NeuroExpress program for analyzing patch-clamp data

** Introduction **

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 (x86)\NeuroExpress. The program can read **Axon Binary files** (ABF) directly. 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 format** in the dialog box. 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 ('Link to Excel' and 'Send'). Also, the content 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 ('Plot').

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** Usage feedback **

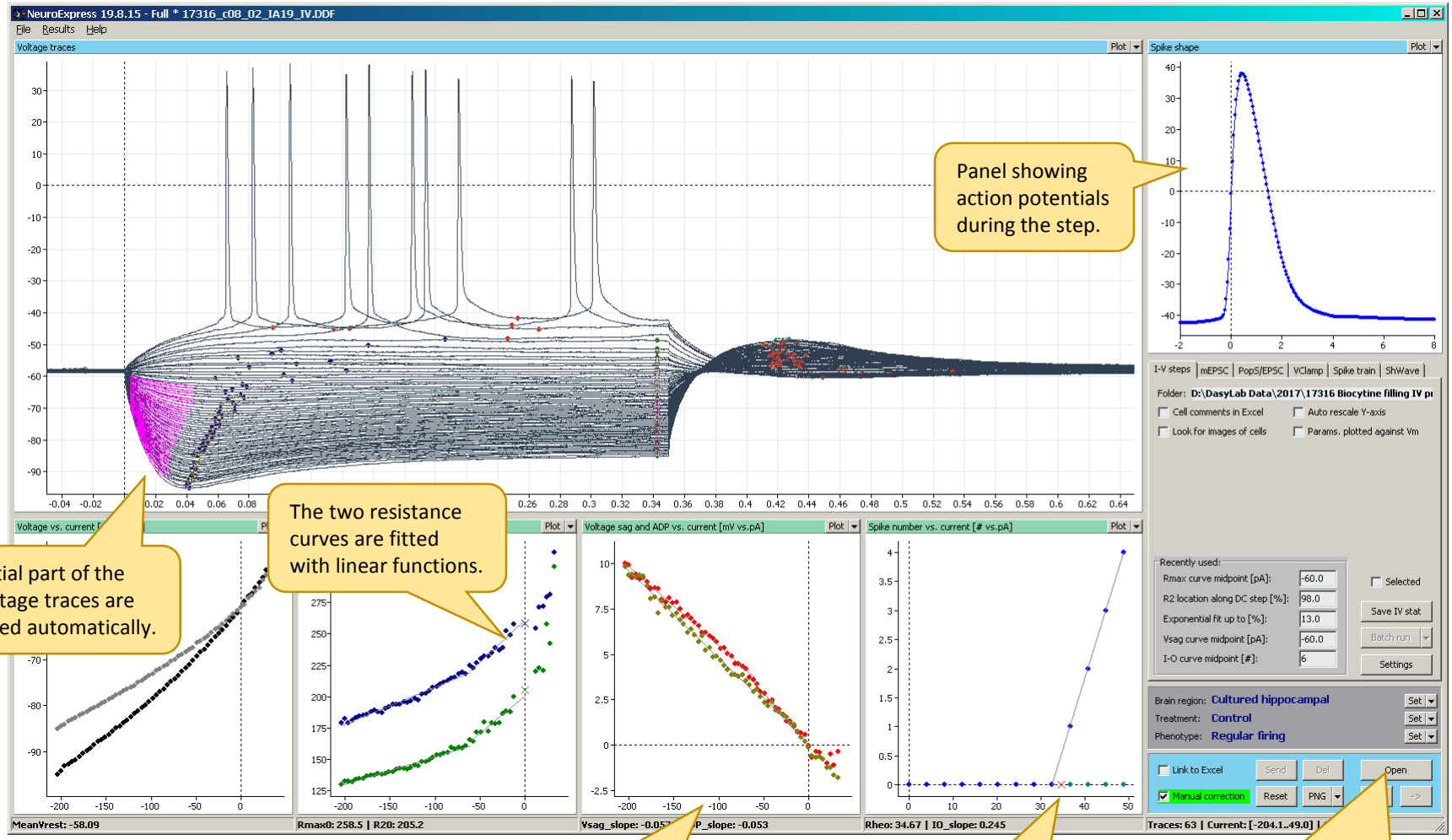
The program has an ability to send a short data packet to the developer that contains information on the number of successfully/unsuccessfully **opened files** and the **type of analysis** performed by the user. This information can be sent via internet when the user quits NeuroExpress, but only if the user allows this feature. The automatic data usage feedback option can be turned off in the **Help menu** if the user prefers not to allow it. The program collects absolutely **no personal information**, names of opened files or parameters calculated by it. The developer would appreciate if the users leave the usage feedback option on, so the program can be **further improved and bugs uncovered**.

Analysis of physiological properties

** General instructions **

To perform analysis of voltage traces evoked by current step stimulation the recording has to 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 has to be **episodic stimulation** for this analysis. Measurement units of the voltage channel has to 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.

Rmax curve midpoint:	This is the midpoint (breakpoint) current level that separates the 2 linear parts of the resistance curves.
R2 location along step:	Percentage of the step duration where the voltage is sampled for the 2nd resistance value (R2). It is typically near the end of the current step (>90%).
Rhpol current level:	A single resistance value is calculated at this current level.
Exponential fit up to:	The percentage of the step duration that is used for the exponential fitting of the onset part of the voltage trace. Typically 10-20%.
Access resist. comp.:	The user can digitally compensate the remaining access resistance (series resistance) in the recording if this value is set to a positive value.
Vsag curve midpoint:	This is the midpoint (breakpoint) current level that separates the 2 linear parts of the voltage sag and afterdepolarization curves.
Post-step duration:	Duration of the voltage trace after the current step that is used for the analysis (afterdepolarization and post-inhibitory rebound).
I-O curve midpoint:	Up to this spike count the I-O curve is considered as linear.
Cumulative spnum I:	Spikes are counted and summed up to this current level.

Analysis of physiological properties

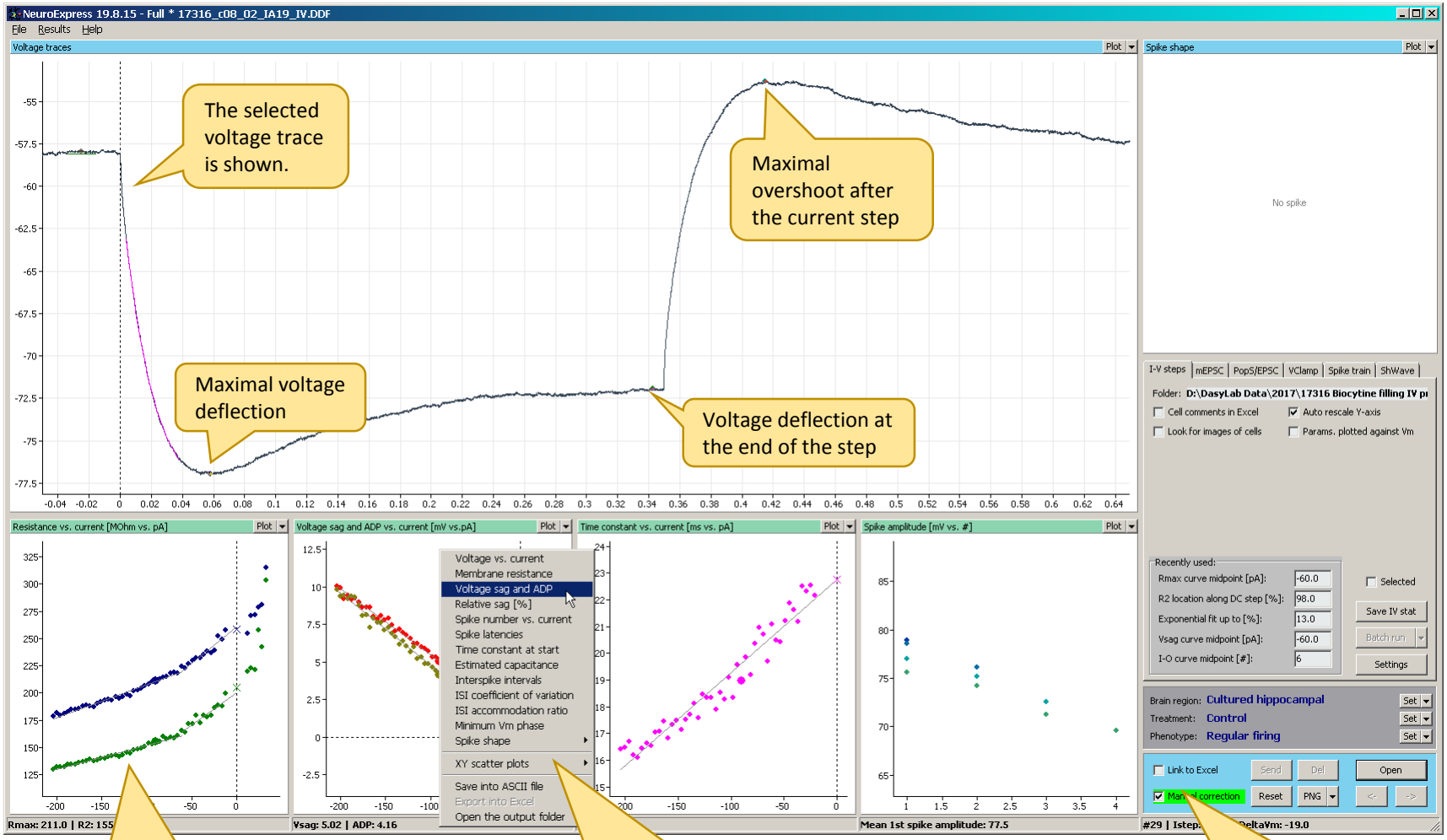
** Cell info **

Brain region:	Use right mouse click to bring up the popup menu that contains the available brain regions or types of preparations.
Treatment:	This is the treatment group, e.g. control, LTP, chronic drug group, etc.
Phenotype:	This is the phenotype of the cell recorded, e.g. fast spiking, pyramidal, PV interneuron, medium spiny neuron, etc.

** Main panel **

Link to Excel:	If MS Excel is installed, the program will open it and starts a worksheet to store calculated parameters and plot data.
Link to Origin:	If Origin is installed, the program attempts to open it and starts a worksheet to store calculated parameters and plot data.
Send:	If pressed, the program sends the calculated physiological parameters into the Excel or Origin worksheet.
Del:	If pressed, the program deletes the last row in the Excel or Origin worksheet.
Manual correction:	When checked, the user can perform correction on the data.
Reset:	When pressed, the correction data for the current file will be deleted.
PNG:	The program saves a screenshot or a GIF animation.

Analysis of physiological properties

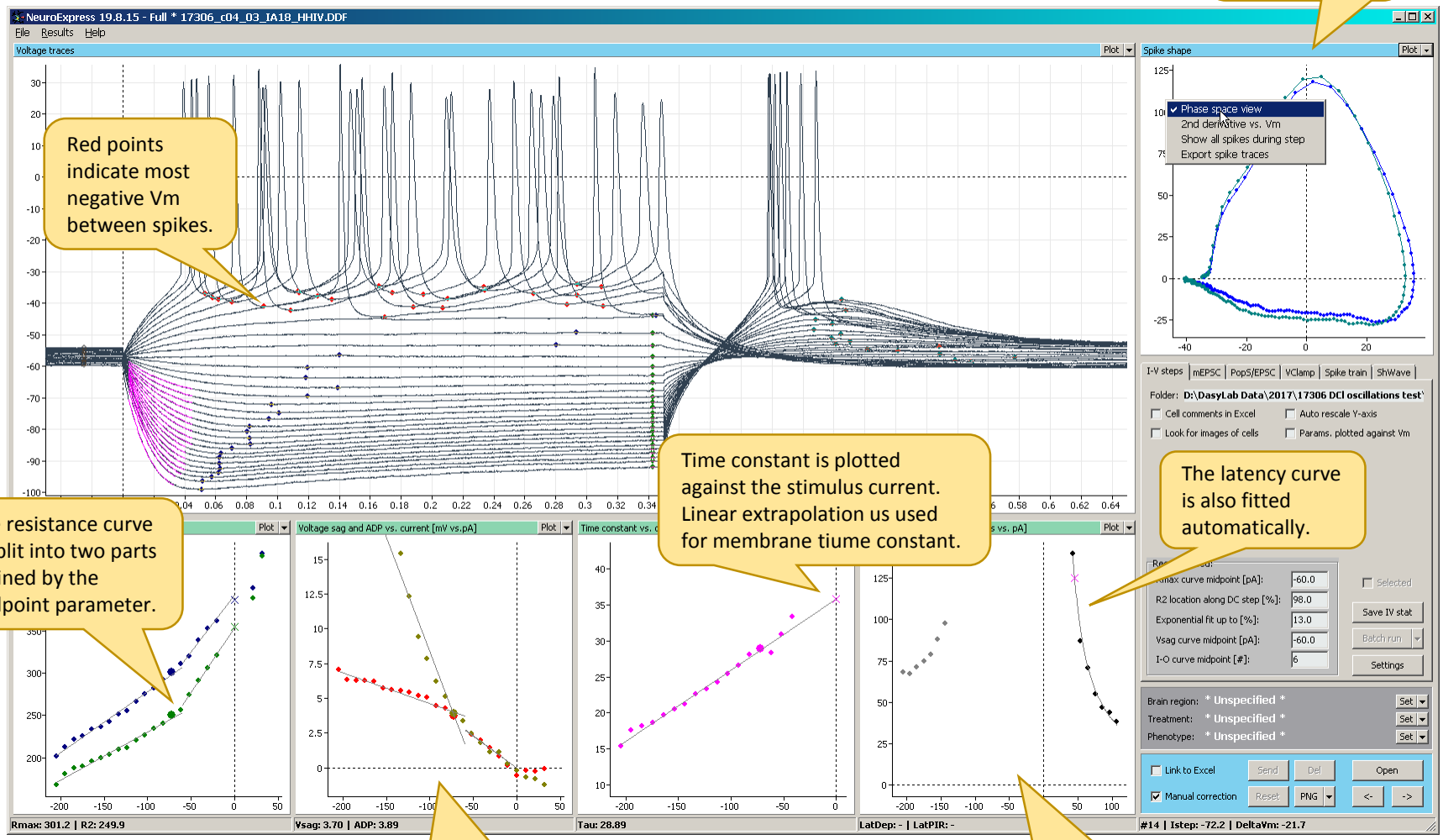


Analysis of physiological properties

** 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 **letter F should be pressed** 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.

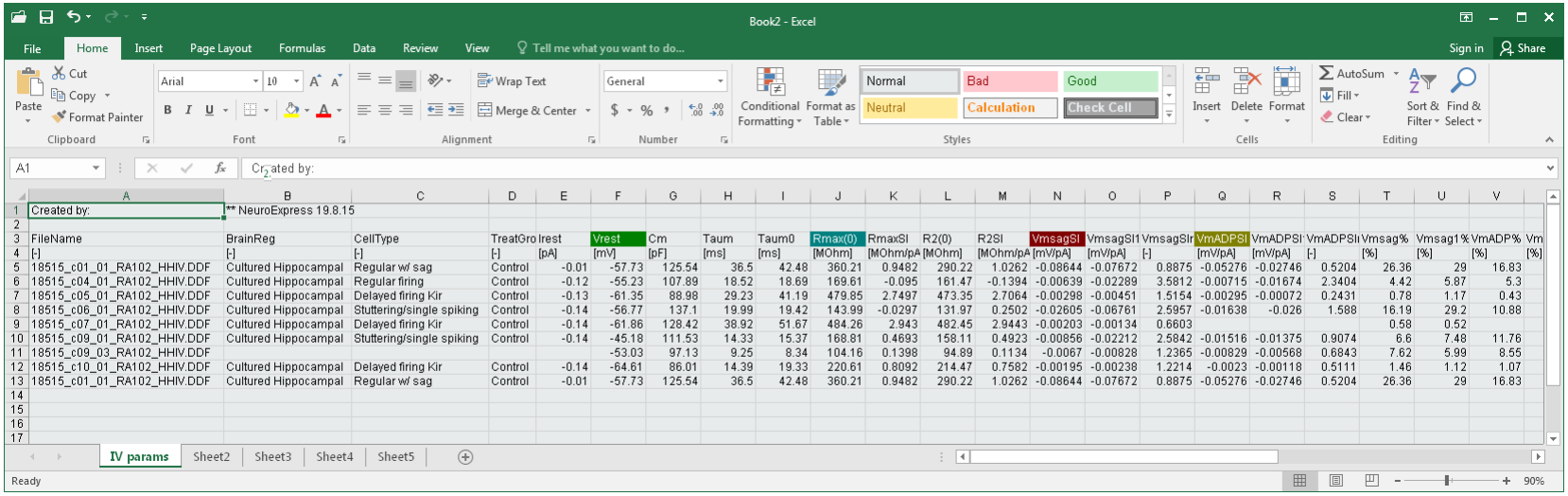
Analysis of physiological properties



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.

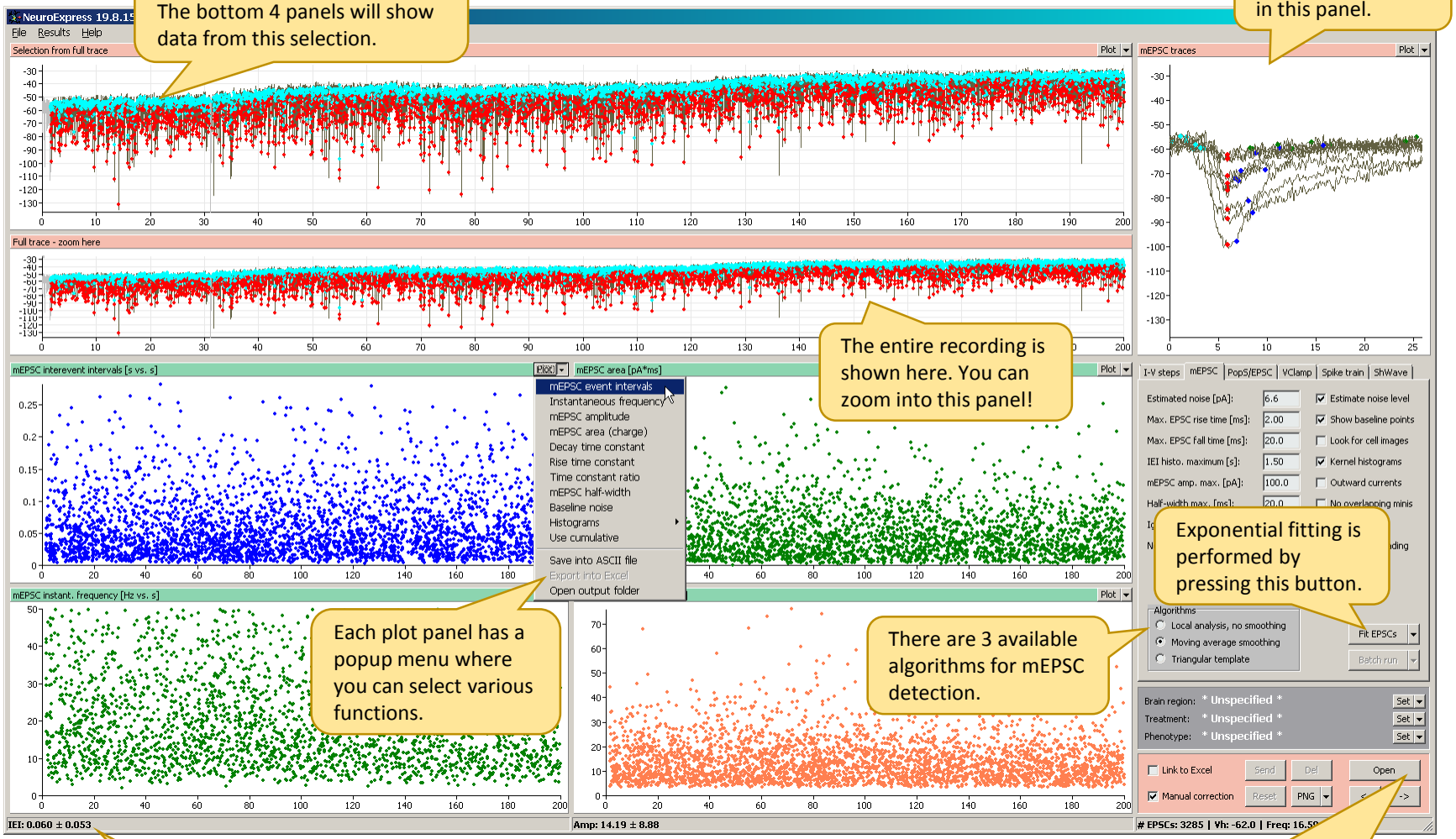


Analysis of miniature EPSCs

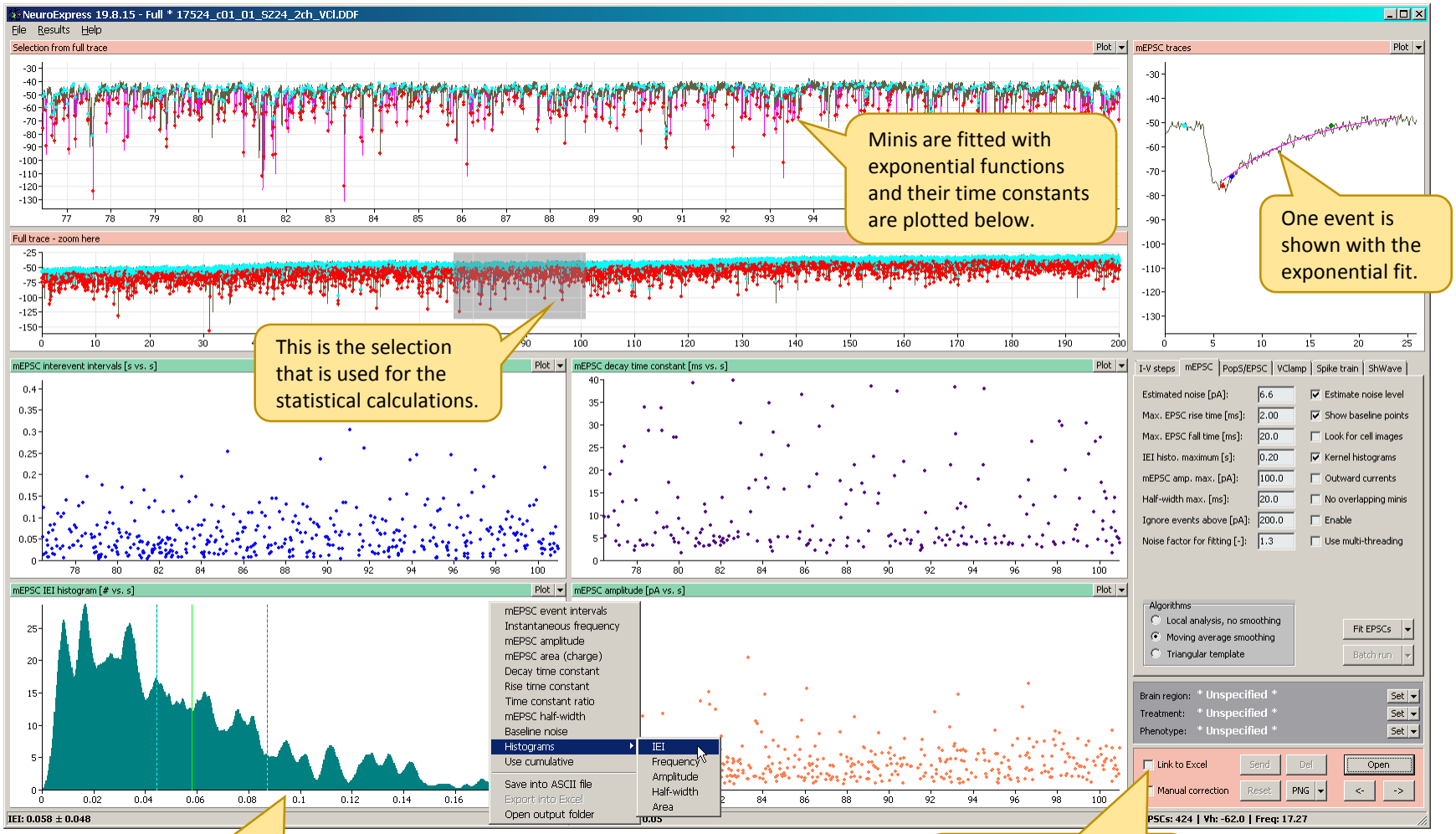
** General instructions **

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 has to 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).

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:	Estimated peak-to-peak noise in the recording.
Max. EPSC rise time:	Maximal rise time of the mini EPSC in ms.
Max. EPSC fall time:	The approximate decay time of the lowest mini EPSC in the recording.
IEI histo. maximum:	Inter-event histogram is calculated from zero to this maximal time interval.
mEPSC amp. max.:	Amplitude histogram is calculated up to this level.
Half-width max.	Half-width histogram is calculated up to this level.
Ignore events above:	If the recording has some high-amplitude events, such as spike mediated post-synaptic currents, those can be removed from the analysis.
Noise f. for fitting:	This factor sets the threshold for fitting the mini events. Mini events with amplitude factor-times the estimated noise level will be considered for fitting. Decay and rise time constants will be determined for events only when fitting was successful (chi-square of fit reduced).

Analysis of miniature EPSCs

Estimate noise level:	Turn it ON for automatic noise level estimation based on standard deviation of the signal in the beginning of the recording.
Show baseline points:	Turn it ON to display the baseline for each mini event.
Look for cell images:	If images of cell are available, the program attempts to display the associated image file (*.JPG or *.TIF).
Kernel histograms:	When checked, the program calculates histograms based on triangular kernels (Parzen-estimation).
IPSC detection:	When checked, IPSCs are detected instead of EPSCs.
Use multi-threading:	Speeds up calculations by using multiple cores of the CPU.
Algorithms:	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.
Fit EPSCs:	Pressing this button will initiate the fitting process. Events will be fitted according to the formula that is selected by accessing the drop-down menu of this button.

Analysis of miniature EPSCs

** Cell info **

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** Main panel **

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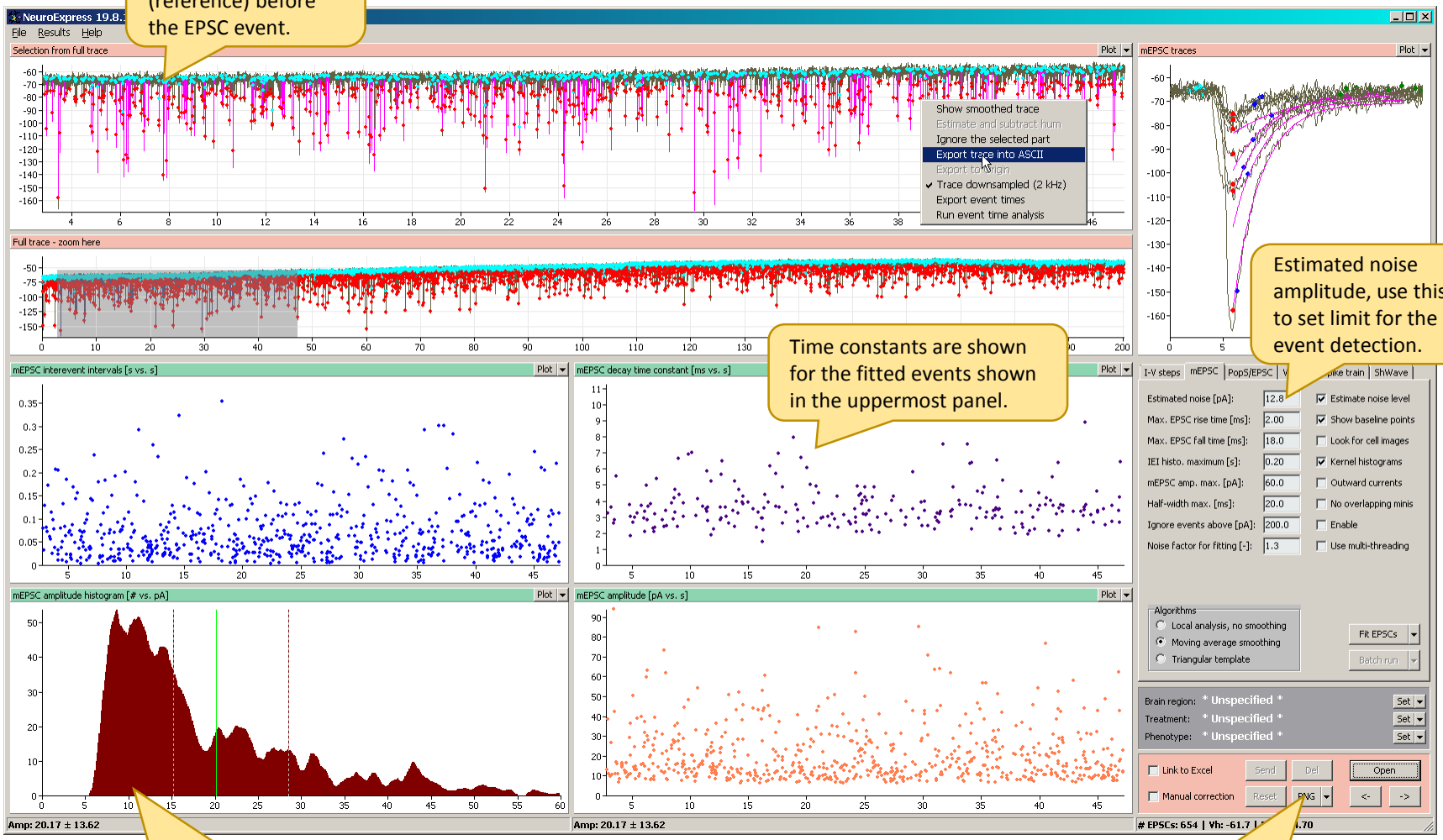
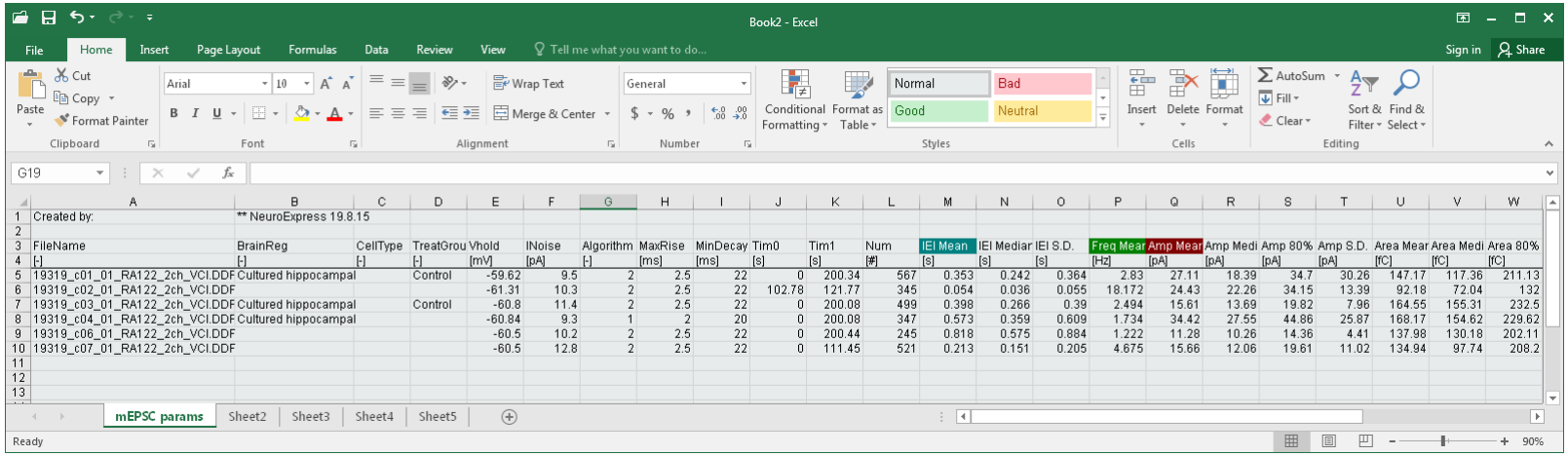
Analysis of miniature EPSCs



Analysis of miniature EPSCs

** Exporting calculated parameters **

The program saves the parameters of the miniature EPSCs/IPSCs into an Excel worksheet. The ‘Link to Excel’ checkbox needs to be checked 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 ‘Cell comments in Excel’ box in order to have descriptions of the calculated parameters as comments appearing in the Excel worksheet.



Spike train analysis

** General instructions **

Spike arrival times are used for this analysis, but the membrane potential or extracellular voltage is not taken into consideration. The input data are simply a list of numbers that represent the arrival times of successive action potentials. The input ASCII or text file should contain a single column of spike times in seconds. The recording starts at t=0 and spike times should be recorded at ms or submillisecond precision.

Spike train analysis