Tutorial 4 Cycle-triggered trace average

This section describes how to average traces based on the step cycle. That means that you take all the traces taken during the flexor or the extensor phase and average them. If you look at figures 2.1 and 2.5 where you had an average of all the traces collected (and not excluded by flagging), you can see that there is a calibration pulse prior to the recorded PSPs. You also may notice that the time scale starts at 20 and not at zero ms. There is also a delay of about 45 ms before the actual PSP=s were elicited and recorded from the motoneuron. The duration of the recorded PSPs is about 20 ms: they go from 45 to 65 ms. There are two important parameters for cycle-triggered trace averaging. One is the delay from the point when the intracellular recording was taken to the point where the PSPs were started to be recorded and the other is the window of the duration of the recorded PSP=s. For example, what if you started to record a trace at the very end of flexion but the PSPs were actually not evoked until later, in the early extension phase. Would you want this trace to be averaged with the traces during flexion or extension? To avoid such errors you can set the delay and also the window to have each trace averaged in the appropriate phase: when the recorded PSPs were actually evoked.

4.1 Set delay and window for cycle triggered trace averaging

Load the c08-01 data file into Analysis. Set the Range for 44 to 94 s and perform a raw waveform display. First you will set the delay based on what you have determined from figures 2.1 and 2.5. From the Top Menu select Set, then Cycles, then In-phase, then Delay. Enter 45 ms for the delay. Then select Window and enter 20 ms for the window. S,C,I,D:45<CR>,W:20<CR>,Q,Q

4.2 Set parameters for cycle triggered trace averaging

You have to select a waveform to be the reference for the cycle triggered averaging. If you have already performed Tutorial 3 and saved the parameters, you

should have waveform 4, LGS, set as your reference waveform. If you do not have the cycles set for LGS and you do not have it as the reference, you should set the cycles (W:4,S,C,V,A,D,M(adjust cycles),D,Q,Q,Q,K4<CR>,Q) and then set LGS to be the reference waveform (S,C,W:4<CR>,Esc)

You also should normalize the cycles for the averaging. Enable Normalize option in the Set/Graph menu (S,G,N:Y,Esc).

Select Set from the Main Menu then Cycles and then Stop-time. Enter NO for stop time. Y doing so, you make the program to look for traces to be averaged that are in the active phases of LGS ENG activity—that is during extension. If you have entered Yes for Stop-time, the program would take the traces collected during the inactive phase of the reference waveform (i.e. that would be flexion in this case). S,C,S:N
Then select Percent from the Set/Cycles menu. Enter 100 for the value. P:100<CR>Esc
By using 100%, you make the program to take the traces collected during the total length of the extensor phase (100%) and use them for averaging. ***Why di we have to set it to the proper % later???

Set the number of bins to be 1 for the averaged data by selecting Set from the Main Menu then Average, then Bins and enter 1. S,A,B:1<CR>Esc You can think of the number of bins as the number of data sets you wish to have or as the number of intervals used during integration. For example if you had it set to be two, then the data for creating the average would be divided up into two sets. You can read more about the function of this option in the on-line documentation.

Check that you have flagged the traces that you wish to exclude from the averaging using the Frmsel program (see Tutorial 2):
M,S,R:10<CR>3<CR>3<CR>D:all<CR>,Y(flag unwanted traces throughout the run),D,Q,Q. The traces you have flagged will not be included in creating the average.
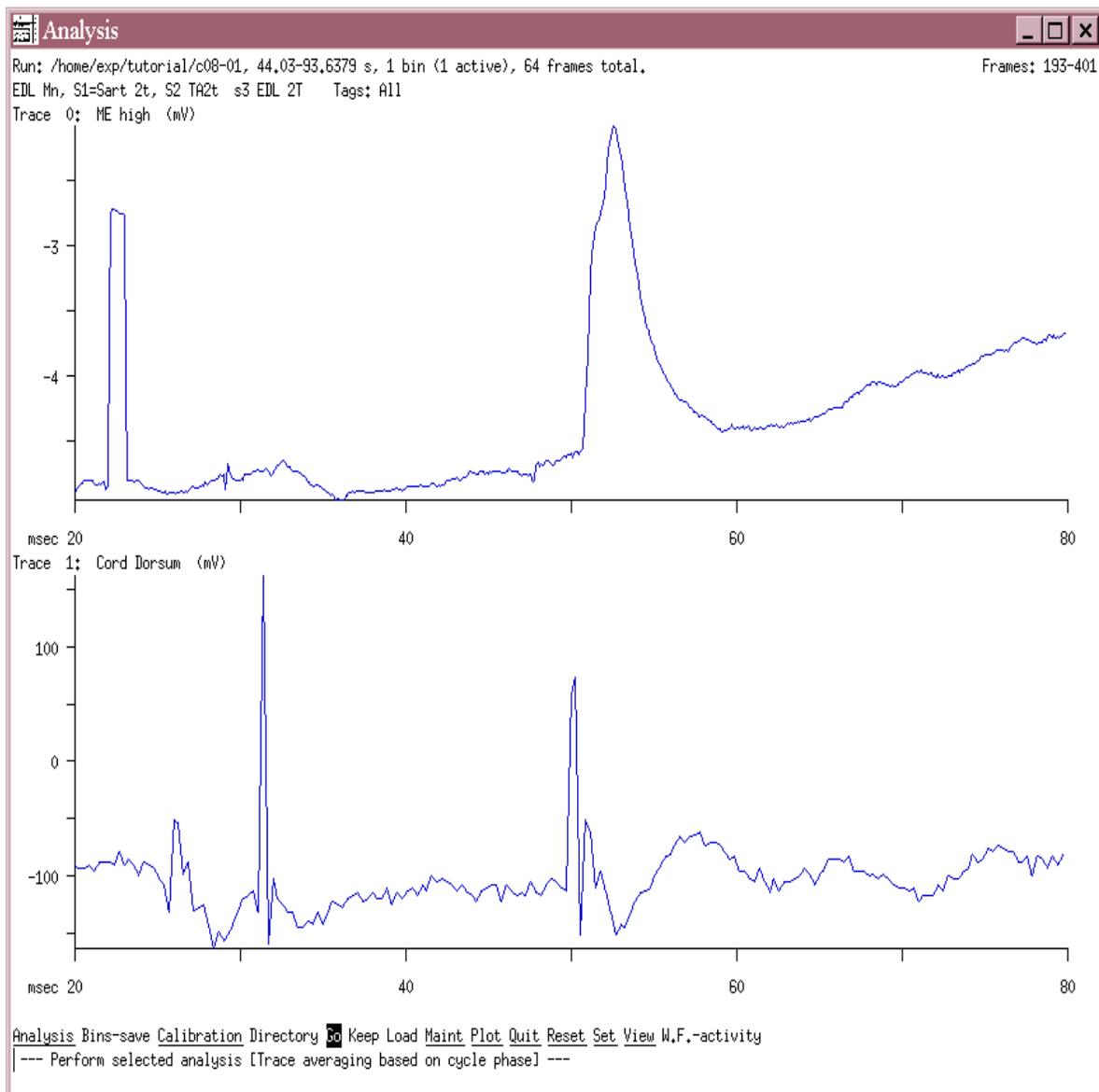
4.3 Perform cycle based trace averaging

 To perform the averaging of the traces during the extension phase, from the Main Menu select Analysis then Trace, then Cycle-triggered, then Go. Esc,A,T,C,G
Now you should see a window that looks like Fig 4.1.

Fig 4.1

This average has been constructed from the traces that were collected during the extension phases occurring throughout the analysis range (not including the ones you have flagged for deletion). At the end of the top row in the right corner of the average display (or Fig 4.1), you should note the number of frames averaged during the extension phase. That should be around 60, depending on how you have set the cycles in LGS.

Next you will perform trace averaging during the flexion phase. From the Main Menu of Analysis select Set, Cycles, Stop-time and enter Yes. Esc,S,C,S:Y,Esc Then do ATCG again. Now you should see the average of the traces collected during the flexor phases throughout the analysis range. Again, in the upper left corner you should see the number of traces included in this average. It should be around 130.

If you calculate the percent of traces collected during the flexion and the extension phases, you will se that about 30-35% of the traces was collected during extension and 65-70% was collected during the flexion. This reflects the ratio that was determined earlier when you constructed the waveform amplitude averaging (because the frequency of stimulation (i.e. the frequency to capture a trace) was constant during the whole run.

## 4.4 Number of bins during the step cycle

Previously when you set the parameter for the number of data sets you wanted for the trace averages (in section 4.2) you set the bins to 1. The Analysis program is able to do the cycle triggered trace averaging in as many intervals during the step cycle as you wish. There may be one problem with selecting a large number (>200) for the value of bins. If the number of bins is large, the step cycle is divided up into lots of small periods during which there may not be any traces captured, so the averaging would not be possible (i.e. that bin would not have any value associated with it). You should arbitrarily select a number for the bins when you wish to do that during the analysis of a data set. Then you should make the average and by displaying it you will be able to tell whether you have too many bins and whether you have to increase or decrease the value of the

bins. In this analysis, we have chosen 6 to be the number of bins for the averaging. That means that the step cycle will be divided up into 6 periods. Since the ratio of flexion and extension is about 65% to 35%, there should be 4 bins for flexion and 2 bins for extension. You should set the number of bins to 4 and perform cycle-triggered trace averaging for flexion. To set the bins, select Set from the Main Menu, then Average then Bins and enter 4. Then Esc back to the Main Menu. S,A,B:4<CR>,Esc Next you should make sure that the Set/Cycle/Stop-time is yes, so that averaging is performed for the flexion phase. Do S,C,S:Y,Esc. Then do ATCG. You should see a window as shown in Fig 4.2.

Fig 4.2
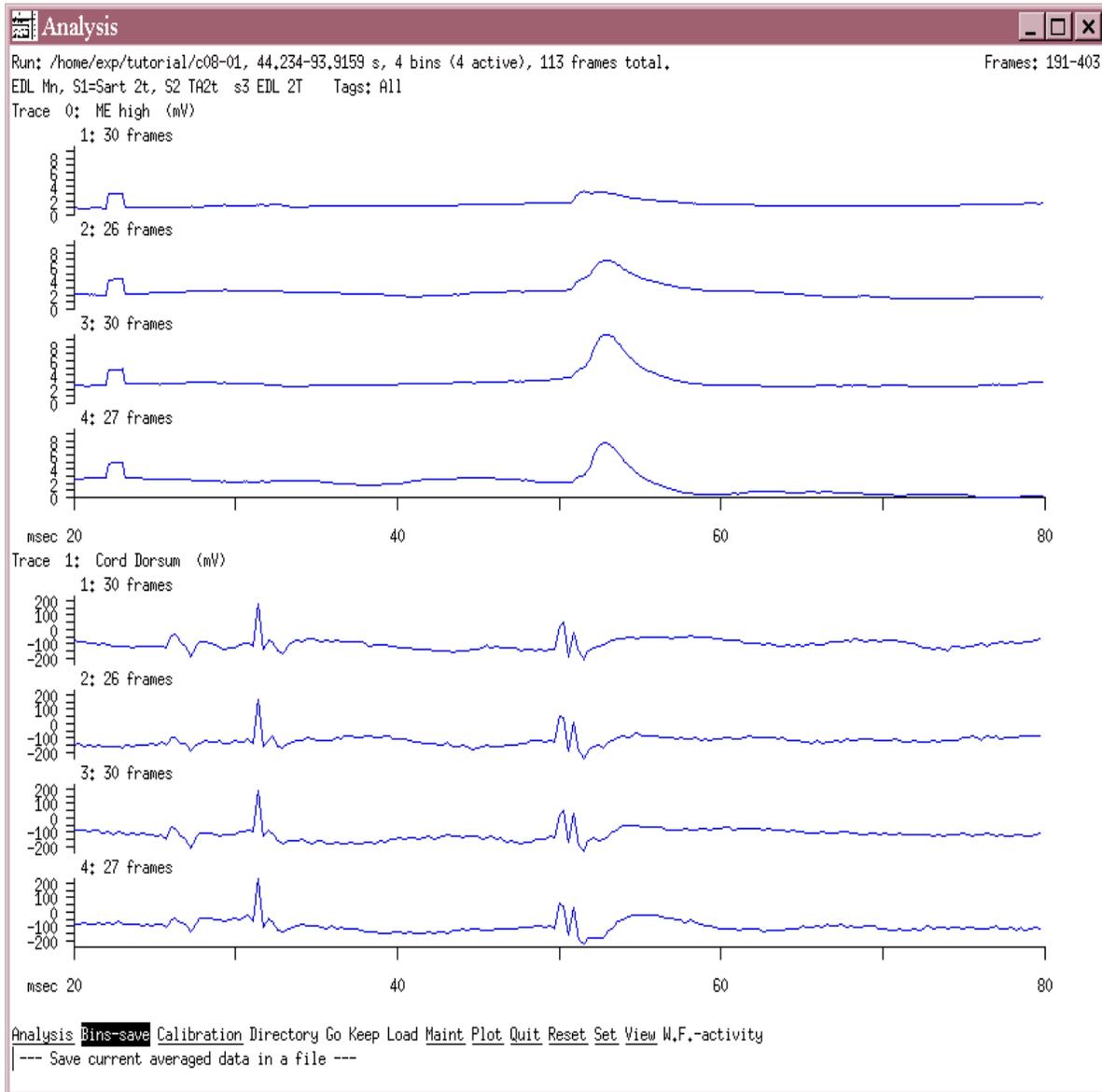
Save the averaged data by using the Bins-save option. From the Main Menu select Bin-save and enter the following for the name of the file: "08-01ina45". Accept the run description that is prompted (it should say the same as the descriptor of c08-01 file). This file name is intended to describe the followings: it has the name of the data file (*08-01*), it has the phase during which the averaging was performed (*ina*, i.e. flexion since an extensor (LGS) is the reference waveform), and it has the value of the delay (*45* ms).

Next set the number of bins to 2 and perform the trace averaging for the active phase (extension). From the Main Menu select Set, Average, Bins and enter 2. S,A,B:2<CR>Esc Then perform A,T,C,G. Then save this average as "08-01act45" using the Bin-save option from the Main Menu.

Next you will combine these two files in order to view them at the same time and take measurements on the PSPs recorded in the motoneurons. You should quit analysis program and save the parameters that you have entered last. Q,Y,Y,<CR>