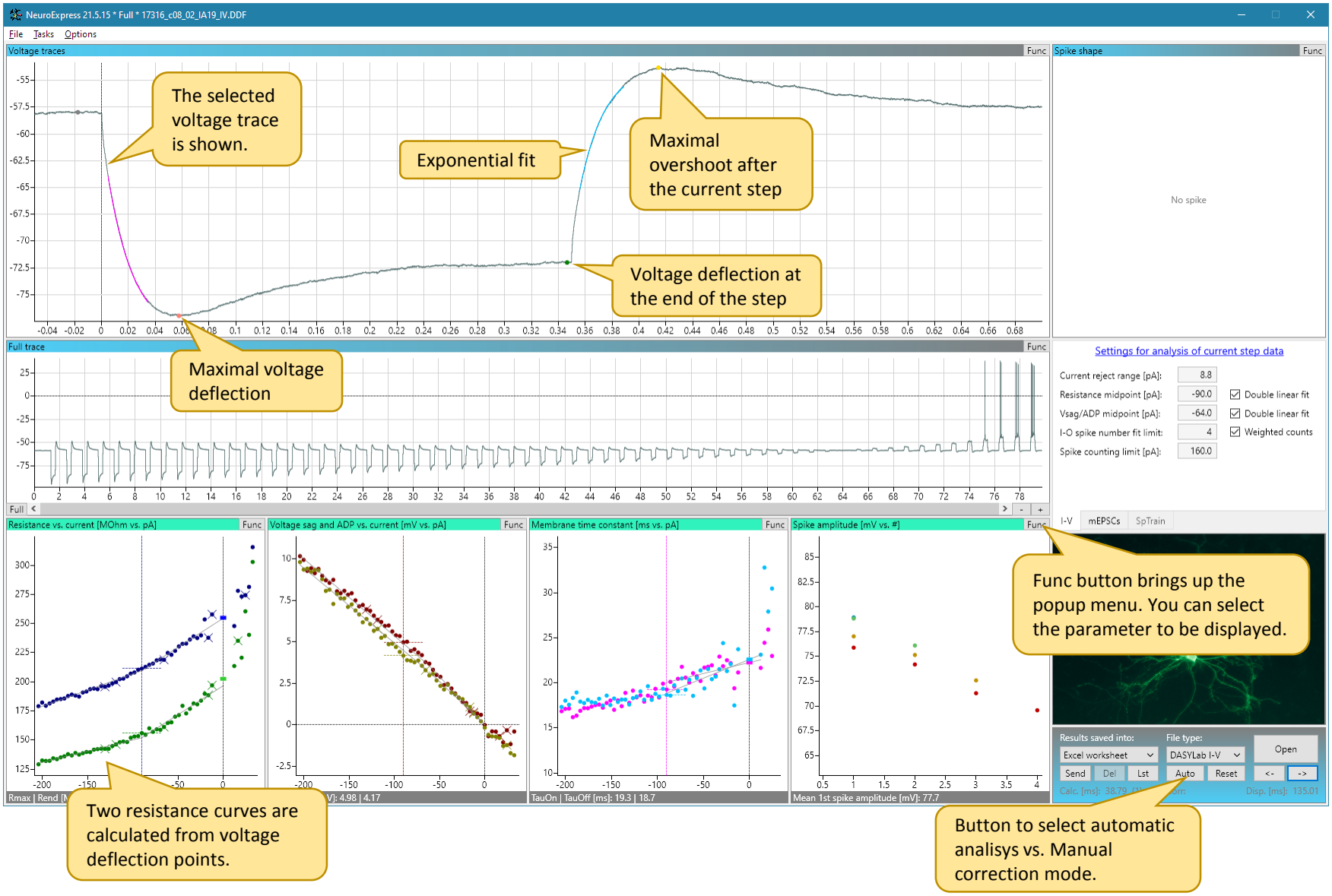
**** Cell info ****

Nervous system/area:	Use the combobox to select the preparation or brain area where the recordings were made.
Cell type:	This is the phenotype of the cell recorded, e.g. fast spiking, pyramidal, PV interneuron, medium spiny neuron, etc.
Treatment group:	This is the treatment group, e.g. control, LTP, chronic drug group, etc.

**** Main panel ****

Results saved into:	The user can select the destination where calculated parameters are saved.
Send:	Pressing this button the user saves the calculated parameters for the current data file.
Del:	When pressed, the program deletes the last row in the Excel worksheet.
Lst:	When pressed, the program lists the event data into the Excel table or text file.
Auto:	Button to select fully automatic calculation vs. manual adjustment mode.
Reset:	When pressed, the correction data for the current file will be deleted.
Open:	The most used button, this will bring up the dialog box to open single data file.

Analysis of physiological properties

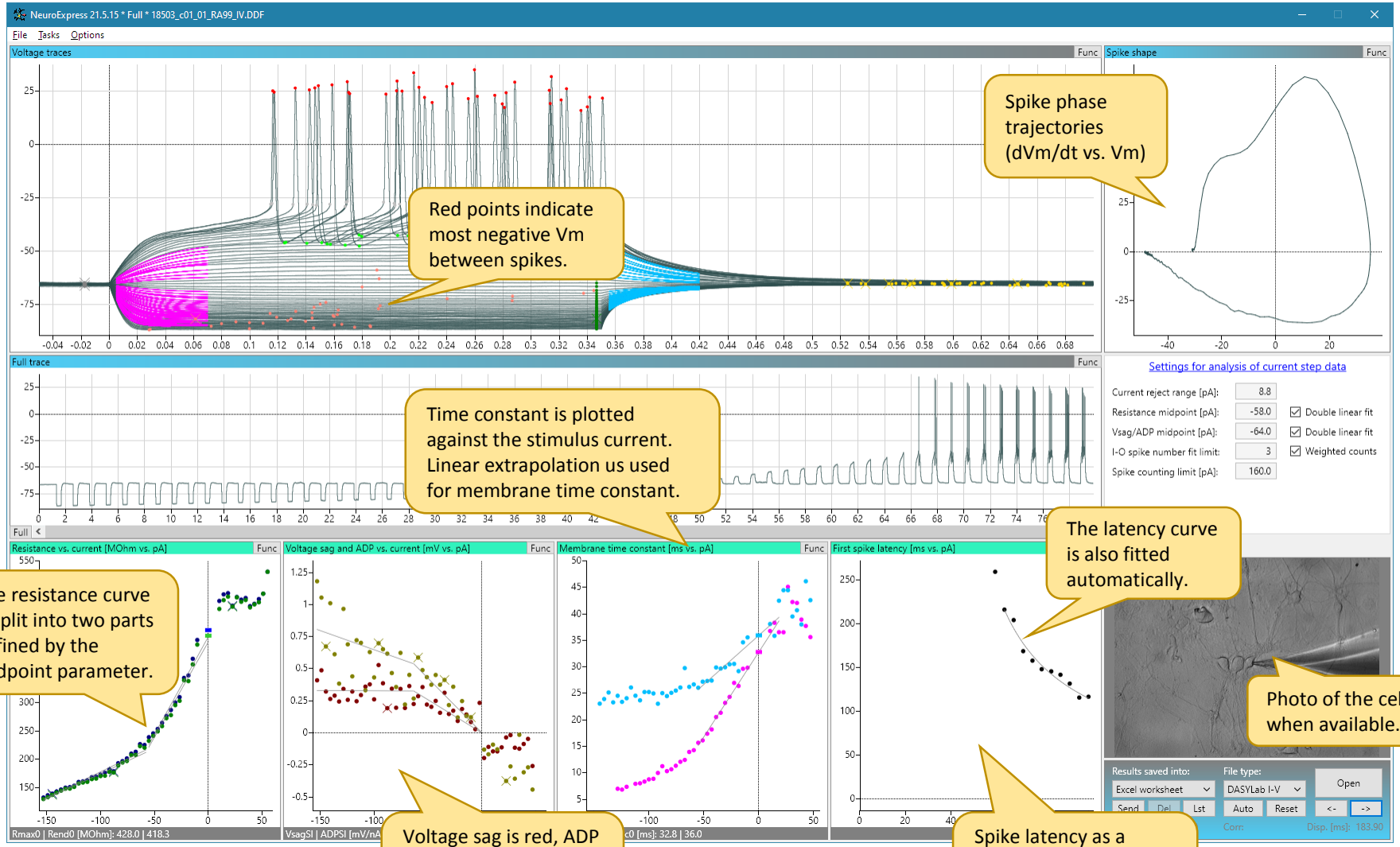


Analysis of physiological properties

** Correcting data **

Voltage traces are often **contaminated** by EPSPs or IPSPs received by the neuron. Other type of noise or transients can appear in the recordings that are usually interfere with the analysis. One example is when an EPSS arrives just before the current step, so the resting membrane potential will be inaccurately obtained. The user can override this problem by **manually moving the datapoint** that is associated with the resting membrane potential. This is a gray colored symbol shown in each voltage trace and appearing before the onset of the current step. The user can check the box **'Manual correction'** in the lower right panel and then move the datapoints freely. First, the contaminated voltage trace is selected by moving the mouse within the plot box that shows the resistance or voltage sag. When the contaminated trace is displayed in the upper panel, the **user can click the left mouse button to make the selection** and the gray datapoint can be grabbed and moved by the user. The same can be applied to the datapoints associated with the maximal voltage deflection, voltage at the end of the step and the afterdepolarization. Moving the datapoints will cause the associated parameters to be **recalculated** and the plot boxes refreshed immediately. A completely wrong datapoint can be erased by moving it outside of the 'Voltage traces' panel.

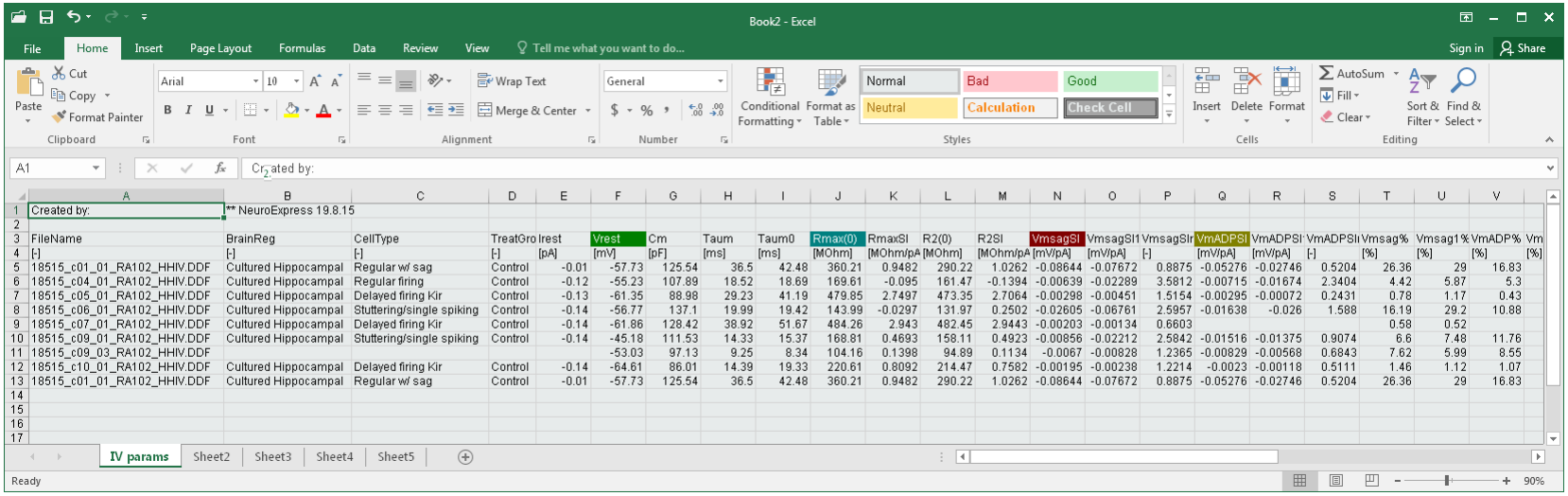
Analysis of physiological properties



Analysis of physiological properties

** Exporting calculated parameters **

The program saves the physiological parameters calculated from the current step responses into an Excel worksheet. The ‘Link to Excel’ checkbox needs to be checked first. Input resistance, resting membrane potential, sag ratio, rheobase and many other parameters are saved just by pressing the ‘Send’ button in the main panel. The user can check the ‘Cell comments in Excel’ box in order to have descriptions of the calculated parameters as comments appearing in the Excel worksheet.

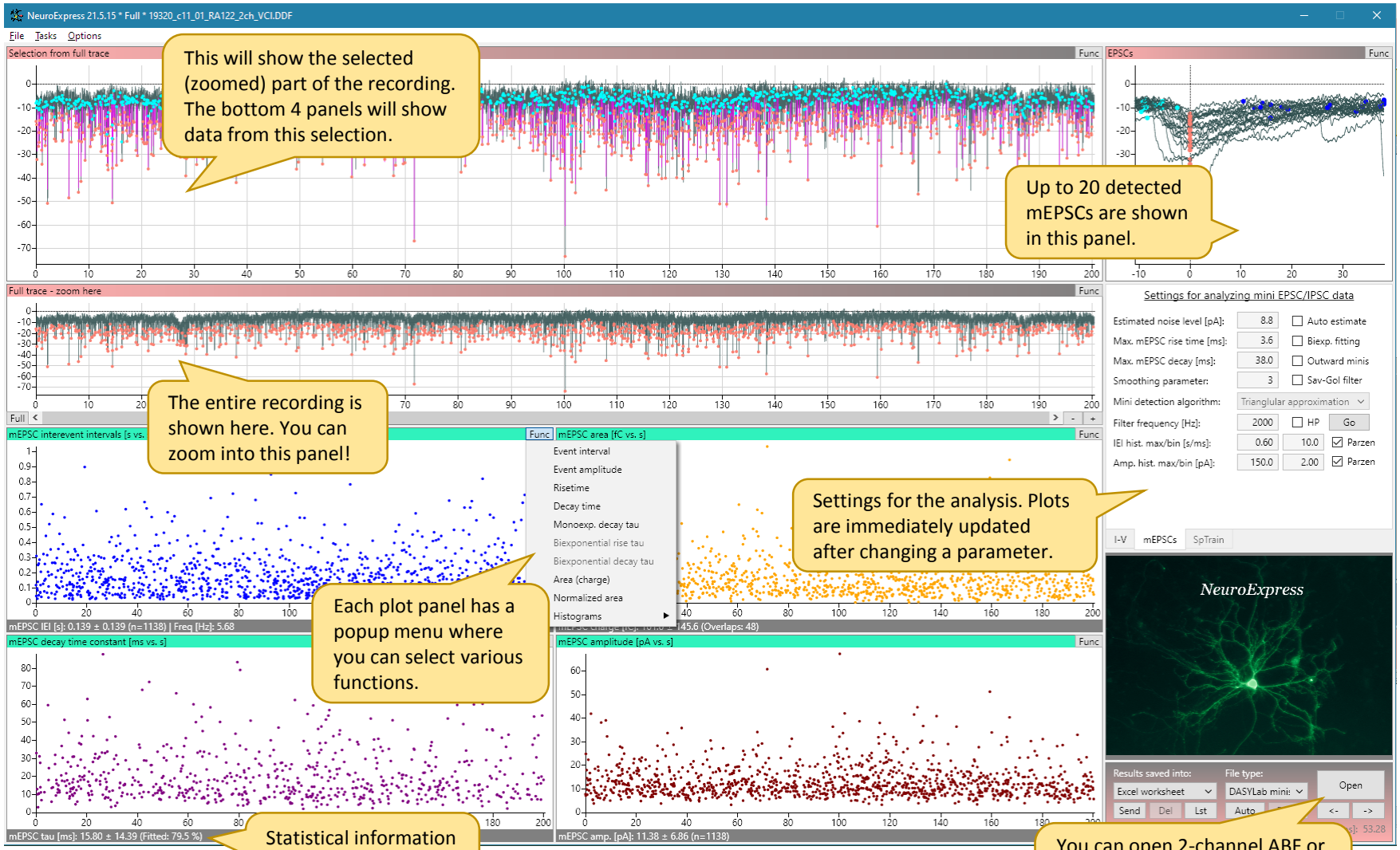


Analysis of miniature EPSCs

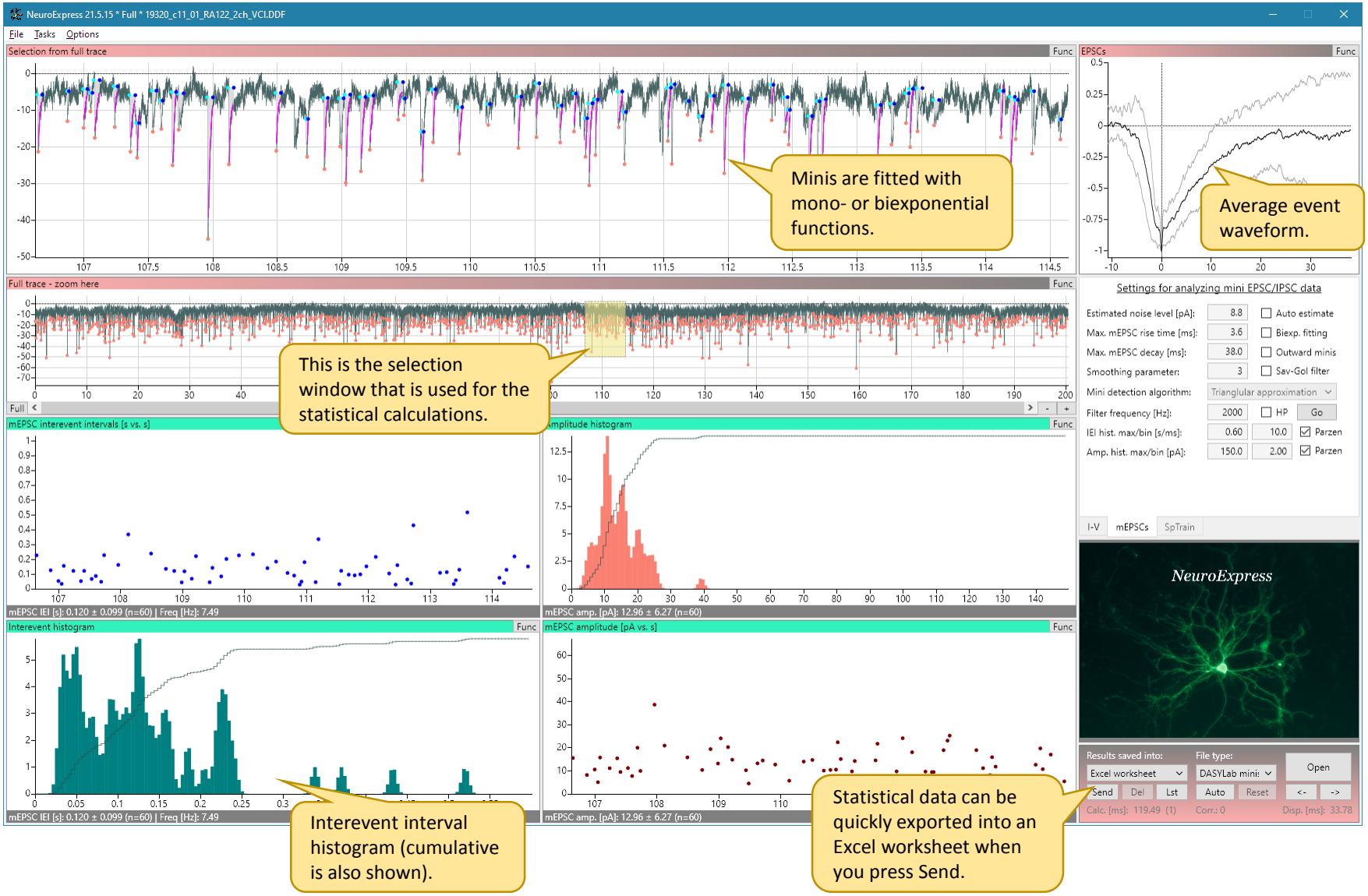
** General instructions **

To perform analysis of miniature postsynaptic currents first open the file to be analyzed. **ABF and ATF** files containing **1 or 2 channel recordings can be analyzed**. The acquisition mode of the recording must be **gap-free** for this analysis. Measurement units of the channels should be **pA or nA** for the current channel and **mV** for the voltage channel. If the 'Estimate noise level' checkbox is checked, the program will attempt to calculate the mean noise floor for the recording and sets the value of the corresponding edit box. After this, events are detected automatically by running one of the 3 algorithms specified in the gray colored group box below. To achieve the most accurate analysis, the Estimated noise, Max. EPSC rise time and Max. EPSC decay time parameters should be set by the user. Noise should be set to a level close to the amplitude of baseline **peak-to-peak fluctuations** in the signal. Max. EPSC rise time parameter should be set to a level that is up to **2-3 times greater** than the visually determined rise time of the mini events. Max. decay time should be set in a way that most of detected mEPSC events can be entirely displayed in the upper right panel (mEPSC traces). Detected events will be indicated by **red symbols** in the trace panels. If **baseline points** are shown, those are indicated by cyan colored symbols. Use the **zoom feature** to check the quality of event detection and change the parameters listed above to get the best analysis. Here, you use the mouse in the Full trace panel and define a rectangle in which the data will be displayed and analyzed separately. The uppermost panel (Selection from full trace) will show the section of the trace you selected. Statistical parameters will be calculated for this selected section, so the rest of the trace is not included. Of course, you can use the entire trace for analysis when you unzoom (just click outside of the grayed rectangle or press the **Full** button).

Analysis of miniature EPSCs



Analysis of miniature EPSCs



Analysis of miniature EPSCs

**** Settings and controls ****

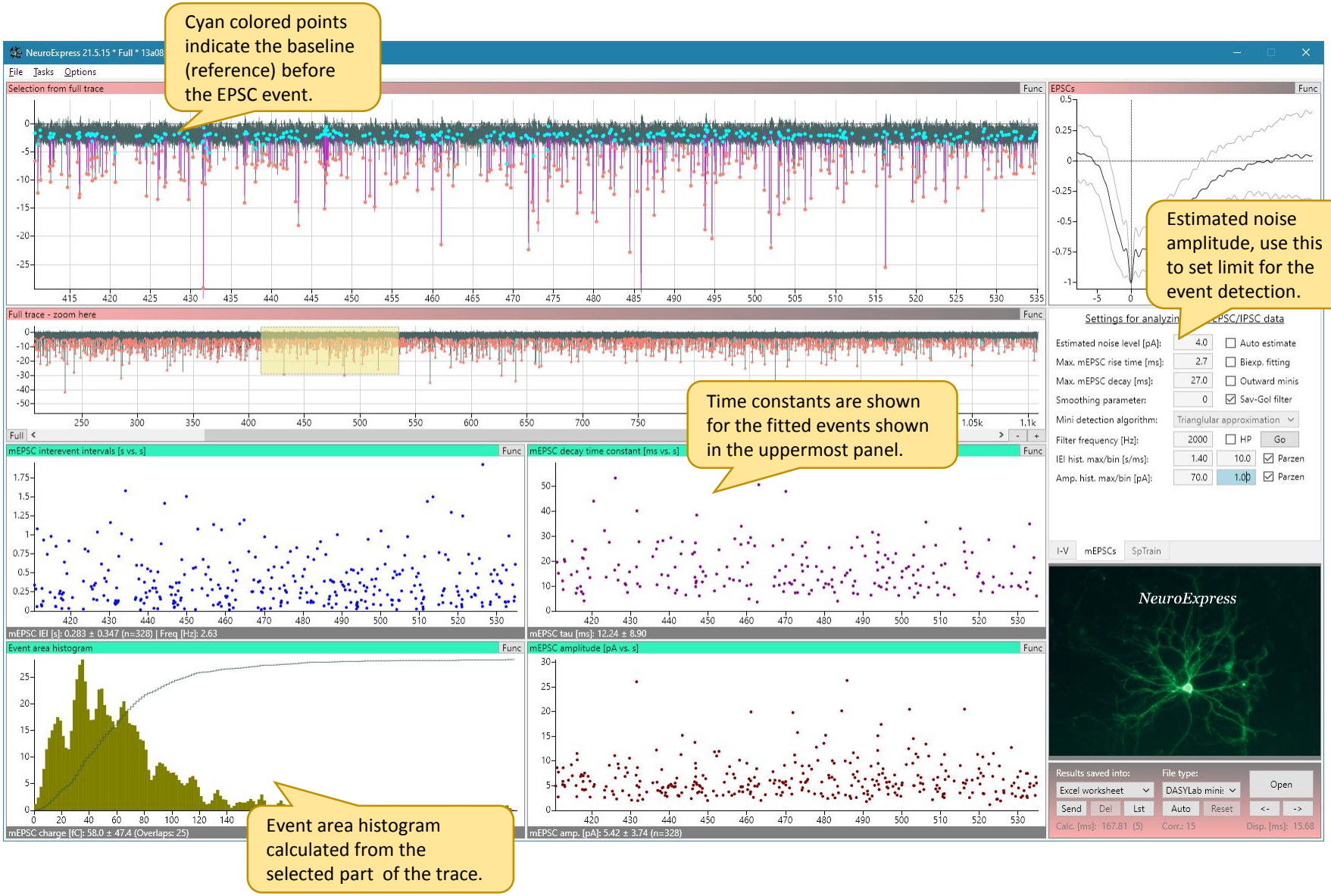
These are parameters for the analysis of mini EPSCs or IPSCs. Plots will be updated when the user enters a new value for a selected parameter and then clicks in an adjacent edit box (light-blue colored edit box is left).

Estimated noise level:	Estimated peak-to-peak noise in the recording.
Max. mEPSC rise time:	Maximal rise time of the mini EPSC in ms.
Max. mEPSC decay:	The approximate decay time of the slowest mini EPSC in the recording.
Smoothing parameter:	This parameter is used for moving average smoothing or Savitzky-Golay smoothing of the current trace. Higher values will perform stronger smoothing.
IEI hist. max/bin:	The width and binsize of the interevent interval histogram.
Amp. hist. max/bin:	The width and binsize of the amplitude histogram.

Analysis of miniature EPSCs

Auto estimate:	Turn it ON for automatic noise level estimation based on standard deviation of the signal in the beginning of the recording.
Biexp. fitting:	Turn it ON to use biexponential fitting of events instead of monoexponentials.
Outward minis:	When checked, IPSCs are detected instead of EPSCs.
Sav-Gol filter:	When checked, the program uses Savitzky-Golay filtering instead of simple moving average filtering of data.
Parzen:	When checked, the program calculates histograms based on kernel estimation algorithm (Parzen-estimation).
Detection algorithm:	There are 3 slightly different algorithms to detect mini EPSCs. Use the first when the data are low-pass filtered and smooth. The second is better for data with high-frequency noise, but it performs slower. Optimal setting for estimated noise level can be different for the different algorithms.

Analysis of miniature EPSCs



Analysis of miniature EPSCs

** Exporting calculated parameters **

The program saves the parameters of the miniature EPSCs/IPSCs into an Excel worksheet. The ‘Excel worksheet’ needs to be selected in the combobox first. mEPSC interevent intervals, amplitudes, decay time constants and many other parameters are saved just by pressing the ‘Send’ button in the main panel. The user can check the ‘Parameter explanation in Excel’ menu under Optionsin order to have descriptions of the calculated parameters as comments appearing in the Excel worksheet.