Tutorial 16: Peristimulus time histograms (PSTHs)
Myriam Lafreniere-Roula

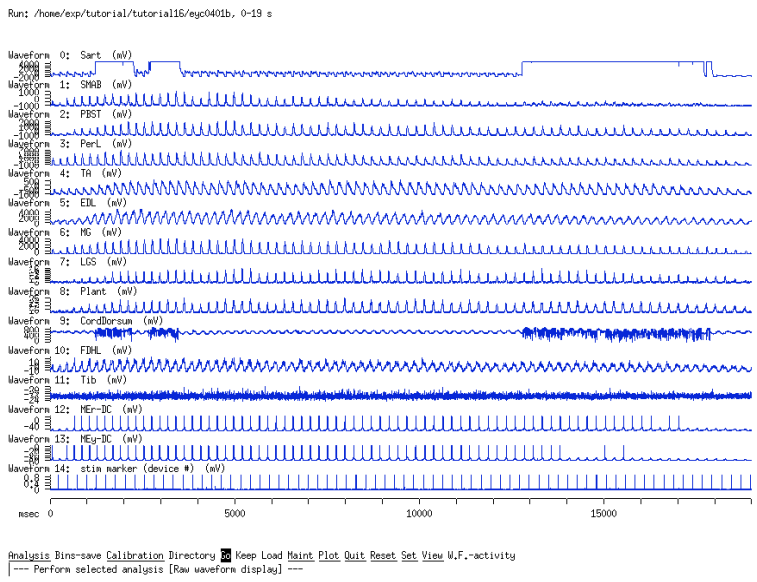
This tutorial uses data file eyc0401b.

A peri-stimulus time histogram is a way to compare two sequences of events (usually action potentials) and to determine whether there is a relationship between the timing of events in one sequence and the the timing of events in the other sequence. For example, you might have intracellular recordings from two motoneurons and want to determine whether the two motoneurons fire action potentials at the same time. You will be able to answer this question by constructing a PSTH. Also you will be able to tell whether one tends to fire before or after the other. The PSTH has some conceptual similarity to the spike-triggered average if you are familiar with this technique. The idea in both cases is to use events in one recording as time points to observe events in another recording. In the spike-triggered average, action potentials in one recording are used to trigger sweeps of data from the other, and the averaged sweeps will show the relationship between firing events in both sequences.

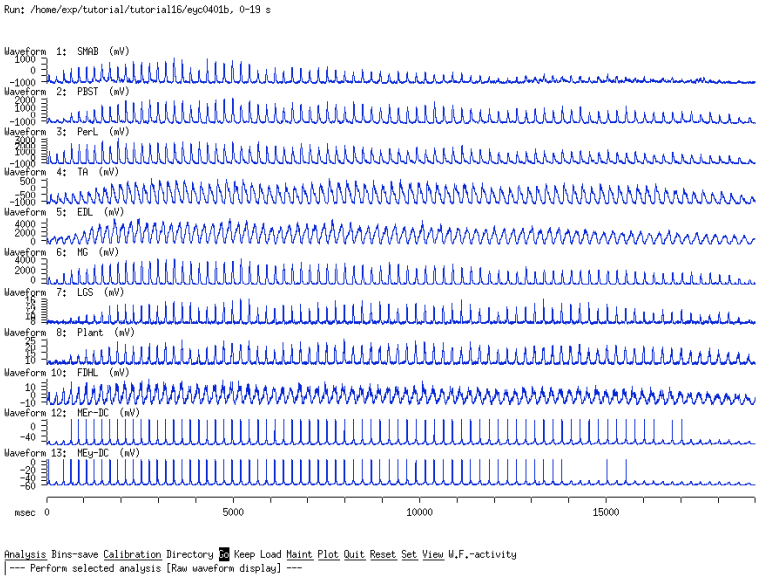
The analysis program uses the term cross-correlation to refer to PSTH which can therefore also be called cross-correlograms. In order to generate a PSTH one needs:

1. Two waveforms where either spikes or units have been defined. Events such as action potentials can be "set". This means that criteria are given to the program to that it can recognize the shape of the action potential. The program then keeps track of where those action potentials are, usually by keeping a record of the time that threshold is crossed. Spikes usually refer to intracellular action potentials. They are defined using 3 parameters: threshold, hysteresis, window discriminator. Each is set as a value in mV. The threshold level is the action potential threshold. The hysteresis is the voltage level that the cell must go back down to. The window discriminator is the maximal height of an action potential: anything taller might be an artifact and should not be taken into account. However, the same parameters can be used to detect the active phase of ENGs as is explained in tutorial 9 of this series. Units on the other hand usually refer to extracellular action potentials. Units from individual cells will have a characteristic appearance. The procedure to set parameters to recognize particular units is explained in tutorial 8 of this series. If the parameter under W.F.-activity/Set/Spikes/Unit/Number is set to zero, then the cross-correlation is performed using the spikes set with the Set/Spikes option. If the parameter under W.F.-activity/Set/Spikes/Unit/Number is set to a number other than zero, say 5, then the cross-correlation is performed using the units set within group number 5.
2. A time window during which significant events can be observed. You will need to consider what time window you want to use. You might find that only events occurring within 10 ms of each other are likely to be related

These are the waveforms contained in the sample data file, eyc0401b, as shown in the Raw waveform display (<*Esc*>**AR G**).

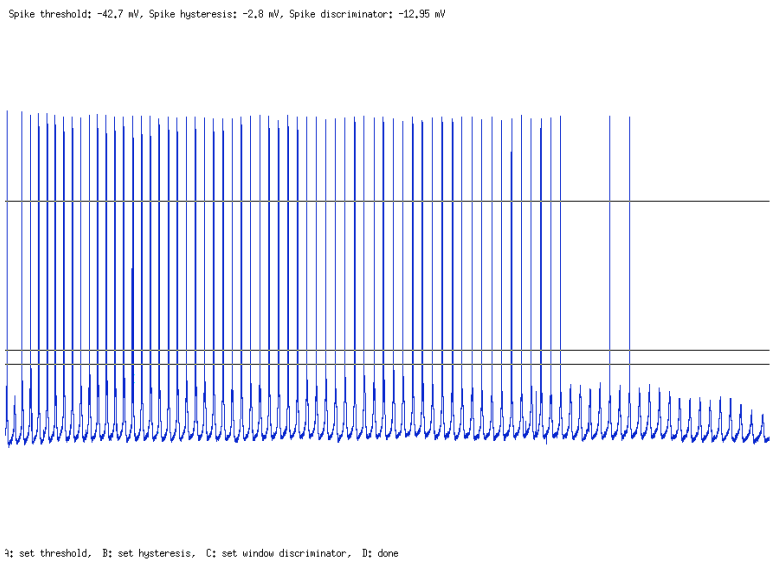


Use the *Set/W.F.-disp/List* command to focus on only the waveforms of interest: <*Esc*>**SWL1:8,10,12,13**<*CR*>**QQG**.



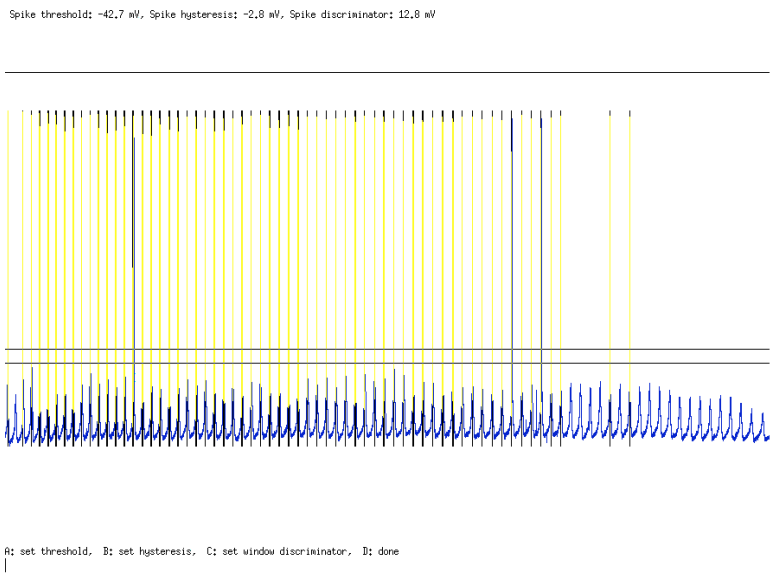
Now, use *W.F.-activity* to set the spike detection parameters for one of these waveforms. In the process, you should also set the "Time" parameter to "max", so that the whole waveform is shown at once in one section.

- **W13**<*CR*>**STmax**<*CR*>**SV**



The spike threshold is set to –42.7 mV by clicking with the leftmost mouse button, spike hysteresis to –2.8 mV by clicking the middle button (wheel button if you have one). The value indicated by the hysteresis is not an absolute value but rather a relative value compared to the threshold. Therefore, in this case, it is 2.8 mV less than –42.7 mV and hence is at an absolute value of –45.5 mV. This is why once you have set both the threshold and hysteresis, the hysteresis will follow every time you change the threshold.

Here no spikes are selected because the spike discriminator (-12.95 mV) is too low. This value means that spikes reaching levels more depolarized than –12.95 mV will NOT be included. In our cases, this means no spike is included. Setting the spike discriminator to 12.8 mV now results in all the spikes being selected.



When you're done setting the spike parameters, press "**D**" to exit, then quit and save the parameters:

- **QQQ**<*CR*><*CR*>

That's the quick tour of spike setting. Now let's back up and take a closer look.

- First zoom in on a few closely spaced action potentials so you can set the values accurately.
- **SRS9400**<*CR*>**E9440**<*CR*>
- This range should contain two action potentials in waveform 13. We will use these two action potentials to set the spike parameters.
- **W13**<*CR*>**SSV**
- There is now a display of two action potentials. The all three mouse buttons will be used to set the threshold (leftmost button), the hysteresis (middle button) and the discriminator (rightmost button). The threshold should be set at the point where the EPSP ends and the fast rising portion of the action potential starts. Do this by clicking the leftmost mouse button once you have placed the cursor at the appropriate level. The hysteresis should be set as the baseline level that the cell returns to after the action potential. The discriminator is the maximal height the action potential can have. This is a useful to discriminate artifacts from real action potentials. Here it is set above both actions potentials so neither is eliminated. This is the proper way. The actual level will have to be verified when looking at the whole range of the data file.

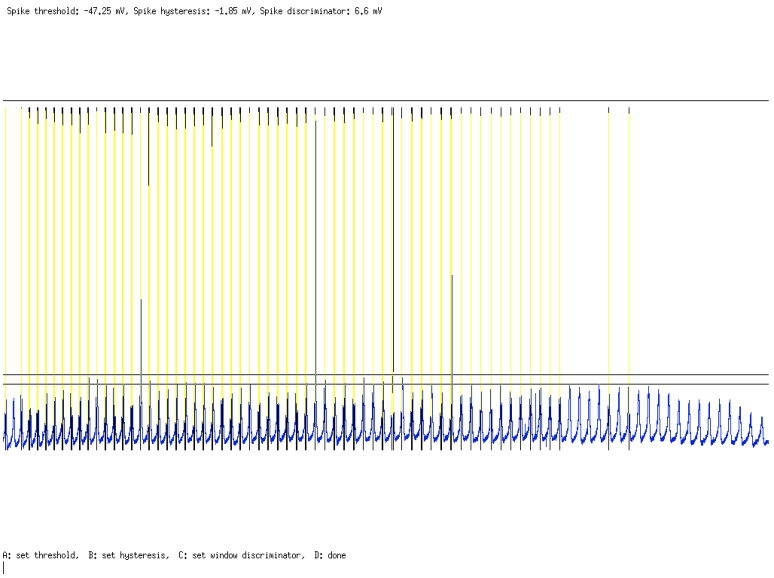
Here the discriminator is set much lower and neither action potential is detected.

Now you can exit (**D**) and set the program to look at the entire range of the file so that you can set these parameters for the entire file and therefore detect all the action potentials and not just those two that we have seen so far.

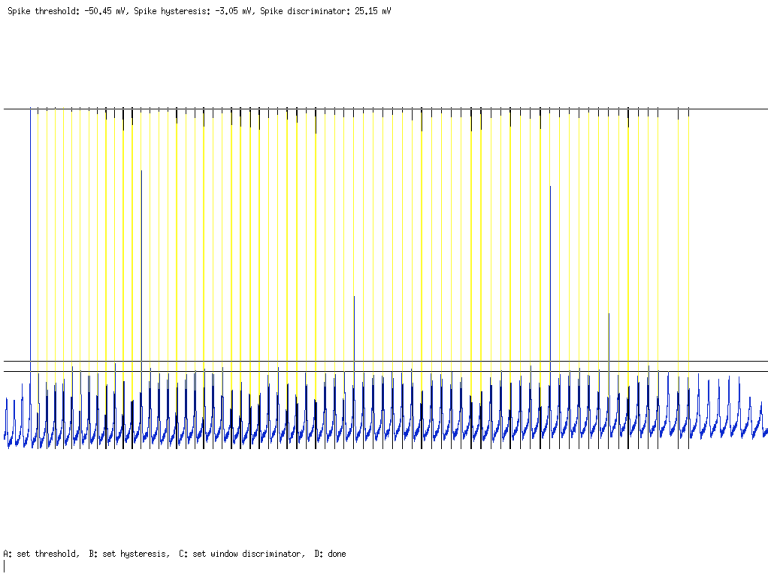
- **QQSAAQSV**

(Note how the analysis display range can be set from the W.F-activity menu as well as the main menu.)

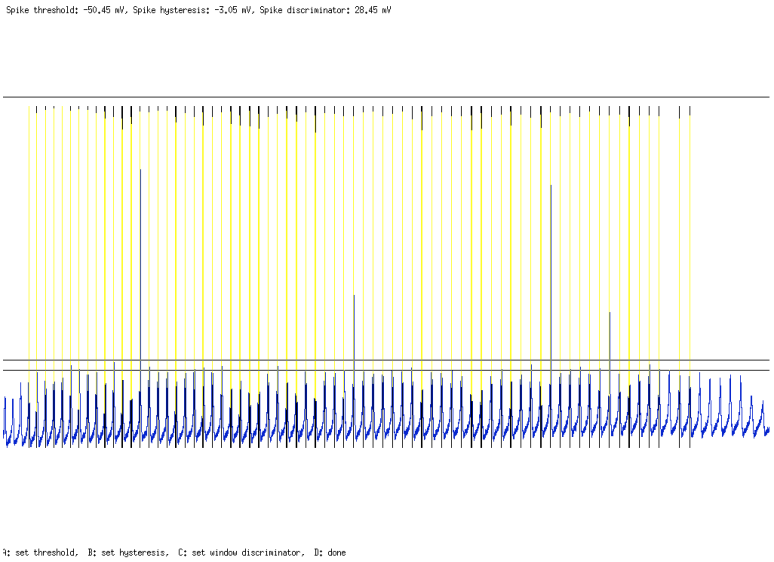
You can now adjust all three spike parameters to get all the spikes, and hit **D** to exit.



- Now you have to do the same thing for waveform 12
  QQQ<*CR*><*CR*>**SRS9600**<*CR*>**E9700**<*CR*>**W12**<*CR*>**SSV**
- Set the threshold to –50.45 mV, hysteresis to -3.05 and discriminator to 25.15
  DQQSAASV



- In this case the discriminator is a little too low (notice how the first action potential is not detected; it stays blue) and close to the most depolarized point of the action potential so it is appropriate to change it here.



- By increasing the discriminator slightly we were able to include all action potentials in the group.
- Now, exit and save: **DQQQ**<*CR*><*CR*>

Now that action potentials were set in both waveforms, we are ready to perform a cross-correlation.

- Select the analysis method: W.F. spike cross-correlation histogram:
  AGCC
- See what analysis parameters are required for this analysis:
  VR
- Set the two waveforms to be cross-correlated:
  SSWN13<*CR*>**QCN12**<*CR*>**QQQ**
- Set the delay and window parameters to indicate the region of interest before and after the spikes (e.g. ±20 ms):
  SAWD–20<*CR*>**W40**<*CR*>**QQQ**
- Set the number of bins, or bars, in the histogram:
  SGB40<*CR*>**QQ**
- Hit "**G**" to "Go" and perform the analysis.
- You can also turn on the "Histogram display" option, which in the case of this particular analysis, changes the display from average samples per bin to total samples per bin:
  SDTHyQQQG