ScienceBeam

Product Manual

eProbe

V 0.1.0.1

Content

eProbe Overview

eProbe is a software designed to work with Electromodule, a ScienceBeam Amplifier-Data Acquisition system. It is downloadable from the download area in our website (*www.ScienceBeam.com*).

eProbe offers the users a collection of electrophysiological toolboxes for both data collection and offline analysis the data.

eProbe version 0.1.0.1 contains the following toolboxes:

1. ***eTrace Experiments:*** Briefly, associated with Electromodule this toolbox planned for acquisition and online monitoring of local field potentials such as: evoked fEPSP and PS, EEG, EMG ….
2. ***eTrace Analysis:*** With or without connection to Electromodule, this toolbox designed for offline analysis of data collected through eTrace Experiments.
3. ***eSpike Experiments***: In association with Electromodule, eSpike planned for acquisition and online monitoring of neuronal unit activity (single or multi unit activity).
4. ***eSpike Analysis:*** This toolbox developed as an offline analyzing program for collected data through eSpike Experiments
5. ***eSorter:*** This toolbox designed for offline sorting and clustering of extracellular recoded action potentials.
6. ***eStat:*** This toolbox offer the user some of commonly used statistical analysis.

In the current manual, I will present you an overview about the Electromodule, then we will review working with *eTrace* and *eSpike*:

Overview to the Electromodule

Electromodule front panel

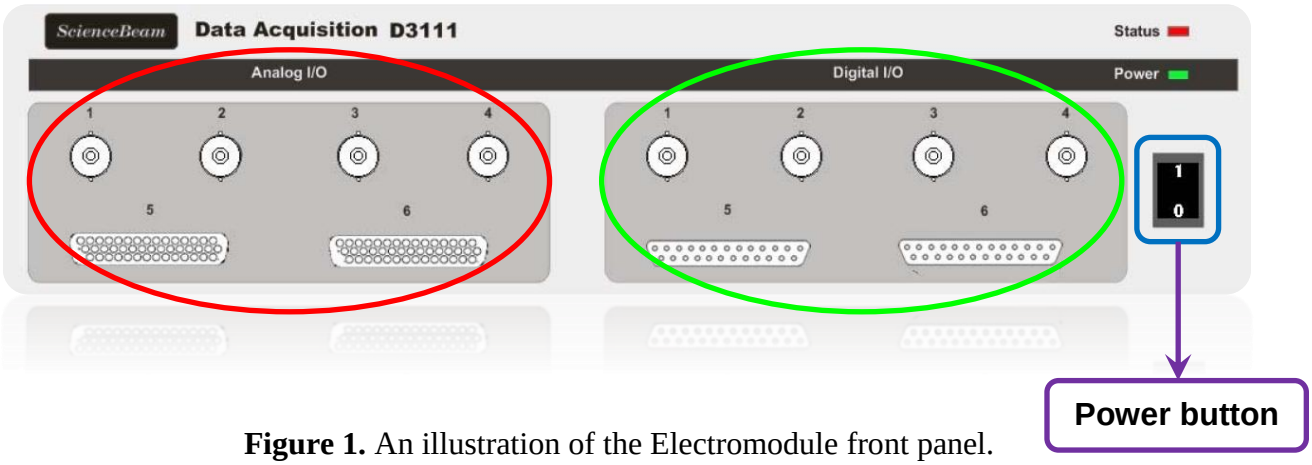


Figure 1. An illustration of the Electromodule front panel.

Figure 1 shows an illustration of Electromodule front panel. As you are seeing Analog I/O, Digital I/O and Power button are the main elements of the front panel. First, I will get you a brief explanation about these elements:

- **Power button:** As I show with a blue circle, you have a power button to turn the Electromodule on or off.

A green light in the top-right side of front panel shows the power state. When you turn on Electromodule this light is also on and vice versa.

Note: *If the red light turns to on, you have a problem in your system. If the red light is flashing and you are using the internal stimulator of Electromodule, so check the stimulation electrode to be sure it is into the electrolyte solution. Also you might check the connections of the stimulator. When the red light turns on continuously (not flashing), there might be a problem in data transferring to the computer (Data overflow). At this situation, you must turn off Electromodule, and then start again.*

- Analog I/O: All analog input/output (I/O) of Electromodule demonstrated with a red circle (figure 1). As you are seeing, four BNC connections and two D-Sub connections are available in Analog I/O.

BNC connections 1 to 4 are designed to be analog inputs or outputs. Potentially, these connections have the capability to use in various ways. However, in our normal version of Electromodule these connections are inactive but we can customize these connections in accord with your demands (see our website for contacting us).

D-Sub connections 5 and 6 are also analog inputs-outputs. You must attach your recording or/and stimulating electrodes to these connections (a shielded cable and connectors are including in the package of Electromodule)

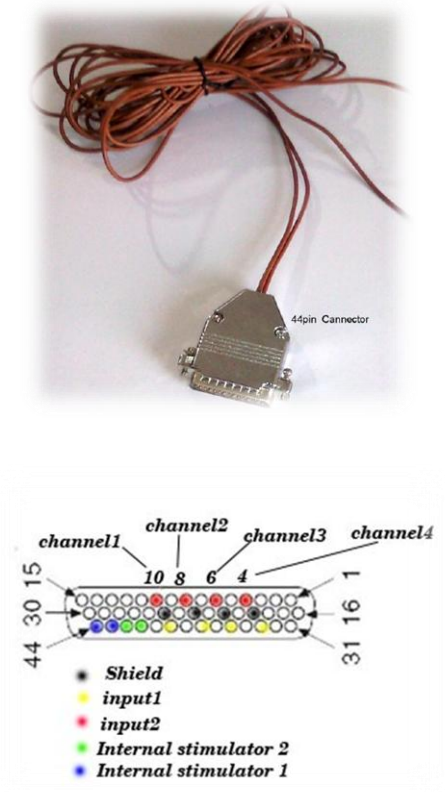


Figure 2. A 44-D-Sub connection (above), also a schematic view of 44-D-Sub connection map (below) illustrated. Four analog inputs demonstrated in red-yellow planned to connect to recording electrodes. Blue and green are showing current stimulators outputs.

- Digital I/O: All digital input/output (I/O) of Electromodule demonstrated in figure 1 with a green circle. As you see, four BNC connections and two 44-D-Sub connections are available. Through these digitally inputs-outputs you could mange or synchronize with another data acquisition or external stimulator. For example, using these BNC connections you could synchronize two or more Electromodule systems together or even synchronize an Electromodule with another digitizer or stimulator.

Note: *The Capability to provide other inputs-outputs according to your desires is always open in Electromodule (contact us for customizing your system).*

Back panel

Figure 3. Back panel view.

- DC input: Use a DC power supply (24V and at least 2Amp) and connect it to your Electromodule through a connector as I show in the figure 4.

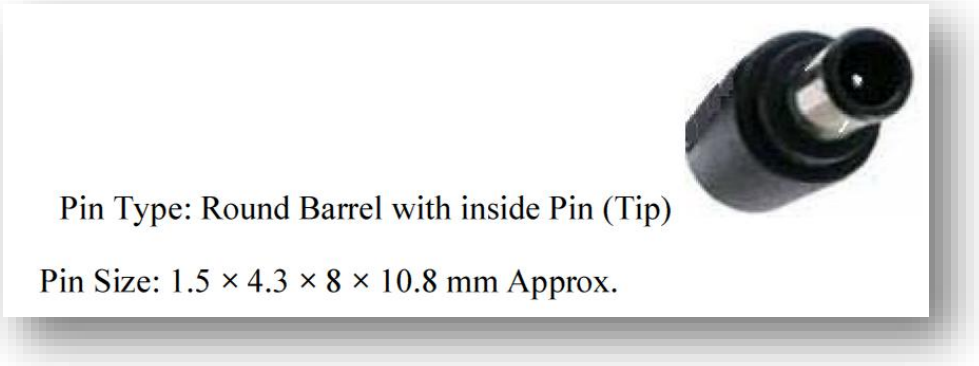
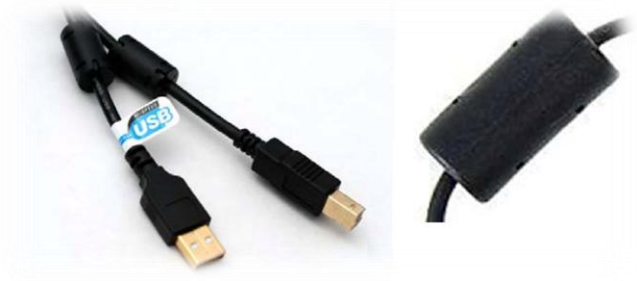


Figure 4. DC power supply connector.

USB input: connect your Electromodule to a computer trough a UBS connector. The cable must be shield otherwise it could bring noise to your system.



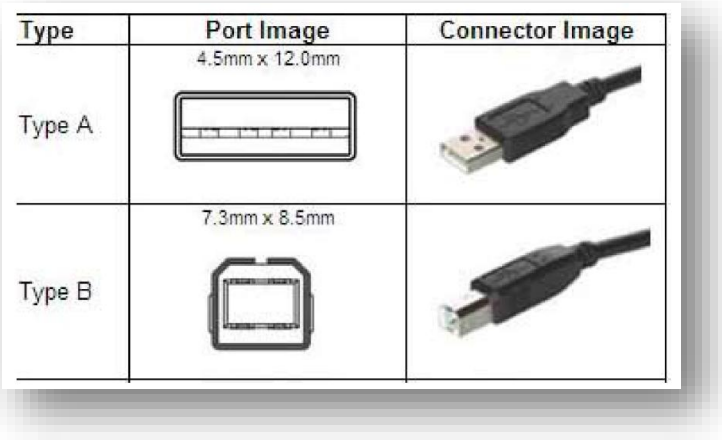


Figure 5. Appropriated USB connections for Electromodule.

LAN input: Designed to use for network connection. If you want to use this item, contact us for customizing the system.

Digital I/O: Designed to use, when more inputs-outputs are desired. We can customize it.

GND: Use this item to ground your Electromodule.

Memory Card: Designed to use, when you need an external memory connected to your Electromodule. If you want to use this item, contact us for customizing the system.

Hardware Installation

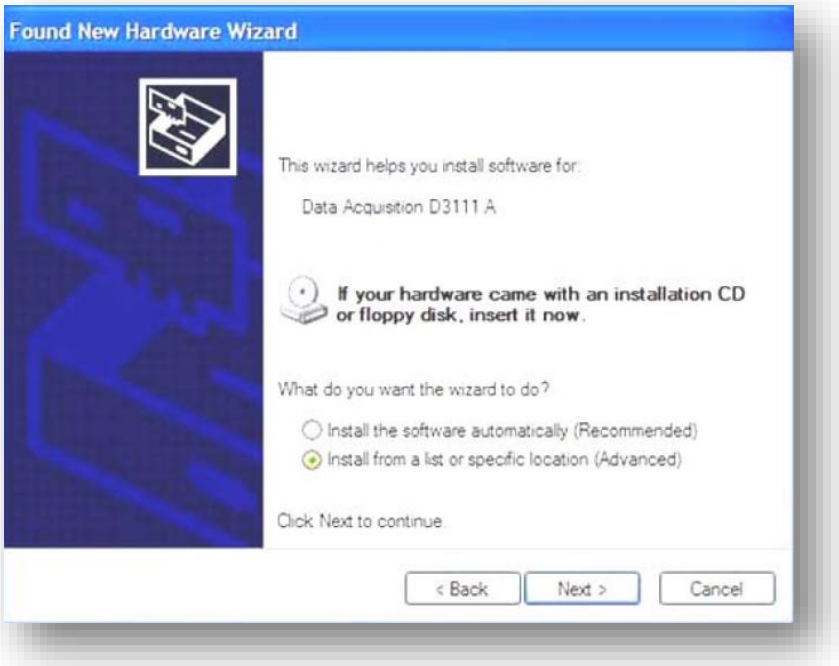
eProbe software is downloadable from the download area of our FTP. Point your browser to *http:/www.sciencebeam.com*. All installers are available at this area.

Note: if you are using Microsoft operating system, which are older than windows 7, you *have to install .NET framework before you install the eProbe software. It is also available in our FTP.*

If you want to us eProbe with Electromodule, you have to install the Electromodule drivers. First, connect the Electromodule to the computer through USB connector (figure). Then turn on Electromodule and wait for this message from your windows "*Found new hardware*".

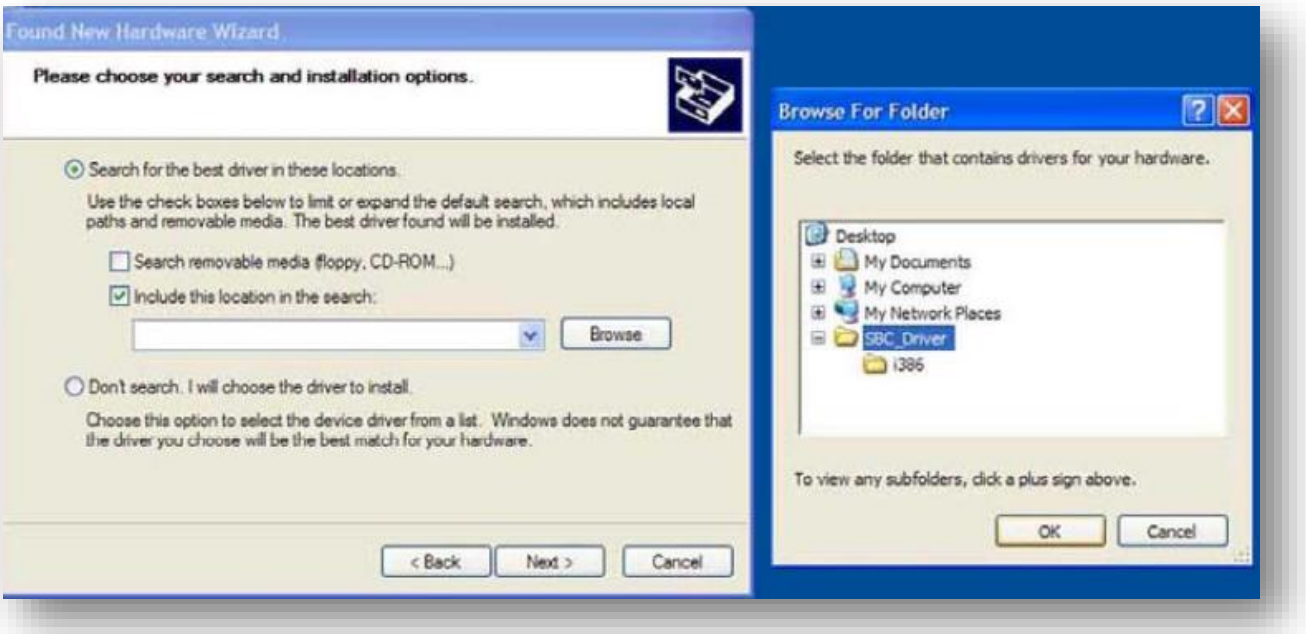
Do not click on this box and just wait to see "*found new hardware wizard*" box.

Select "*Yes, this time only*" and go to the next. You will see another box like the following figure:

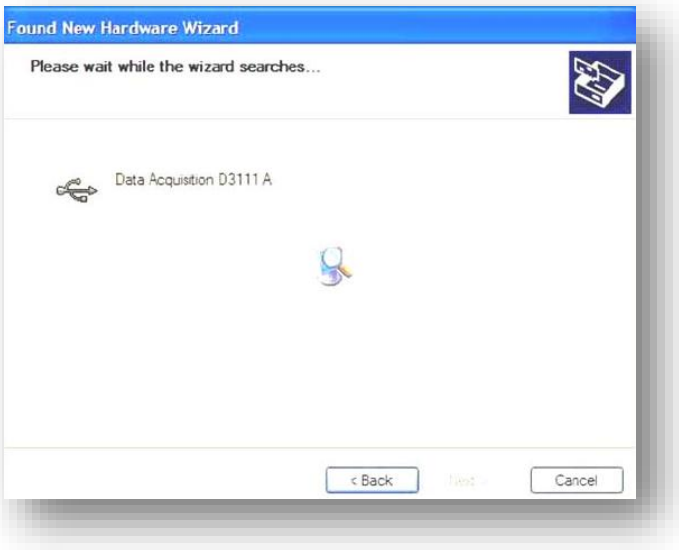


Select the second option "*install from a list or a specific location*" and then click on next. Then you will see a new box as below:

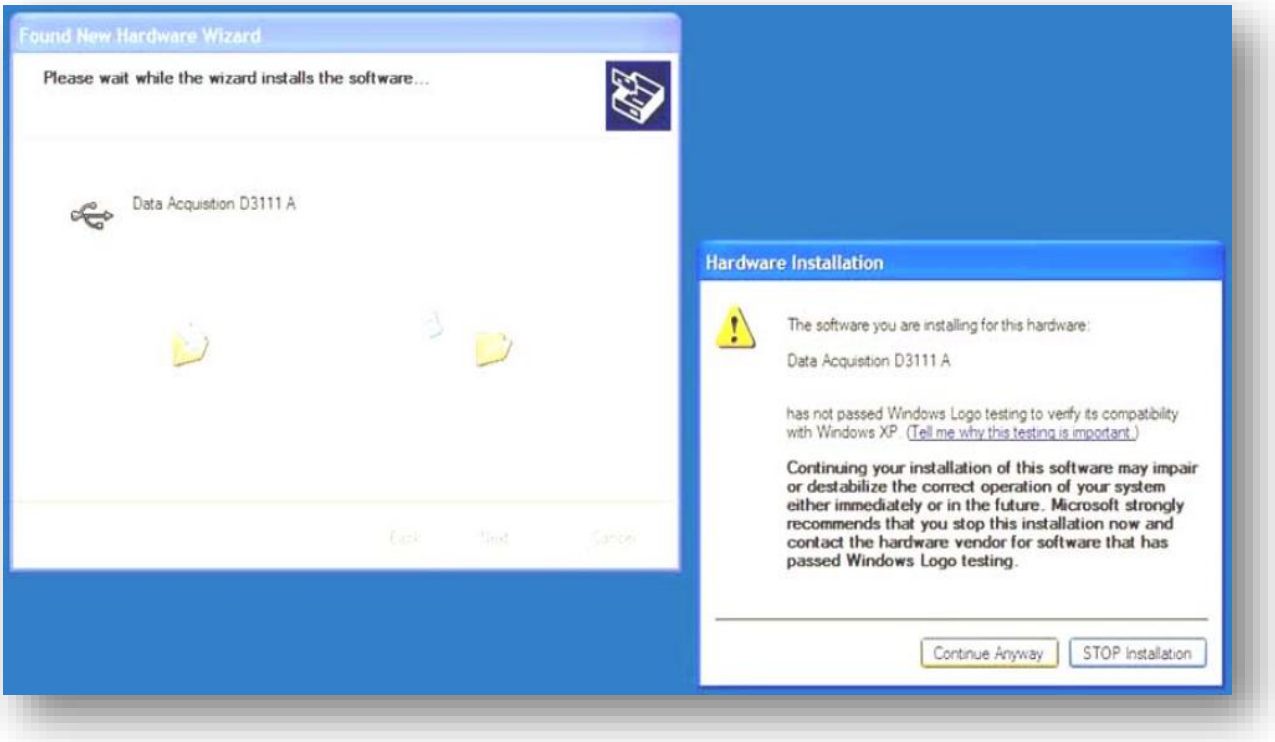
From this new box browse the location that you have saved SBC driver, then choose the driver and click to make it "*ok*" then go to the next.



Wizard will automatically search to find and install the driver A.



During the installation you may see a message as below:

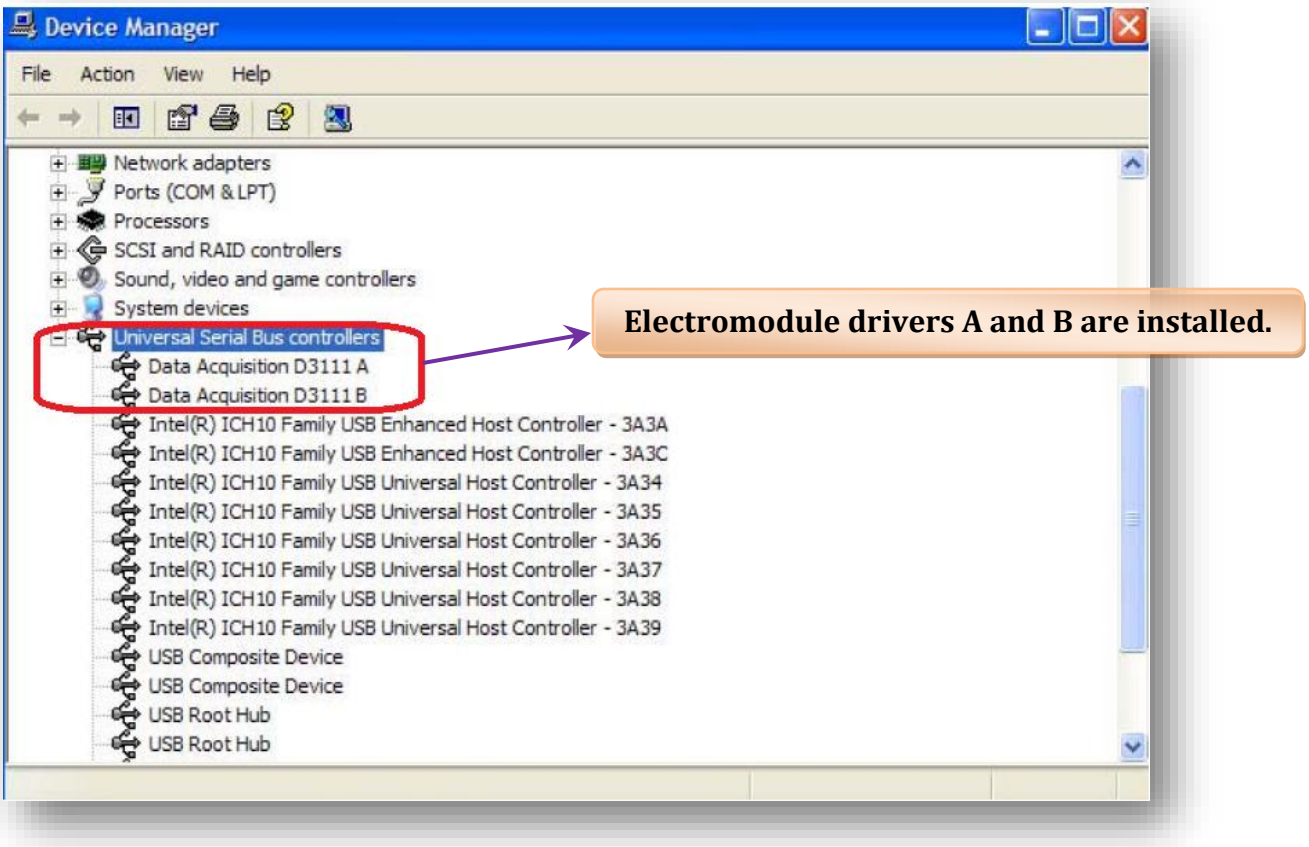


Click on "*continue anyway*" to finish the installation. Thereafter you will see the completing message, now you have driver A installed on your computer, click to "*finish*" this part.

Then you will see another message from your windows which ask to install deriver B. Continue and do as you did previously for driver A.

After installation both A and B drivers, you could check them on your computer. For this, follow this pathway:

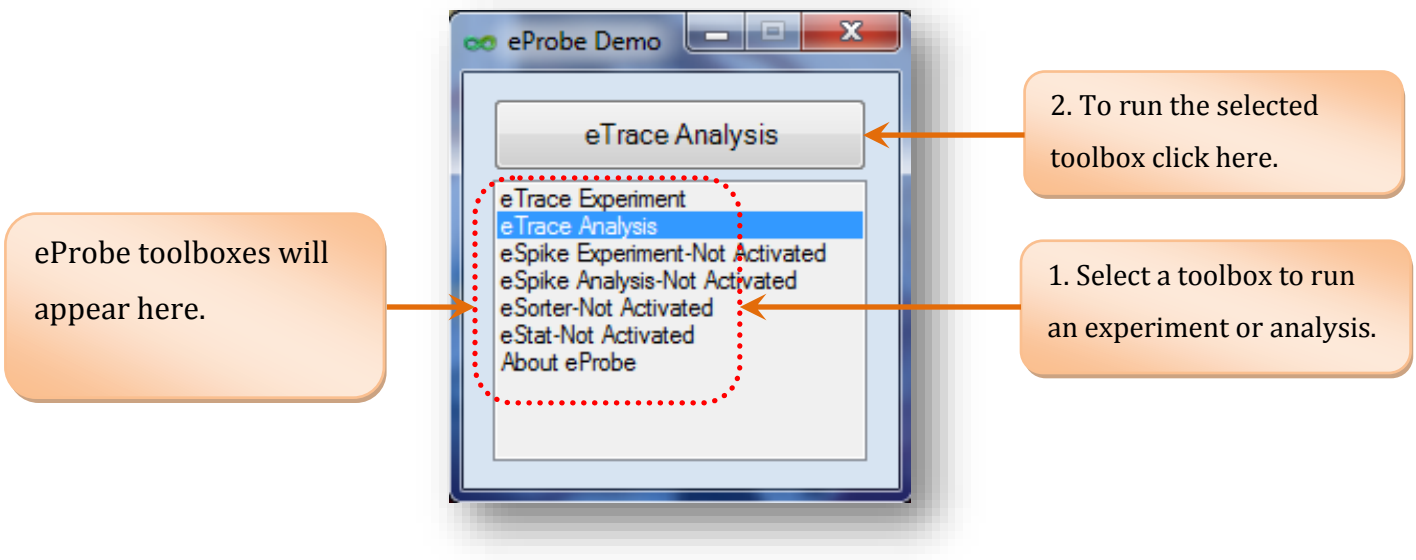
Go to the *Control Panel* > click on the *System* > select *Hardware* > go to the *Device Manager* > double click on the *Universal Serial Bus controllers* > find your *Data Acquisition A and B*.



If you was not able to see your data acquisition A and B from this menu, probably you did an incorrect installation. Turn off your Electromodule and restart your computer. Then start the procedure again.

Installation the eProbe software

Copy eProbe files to a new folder in your computer. In the eProbe folder you will have this icon “ ”. eProbe will be easily run up when you double click on this icon and then you will see a box like you are seeing in the figure below.



eProbe version 0.1.0.1 have six toolboxes. To active and run up each toolbox, you have to buy a license key by contacting us.

eTrace

eProbe version 0.1.0. has two eTrace toolbox for stimulation, data acquisition and off-line analysis of a wide range of extracellular electrophysiology studies, such as Long-Term Potentiation (LTP), Long-Term Depression (LTD), EEG, EMG and epileptiform bursts activity.

eTrace records extracellular signals at 10KHz/channel. Users are able to run up eTrace through two different toolboxes simultaneously and independently (as appeared at the above figure):

1) ***eTrace experiment*** for online Viewing/Stimulation/Acquisition the traces or continuous acquired data

2) ***eTrace analysis*** for off-line data analyzing.

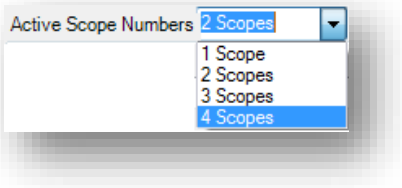
In addition, *eTrace experiments* toolbox has a protocol builder (named as: *design protocol*) to produce complex stimulation protocols.

eTrace analysis includes all basic analyses of synaptic potentials (Slope, Peak Amplitude, Latency, Area, PopSpike Amplitude, …) also analyzing EEG, EMG, phase and frequency of signals.

First of all, I will explain how to do your experiments with "eTrace experiments" then I will go to the "eTrace analysis".

eTrace experiments

Recoding panel of eTrace will appear by clicking on the "*eTrace experiments"* button. You will see a panel as shows in the figure 6. By default, this panel will open with two scopes, each one from a separated recording channel. However, you are able to change it into one, two, three or four scopes through "*active scope numbers*".



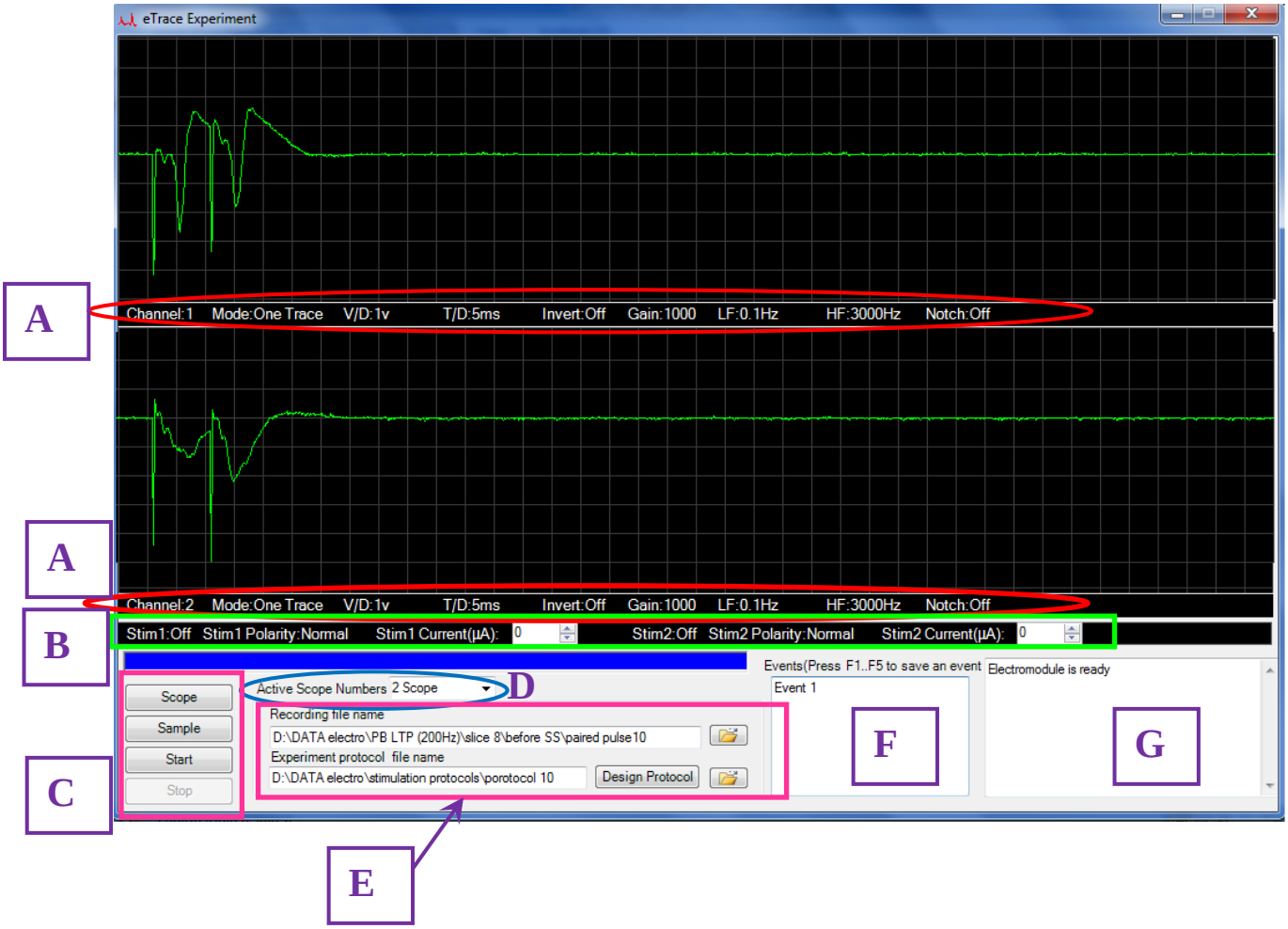


Figure 6. A demonstration of eTrace experiments panel. Two scopes each one has a menu bar at the beneath (red lines, A). Using this menu bar user is able to set both the scope and amplifier, in accord with the recording situation. Another menu bar (green line, B) is provided to set the stimulation. Using these buttons (pink line, C) you could run the program in scope, sample or saving modes or stop each running mode. Through this item (blue line, D) you can change available scope numbers from one to four scopes. From here (pink line, E) you could select a pathway and a filename to save each recording, also you can choose to apply a previously made up stimulation pattern. Using this box (F) user is able to save occurrence of one to five different events. Finally, through this box (G) you could see the running status.

As you have two channels of amplifier-digitizer in your Electromodule, so you usually need one or two scope to see and follow your experiments online (figure 6 and 7). However, in eTrace, you are able to run up three or four scopes in your *eTrace experiments* and then set each scope **separately**. By this way you can see each input channel of your Electromodule into more than one scope (figure 7).

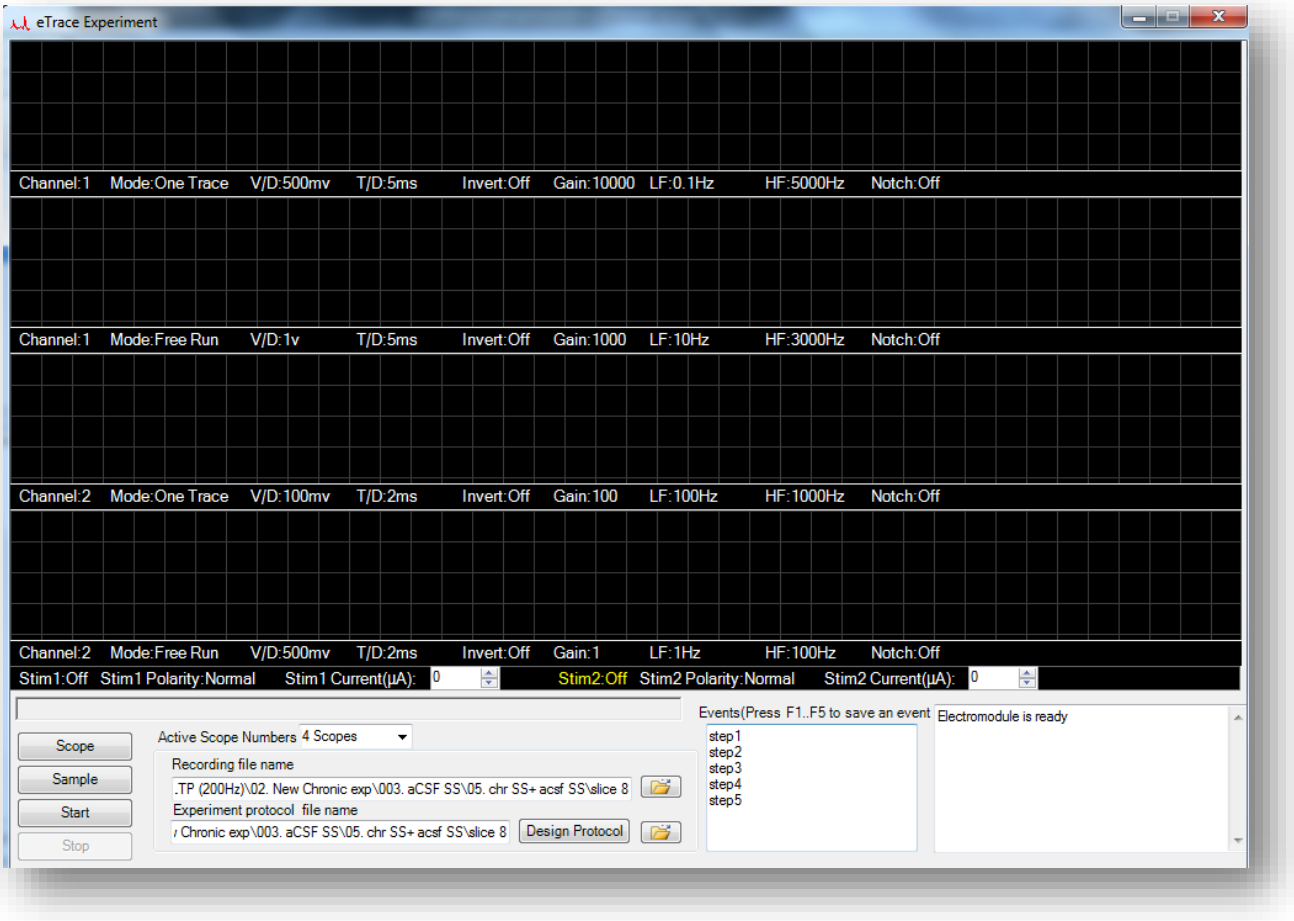


Figure 7. As demonstrated in the figure you could run up eTrace in four scopes and set each one independently.

As in the previous figure showed, each scope has a menu bar placed below it (red lines or A in figure 6). This menu bar contains a number of parameters which will let you to set both the scope demonstration and the signal acquisition, according to your work. You can use this menu bar to set the parameters of each scope separately. To change the values of a parameter just right/left click on its item.

Now I am going to describe this menu bar:

Channel: Electromodule has two channel of amplifier-digitizer. In this version of eTrace you can switch between these two channels. You are able to see channel 1 in one, two, three or four scope.

Mode: For each scope, there are three different modes. *One trace*, *free run* and *off*. If you choose "free run" the scope will continuously show the signal. If you choose "one trace" then you will have a triggered signal in each trail. Choose "off" to turn the scope off.

V/D: This item (Voltage/Division) shows you voltage scale. Using this item, you can set the voltage values of vertical division of your demonstration.

T/D: This item shows you Time/Division. Just like above you can adjust time values of each horizontal demonstration.

Invert: If you turn on this item, your signal will appear inversely.

Gain: Using this item you are able to change the amplification level of the signal. You have many options from 1 to 10000.

LF: This item offers you a *Low cut Filter*. By clicking on this item, you can change low cut filtering of you signals. You can choose one of these filters: 0.1, 1, 10, 100, 300 Hz in accord with your work.

HF: Using this item you can adjust *High cut Filter* setting. *A* high cut filter will eliminate the frequency bands beyond the filter cut off. You can choose a HF from the list: 5000, 3000, 2000, 1000, 100, 50, 40, 30 Hz.

Notch: This is a filter designed for 50 Hz noise. You can switch it on or off by clicking on this item.

Electromodule Amplifier- Digitizer

Electromodule Amplifier-Digitizer has the following properties (you could also use your own microelectrode amplifier or preamplifier and make a connection with Electromodule):

Microelectrode amplifier (U3022)

Type: Differential, Isolated, Extracellular

Number of channels: two

Low cut filter setting: 0.1, 1, 10, 100 and 300Hz

High cut filter setting: 1000, 2000, 3000, 5000 and 10000Hz

Notch filter setting: 50Hz

Gain: 1, 2, 5, 10, 100, 200, 500, 1000 and 10000.

Input voltage range: ±5V

Maximum analog input voltage: ±5V

Input Impedance: $10^{12}\Omega$, common mode and differential

Input leakage current: 60pA (typical)

Input capacitance: 8pF

Common mode rejection ratio: 75dB @ 50/60Hz

Isolation type: Optical

Isolation voltage: 2500V

Isolation resistance: $10^{12}\Omega$

Digitizer

Coupling: DC

Analog input range: ±2.5V

ADC resolution: 24bits

Linearity error: ±7.6ppm (maximum)

Maximum sampling rates: 50 kHz, each channel

Number of analog input channels: Two

Electrical current stimulation

Electromodule has an internal current stimulator with the following properties (however eTrace is able to run an external stimulator):

Constant current stimulator (U3022):

Type: Constant current, Unipolar, Isolated

Number of channels: One

Polarity inversion: Yes, controlling by software

Isolation type: Optical

Isolation voltage: 2500V

Isolation resistance: $10^{12}\ \Omega$

Current control: Digital, controlling by software

Current range: 1µA to 4095µA

Current resolution: 1µA

Output waveform: DC or current pulse

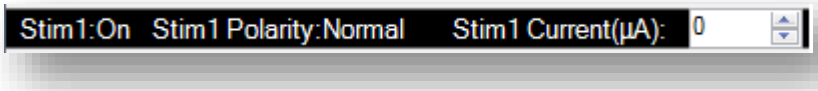
Current amplitude error: 3LSB (maximum)

Output voltage compliance: 150V

Current rise time and delay: 5µs, typical (1KΩ load)

Current fall time and delay: 5µs, typical (1KΩ load)

To use our internal stimulation, there is a menu bar as I show in the below.

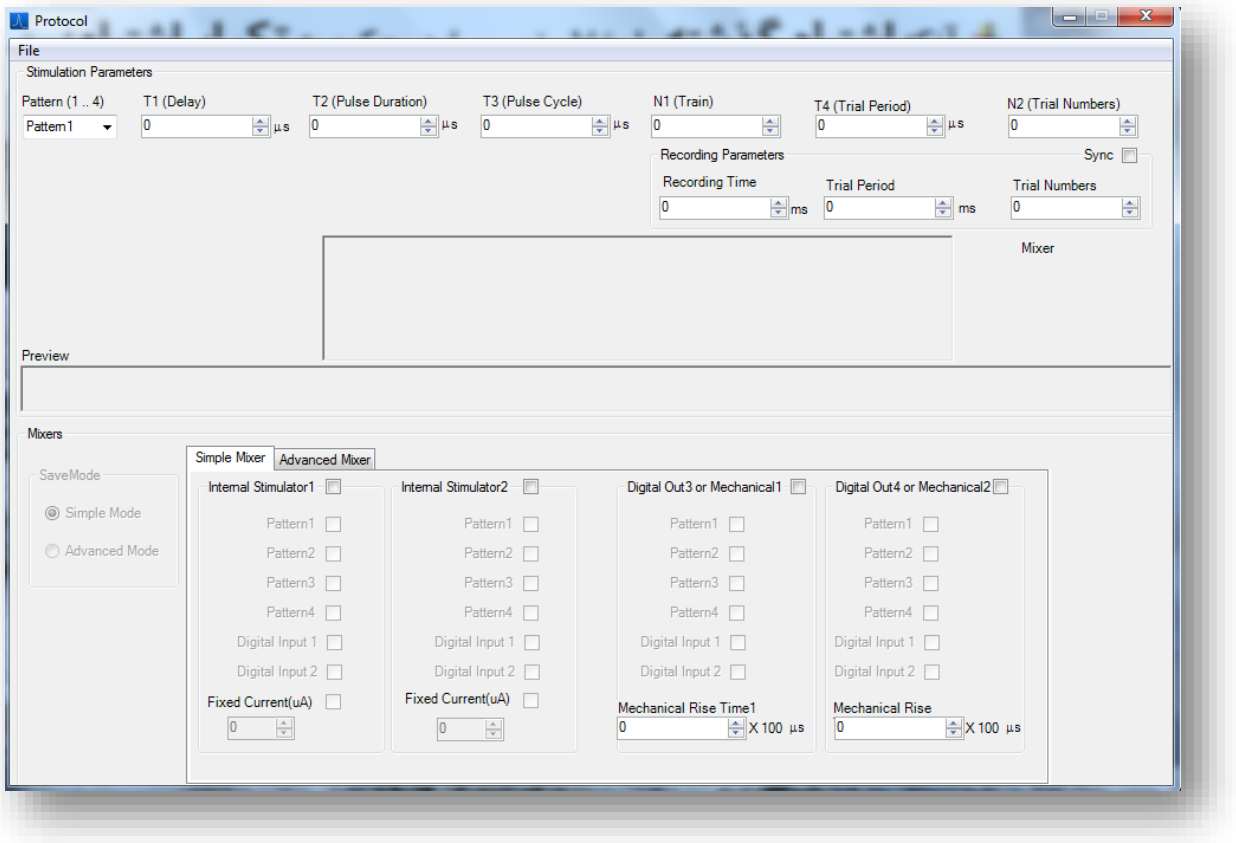


Using this menu bar you can turn the internal stimulator *on* or *off*. You are also able to change the stimulation polarity between normal and invert (as this is a monopolar stimulator, sometimes you might need to change the polarity).

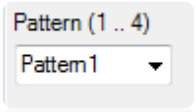
You can write a desired current intensity in this menu bar. eTrace stimulator just apply current (you can not apply voltage). To set the other properties of your stimulation (such as pulse duration, frequency) or describe a pattern for your stimulation, you must go to "*design protocol"* 

, see also E in figure 6.

In the *eTrace experiments* panel there is a button named "*design protocol".* Click on this item then you will see another window. Through this panel you are able to design and save a simple or complex stimulation pattern to use during an experiment.

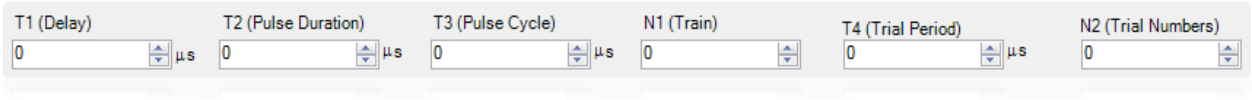


First, I will explain you all contents of this panel then I will give you a few examples.

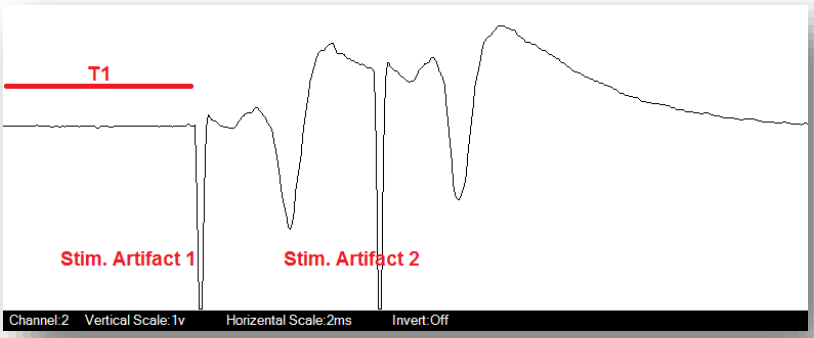


Using this item you are able to make four different patterns of stimulation. Then through "*Mixer*" you can mix these patterns to produce a complex protocol.

To create a pattern using this menu:



T1 (Delay): Latency between starting the recording time and applying the first stimulation pulse of each train. This latency will appear before the first stimulus artifact of each train. I show you an example in the following figure.



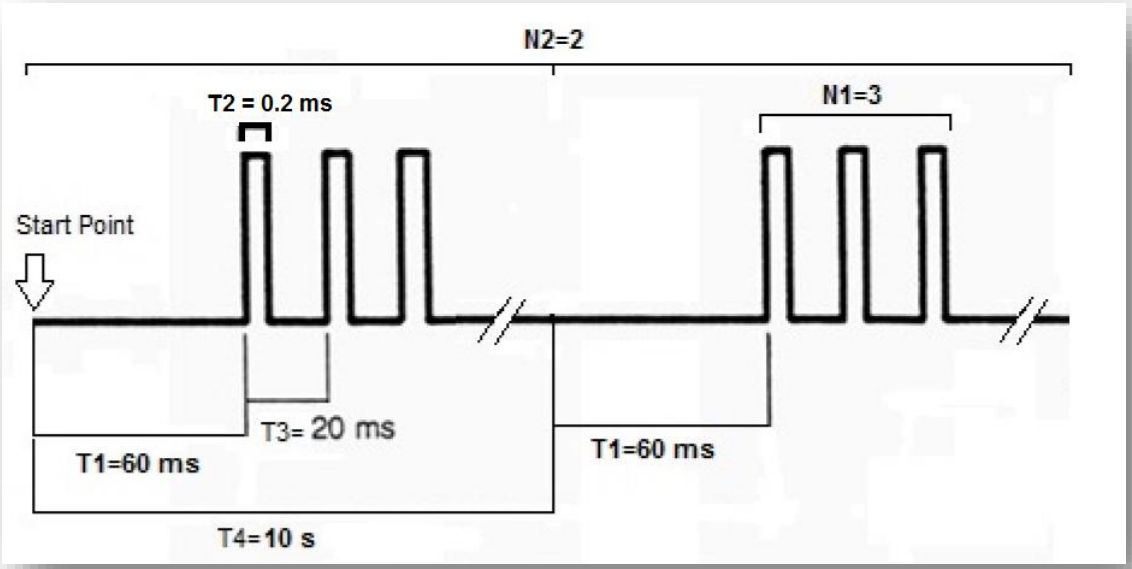
T2 (Pulse Duration): Duration of a single stimulation pulse. (See T2 at the next figure)

T3 (Pulse Cycle): Duration from starting a single pulse to starting the next single pulse.

N1 (Train): Number of pulses in a *trial period.*

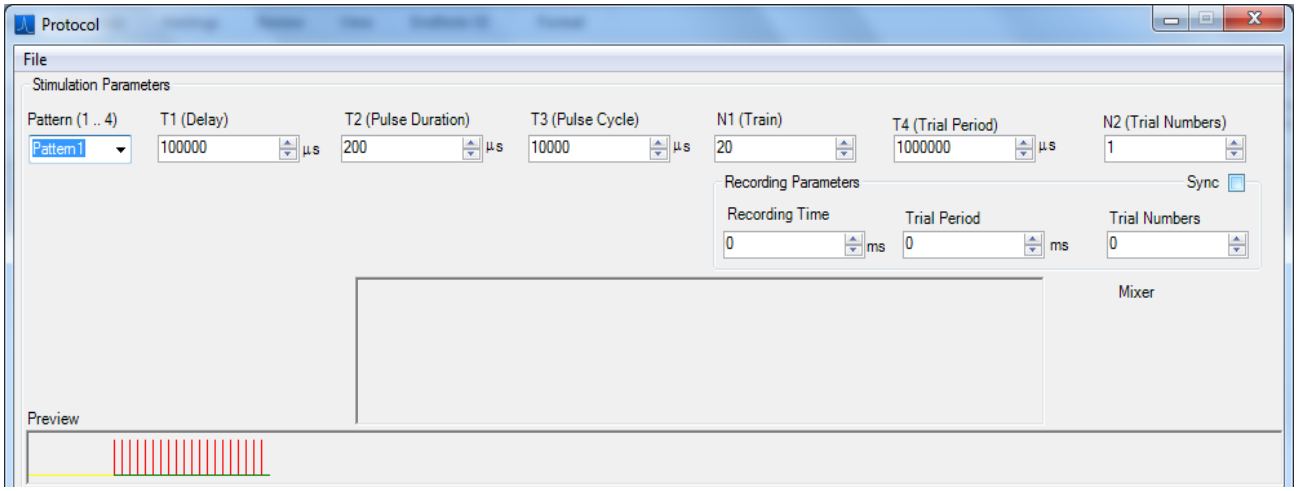
T4 (Trial Period): Duration from starting a trial period to starting the next one.

N2 (Trial Numbers): To determine how many times you want to repeat a desired trail.

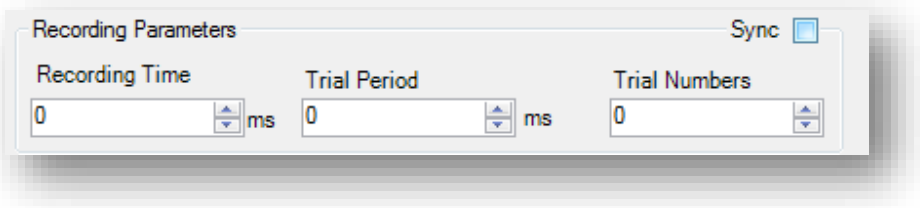


To show you an example I made a pattern as: A train of 20 single pulses at 100 Hz, each single pulse has 200µs duration.

When I want a stimulation pattern in 100 Hz (100 pulses/second), so the pulse cycle must be 10ms (1000ms/100pulse). As you see, through the pulse cycle you could establish frequency of a train. Also, N1 will explain how many pulses you want to have in a train. According to T1, T3 and N1 you must write a value for T4.



Usually, following the stimulation, you have an electrophysiological response and you want to save it. Save your data using the below menu:



Recording time: It is part of trial period, which you wish to save it on the computer.

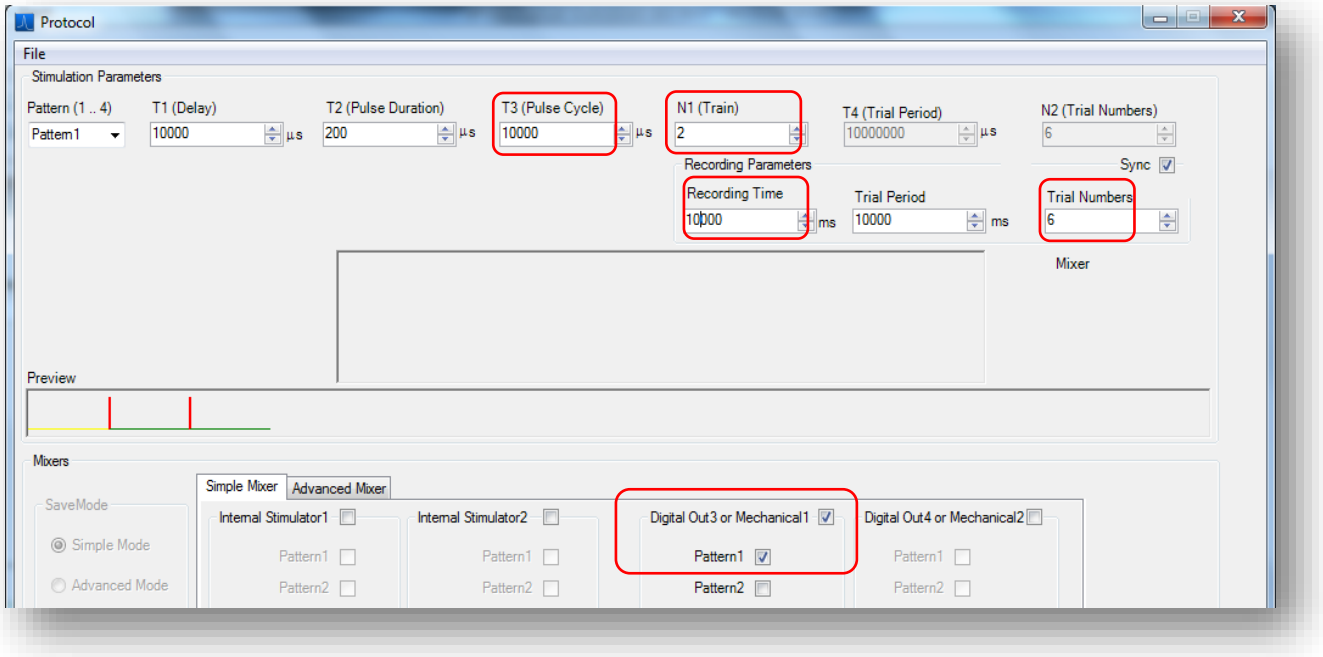
Trial period and ***Trial Numbers*** are the same as described above. Recording time must not be longer than the trial period (could be lesser or equal).

You can check the sync box to equalize the values of *Trial period* and *Trial Numbers* in both Menu bars.

Example 1:

I want to apply two single 200 µs pulses with 10ms interpulse interval, then repeat this pattern 6 times every 10 seconds. I also want to save all of the time of each trial.

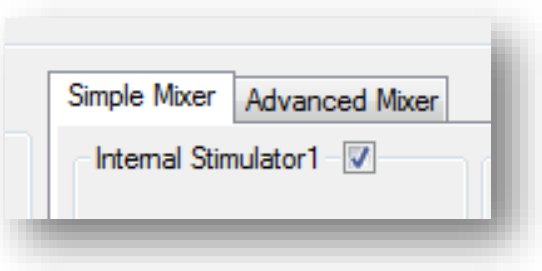
See the following figure:



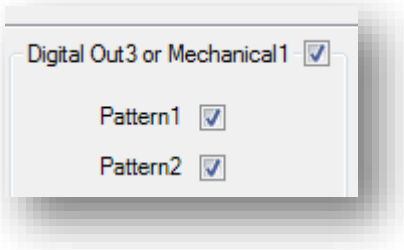
Note: When you are going to apply more than one plus in each train then pulse cycle is just equal with interpulse interval.

Mixer: sometimes you need to make a complex protocol for stimulation so you will need more than one pattern. Mixer item in the panel provides you an opportunity to do this.

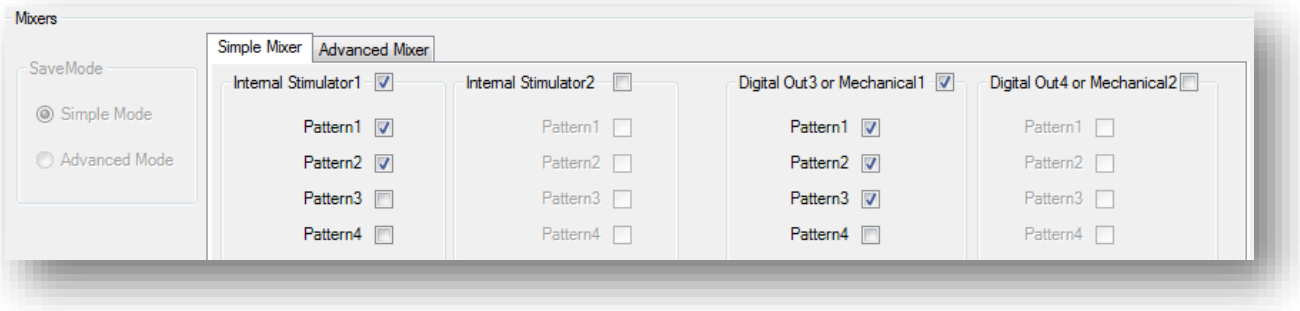
If you are going to use Electromodule internal stimulator, check the box next to the internal stimulator1, to active this option. You can design more than one pattern then select them in the mixer.



If you are going to use your external stimulator check, the "*Digital out3"* box to active it. Now select one or more pattern, which, you wish to mix them as a stimulation protocol.



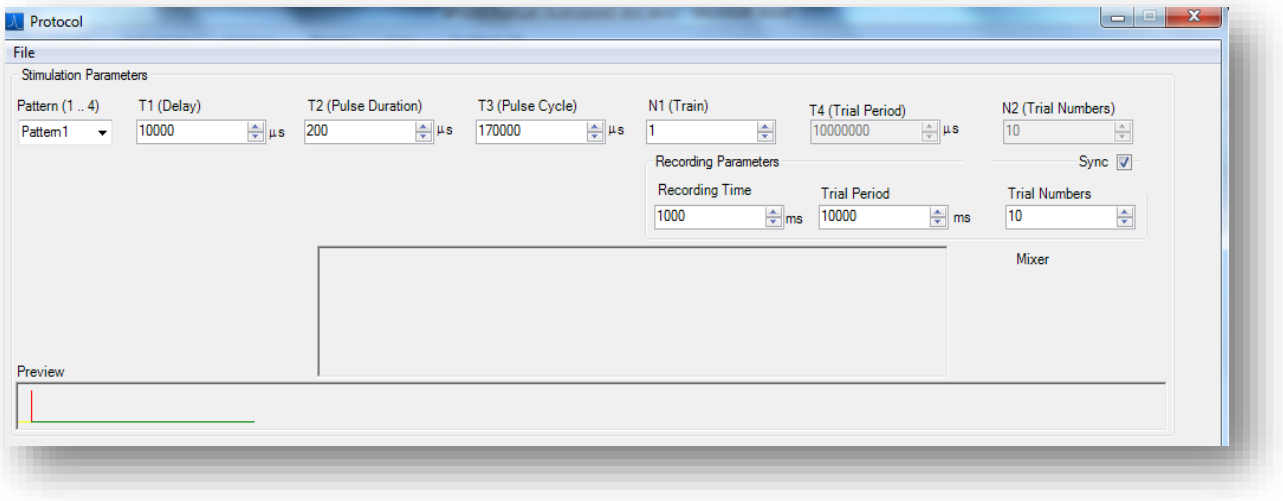
This Mixer will also let you to use both internal and external stimulator simultaneously (for example if you want to use two stimulation electrodes)



In the following example, I am going to make a complex protocol using two patterns.

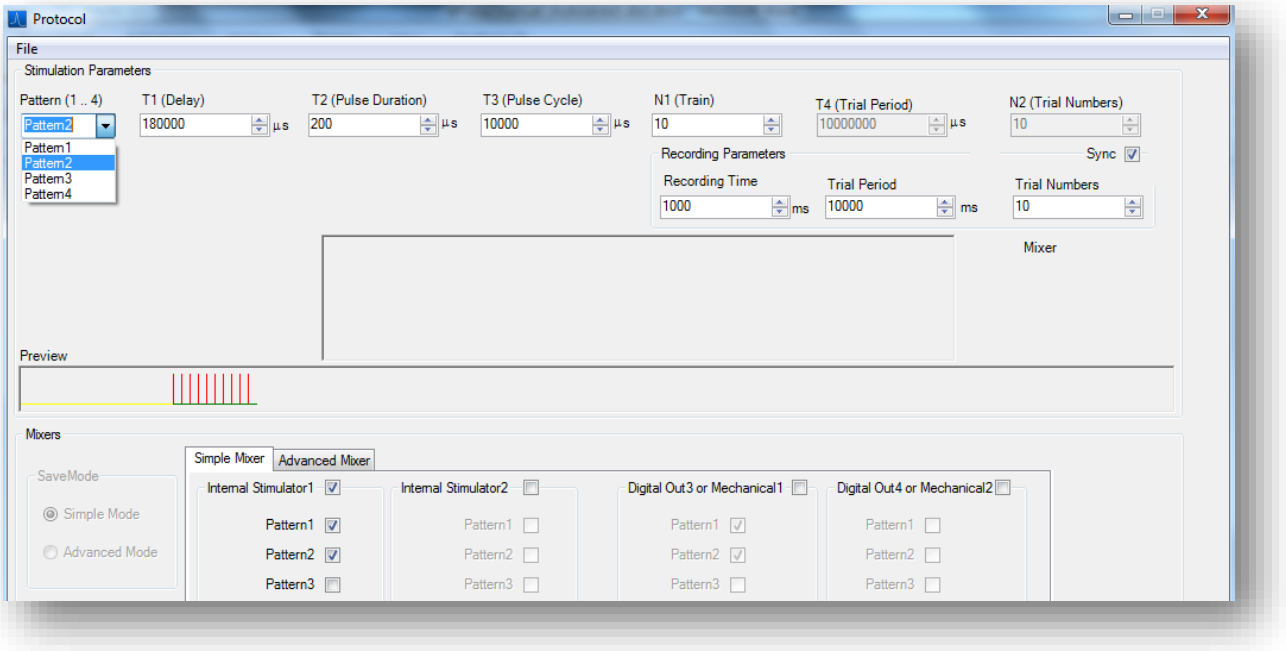
Example 2: I want to induce LTP using primed burst stimulation. This protocol contains a primed single pulse followed 170ms later by a burst of 10 pulses in 100 Hz. I also want to repeat this 10 time each 10 seconds. See the below figure:

In the first step I defined pattern 1, in this pattern a single pulse (200µs duration) repeat 10 times each 10 seconds. (As I have one train of this single pulse the pulse cycle is not important in pattern 1 but be careful to write it correctly when you have more than one pulse in a train)



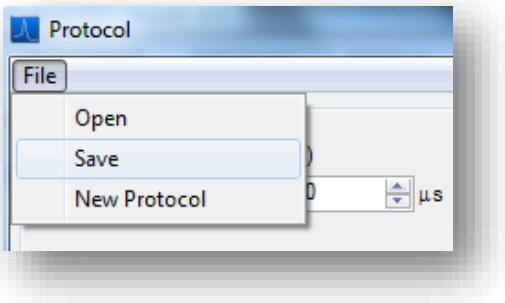
In the second step I am going to define pattern 2 with a burst of 10 pulses in 100 Hz and repeat it 10 times each 10 seconds.

I also set the recording time to save 1000ms of each trial period. See below;



To apply both pattern 1 and 2 simultaneously through the internal stimulator I made the mixer active. See the previous figure.

To save the protocols use save icon from the file menu, like the figure:



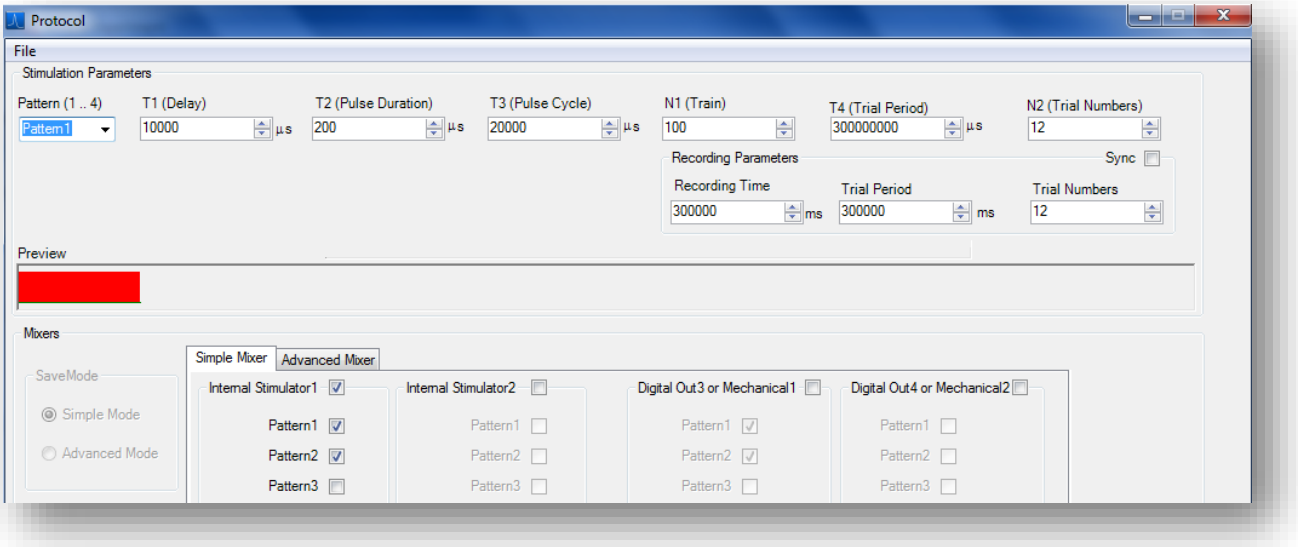
I recommend you to save all your protocols with a specific name in a folder with a specific name like “my stim protocols”. By this way you can reach them always.

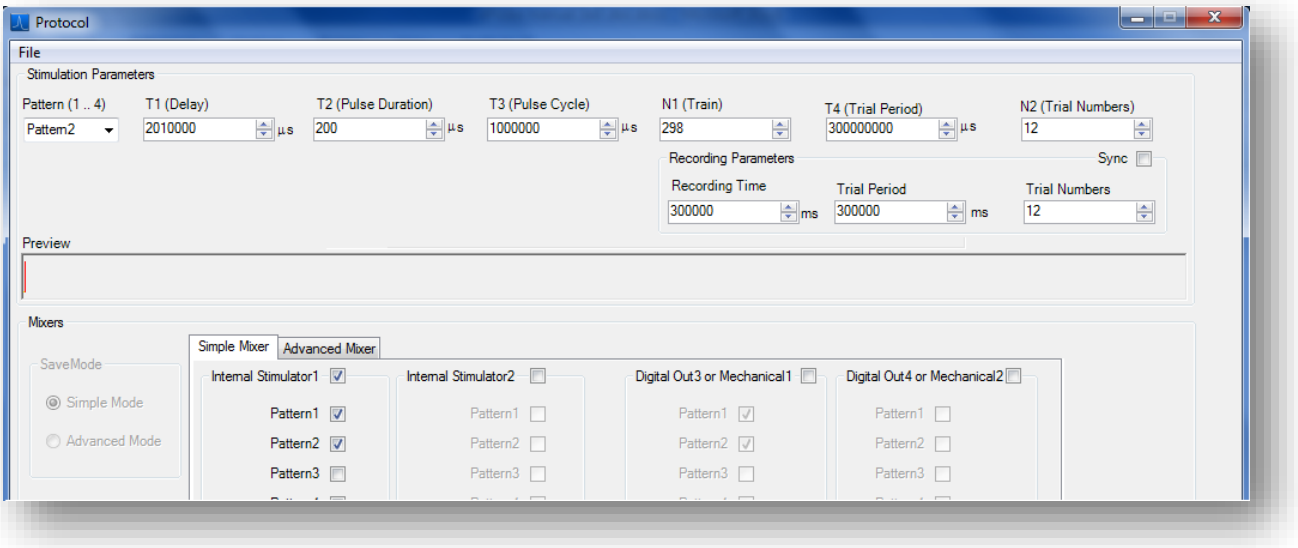
- *Note:* if you are going to use Electromodule internal stimulator it is better to set the current intensity form the eTrace experiments window even you could fix the current from the “design protocol”.
- If you want to use an external stimulator, you must set the current intensity and polarity of your stimulus through your stimulator front panel.

Example 3: An experimenter needs to make a protocol for epileptogenesis.

He wants to apply 2sec of 50Hz stimulation and repeat it each 5min for 60min. He also wants to apply low-frequency stimulation in 1Hz between (not within) all those 50Hz stimulations.

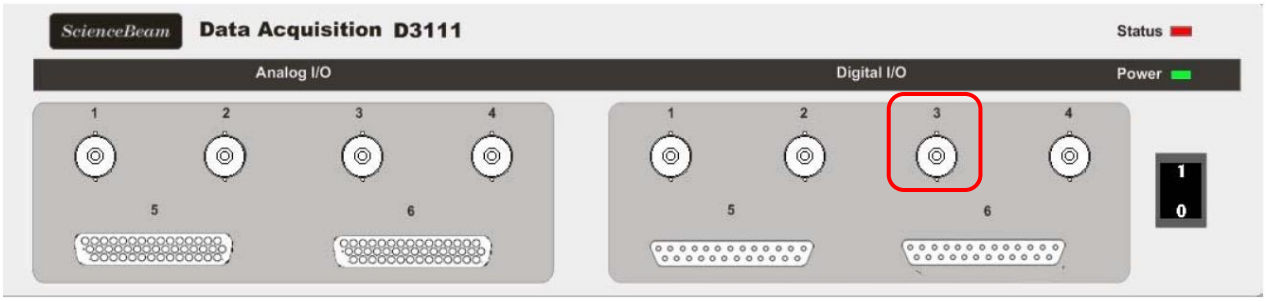
See the following figures: pattern 1 shows 2sec of 50Hz stimulation and pattern 2 shows 1Hz stimulation. Like before, use mixer to combine these two patterns. And then save it as a protocol for the future use.





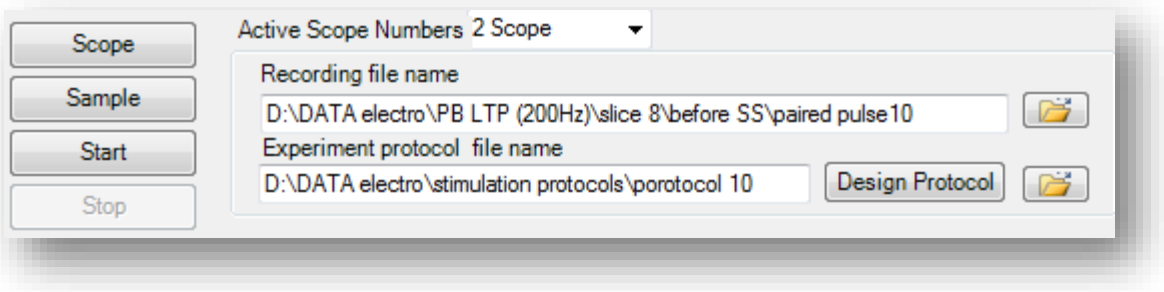
How you could connect an external stimulator with your Electromodule?

You just need a BNC connector. Digital output 3 in the front panel of Electromodule designed to run up an external stimulator (red circle in the below figure). Then connect your stimulator through a BNC connector to the Digital output 3.



Data collecting with eTrace

Using the following menu you are able to start the eTrace and see your electrophysiological records.



First, set a pathway through the "*Recording file name*" and give a file name to save your recording in a preferred location. You have to apply a protocol for recording and/or stimulation.

Clicking this icon,  you can set these mentioned pathways and file names.

There are also, four buttons in the left side of the panel which work as below:

Scope: Without applying a stimulation protocol, scope will show you a preview from the basal electrical activity in the recording site. (e.g. you could check the noise).

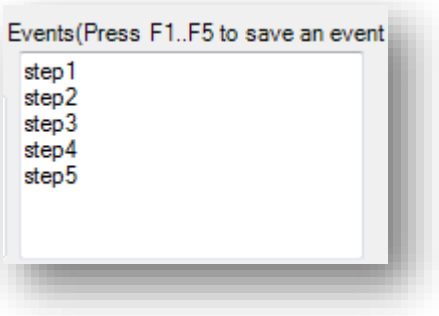
Sample: This icon offer you to test one train of your stimulation protocol as a sample. Using this icon you could test the stimulation protocol and if it is working. Also you could check response(s) to the stimulation and if you have the response (e.g. if you are in a correct place). You cannot save these samples.

Start: By clicking on this item, stimulation and data acquisitioning will start according to the defined protocol. eTrace will save these collected data in file with the name and pathway you were defined before.

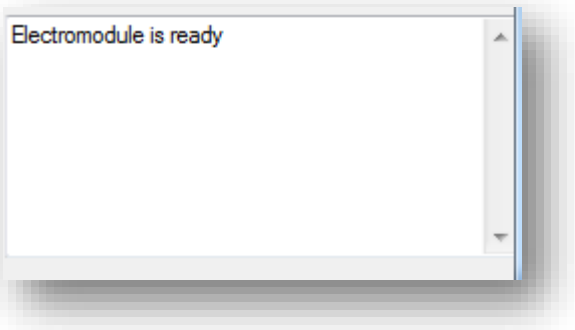
Stop: Push this button to stop an experiment before its ending

Events box

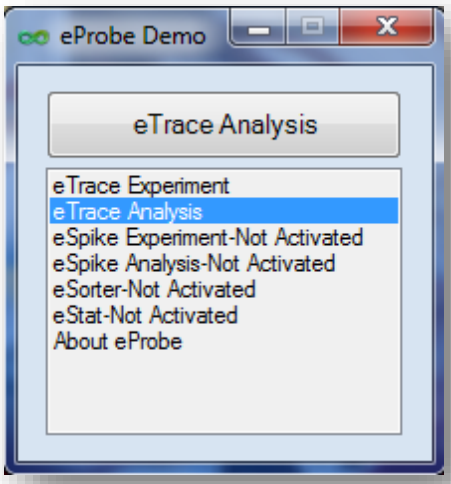
Sometimes one or more event might happen during you recording and you need to save time of occurrence for each one. eTrace offer you to save time points of five distinct events while your program is running. Using F1-F5 in your keyboard you could save these five events. Write those events in the following box, when an event appeared just push F button to save it in a text file.



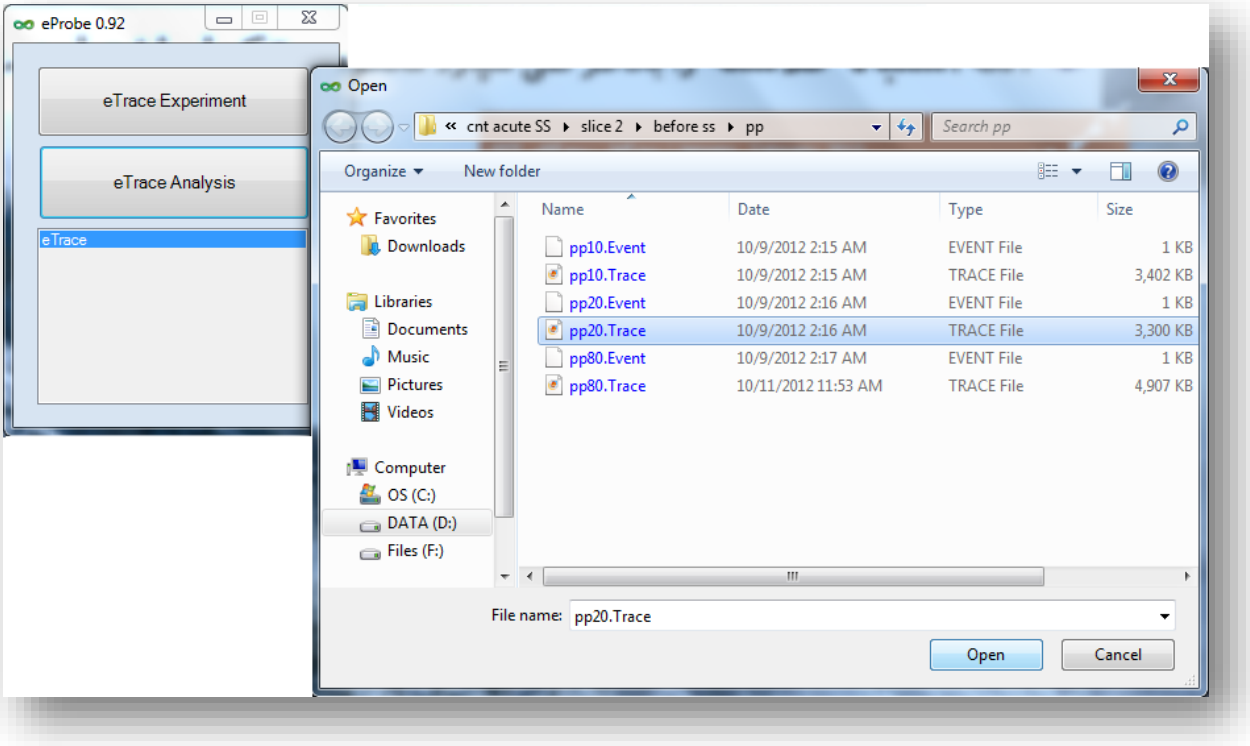
The box in the next figure also displays status the running status of *eTrace experiments*. During the recording you could check the recording time or how many trials passed.



eTrace analysis



By clicking on the "eTrace analysis" from the eProbe panel, you will see a new window (like the next figure), which will ask you to choose the file you intended to see and do analysis on that. Then go ahead and select a previously recorded file.



eTrace analysis window

eTrace analysis window will appear like the following figure:

Figure 8. An illustration of eTrace analysis window presented. As you see, the main part of this window includes a scope panel, which provides you to see a recorded file offline. Each letter in this figure shows different parts and options of analysis window.

Note: When you start a recording with *eTrace experiments* the software will save two separated file: a file with *.Trace* and a file with *.Event*. Event files (*.Event)* are text files. *eTrace analysis*, just able to open files with *.Trace*.

Now, I will explain all the elements that are illustrated in the eTrace analysis window then I will show you data analyzing with *eTrace analysis*:

The scope panel has a menu bar (figure 8, B or likewise the next figure), using this menu bar you could do setting on the scope.

Channel: If you had collected data in more than one channel you could use this item to select the channel for analyzing.

Vertical scale: Using this item you can change vertical scale of the scope or volte/division.

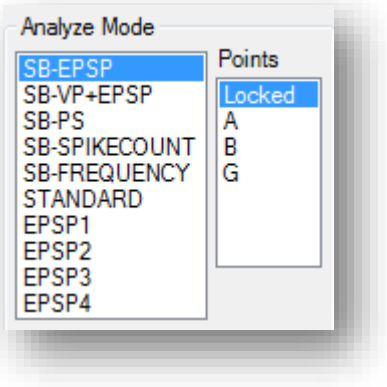
Horizontal scale: Using this item you can change horizontal scale of the scope or time/division.

Invert: If you keep it “off” you will see a recorded signal as you saved it. If you turn it to “on” then you will see that signal inversely.

Start Point: You could select the start point of your signal in the scope. Actually, through this item you are able to do scanning on each recorded epoch. I will provide an example for application of this item in page 41 (with SB-PS mode analysis).

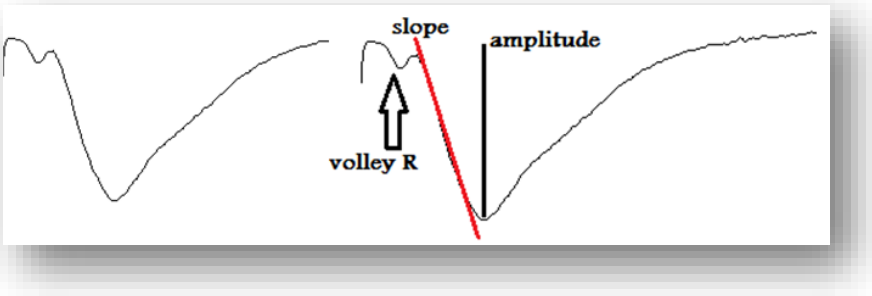
Analyze modes panel

To analysis field neuronal responses, eTrace offers a panel including a box of different "analyze modes" plus a box of various pointers, as illustrated in the following figure (also figure 8D), to do a correct analysis you have to select an appropriated pointer then put it on a correct position by clicking on your scope (see the following explanation):



Each analyze mode designed to analyze a specific type of field responses. The most common field activities are: fEPSP, Population Spikes, unit spikes and local oscillations.

1. SB-EPSP mode: this mode planned to extract main features (amplitudes, slopes and times) of a simple fEPSP.

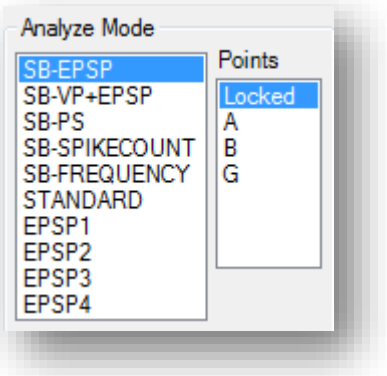


An example fEPSP (evoked field excitatory post-synaptic potential recorded from the dendrite layers) is demonstrated in the above figure. As demonstrated in the figure slope of the red line which is overlapped with descending part of fEPSP, will considered as slope of fEPSP. Both slope and amplitude of fEPSP are important parameters for assessment of fEPSP.

Usually, a *volley response* (see the arrow in the figure) would appear before the descending part of fEPSP however you might record fEPSP without this volley. eTrace analysis offer to different modes for analyzing a fEPSP with or without volley.

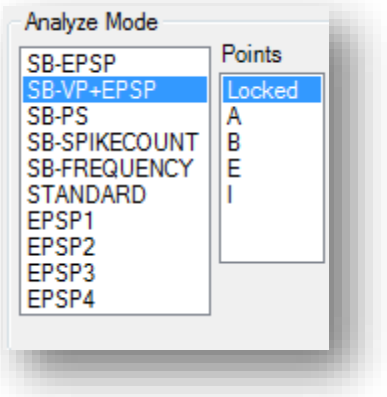
If you had a fEPSP without Volley response then use “SB-EPSP mode” and if you had volley as a part of your signal, then go to the “SB-VP+EPSP mode”.

If you choose “**SB-EPSP mode”** then at the “pointes box” you will have three points as “A, B, G”



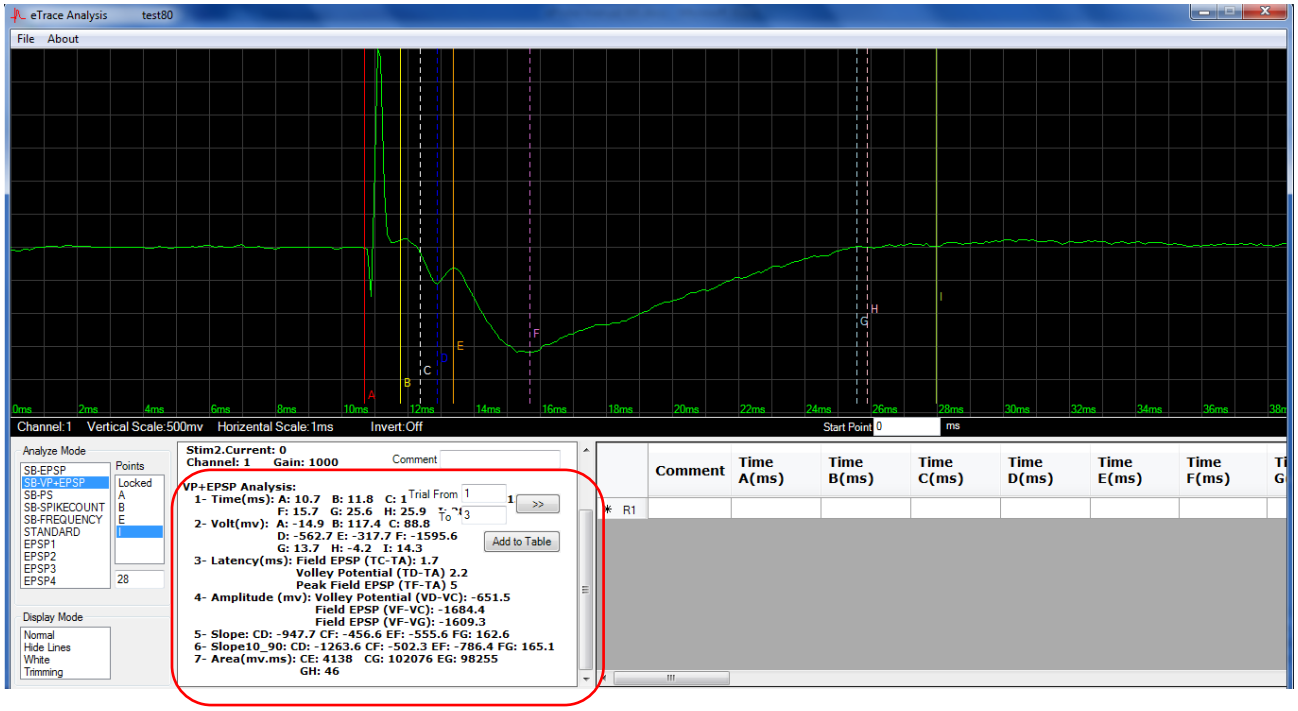
Put point “A” right before the stimulus artifact, put point “B” after the artifact and before the descending part of the EPSP. Finally put point “G” after EPSP on the baseline.

If you went to “**SB-VP+EPSP mode”** then you will see four points at the “pointes box” as “A, B, E and I”



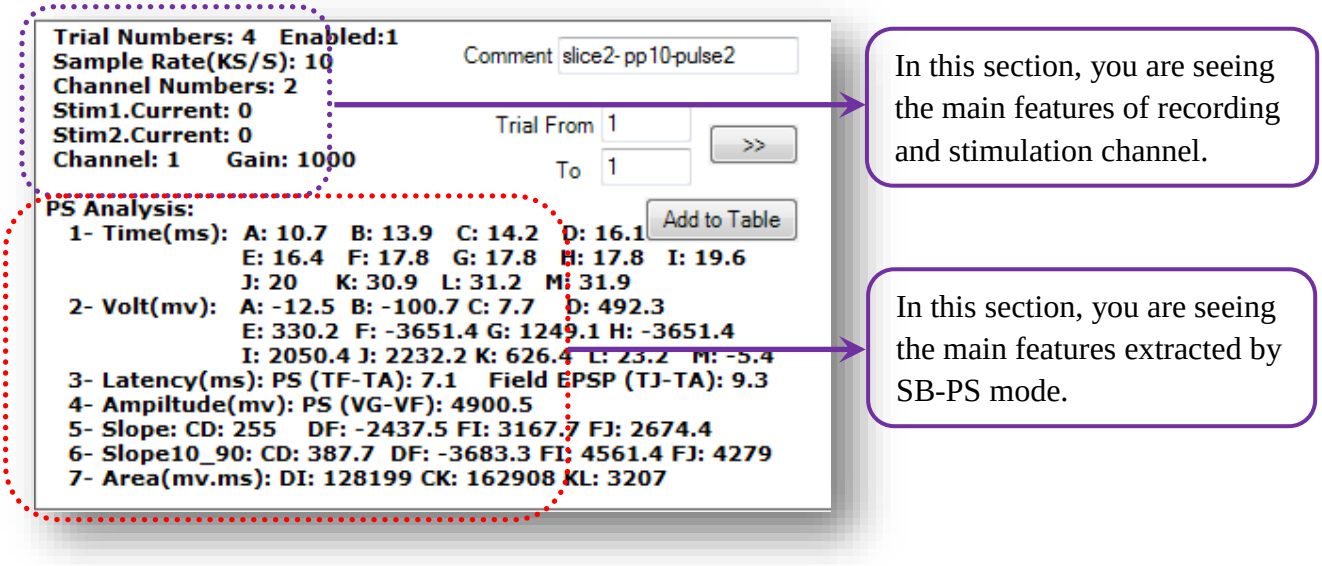
Put “A” and “B” likewise you did in the EPSP mode, after that put “E” just at the turning point of Volley to EPSP, then put “I” at the end of the response (see the next figure).

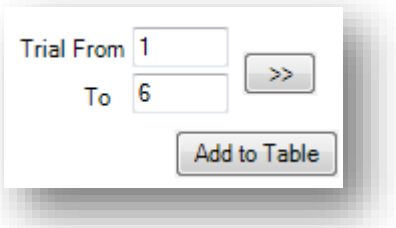
As you are seeing (in above figure) fEPSP slope is equal to the EF slope. After putting the pointers on the correct positions, all parameters of EPSP will appear in a separated box below the scope (see the red line on the next figure).



Analysis box

A box below the scope panel, which shows the extracted data, based on selected analysis mode.

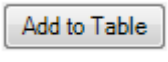




Also using this option, you could choose those trials, which you want to use for averaging (to see their average or analyze it).

For example if you have 30 minutes recording which includes 0.1 Hz stimulations and recorded responses (1 evoked response/10 seconds) and you want to present it as 30 points, each point averaged from one minute recording, then you will need to average from each six trials. Through this item 

you can go to the next six trials.

If you put the pointers correctly and choose your trials then you will see several parameters of your signal in a box below the scope. In addition, to save data from each averaged epoch, user could use “add to table” button 

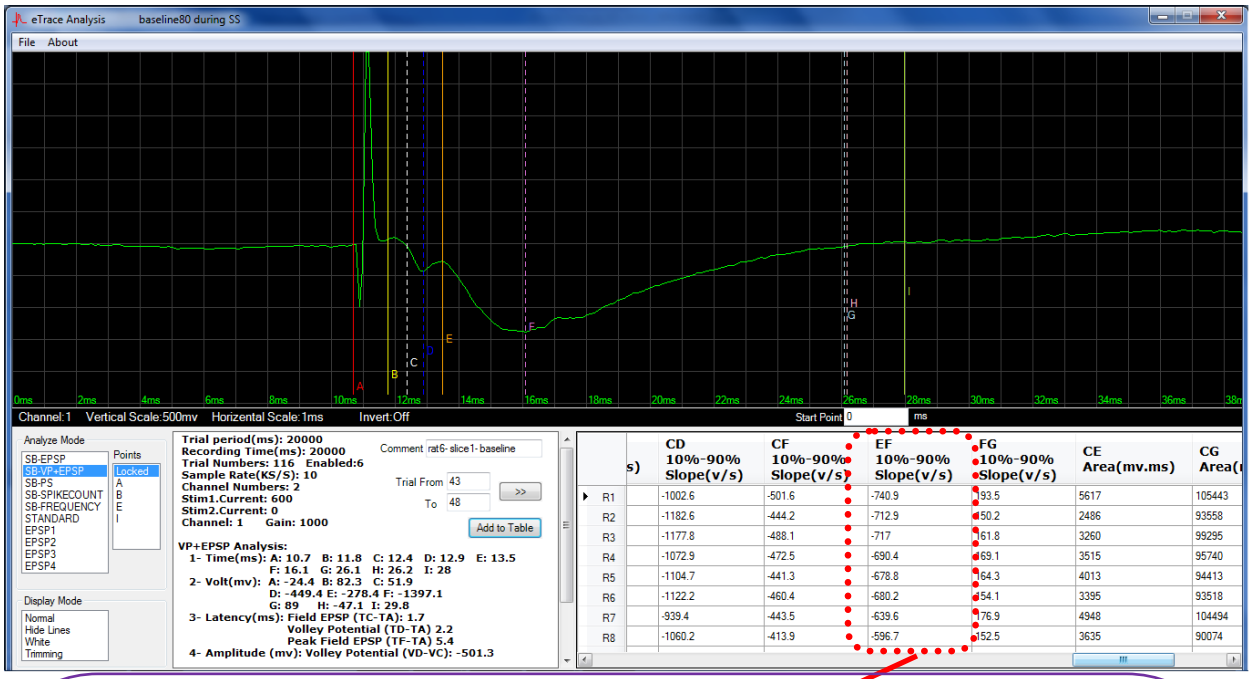
. Click on this button and then you will have a row

of values which are extracted through averaging of that selected trials. Data from this table could copy to any data sheet file such as excel.

Trough this comment box 

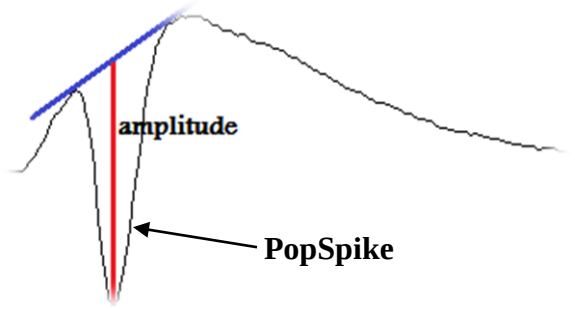
you are able to add a comment about the analyzed traces. The commented text will appear as a column in the data table.

In brief:

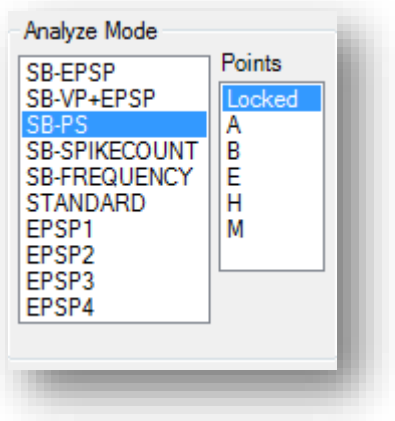


1. Open the file
2. Choose a channel
3. Select “Normal” from display mode
4. Select suitable analyze mode
5. Put pointers in their correct places
6. Choose desired trials
7. Click to add extracted data to table
8. Move to next trial(s) and add them to table
9. You can select one or more columns or rows from this table and copy it to another data sheet file.
10. In this figure, values of fEPSP slope are demonstrated by red dotted line.

SB-PS mode: If you had evoked responses were recorded from the somatic area and if that evoked field responses were include Population spikes (PS), then you could calculate the amplitude of that PS.

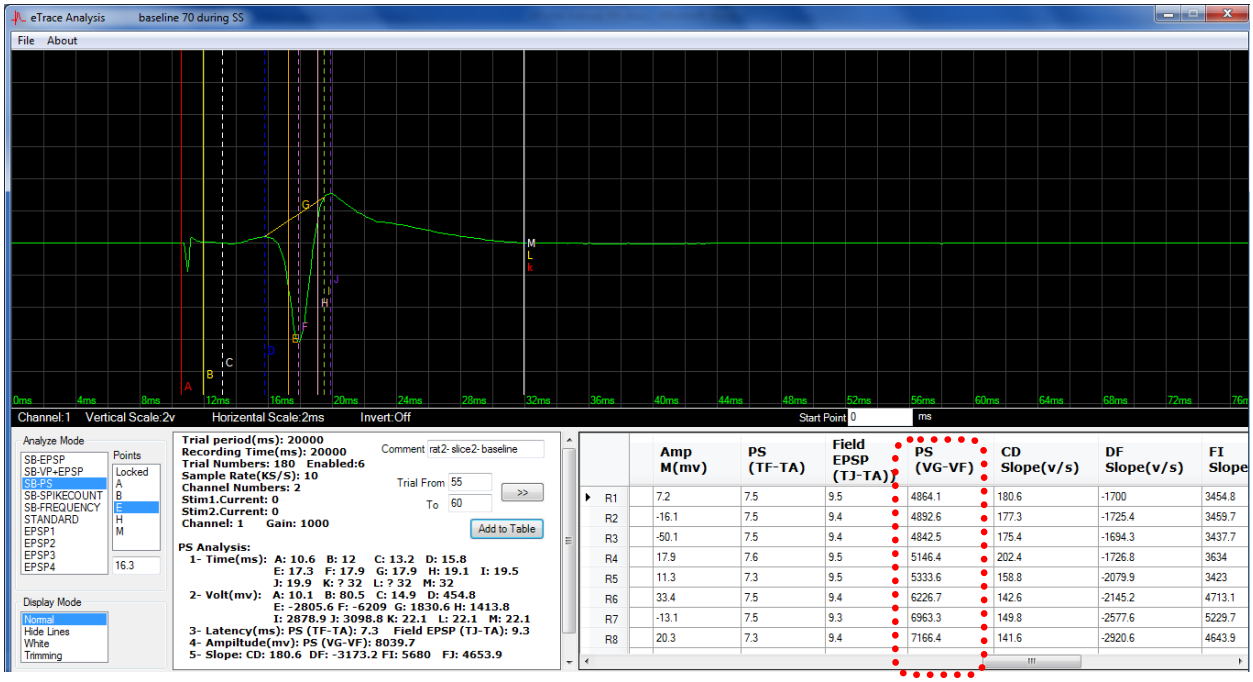


To measure the amplitude of PS you must use **SB-PS mode**. If you choose PS mode then at the "pointes box" you will have five points as "A, B, E, H and M"



At the next figure I will show you how to put the pointers on a PS signal, to measure the amplitude of PS. In addition, you will have the other parameters of PS likewise: delay times or slopes in the box (like that I explained in the previous section). You can do averaging and add the values to the table, just like as we did for fEPSP in the previous section.

Put “A” before the artifact and then put “B” after the artifact and before the signal. Put “E” and “H” at the downward and upward parts of the PopSpike, respectively. Finally, put “M” after the signal when it comes back to baseline. The **red arrow** in this figure shows PS amplitude which will appear in the box or in the table as *PS (VG-VF).*

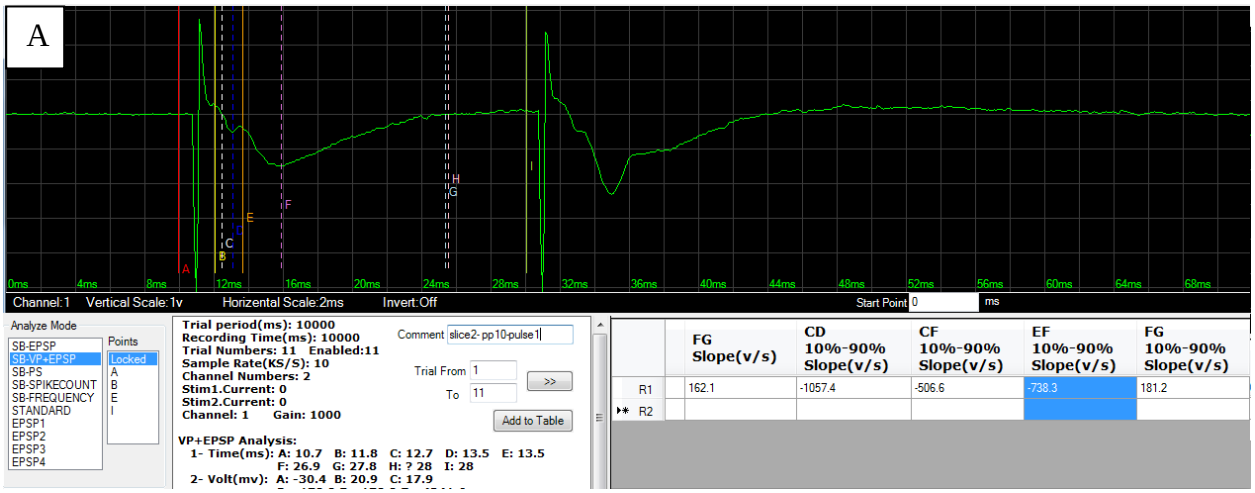


To analyze PS from a recorded file, do as the same as I explained for fEPSP. Red dotted line in this figure show averaged values of PS amplitude. You can copy these column or whole the table to a data sheet file for more analysis.

Example:

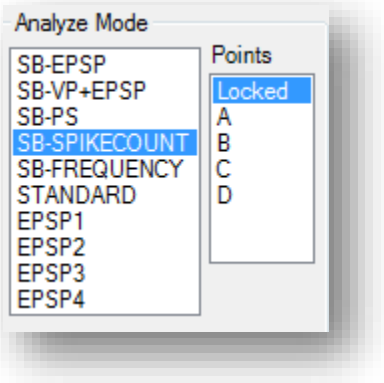
Suppose you are going to analysis a paired pulse recording (two subsequent stimulations with a defined interval. Each pulse will produce a response, as an example see the figure 8.

To analyze these two succeeding responses, you could fix your pointers on the first response and then add its features to the table (as I will explain to you in the next part). Thereafter go to "start point" and give it the paired pulse interpulse interval, you will jump to the second pulse while you will have pointers up to the second plus (without moving the pointers you just moved the signal) (see two next figures).

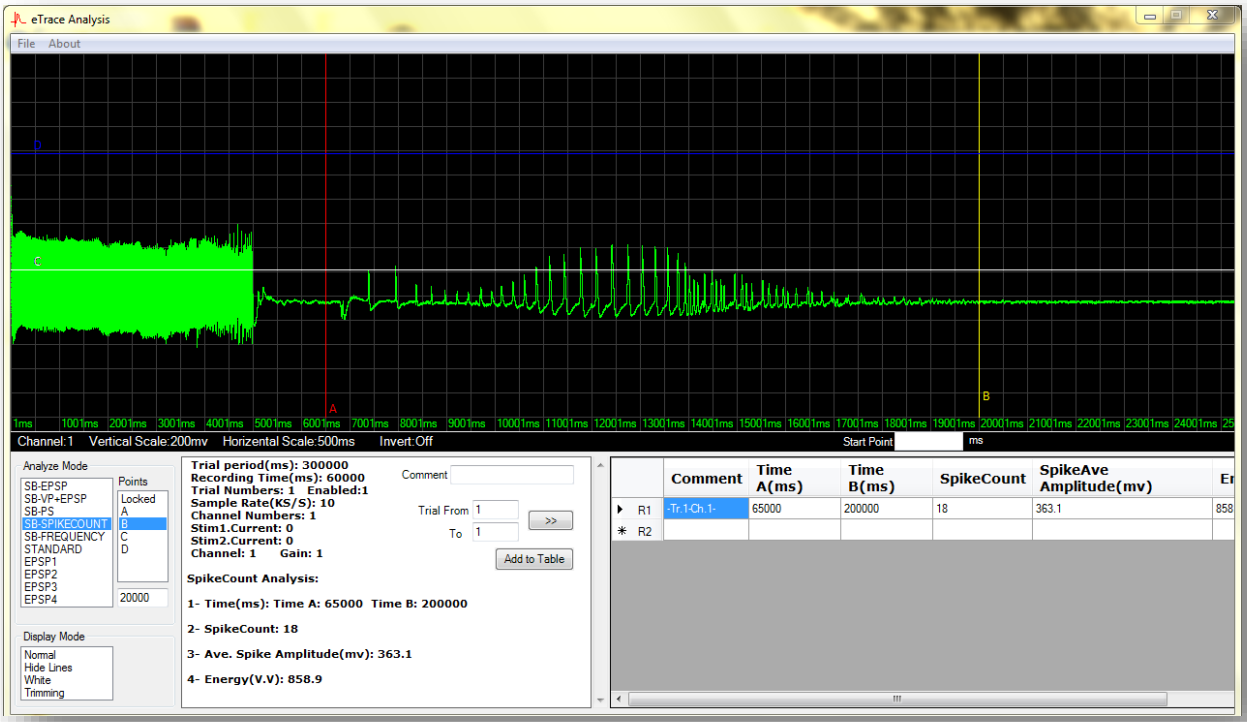


Here, in the figure (A) we have two evoked responses with 20 ms interval. I adjust all pointers on the first response and at the correct positions and then add the data to the table (you will see how to do this in the next part). In the figure (B) I just give the start point value of 20ms. As you are seeing, second response will appear instead of first one. Now, you must check the pointers and if they are in right place, you could add this data to the table.

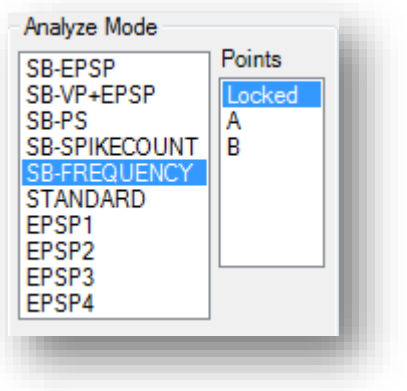
Spike Count: Using this item you can calculate the number of spontaneous spikes in each recording epoch. For example, this mode make possible to count interictal spikes in epileptic models, counting QRS peaks in and EKG or other spiking activity (regardless to their shape) are countable through this mode. If you choose this mode from the "analysis mode" you will have four pointers.



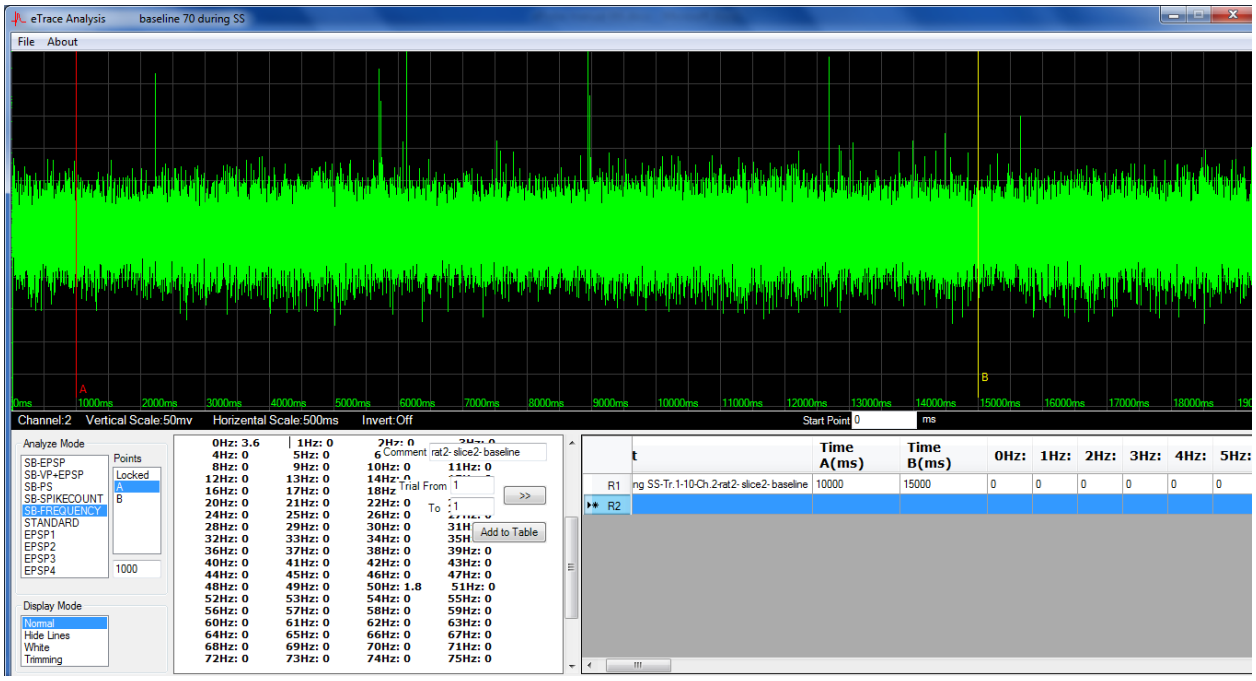
Put "A" and "B" in both sides of an epoch which you want to count its spikes. As "C" and "D" are horizontal pointers, put them as you wish to limit the spikes amplitude.



Frequency: to extract and analyze the frequency of oscillatory activity of a neuronal population you can use this item. Choose “Frequency” from the analyze modes panel then you will have points “A” and “B”.



Using these two pointers, you can select an epoch and see the frequency powers in the data box below the scope. You could add these data from each separated epoch to the table by clicking on “add to table”.



UserDefiend templates: eTrace also offer the users this ability to create a new mode (in accord with their needs) and then save it for future applications. When you download eTrace to your computer, you will find a text file located in the folder of eTrace and entitled as "**eTraceTempleates.text**". You could use this text file to produce a new and desired mode of analysis for your signal. If you create and save a new analyze mode, you will see it at the "analyze mode" panel of eTrace analysis.

Note: If you want to **inactive** a previously created analysis mode, you should type a "//" right before its name in the "eTraceTempleates.text file".

```
template,EPSP4
point,A,0,var
point,B,0,var
point,C,0,var
point,D,0,var
value,START,6.6,
value,END,40,
value,Slope,1,
value,Slope1090,1,
value,Latency,1,
value,Duration,1,
value,Amplitude,1,
min,C,START,END
Search,D,C,END,A,UP
max,B,START,C
slope,Slope,B,C
slope1090,Slope1090,B,C
sub,Latency,A,B
sub,Duration,B,D
sub,Amplitude,B,C

result,1- time(ms),time,A,B,C,D
result,1- Amp(mv),value,A,B,C,D
result,3- Slope,value,Slope
result,4- Slope1090,value,Slope1090
result,5- Latency,time,Latency
result,6- Duration,time,Duration
result,7- Amplitude,value,Amplitude
end of template
```

Figure 9. An example template for eTrace analysis demonstrated.

To write a new template just follow the following rules:

- Each template must start with a command as: “template, name of template” (for example in figure 9: **template, EPSP4**). Also must ends up with this command “**end of template**”.

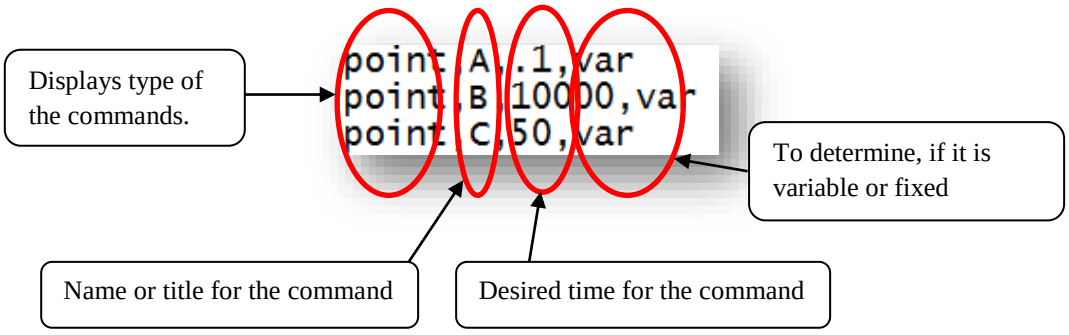
Note: Use “,” to separate each phrase from the next one in a command

1) **Points:** To measure times and values through a template you should define some pointes.

You could define one or more pointer as the following instruction:

`Point` **`(`***means this a command to define a pointer*`)`**`, name of pointer, preferred time for this pointer, “Var”`**

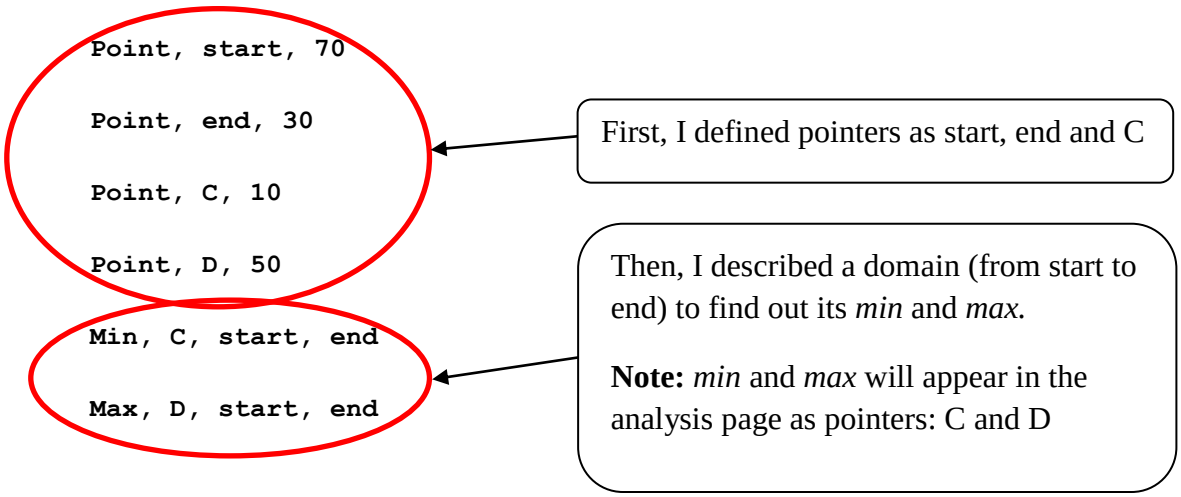
“Var” means this pointer is variable (if you do not want it variable, do not write anything so the pointer will be fixed). In the following figure points A, B and C are variable pointers which mean you are able to change the position of these pointers on the analysis scope.



Note: Pointers which define as “var” will appear at the point box, while fixed pointes will not appear in the point box, also they will appear with dotted line in the scope panel.

2) Min or Max: To find minimum and/or maximum during a recording period you could define it as a command in the eTraceTempleates.text.

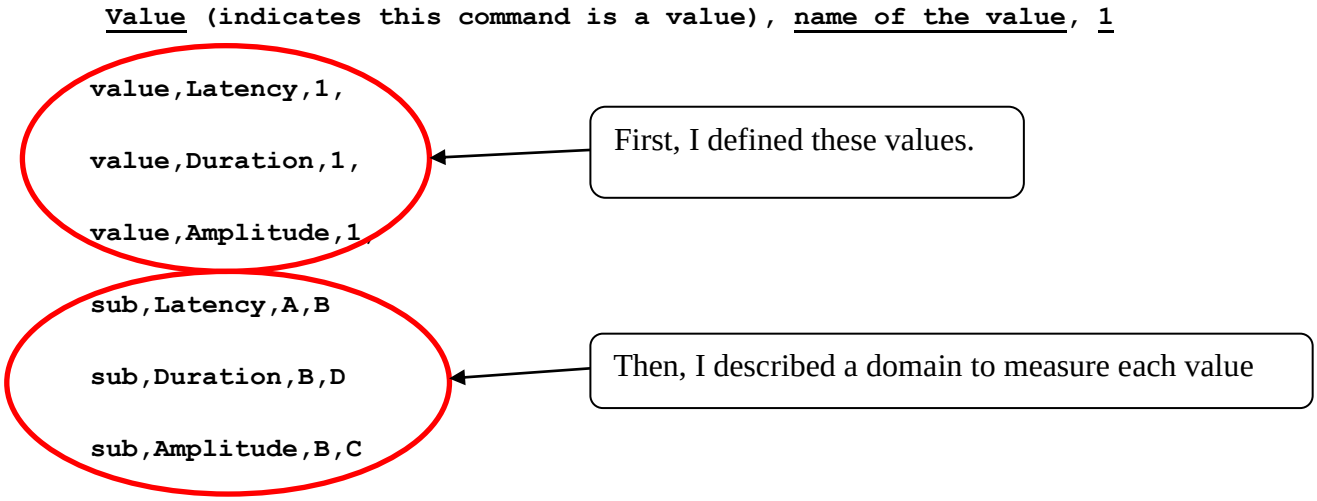
First, define a command to have a pointer then in another command describe the domain that you want to find its min/max. See the following example.



3) Values: User could also define some values (amplitude, slope, frequency, phase et. ac) in the eTraceT*empleates.text*:

First, write commands(s) to define a name for each value, and then describe each value at another command.

For an example in the next figure we have defined values as: slope, amplitude, latency, frequency.



- Slope: To measure slope for between two previously described points follow the next command:

Slope (command), slope (value's name), B (first point), C (second point)

It means I want to measure slope of the

slope1090 (command), Slope1090 (value's name), B (first point), C (second point)

Slope 1090 = slope of the line which connects B and C, but you want to ignore the first and last 10% of this line.

- Sub: To do a subtraction between two points or values, follow the next command:

Sub (command), latency (value's name), A (first pointer), B (second pointer)

This command could also use to describe "duration" among two points or "amplitude" of two points.

sub,Latency,A,B

sub,Duration,B,D

sub,Amplitude,B,C

- Freq: When you want to extract data from an oscillatory signal (like EEG), you could define frequency as values and then describe these value for measurement.

In the previous figure, theta and alpha are values. To describe these values see the next command:

Freq (command), name for freq band, starting point, end up point, start point of freq band, end of freq band, frequency step.

For example:

Freq, gamma, A, B, 15, 30, 1

At this command, I want to measure a frequency value called gamma, between two points A and B, with frequency domain 15 to 30 Hz and with frequency step 1.

4) Result: To see times or values as analysis output in the analysis box of eTrace you should define them with the following command:

Result (command), result title (result will appear with this name), result type (it might be time or a value, values name (must described earlier).

As examples:

result,1- time(ms),time,A,B,C,D

result,1- Amp(mv),value,A,B,C,D

result,3- Slope,value,Slope

result,4- Slope1090,value,Slope1090

result,5- Latency,time,Latency

result,6- Duration,time,Duration

result,7- Amplitude,value,Amplitude

5) Search: to find a desired point or value during a time episode, user could us this command:

Search (command), name of searched item, time domain (starting point, end up point), threshold (as a guide point), up or down (shows direction)

For example:

```
Search, S, C, B, A, UP
```

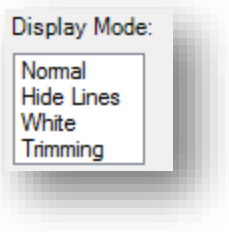
This command means, I want to find a point and name it “S” between points C and B when data grows up beyond the point A.

Internal patterns: the following patterns are internal patterns of eTrace analysis. You could change their order in the analysis mode panel or inactive them temporarily (using “//”).

```
SBtemplate,SB-EPSP

SBtemplate,SB-VP+EPSP

SBtemplate,SB-PS

SBtemplate,SB-SpikeCount

SBtemplate,SB-Frequency
```

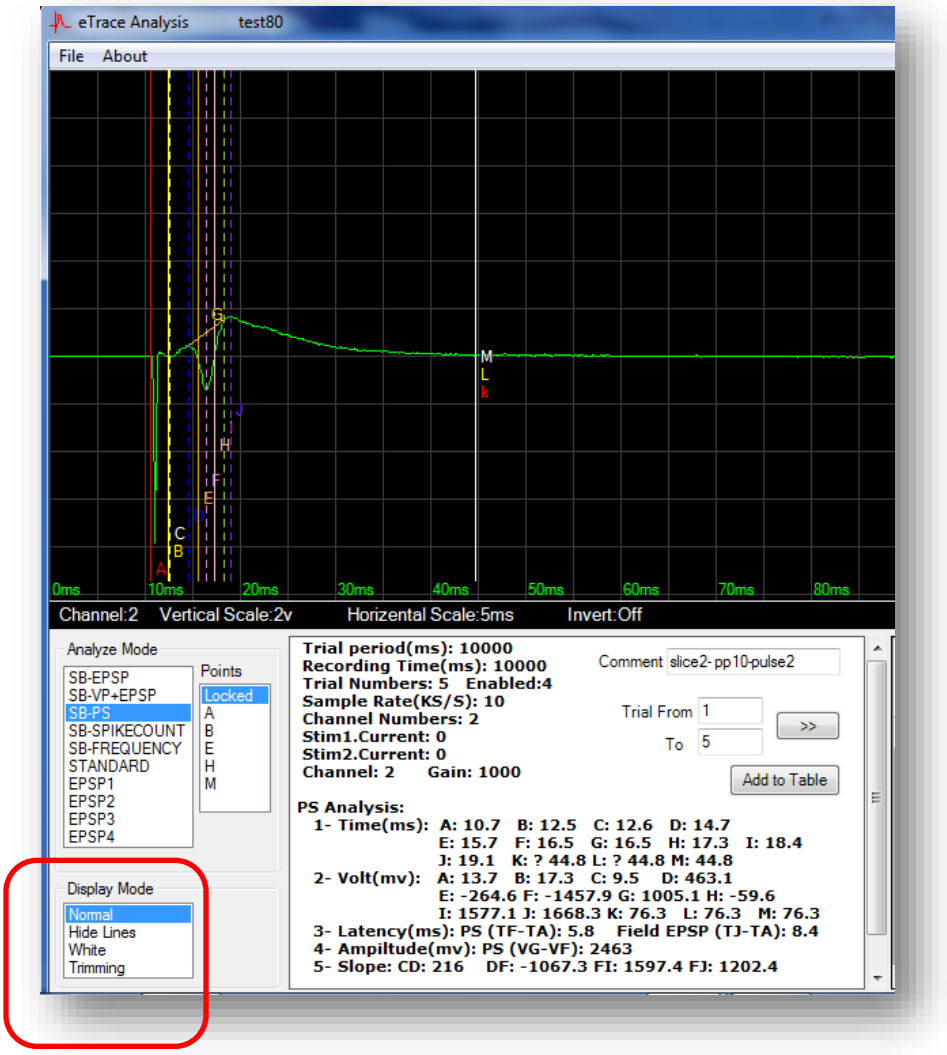
Note: you could also inactivate each command just by typing a “//” just right before it.

Display mode panel

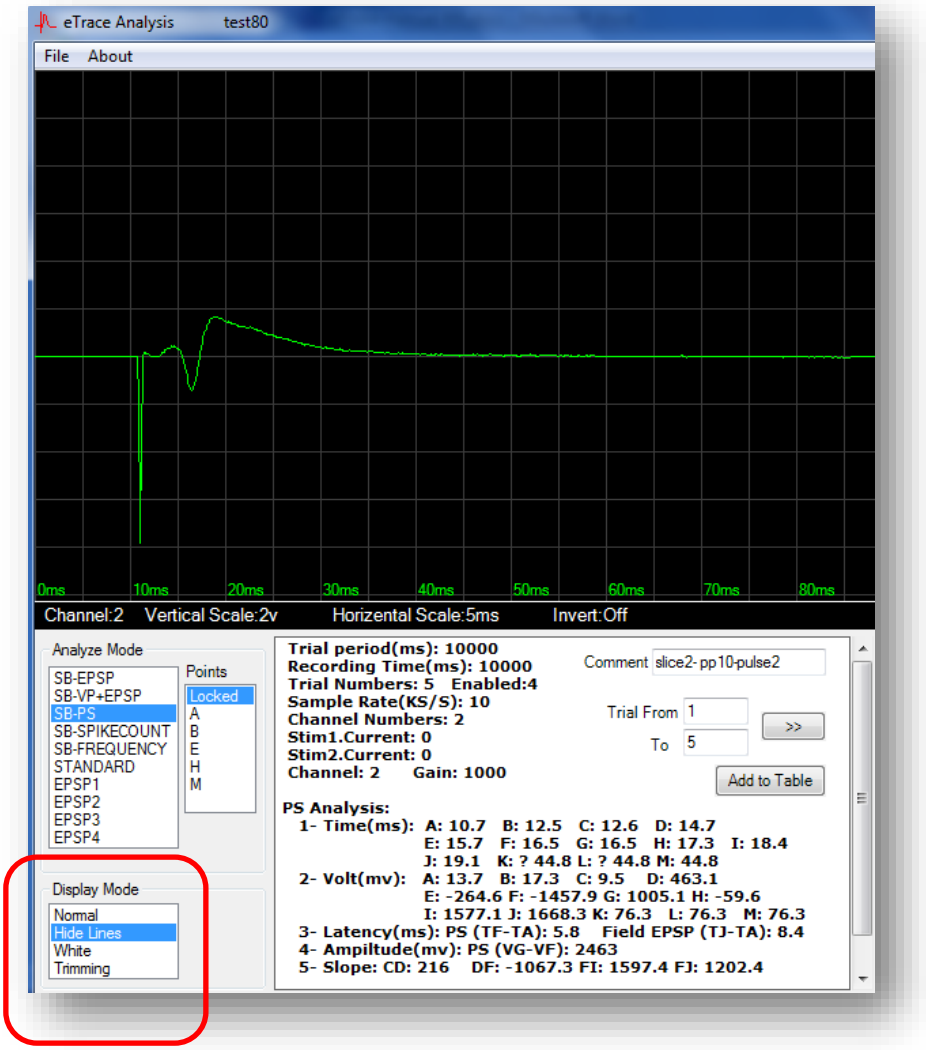


Through this panel you could choose who to see the scope window.

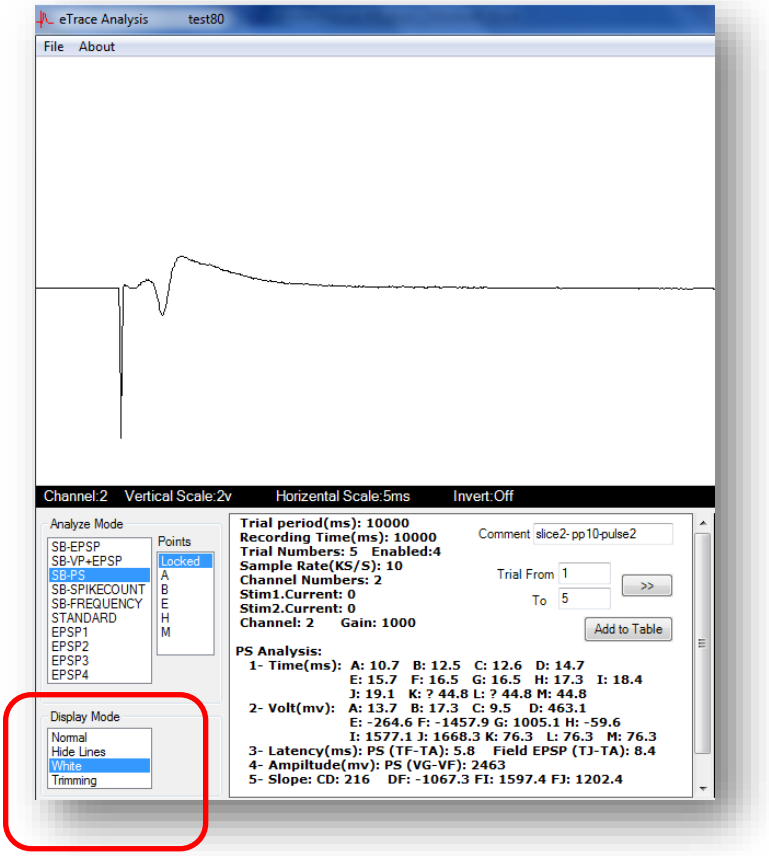
In "**Normal**" mode you will see the recorded signal plus those pointers which you were selected in the analyze mode.



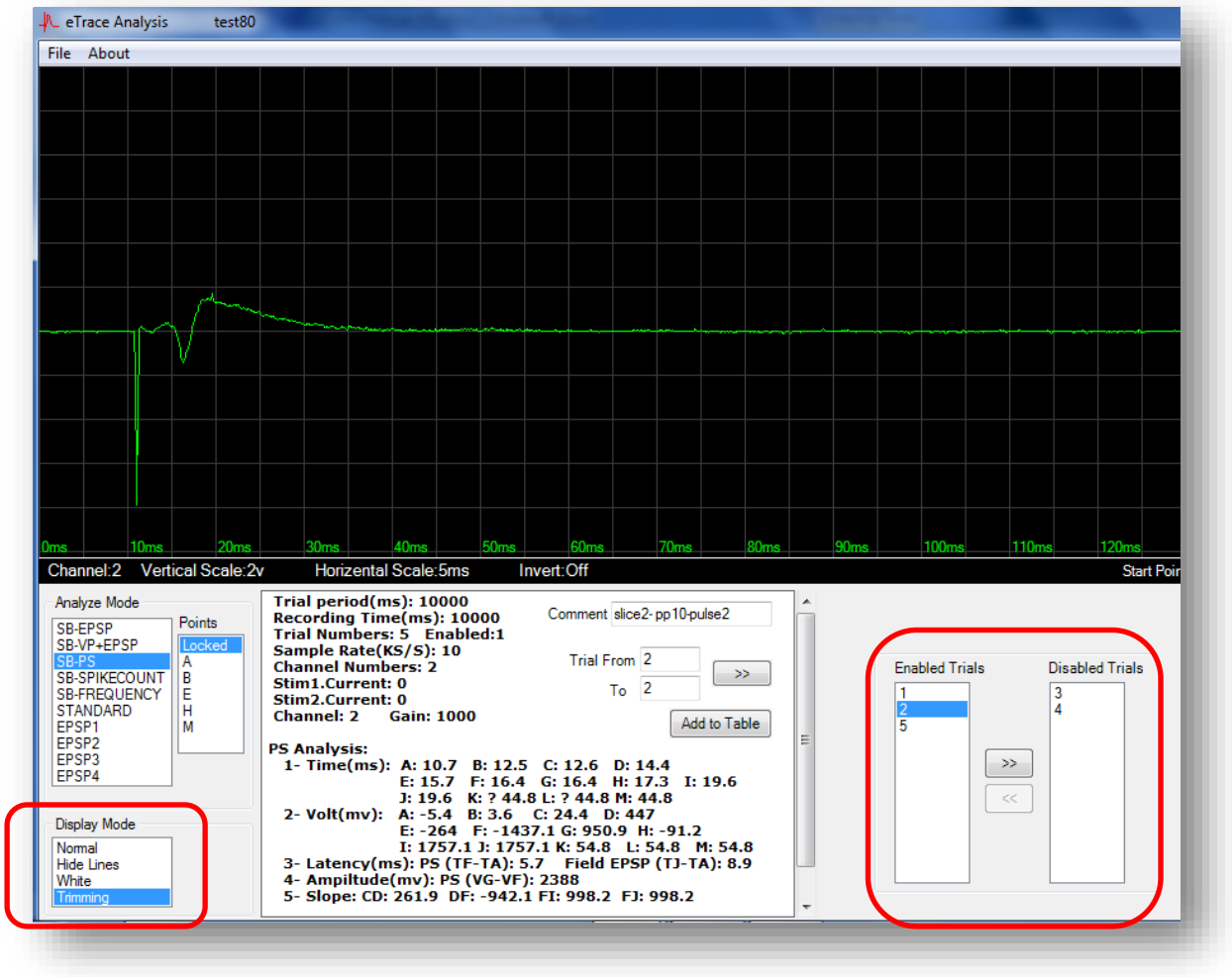
If you choose "**Hide lines**" then you will have just the signal without analyze pointers.



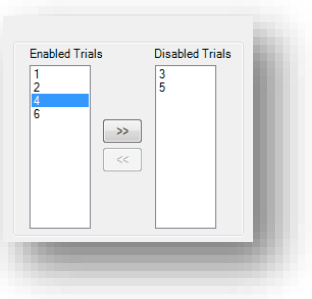
In “**white mode**” grid lines of the scope and black background will go and you just see the signal in a white background (such as the next figure).



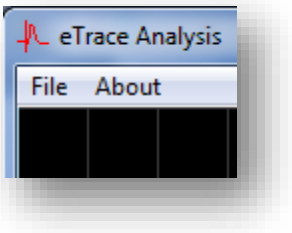
"**Trimming mode**" will let you to see all "enabled trials" of a recorded file. Likewise the next figure when you choose "Trimming" then you will have two separated columns. In one column you have all enabled traces (you can click on each trace to see it in the scope), while at the other column you have disabled traces. If you see a trace from the enabled box is not suitable for averaging, you could disable it.



To disable a trial, first click on its number (select it) from the "enabled trials" then click on this item  to move that selection into the "disabled trials". Disabled trials are returnable.

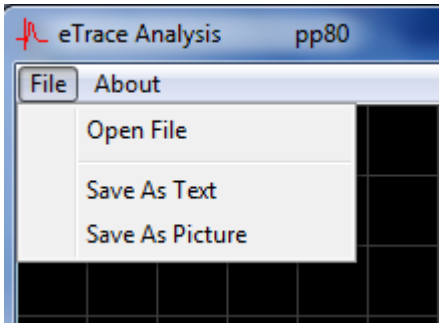


eTrace analysis panel also have a top menu including two options: “File” and “About”



Through “About” you could find our website if you want to visit us or ask a question.

If you click on “**File**” you will have a few items.



- Through “**Open File**” you could go to a data folder and select another file to open it.

- Trough “**Save As Text**” you are able to save all data points of a single trace or an averaged trace to a text file (as a row of points). You can open this text file in a data sheet like excel. When you want to reproduce a trace again in another file or sheet, you could use this item.

- “**Save As Picture**” make you able to save a picture from an existing scope of eTrace.

eSpike

In the eProbe panel, there are two toolboxes for eSpike. eSpike toolboxes are designed for single or multi unit recording of neuronal activity and analyzing these signals.

eSpike experiment provides online monitoring and recording the signals from neuronal unit activity. While ***eSpike analysis*** designed for offline analyzing and data extracting from these recorded signals.

Spike sorting in single unit recording

Extracellular recordings are usually performed by inserting microelectrodes into the CNS. After amplification and band pass filtering, firing activity of the nearby neurons will appear as spikes on top of background activity. According to the literatures, for neurons close to the electrode tip about 100 microns signal-to-noise ratio is good enough to distinguish the activity of each single unit. For more distant neurons (up to about 150 microns), spikes can be detected but the difference in their shapes is masked by the noise (multi-unit activity). Spikes from neurons further apart cannot be detected and they contribute to the background noise activity.

Spike sorting

The procedure of estimating one or more single cell point processes from a noisy time series is known as spike sorting. In fact, spike sorting refers to the process of assigning spikes to different neurons. A range of different approaches is used to address this problem. In eSpike we sorts the spikes according to their amplitudes.

eSpike Experiment

To run the ***eSpike experiment*** or any other toolbox from eProbe menu, you should select it from the eProbe panel (step 1 from the figure 10). A button on the eProbe panel will show the name of that selected toolbox. Run the toolbox by clicking on this button (step 2 from the figure 1).

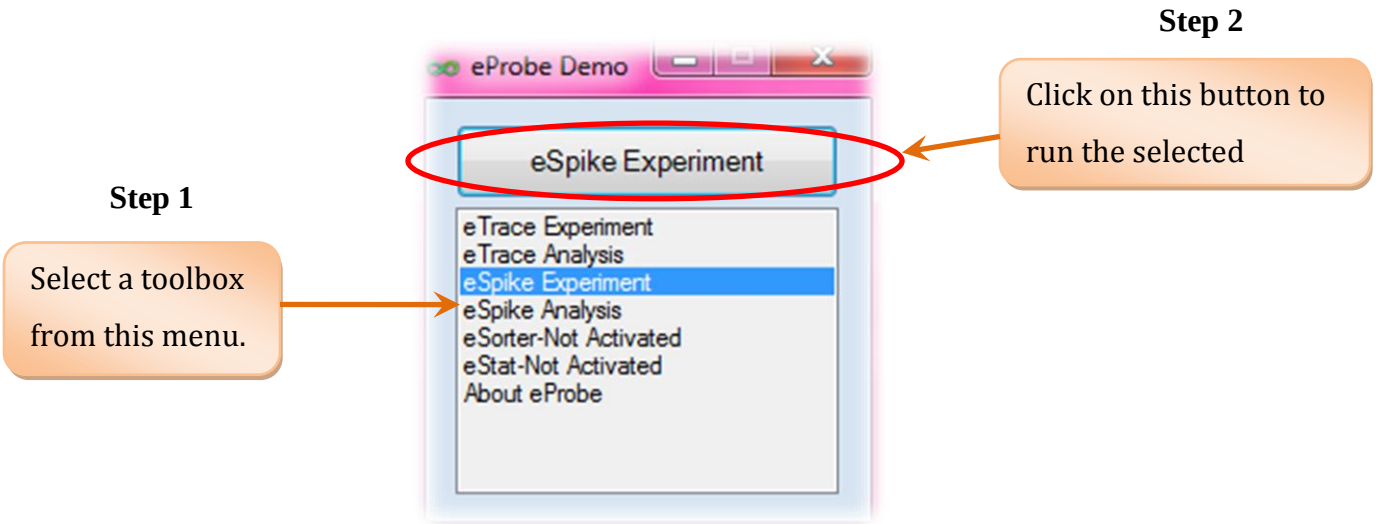


Figure 10. eProbe panel which includes some activated and non-activated toolboxes. you could open activated toolboxes from this menu as showing in the figure.

Subsequently, another panel will appear according to the selected toolbox (as shown in the figure 11). Now, we are going to review ***eSpike experiment*** panel and its components. After that, we will speak about ***eSpike analysis***.

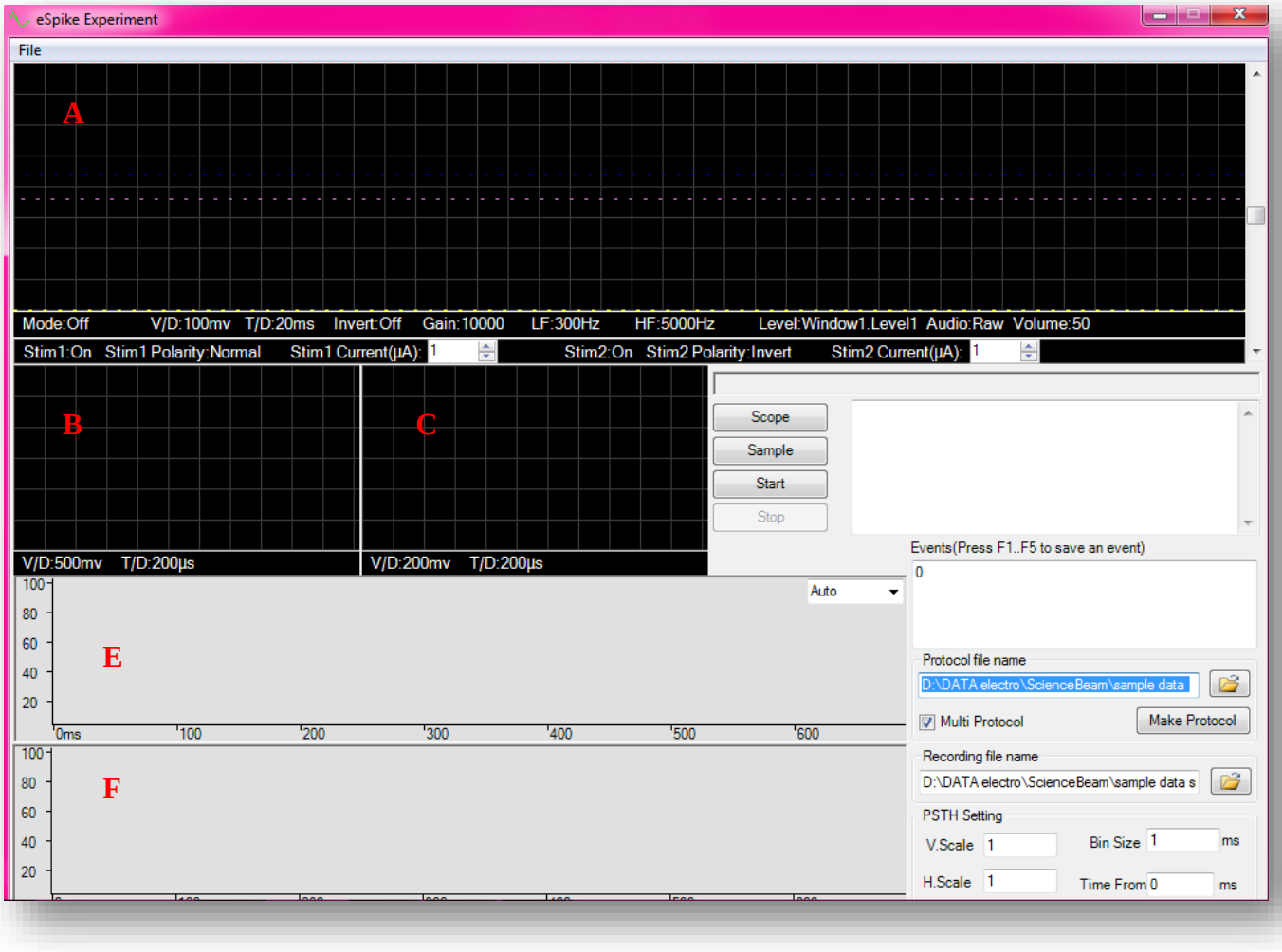


Figure 11. *eSpike experiment* panel before starting a recording task.

When you run ***eSpike experiment*** from the eProbe menu, you will see a window as demonstrated in figure 11. The eSpike experiment panel designed for online monitoring and sorting the signals comes from neuronal unit activity through one electrode placed in extracellular medium. As it seems in the figure 11, eSpike experiment panel includes some components. There are three oscilloscopes in this panel. One of them is the main oscilloscope (A) for monitoring and managing the recording signals. Each one of the other two small scopes (B and C) shows one type of the sorted spikes with two available discriminators. Two PSTH (E and F), each one related to one of the discriminated signals, will also appear in this panel and show the online-discriminated signals.

Components of eSpike experiment panel

Oscilloscope

The main oscilloscope is a gridded page with a menu bar at the beneath of it, as shows in the figure 3. This menu bar includes items, provided to manage the oscilloscope, amplifier, window discriminators and audio (colored lines in the figure 12).

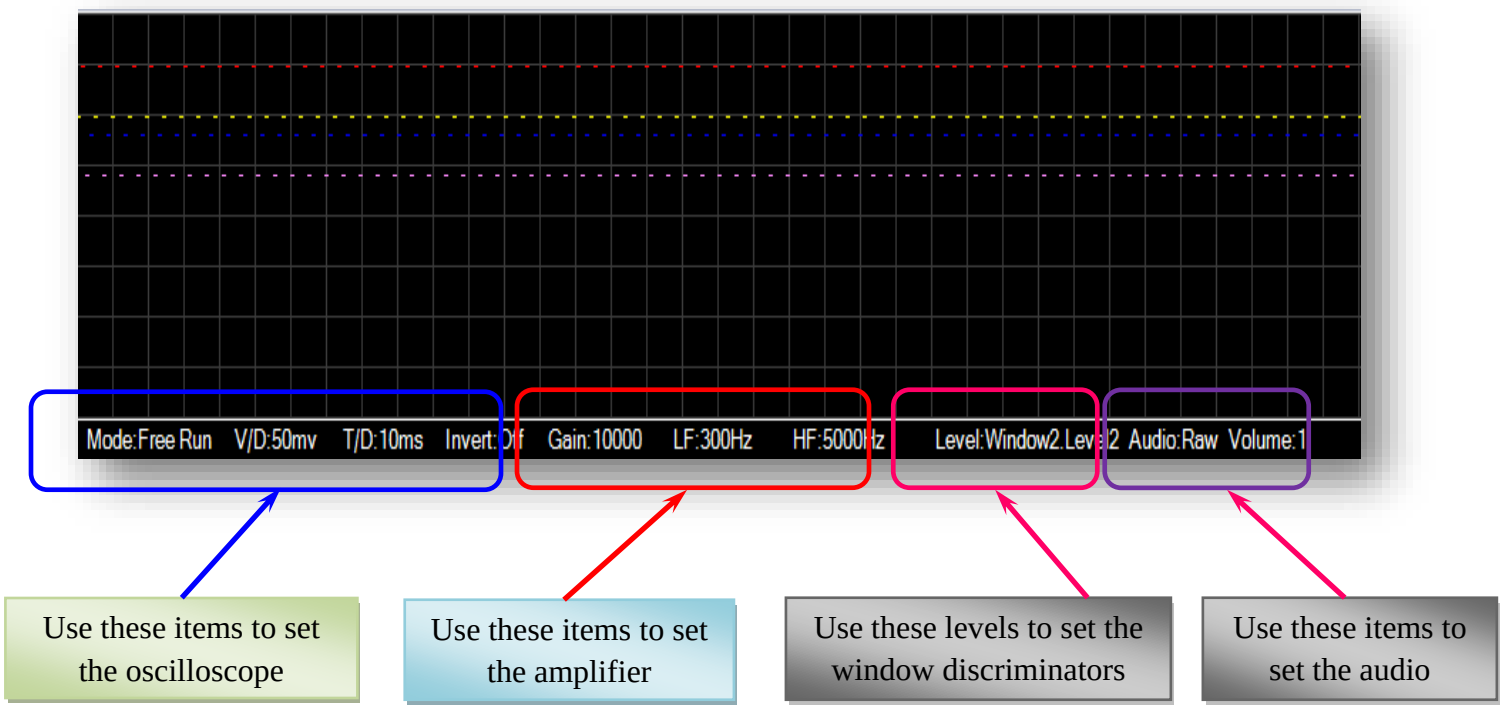


Figure 12. Main oscilloscope of eSpike experiment is showing. The menu bar let the user to set the oscilloscope display, signal amplification and filtering, window discriminators and audio. To change the setting for each item, use right and left click.

Set the oscilloscope from the menu bar

Mode: Three different modes are available through this item: ***off, one trace***, ***free run***.

- Select "off" to turn the recording off.

- Select “one trace” to have a triggered signal in each trail. Triggered signal will appear according to your recording protocol (later in page 15, you will see, how to make a recoding protocol).

- Select “free run” to see continues online recording of the signal.

V/D (Voltage/Division): This item shows voltage scale (in y-axis of graded page). Using this item, you are able to change the voltage values demonstration.

T/D (Time/Division): This item shows time scale (in x-axis of graded page). Through this item, you can adjust time values of each horizontal demonstration.

Invert: To see the signal inversely on the scope, you should turn this item “on”.

Set the amplifier from the menu bar

Gain: Using this item, you are able to change the amplification level of the input signal. There are many gain options (from ×1 to ×10000). You can choose a gain, according to your recording situation.

LF (Low cut Filter): Through this item, you are able to set low cut filtering of the input signals. You can choose one of these filters: 0.1, 1, 10, 100, 300 Hz in accord with your circumstances.

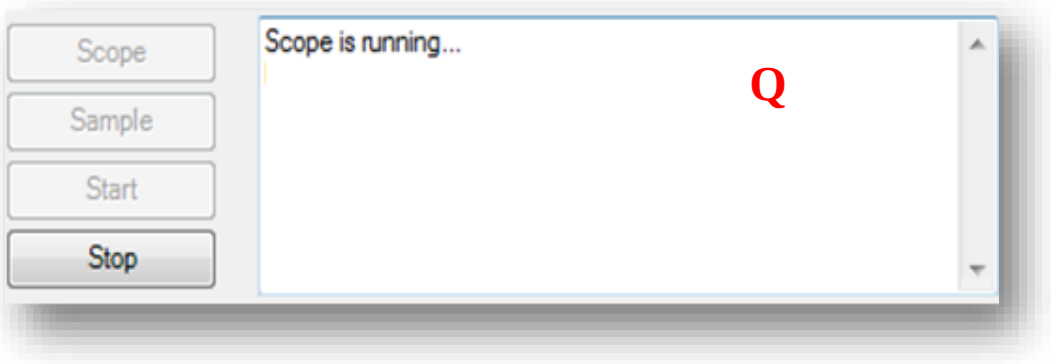
Note: If you have a 50/60Hz noise during your recording, it is highly recommended to select 100 or 300 Hz as LF. As spike discrimination in *eSpike* is according to the amplitude, so fifty-cycle noise might affect the real amplitude of the input signal. It is better to remove this noise before discriminating the signals.

HF (High cut Filter): Use this item to adjust a high cut filter. A high cut filter will eliminate the frequency bands beyond the described filter. You can choose a HF from a list: 5000, 3000, 2000, 1000, 100 Hz.

Note: Using right and left click, users would be able to change the values of these items. Values will increase with right click while will decrease with left click.

Running the oscilloscope status

Another part of the *eSpike experiment* panel illustrated in the next figure and includes four buttons and a status box (marked with Q). Through these buttons, you can manage status of the program. Status box will show the program state.



- **Scope:** You could use this button, if you want to see a preview of basal electrical activity in the recording site (e.g. you want to check the signal/noise ratio or you are going to move the electrode up and down until you find a neuron).

Note: You cannot save a recording file, when you are running in scope mode.

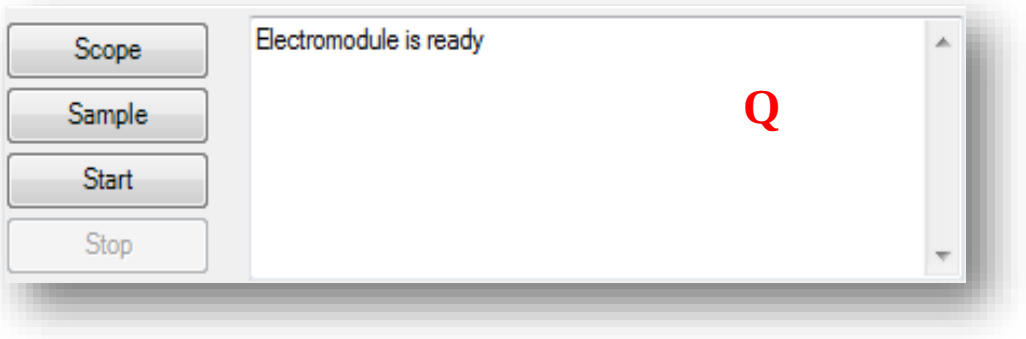
- **Sample:** Through this item, you could test one train of your selected protocol as a sample. Using this icon you could test the stimulation protocol and if it is working. Also, you could check response(s) to the stimulation and if you have the response (You cannot save these response).

- **Start:** By clicking on this item, stimulation and data acquisitioning will start the procedure according to the defined protocol. eSpike will save all collected data in a file with name and pathway you were described.

- **Stop:** To stop an experiment before its ending you could use this button.

In addition, inside the status box (marked with Q), user can see the situation of the program. For example in the next figure, eSpike is running on the scope mode, so in the box you can see it is running.

If you push the "stop" then you see this message "Electromodule is ready" (see the next figure). This message means Electromodule it is not running but it is ready to run up.



When you push the "start" to save a recording file, in the box you will see the elapse time of your recording.

Window discriminators

Level: This item includes four different levels indicated with "window 1-level 1"; "window 1-level 2"; "window 2-level 1"; "window 2-level 2". Each level presented in the oscilloscope with a different dashed line. When you select one of these levels from the menu bar, its position on the oscilloscope will be variable. Therefore, you can put that line in a desired place or change its position by clicking on the oscilloscope to set a window discriminator. Moreover, using the scroll of mouse, you can move the selected level precisely

Spike sorting in the *eSpike experiment* is based on the amplitude of spikes. In extracellular recording, spike amplitude is usually depends to the distance between microelectrode tip and the firing neuron. Therefore, usually highest amplitudes come from nearest neurons, while faraway neurons almost contribute in the background noise activity.

To do amplitude discrimination, a window discriminator should arrange. In this version of eSpike, you can set two separated window discriminator. Using two dashed lines (levels 1 and

2), it will be possible to describe a window discriminator, and each one will discriminate a typical spike.

To have a better illustration from each discriminated spikes, another two small scope provided below the main scope. Sorted signals in each described window discriminator will appear at one of these scopes. Therefore, each scope will shows sorted signals from one window discriminator, these spikes will appear superimposed on the scope and will have refreshment each 1 sec (see the figure 3). Superimposed traces in green color show activity in a single unit. In addition, white traces in the scopes show bad position of levels described for window discriminators (might two described windows overlapping).

Notice 1: Level-1 (yellow in window 1 and pink in window 2) for each window must placed closer to the baseline rather than level-2 (red in window 1 and blue in window 2).

Notice 2: You should put the levels in order to; chosen spikes pass the level-1 but do not pass level-2.

Notice 3: it is possible to set a window discriminator in above or in below the baseline.

Notice 4: when a window discriminator built with level 1 and 2 on the main oscilloscope, in the small scopes you could see the discriminated spikes. (See the figure 13)

Notice 5: non-superimposed traces in the scopes show simultaneous activity in another neuronal unit. Because its amplitude was not matched with the amplitude of described window discriminators, it will not appear as a superimposed signal. However, since it was happened coincident with the selected neuronal units, so you will see it in the scope.

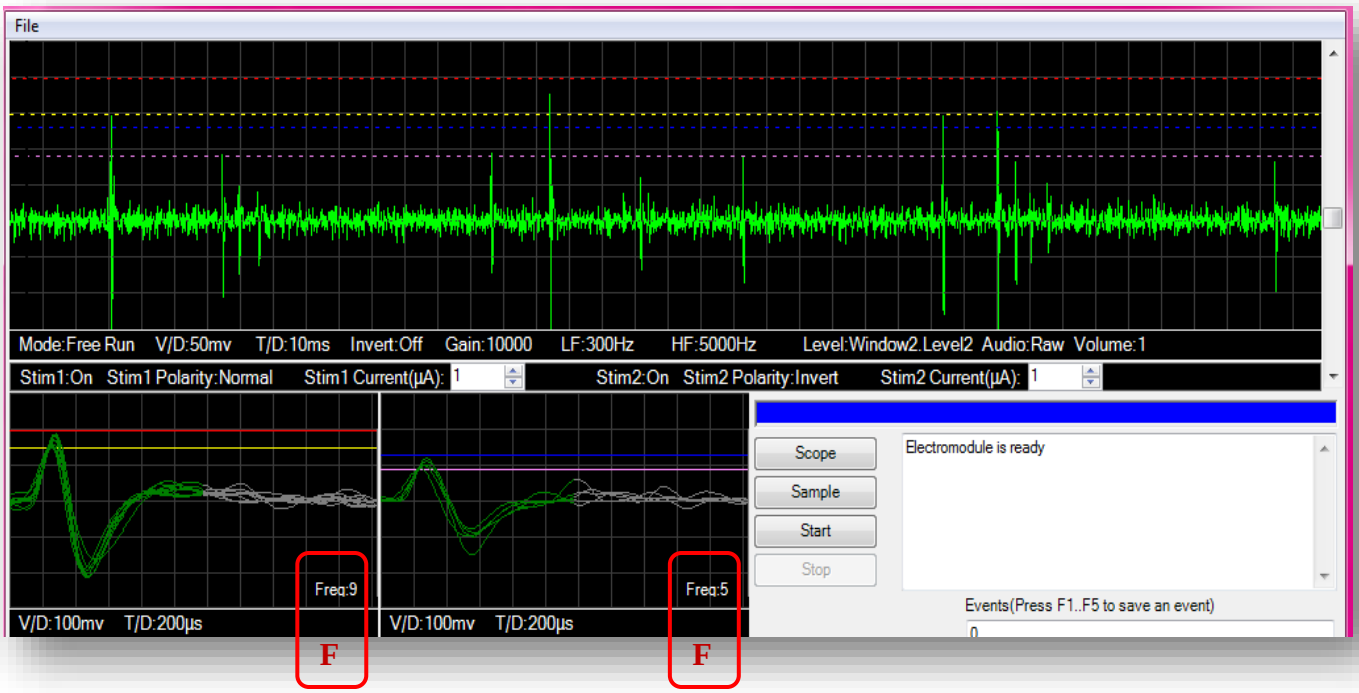


Figure 13. Two separated discriminators are organized on the main oscilloscope using four different levels (as showing with colored dashed lines). Simultaneously spikes from each discriminator will appear in the one small scope. In fact, each scope shows superimposed spiking activity of a single unit and has refreshment every one second. Frequency of the activity for each unit will appear on the scope (F). V/D and T/D show the time and voltage scale and are variables.

Example: In figure 13, a sample data are running in eSpike experiment. With a little attention to the signals and according to the signals amplitude, you will find two different types of spiking activity. One unit produces spikes with larger amplitude. To sort these two types of spikes, we need two independent window discriminators. Therefore, as it demonstrated in the figure, two discriminators built on the main oscilloscope and each one is able to sort one type of neuronal activity. Superimposed spikes at each small scope will refreshed every second.

Audio

Traditionally in single and multi unit-recording experiments, researchers prefer to use speaker and hear the song of the signal. The sound gives them some useful information for example: this sound talk about impedance at the tip of the electrode. Also, help to know about the noise and the environment electrical activity at the tip of electrode. The sound will also talk about distance from an active neuron.

In audio four options are available.

- Using “mute” you can turn the audio off.

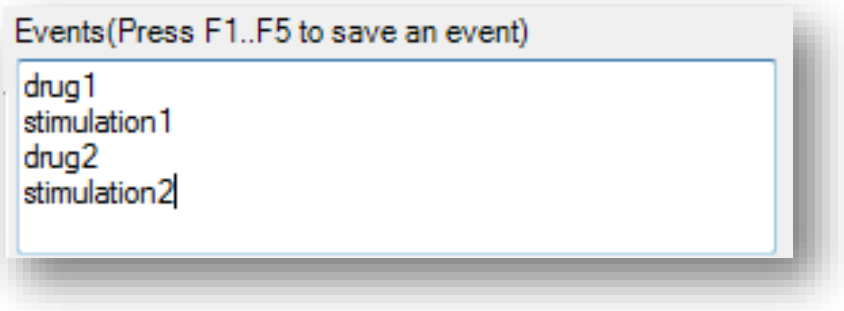
- Through “row”, you will hear sound of the row signal (noise and signals, not filtrated).

- select “window-1” or “window-2” you will have the song of only one unit.

Volume: Through this item, adjustment of the sound volume would be possible.

Events box

During the recording of neuronal unit activity, you might need to mark and save the occurrence of some events on your recording file. In eSpike experiment there is a event box as demonstrated in the next figure. Users could save time points of five distinct events while the program is running. First, you should write those events in the event box. Then using F1-F5 keys on keyboard, you could save these five events. When an event appeared, push F button to save it in a text file.

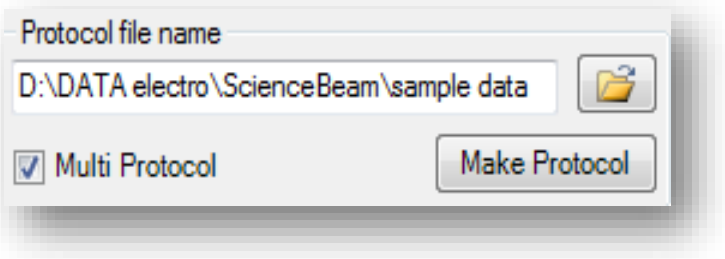


Internal stimulator

The following menu bar provides two channels of stimulation.

Using this menu bar you can turn the internal stimulator "on" or "off". You are also able to change the stimulation polarity between normal and invert. In addition, you can also write a desired current intensity in this menu bar.

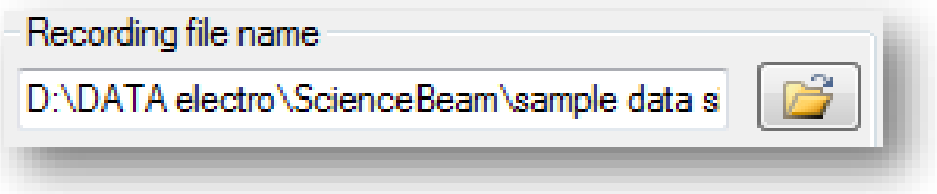
To set the other properties of your stimulation (such as pulse duration, frequency) or describe a pattern for your stimulation, you must use the button of "  ".



Actually, if you need to apply a stimulation protocol during the recording and you have a stimulation protocol use this icon "  "to employ and use it. However, if you already have not a stimulation protocol, click on this button  to make a new protocol for stimulation. After clicking on this button, you will see another panel as demonstrated in figure 5. Through this panel, you are able to design and save a simple or complex stimulation protocol. You can use this protocol during an experiment by choosing its pathway through this icon  .

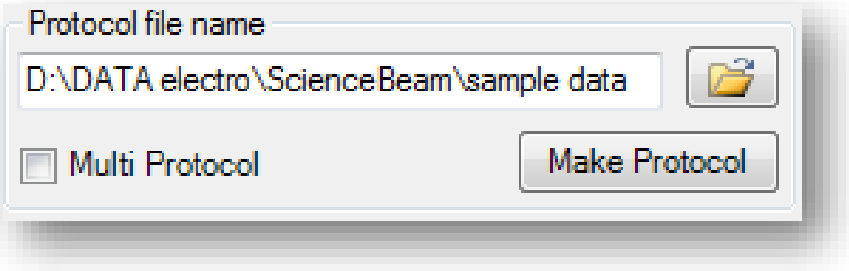
Recording file name

To save a recording file you must push the "start" button but before doing that, you must set a file name and a pathway through the "Recording file name" to save your recording data into the preferred location.



Using this icon,  you can set the pathway and give it a file name.

In addition, you have to set a protocol for recording and stimulation from "protocol file name". In this protocol, you must describe the recording parameters. In addition, if you have to apply a stimulation pattern you should describe it in this protocol (see the figure 14).



Click on this icon,  to select a previously built protocol.

If you do not have a prepared protocol or if you need to make a new protocol, then click on this button,  and continue to make a new protocol.

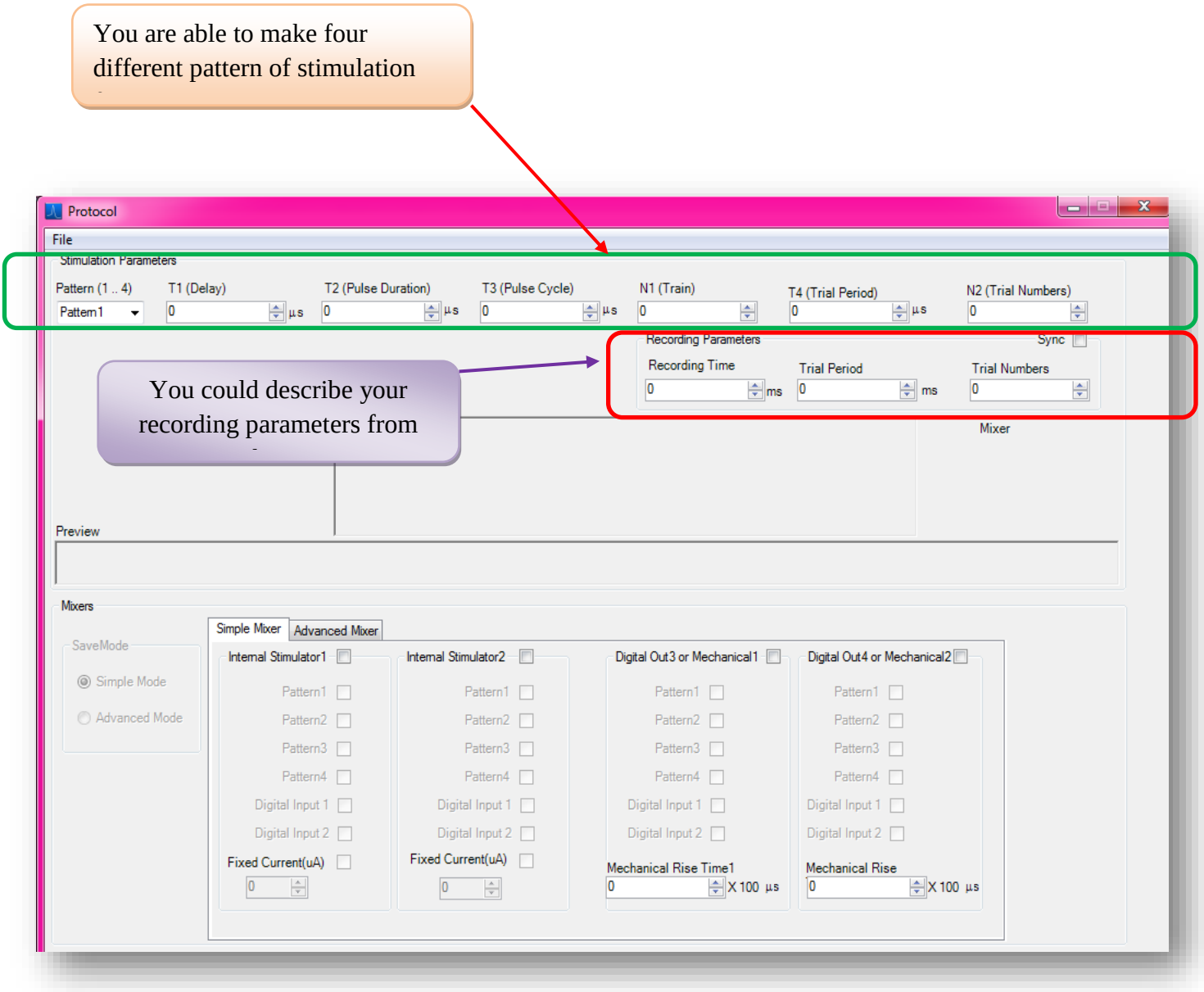
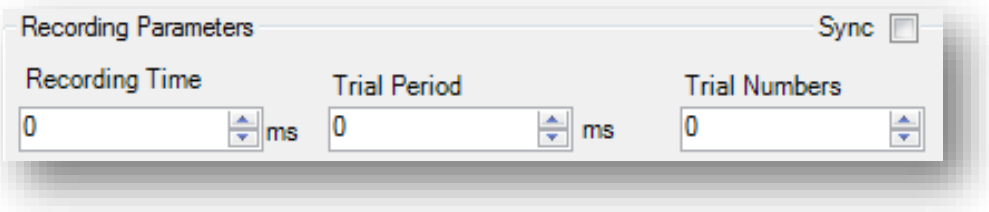


Figure 14. This panel designed to make a protocol for recording and for stimulation. This panel has many components. Through the "stimulation parameters", users are able to make a new stimulation protocol or edit an old stimulation protocol. Using the "recording parameters" users are able to describe the recording parameters. In addition, using mixers you could make a complex protocol for stimulation.

Set up the recording parameters



- Recording time: It is part of a trial period, which you wish to save it on the computer.

- Trial period: Time from the beginning a trial period to start the next one.

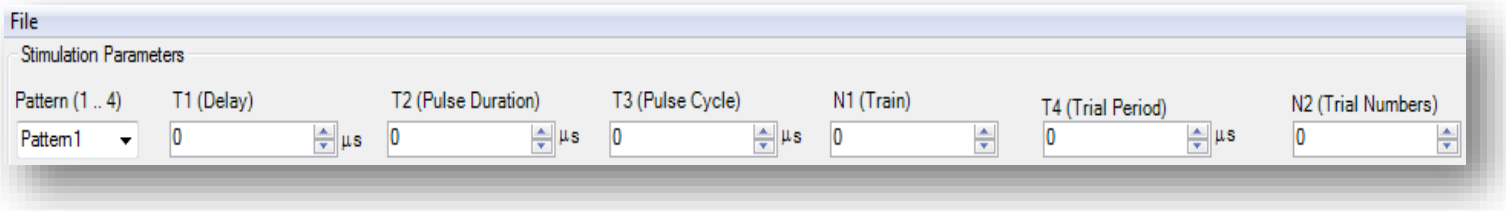
- Trial Numbers: Describes how many times you want to repeat a desired trail.

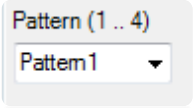
Sync: check this box to synchronize (make equalize), recording parameters with stimulation parameters.

Notice: Recording time must not be longer than the trial period (must be ≤).

Note: chosen time for a trial period depends on the duration of stimulation and its effects. For example an electrical or mechanical stimulation usually have a tiny pulse duration and produce an immediately and short-lived response, so the trial period could describe short. However a chemical stimulation produce a mid- or long-lived response with delay, so a trail period should be longer to overlap with the response.

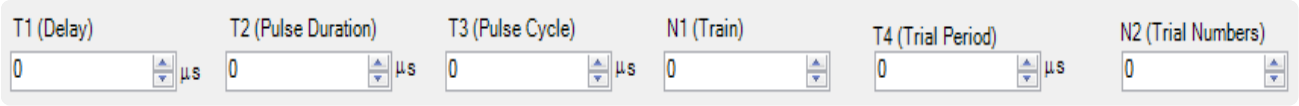
Set up the stimulation patterns





- Pattern (1…4): From this option, you are able to make four different patterns of stimulation. Then through "Mixer" you can mix these patterns to produce a complex protocol. You can also mix the patterns of internal stimulation with the patterns of an external stimulator.

To make a pattern you should set these items:



T1 (Delay): Latency between pushing the "start" button until start the stimulation.

T2 (Pulse Duration): describes the pulse duration of a single stimulation.

T3 (Pulse Cycle): Time from starting a single pulse to starting the next one.

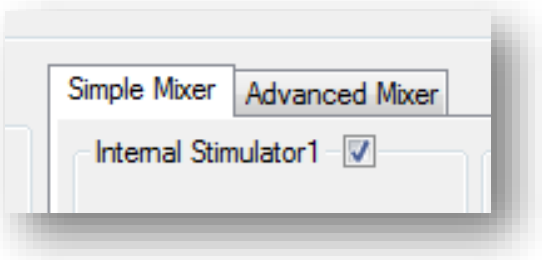
N1 (Train): Number of pulses in a *trial period.*

T4 (Trial Period): Duration from starting a trial period to starting the next one.

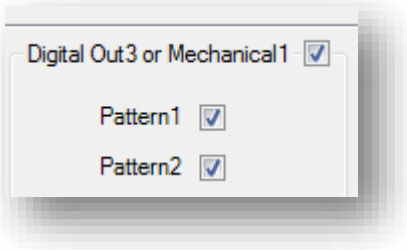
N2 (Trial Numbers): describes how many times you want to repeat a desired trail.

Mixer: The "Mixer" in the protocol panel provides to make a complex protocol for stimulation through more than one pattern or even more than one stimulator.

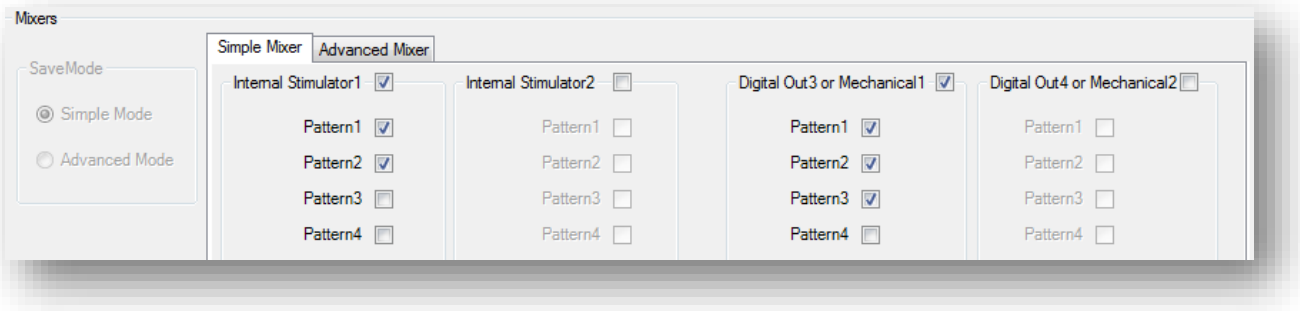
If you are going to use Electromodule internal stimulator, check the box next to the internal stimulator1, to active this option. You can design more than one pattern then select them in the mixer.



If you are going to use your external stimulator check, the "*Digital out3"* box to active it. Now select one or more pattern, which, you wish to mix them as a stimulation protocol.



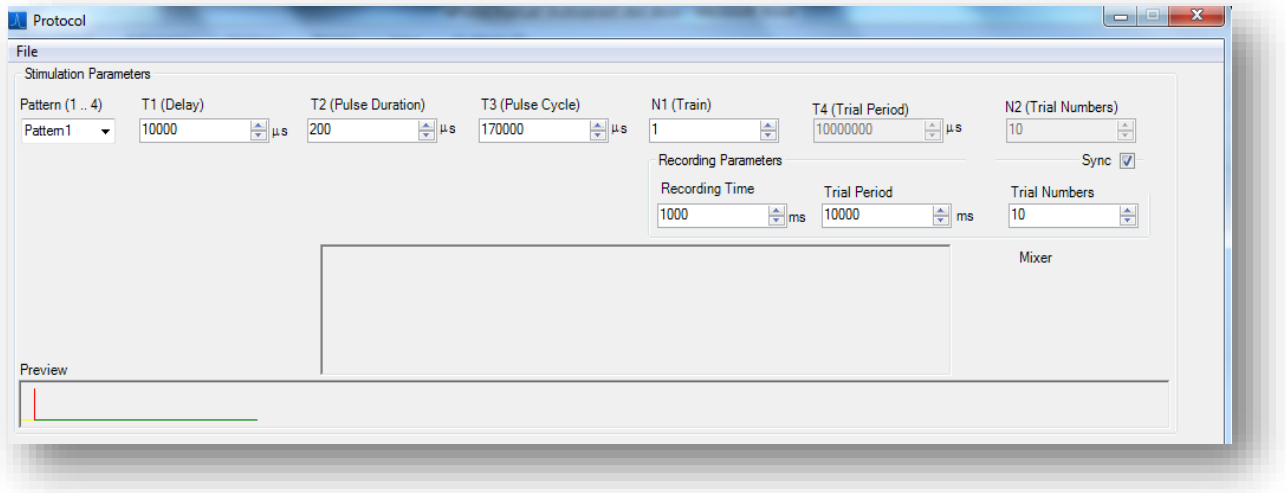
This Mixer will also let you to use both internal and external stimulator simultaneously (for example if you want to use two stimulation electrodes)



In the following example, I am going to make a complex protocol using two patterns.

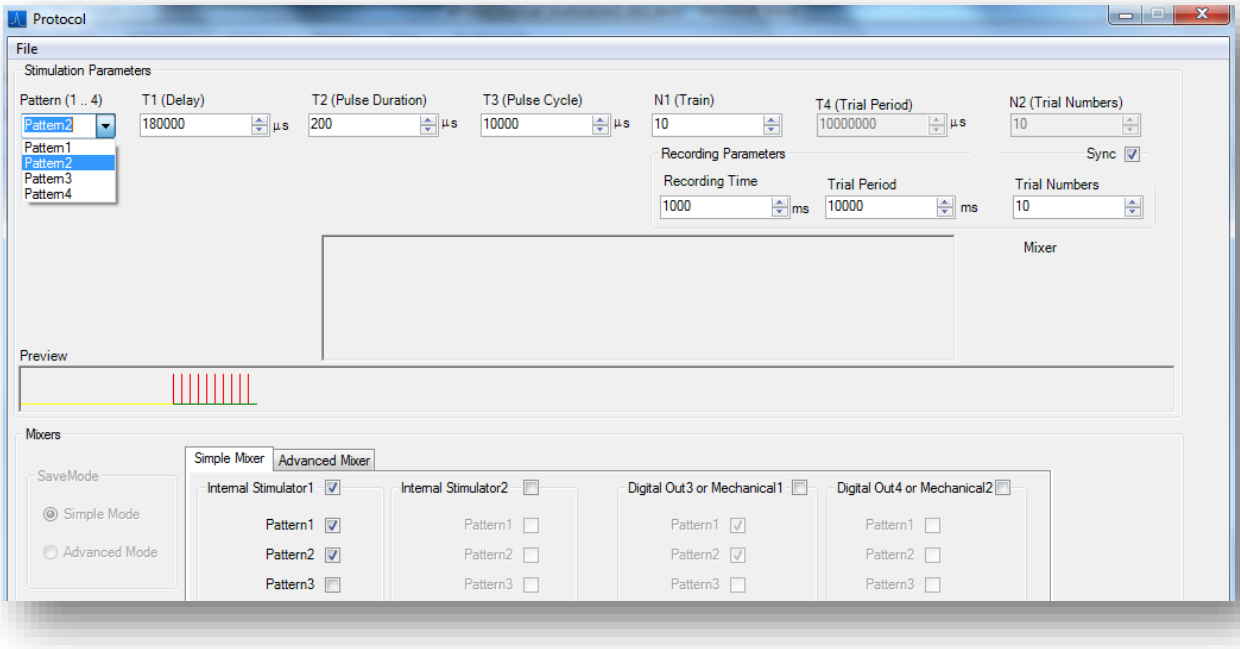
Example: I want to induce LTP using primed burst stimulation. This protocol contains a primed single pulse followed 170ms later by a burst of 10 pulses in 100 Hz. I also want to repeat this 10 time each 10 seconds. See the below figure:

In the first step I defined pattern 1, in this pattern a single pulse (200µs duration) repeat 10 times each 10 seconds. (As I have one train of this single pulse the pulse cycle is not important in pattern 1 but be careful to write it correctly when you have more than one pulse in a train).



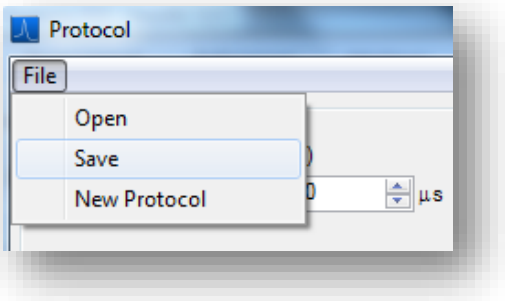
In the second step, I am going to define pattern 2 with a burst of 10 pulses in 100 Hz and repeat it 10 times each 10 seconds.

I also set the recording time to save 1000ms of each trial period. See below;



To apply both pattern 1 and 2 simultaneously through the internal stimulator I made the mixer active. See the previous figure.

To save the protocols use save icon from the file menu, like the figure:

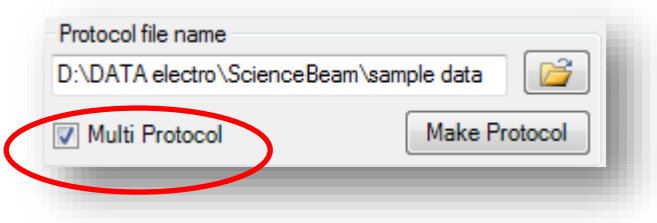


Multi protocol

Multi protocol is an option (as shown in the next figure with red circle) to apply different types of stimulation protocols in a random way. Sometimes you need to use a mixture of different protocol of stimulation or even various types of stimulation. In this situation, stimulations should apply in a random manner to avoid adaption in the responsiveness of biological system. For example in research on sensory system different protocols of stimulations (mechanical, electrical, visual …) apply in a randomly order and behavioral or electrophysiological responses save on the computer. Doing this kind of stimulation is available in eSpike experiment through multi protocol.

First, you need to describe those patterns or protocols that you want to apply, each one in a separated file with an individual name. Then you should write the name of these files inside a text file (aaa.txt) and save it into the computer. Finally you can call this text file as your stimulation file, if you check the multi protocol box.

Check the box next to the multi protocol, and then call the text file that is contain stimulation protocol names. You could call the text file with this  icon.



PSTH

Peri-Stimulus Time Histogram (PSTH) represents a histogram form neuronal unit activity before and/or after a stimulus.

Histogram is a bar graph but especially plot frequency distributions. In the figure 15, an instance histogram illustrated.

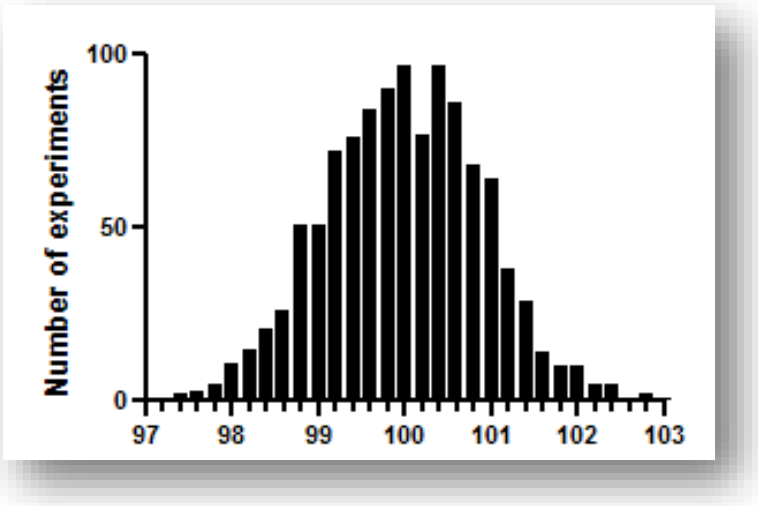


Figure 15. A histogram that is showing frequency distribution on x-axis.

Using a PSTH an experimenter could follow neuronal unit activity during the time and compare this activity before and after a treatment or stimulus. In fact, a PSTH represent frequency distribution of neuronal unit activity during the time.

In the eSpike, spiking activity in each window discriminator will also appear as a PSTH below the scopes (as demonstrated in figure 16). Therefore, when you start the recording and set the discriminators you could see and follow them in two online and separated PSTH.

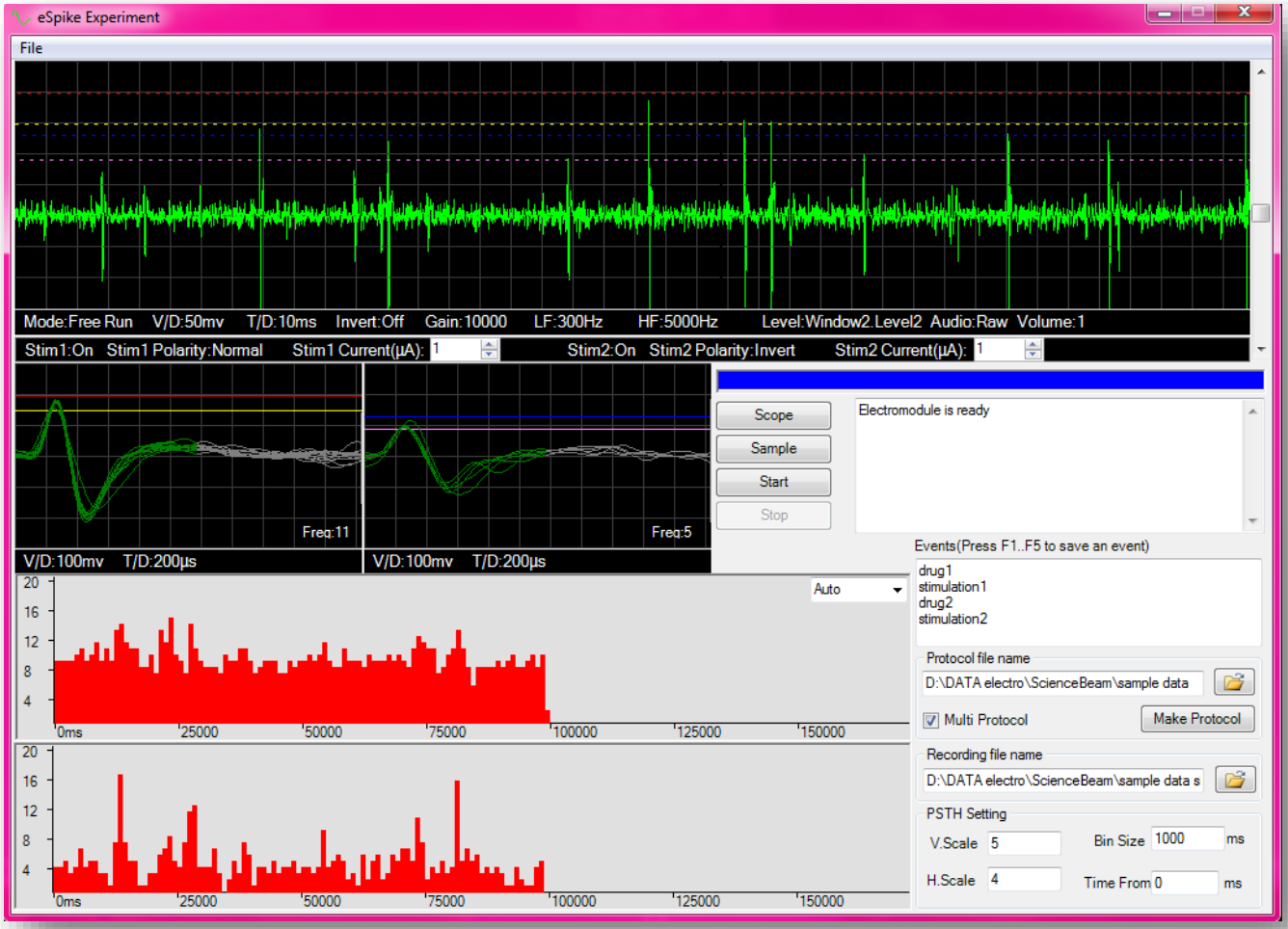
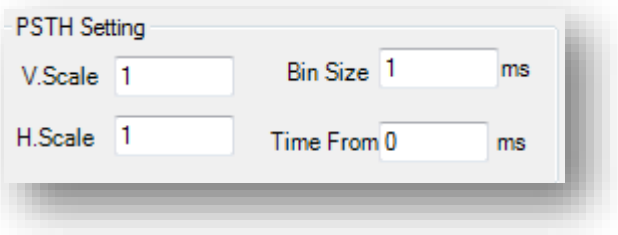


Figure 16. Two online PSTH are demonstrated, each one shows frequency distribution of neuronal unit activity from its related window discriminator. Bin size, vertical and horizontal scales are controllable from a setting box named as “PSTH setting”.

PSTH setting

From this box “PSTH setting”, it will be possible to set the PSTH illustration. V/D and T/D are using to change the resolution of the PSTH.



V.Scale: Use this item to set the vertical scale of the PSTH. The V scale shows spiking activity of the bin sizes.

H.Scale: Use this item to set the horizontal scale of PSTH. This H scale shows time.

Bin Size: Use this item to set a bin size for PSTH. Bin size will plot in the X-axis. Through choosing bin size, users could define the time resolution of PSTH demonstration. Bin size could have a value from 1ms to several minutes.

Note: It highly recommended, to use a wide bin size when the spontaneous activity of the unit is low or when the spiking activity in response to the stimulus is low.

Time From: Choose a time to start the PSTH online illustration from that point.

Play eSpike in demo

Here, we offer the user to try *eSpike experiment* in demo mode. Therefore, they are able to test and see how it will work during a real experiment.

To play eSpike in demo, we provide a sample data file from a previously experiment. When you download eSpike you will find this sample data file among the other installation files. You can run this file in demo mode and test window discriminators, PSTH representing and scopes.

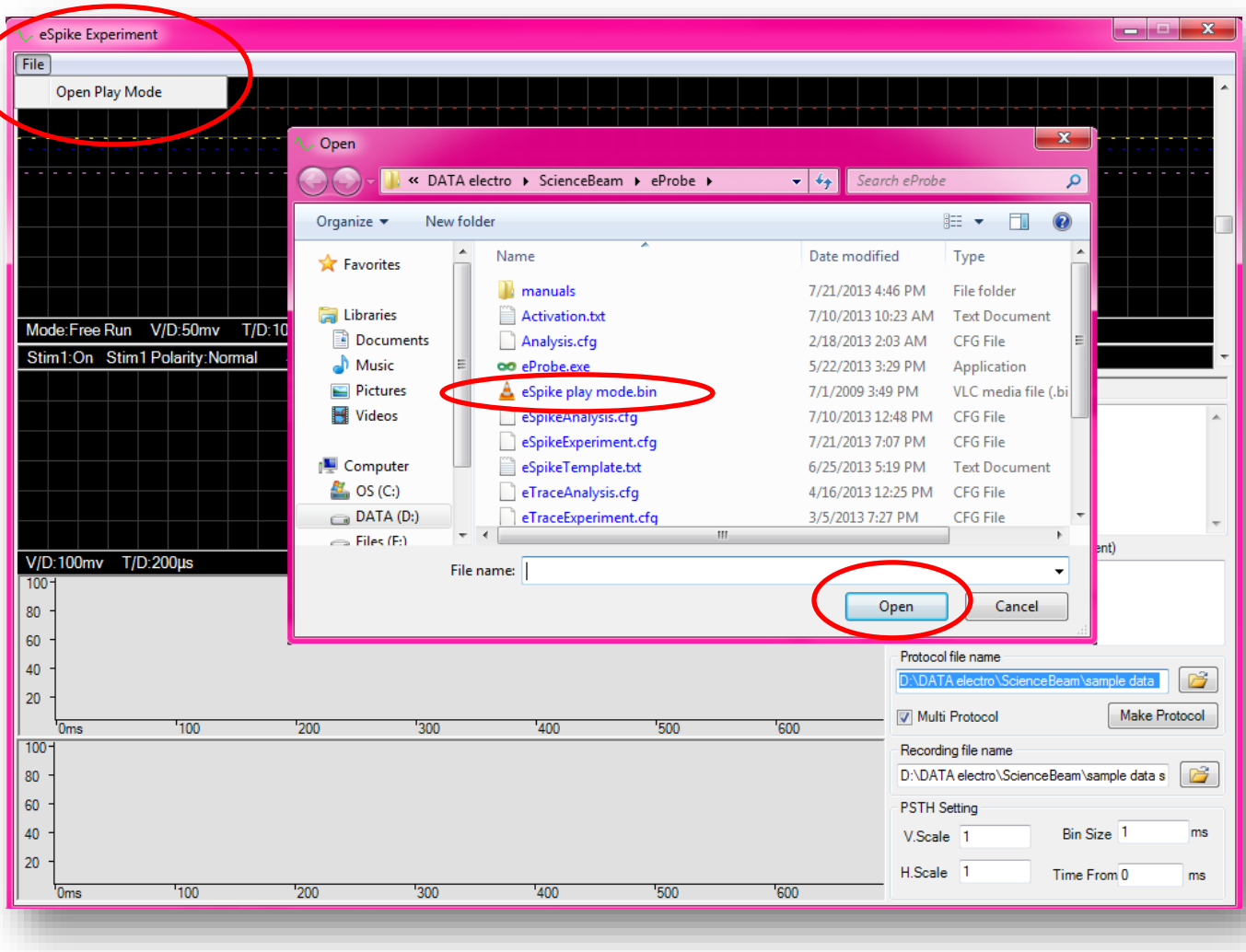
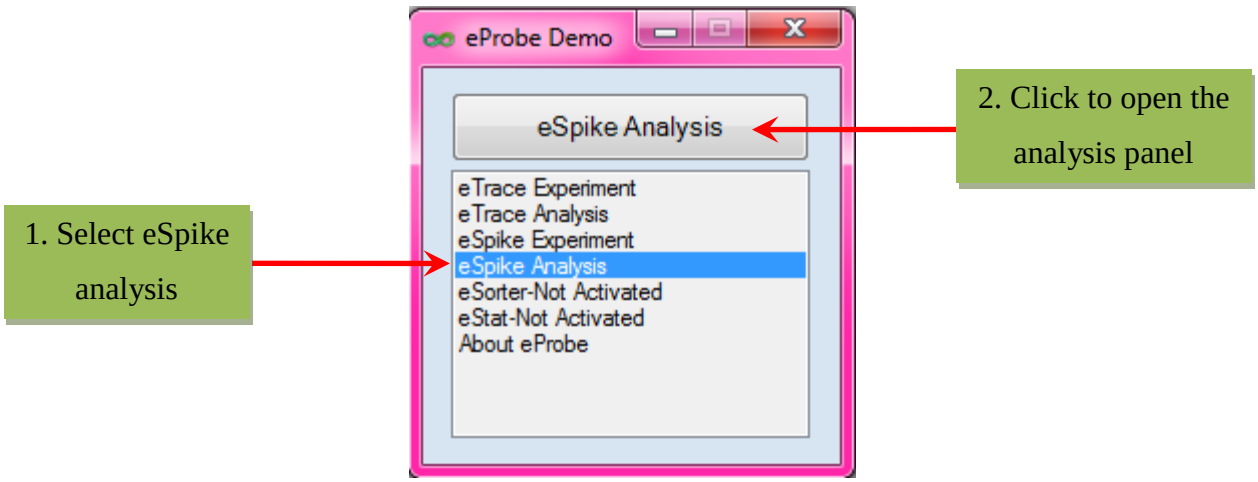


Figure 17. From the file option in the eSpike panel, you can play eSpike in demo. First click on "Open Play Mode" then select the sample file from eSpike folder and click to open it (as demonstrated with red circles). After that click on "Scope" button on eSpike panel to run the sample file.

eSpike analysis

The present version of eSpike is based on amplitude sorting the signals. When you run ***eSpike experiment*** and set window discriminators appropriately, you should click on "start" button to start saving the recorded data. Data will save on your computer in three different formats ".Stream", ".PSTH", ".Event". ***eSpike analysis*** is able to open the files in ".PSTH" format.

To open eSpike analysis, just go to the eProbe menu and open it as demonstrated in the next figure.



eSpike analysis panel demonstrated in the figure 18. As seems, it has a graphic part, which includes two x- and y-axis and showing a histogram which is extracted from one or multi trial recorded file.

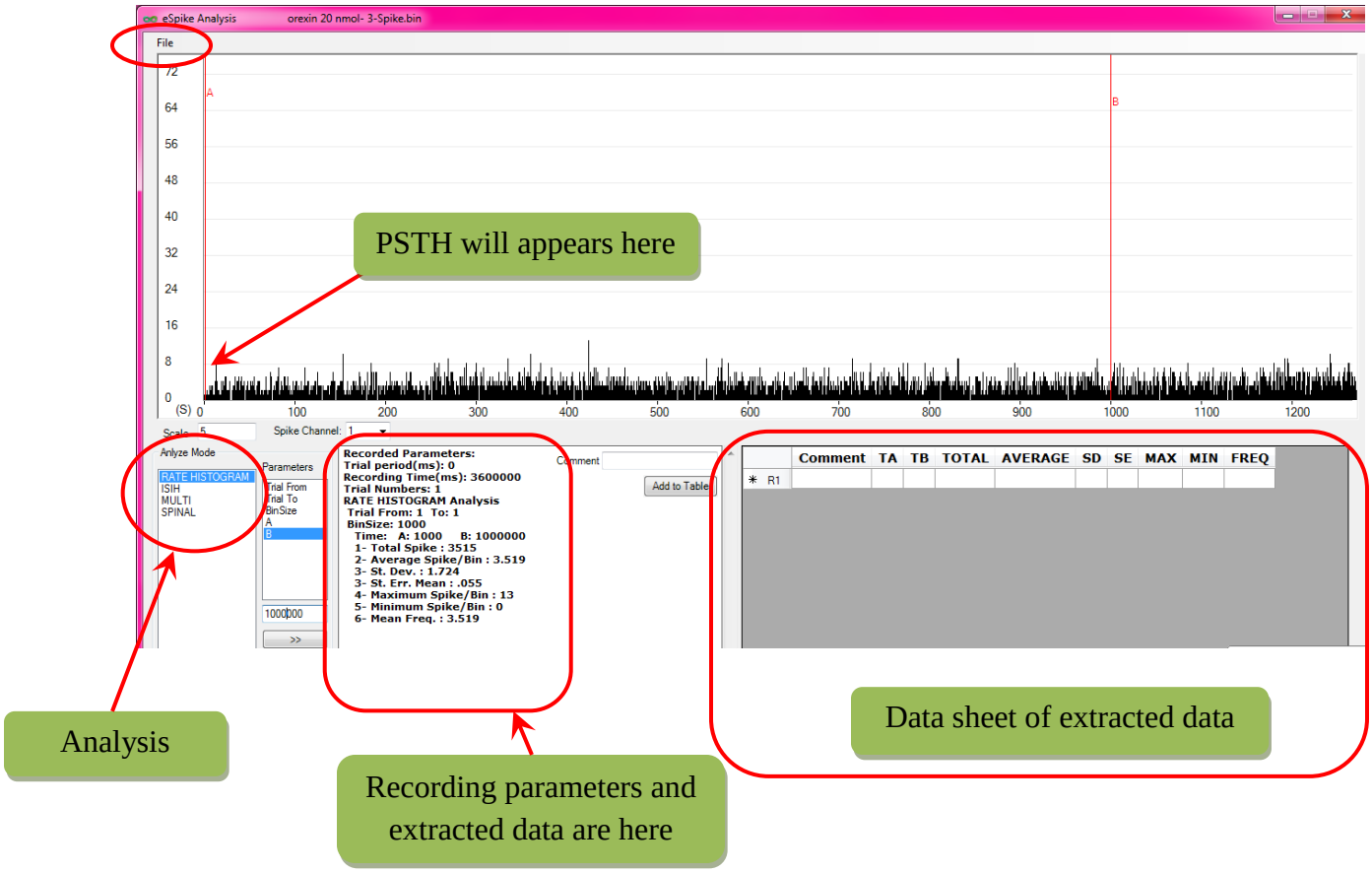
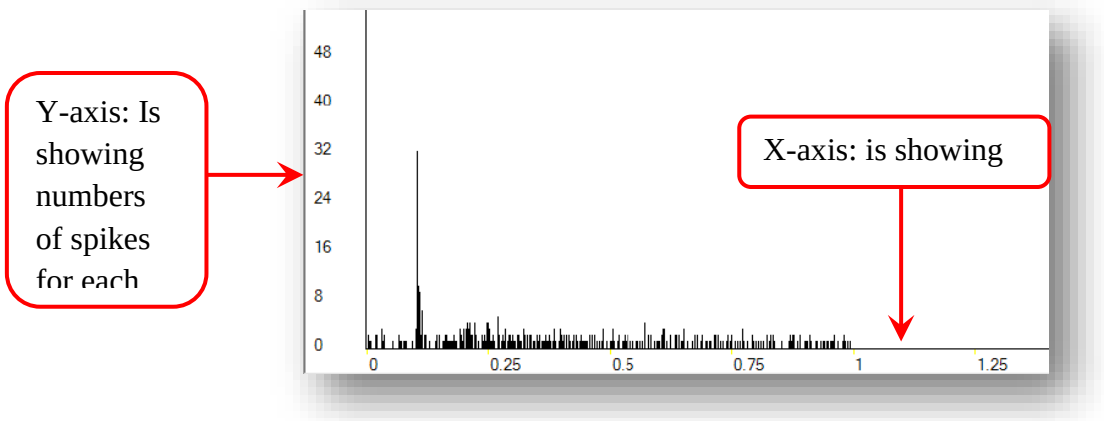


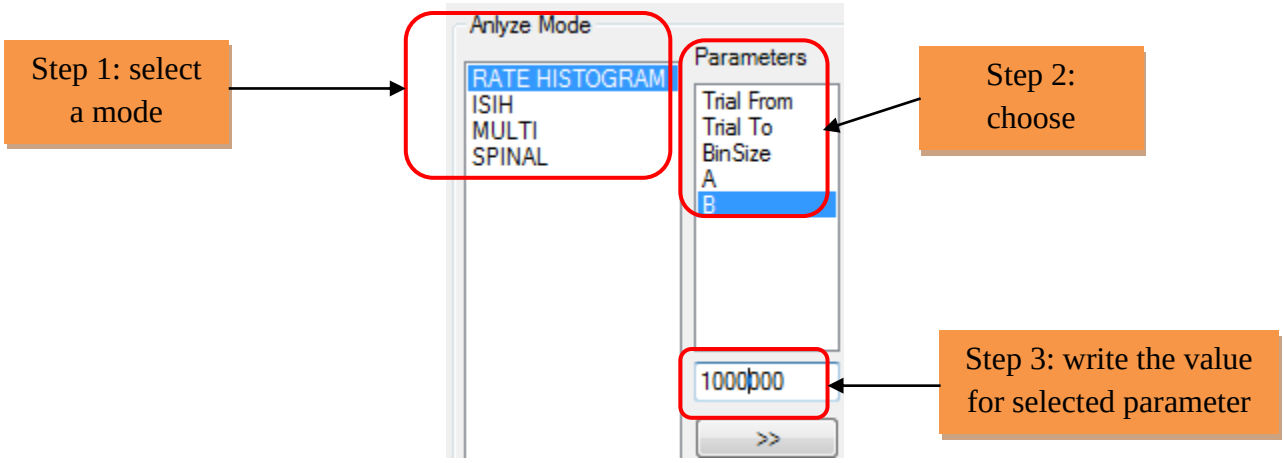
Figure 18. Different components of the eSpike analysis panel described briefly with labels.



Analyzing modes

In the ***eSpike analysis*** four different modes (as shown in the next figure) are described for data analyzing. However, it is possible for users to define one or more new modes in accord with their needs. To make a new analyzing mode you should use "eSpikeTemplate.text" file and describe a new mode in that. The "eSpikeTemplate.text" file located in eSpike folder. Later, I will explain who you could define a new mode in this file. When you save a new described mode in this file, it will appear in the analyzing box.

As demonstrated in the next figure (with steps) first, you should select a mode for your analysis then you should set the parameters from the next box and write its values.



Rate histogram

Using this mode, it is possible to offline drawing a PSTH for one or more recording trials. As it were explained previously, PSTH will show the frequency distribution of the spikes during the time. In the next figure, "rate histogram" is selected as analyzing mode. Then from the next box, histogram parameters were arranged.

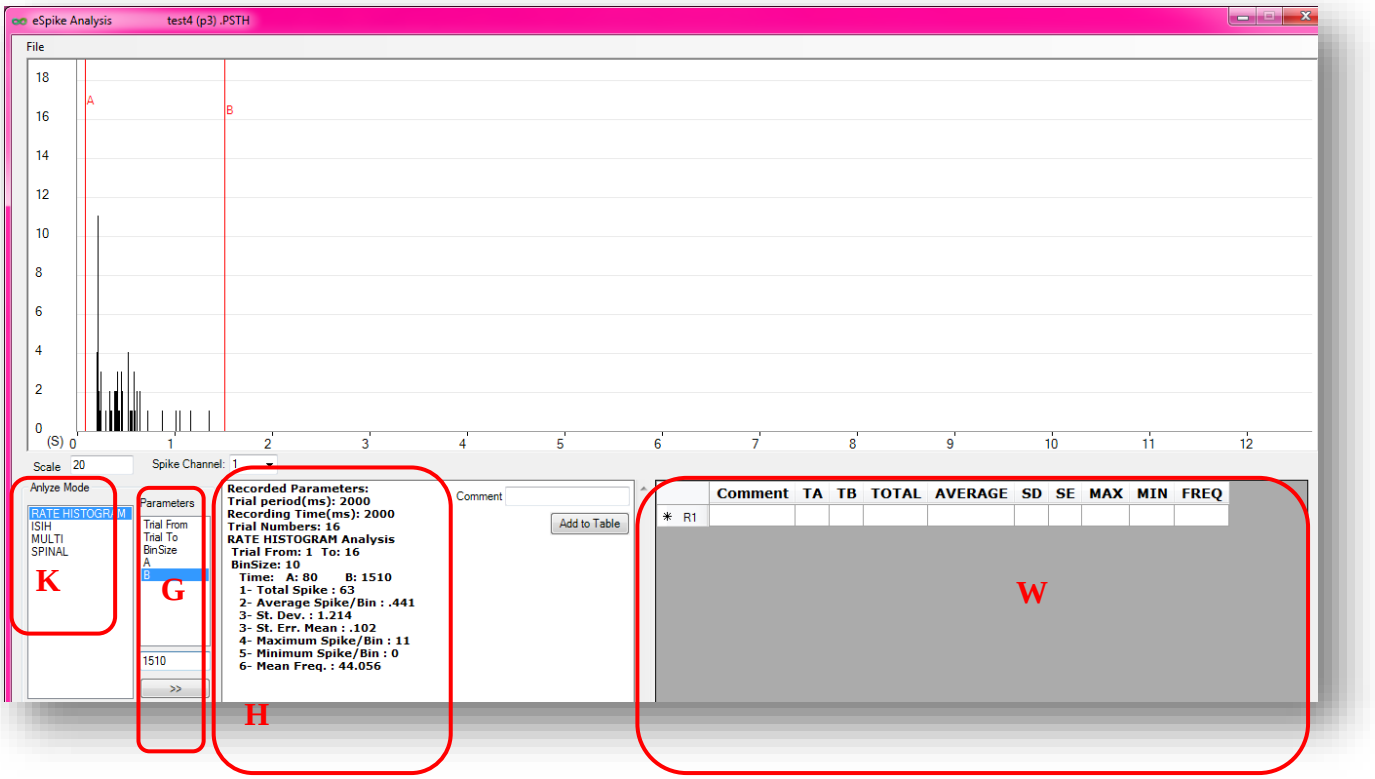


Figure 19. A PSTH were prepared with "rate histogram" is showing. As analysis mode "rate histogram" was selected from the analysis box (K). After setting the parameters from box G the extracted data will appear in the box H. Using "add to table" button, user could add the data to the box W and make a data sheet. A and B (box G) are pointers which show start and ending points of each train that must be analyzed.

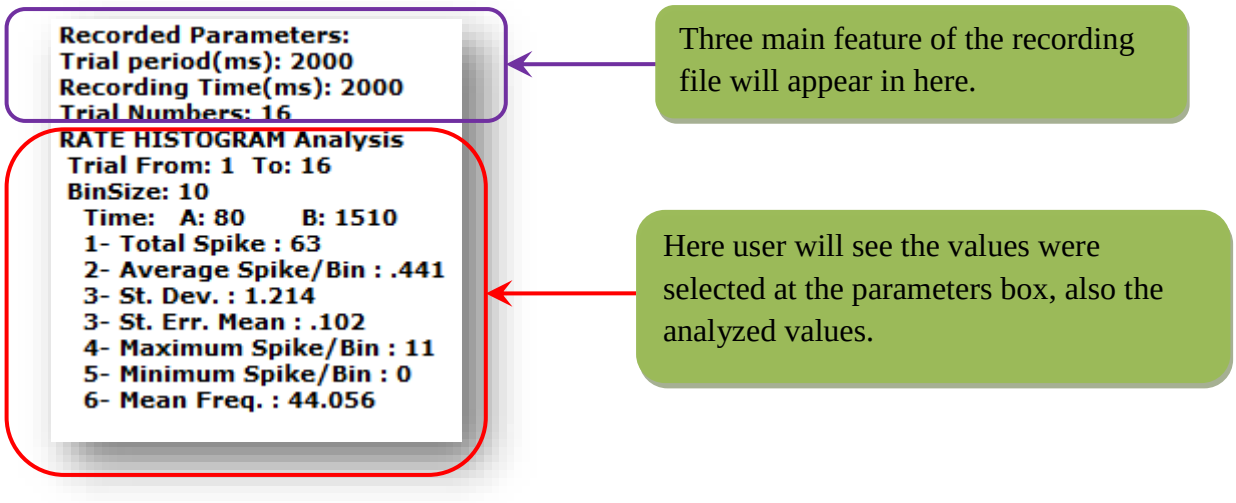
"Trial from" and "Trial to": If there are more than one trial in the recorded file, you can choose number of trials should be analyzed.

For example: to analysis just the first trial, you should write "trial from: 1" and "trail to: 1". If you have 16 trial but you want to analysis trial one to eight so you should write "trial from: 1" and "trail to: 8"

Bin size: In a PSTH, x-axis shows time and divided into bins. Therefore, each bin is a division of time and appears as a column in the PSTH. The altitude of each column shows frequency of spikes in that bin size.

Notice: If you chose small bin size, you will have high-resolution of spike frequency in time. However, if you chose a big bin size, time resolution will decrease although frequency values will increase.

A and B: these two are points which will are appear in the graphic section as red lines. Using these points user is able to define the limits at the x-axis for their analysis.



Rat histogram analysis:

Trial from: shows the numbers of trials were selected for analysis. If you have more than one trial in the recording file, from the parameters box you can choose which trials you want to be analyzed.

Bin Size: shows the chosen bin size for analysis.

Time (A, B): shows the time domain were selected trough A and B pointers.

Total spike: shows total counted spikes in time between two A and B pointers.

Average spike/bin: give you the average of spiking activity per each bin.

St. Dev.: shows statistical index of standard deviation of mean for spiking activity per bin.

St. Err. Mean: shows statistical index of standard error of mean for spiking activity per bin.

Maximum spike/bin: shows highest spiking activity between all selected bins.

Minimum spike/bin: shows lowest spiking activity between all selected bins.

Mean freq: shows the mean frequency of spiking activity between all selected bins.

ISIH (inter-spikes intervals histogram)

An inter-spike interval (ISI) is time duration between occurrences of two subsequent spikes. ISIH will represent a histogram of distribution of these intervals (as represented in the next figure). In this analysis mode, each column shows an inter-spike interval time (on x-axis), in addition the altitude of the column (y-axis) shows its frequency.

ISIH could give an explanation about the changes in the neuronal pattern activity although there might be no changes in the frequency of spiking activity in PSTH. In fact, changes in the spiking rate of a unit will appear in the PSTH however PSTH could not show changes in the pattern of the activity.

Neuronal synchronization in a neuronal unit will increase the ISIH in a particular interval.

Notice: ISIH could show 50/60 Hz noises. If the ISIH show big histogram around the 20ms, that could means you recorded 50 cycle noises.

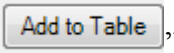
Figure 20. ISI histogram is demonstrated.

"Trial from" and "trial to": If you have more than one trial in the recording file, you can choose which trials you want to be analyzed. For example: your recording file contains 30 trials but you want to do analysis on first 10 trials, so you should write "trial from: 1" and "trail to: 10".

"Time from" and "time to": Through these two items, you are able to choose which part of a trial should be analyzed. For example, you had recorded 60 min trials but you want to do analysis on activity during 20 min to 40min. Then you should write "time from: 120000ms" and "time to: 240000 ms".

"ISI from" and "ISI to": these items make you able to select the desired intervals.

When you set the parameter from the box G, in the box K you will see information about the recording file. In addition according to the analysis mode you will see extracted data.

Likewise the previous, using “ 

” button, you will be able to add the extracted data to the box W and make a data sheet.

Recording parameters: These parameters were described in the recording protocol before starting the recording (with eSpike experiment). eSpike analysis also show these information to help analyzing process.

Trial period (ms): it shows the time duration for each single trial.

Recording time (ms): it is part or whole time of the trial period which is saved on to the computer.

Trial number: it shows how many time a trial were repeated.

ISI analysis:

ISI from: … To: ...

This item shows that ISI ranges were selected to be plotted on x-axis.

Mean ISI: shows the average of all inter-spikes intervals

St. Dev. ISI: shows the standard deviation of mean of all inter-spikes intervals

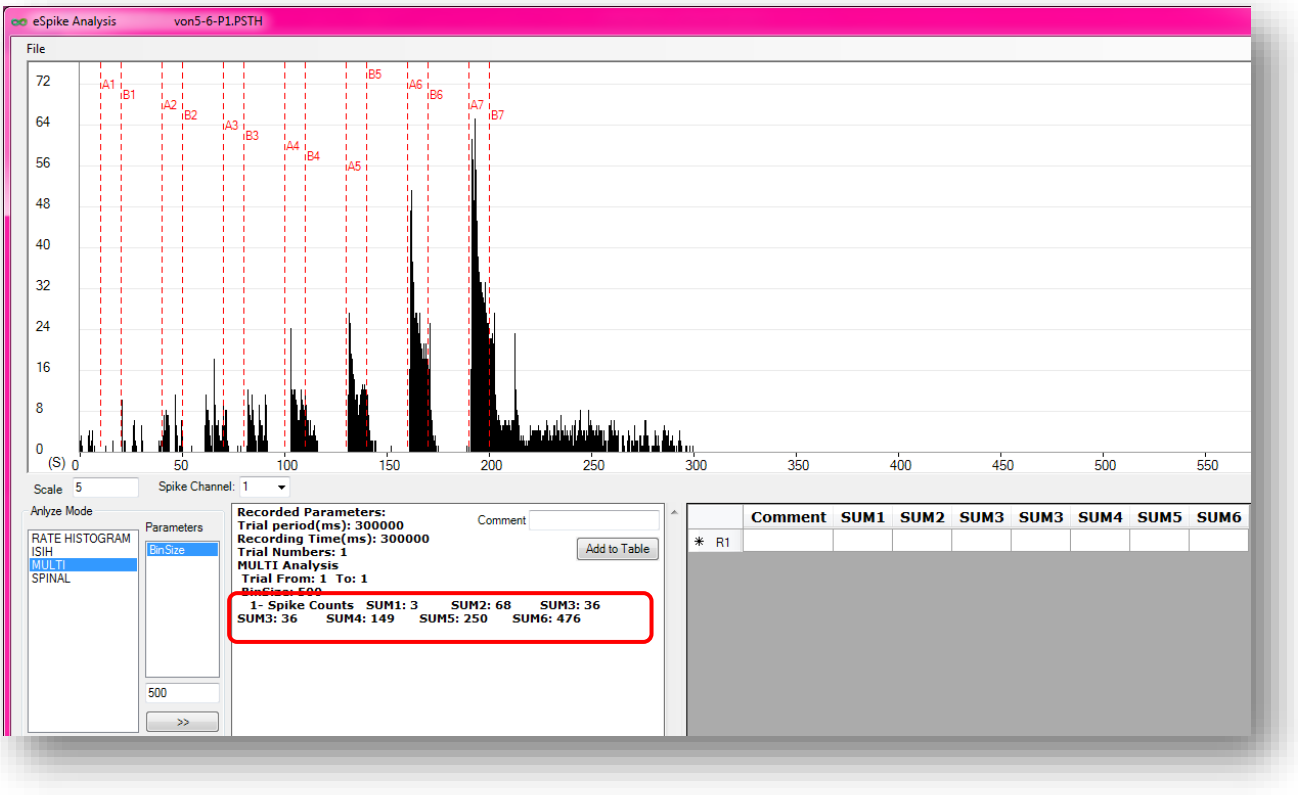
Coeff. Var. ISI: shows the coefficient variant for all inter-spikes intervals

Mode ISI: shows the maximum inter-spikes interval between all ISI.

Median ISI: shows the median of all inter-spikes intervals

Multi Event

This is analysis mode designed for a particular type of recorded file with a special pattern of stimulation. As demonstrated in the next figure when “Multi” were selected from the analysis box a PSTH will appear. This PSTH show a special pattern according to the stimulation pattern was applied during the recording. To do analysis on the PSTH analysis mode “multi” includes different constant pointers. These pointers planned according to the stimulation pattern. When you choose “multi” and set the bin size, the software automatically will calculate the spiking activity between each two pointers and will show it as a “SUM” in analysis box.

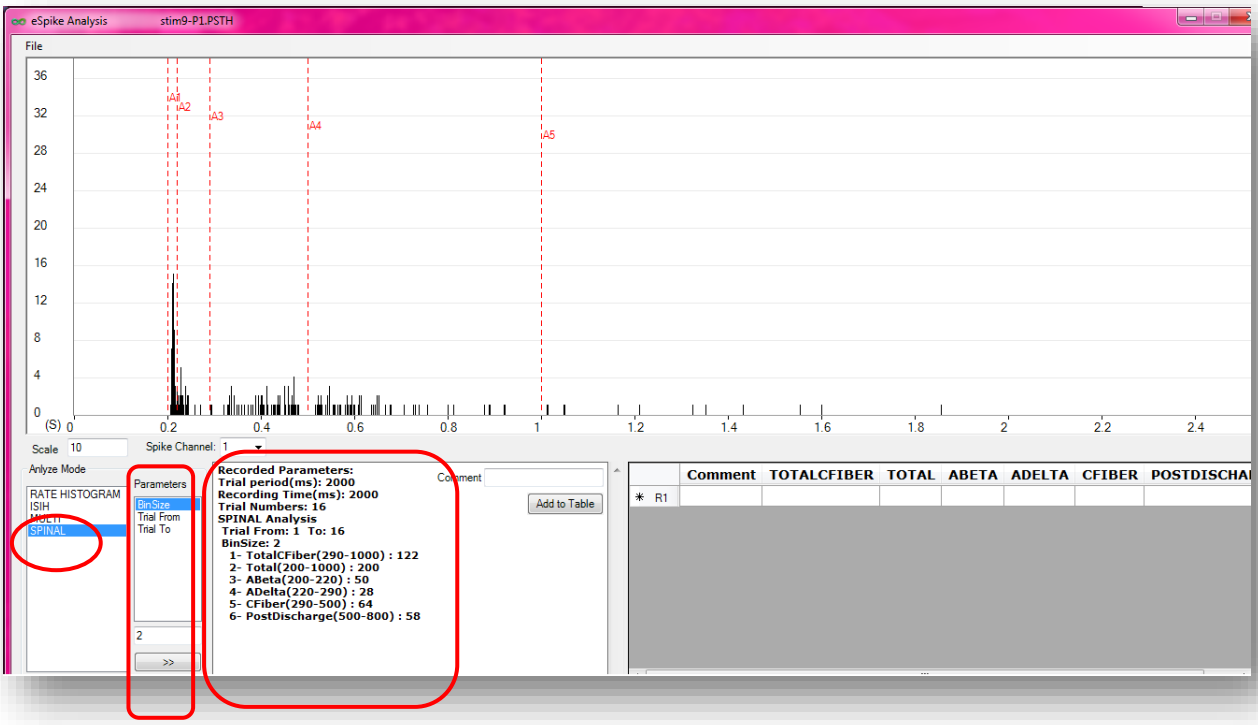


SPINAL

This analysis mode provided for analysis single of neuronal unit activity in spinal cord trough a peripheral stimulation.

Select spinal from analysis mode and set the parameters from the next box. The parameters includes: “bin size”, “trial from” and “trial to”. You should set them as previously explained for other modes.

This analysis mode have five pointers (A1-A5) in the graphic page, likewise the other pointers in eSpike they show starting and ending point of a time, but they are fixed and you cannot change their positions.



Total C-fiber (290-1000): to calculate spiking activity related to C-fibers, eSpike do counting the number of spikes between pointer s A3 (290ms) and A5 (1000ms).

Total (200-1000): total spiking activity will counted between pointers A1 (200ms) and pointer A5 (1000ms).

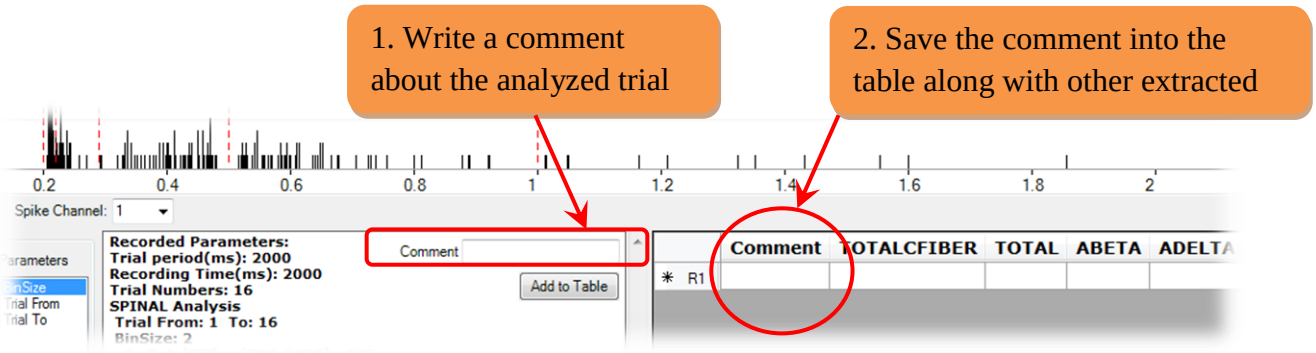
A Beta (200-220): spiking activity in A-beta fibers will counted from pointer A1 (200ms) to pointer A2 (220ms).

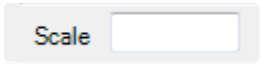
A Delta (220-290): spiking activity in A-delta fibers will counted from pointer A2 (220ms) to pointer A3 (290ms).

C fiber (290-500): C-fibers spiking activity will counted between pointers A3 (290ms) and A4 (500ms).

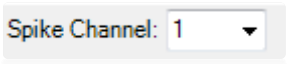
Post-discharge (500-800): to calculate post-discharge activity eSpike will do counting from pointer A4 (500ms) to pointer A5 (1000ms).

Comment: as demonstrated in the next figure there is a comment box on the eSpike panel. When you are analyzing a data file and adding the extracted data to the table, you can write a comment about and save it into the table.



Scale 

: Using this item you are able magnify or shrink demonstration of the graphic part of *eSpike analysis*. For example if you write 10 in this box your PSTH will magnify 10 times and if you write 0.1 in the box your PSTH will appear 10 tomes smaller.

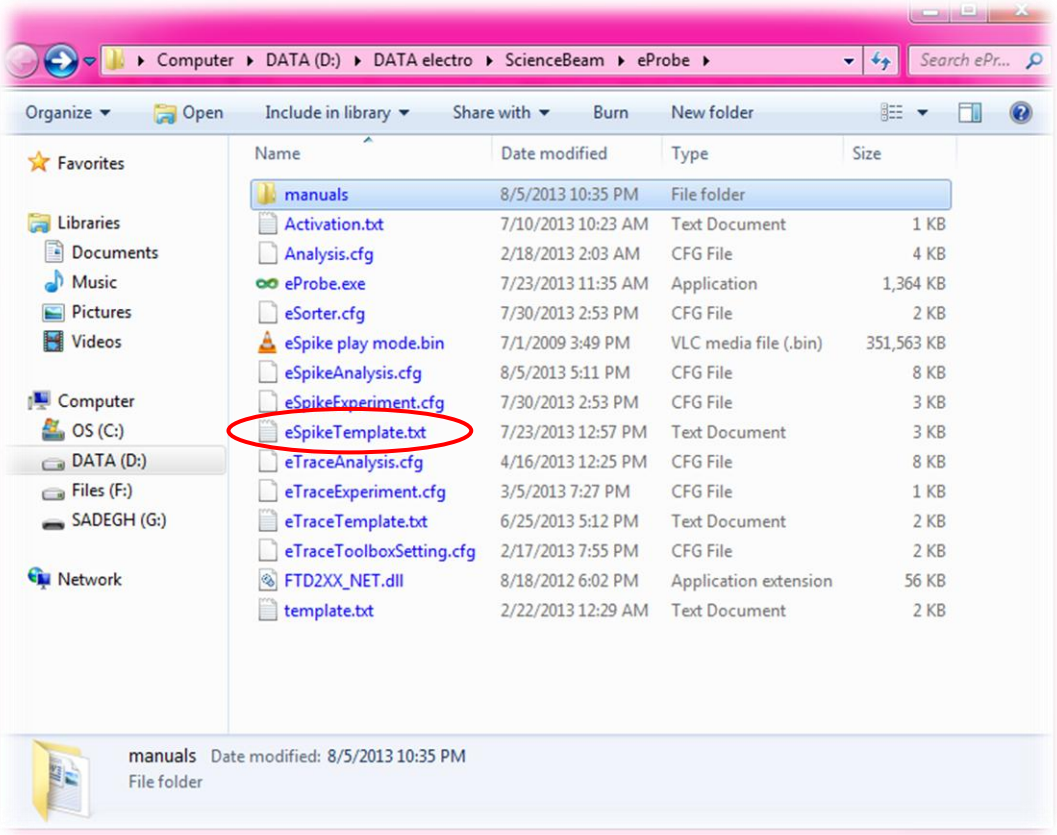
Spike channel 

:

It is showing the channels of your sorted spikes (which discriminated windows choose for analyzing).

Templates

In the eSpike folder (as showing with a red circle in the next figure) there is a text file named as "eSpikeTemplate.text". You can open this file and write a new template in accord with your need. This new added template will appear in the analysis box of eSpike analysis panel and it will be functional.



Note: If you want to inactive a previously created analysis mode, you should type a "//" right before its name in the "*eSpikeTempleates.text*".

How to make a new template?

In the following pages, I will explain how to write a new template. For this purpose, I will use default analysis mode of eSpike analysis:

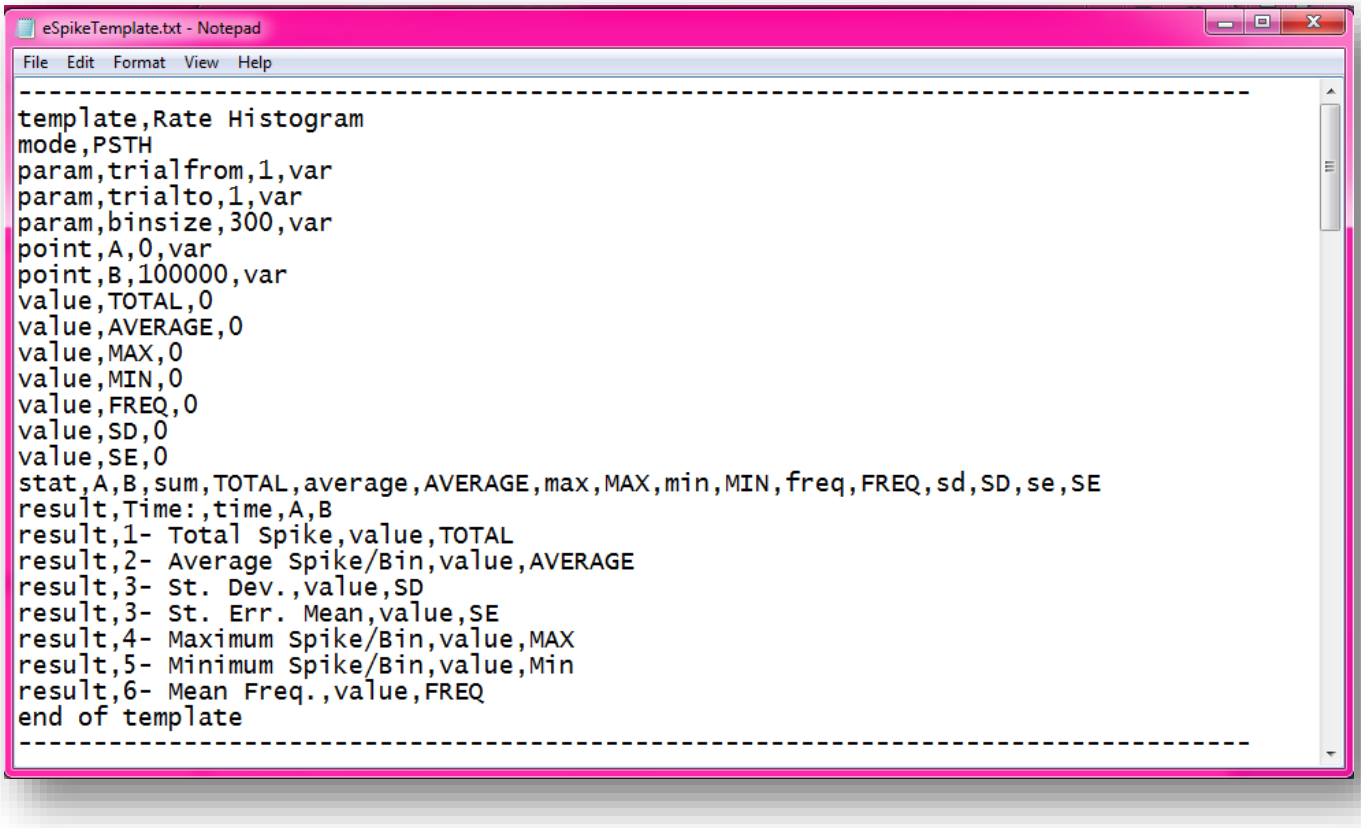
- Each template must start with a command like this "template, name of template". For example in the next figure, you are seeing this command: `template, Rate histogram`.

In addition, it must ends up with this command: `end of template`

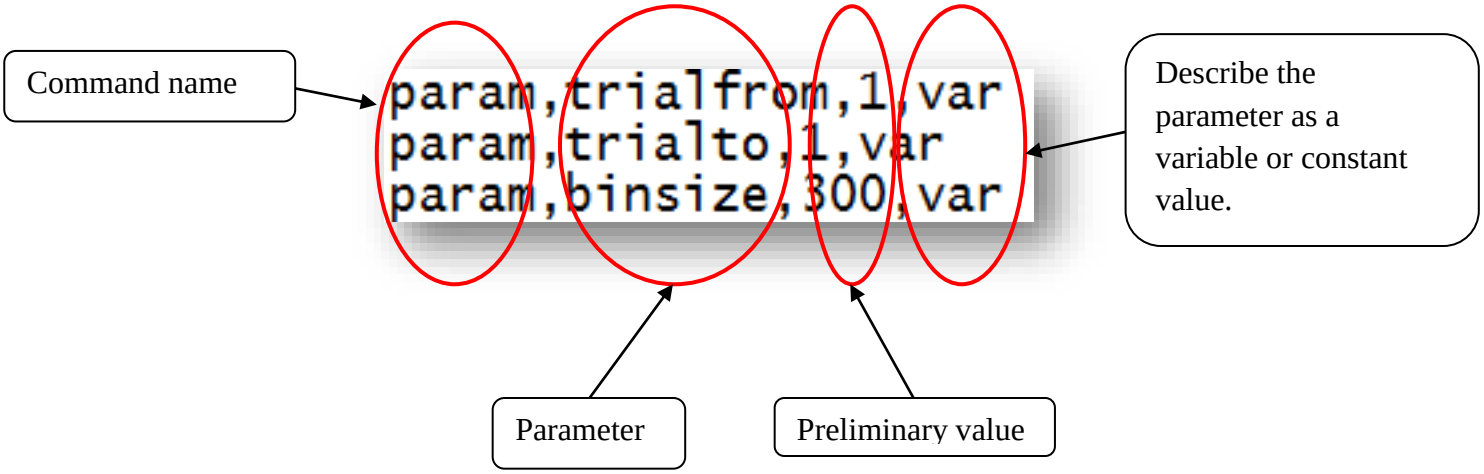
Note: Use “, ” to separate each phrase from the next one in lines of your template file.

After start command, you should write a command for analysis mode. Analysis mode could be PSTH or ISIH. According to the selected mode, in the graphic part of eSpike panel you will have a PST histogram or ISI histogram.

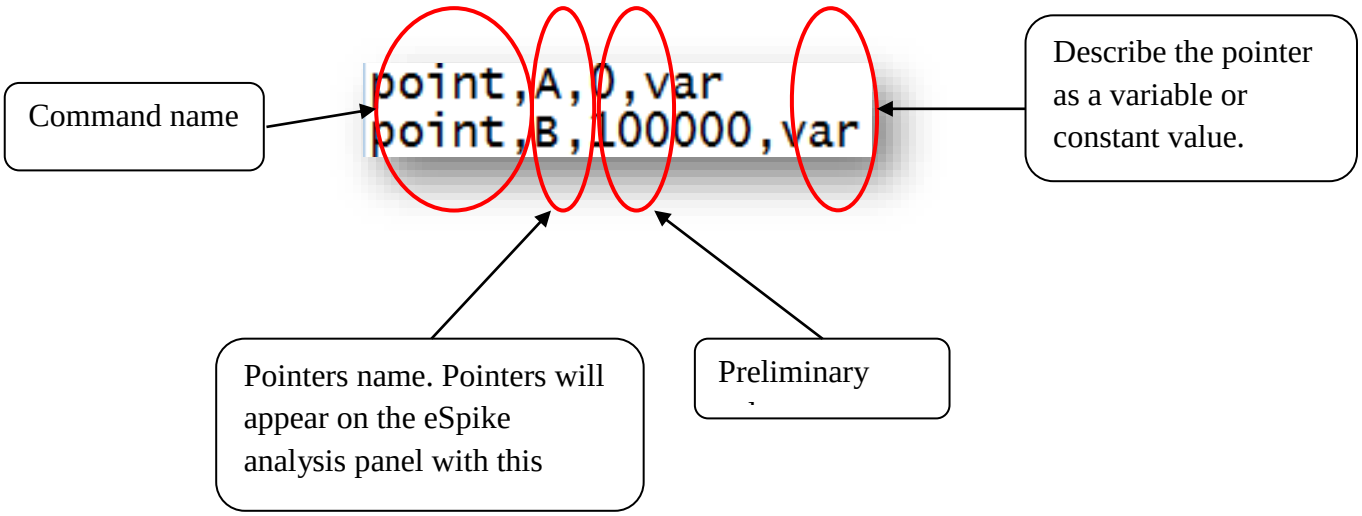
PSTH template:



Param: It is parameter. For each analysis mode, parameters could be constant or variable. For example in the PSTH mode as showing in the above figure, we have these three parameters: "trial from" "trial to" and "bin size"

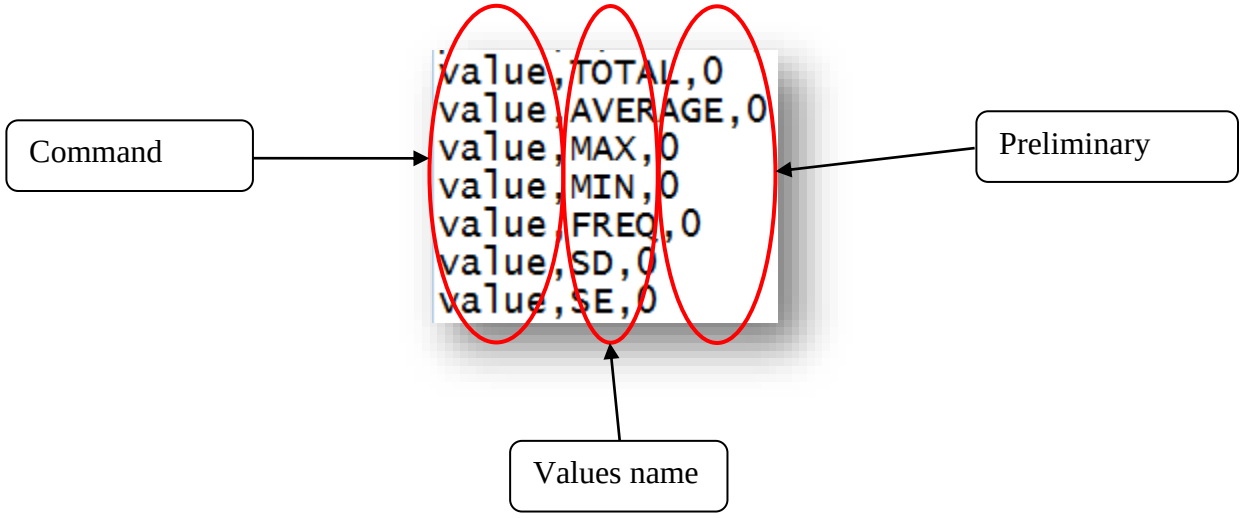


Point: these are pointer and describe the time points on analysis page.



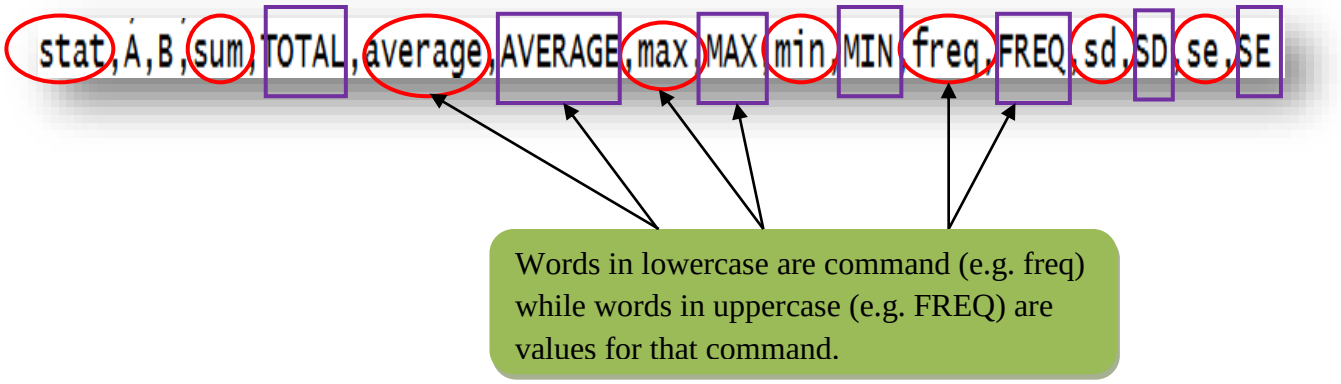
All described "**Param"** and **"pointer**" will appear in the parameters box next to the analysis box.

Value: These items show the values you want to do assessment.



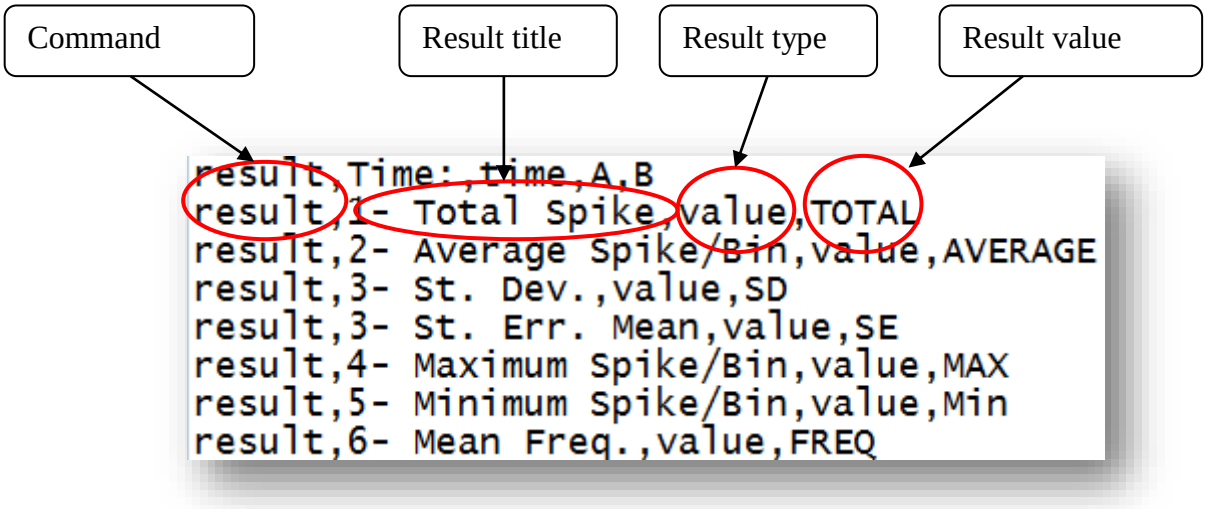
Stat: statistical parameters that you want to do calculation shown.

Note: "stat" is command. In addition, the other terms in lowercase are commands (in next figure with red circles. e.g. "sum, average, max, min, freq, sd, se"). While words in uppercase (purple quadrangle) are values and will save in their related commands. A and B are pointers. "TOTAL" is value of counted spikes between A and B pointers for command "sum".

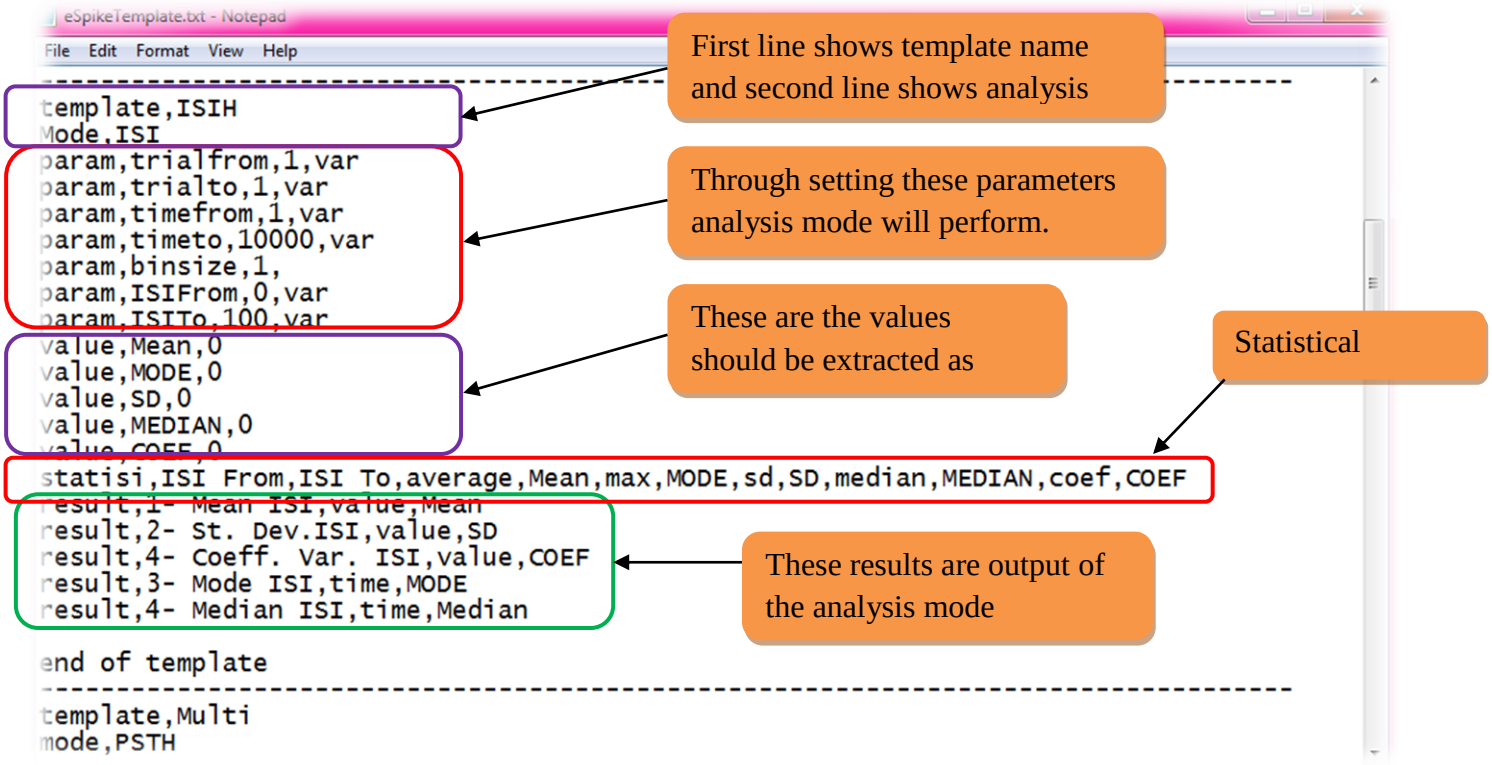


Result: shows the results of the analyzing process. To have a result, a command should write with this order:

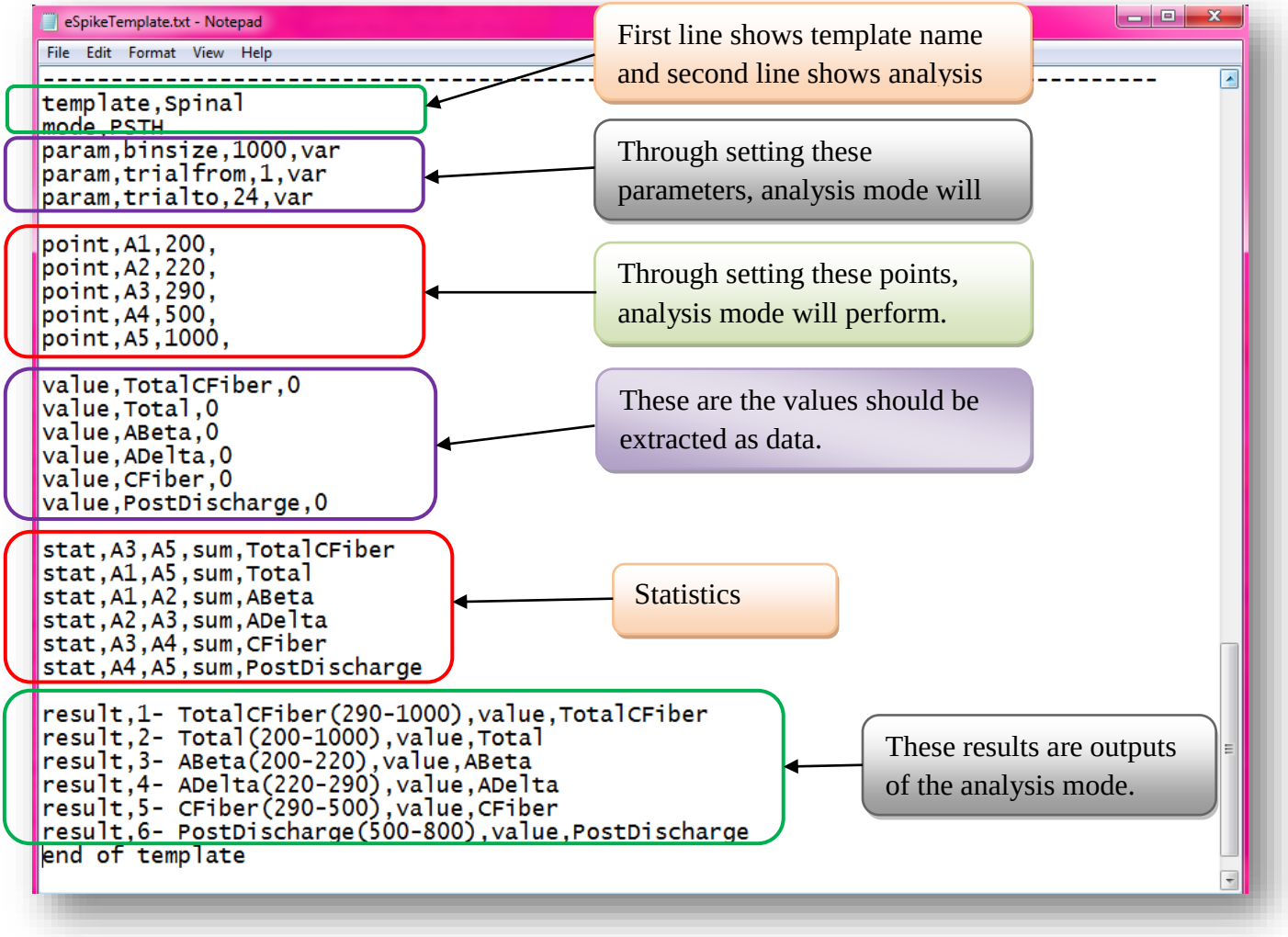
`Result` (command name), `result title`, `result type` (time or value), `result value`



ISIH template:



SPINAL template:



File

Next figure is showing the location of "**File**" menu bar on the ***eSpike analysis*** panel. In this menu bar you have three different options.

- **Open:** Using this option, you are able to open a new file to do analysis. Click on "open" and select the file you want to open it.

Save as picture: To save the graphic panel and the inside graph as a picture, you can use this item. Click on this option and select a path to save the picture file. You can also choose a format for your pictures.

Save as text: If you want to save the graphic panel as a text file, you could use this item. The text file will save on your computer. You are able to open this text file with *MS office excel* as a data sheet. Just open a new *excel panel* and drag the text file into it.