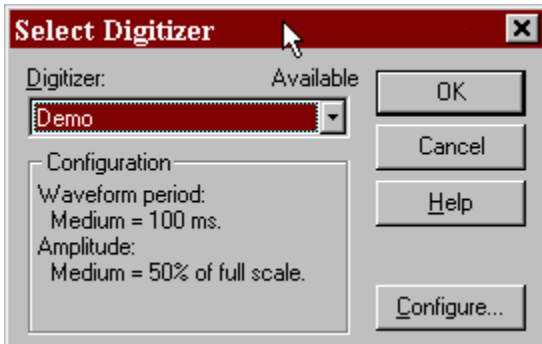


## Creating and editing a Stimulus File:

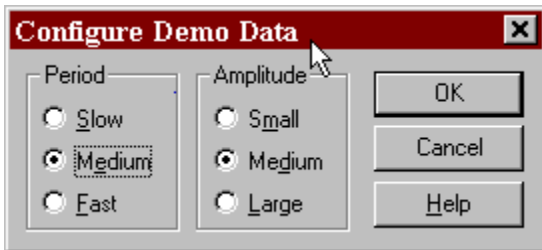
For the purpose of this tutorial, Clampex will be used in the Demo configuration.



*The demo driver behaves differently depending on the acquisition mode*

In episodic mode, it is related to the output waveforms:

1. IN 0 is a noisy version of the OUT 0, IN 2 is a differentiated version of IN 0 etc.
2. If there is only one output channel, IN 1 is an inverted version of IN 0, IN 3 is an inverted version of IN 2 etc.
3. If there are 2 output channels, IN 1 is a noisy version of OUT 1 and so on for the other odd numbered channels.



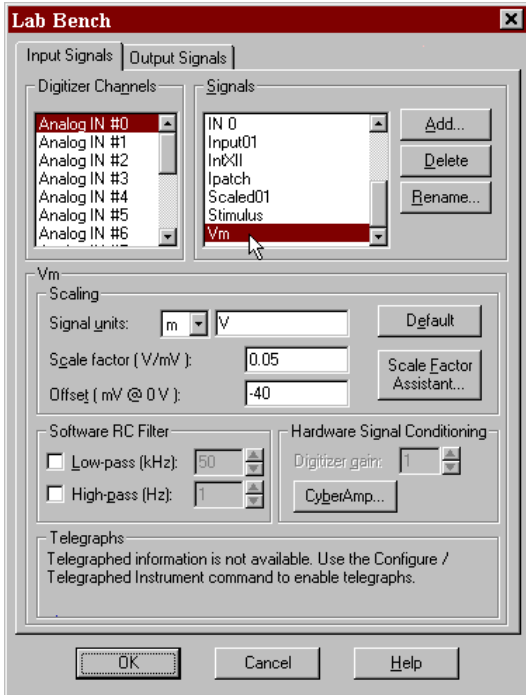
In all other acquisition modes:

1. IN 0: An AP - like waveform
2. IN 1: Square wave
3. IN 2: Sine Wave
4. IN 3: Ramp

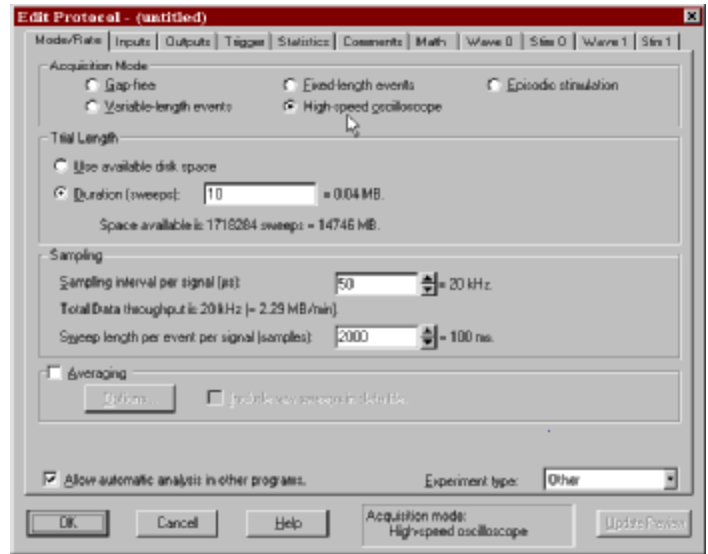
This sequence repeats for channels IN 4 .. IN 15. This behavior is unchanged from earlier versions of Clampex.

The Demo protocol that was used for this exercise was set up in the High-speed oscilloscope such that it would produce an action potential (AP) like waveform. To mimic a cell that is resting near 76 mV, inside negative with respect to a bath defined as ground, and firing APs that rise to near 60mV, inside positive, it was necessary to create a signal in the Lab Bench which designated Vm. The scaling and offset were chosen to most closely simulate the conditions noted.

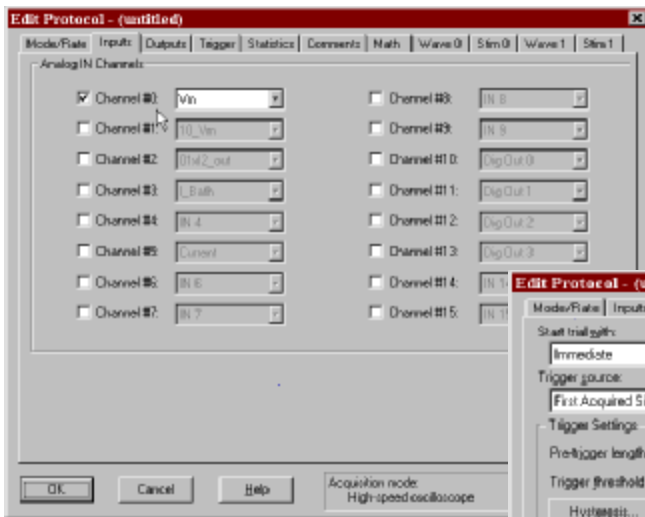




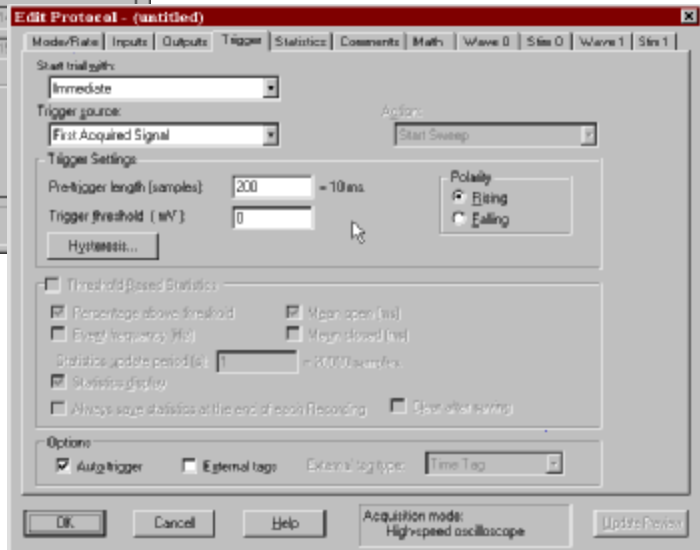
The protocol that was used to mimic collecting data from a real cell was set up in the High-speed oscilloscope mode, sampling at 20 kHz and collecting 10 sweeps.



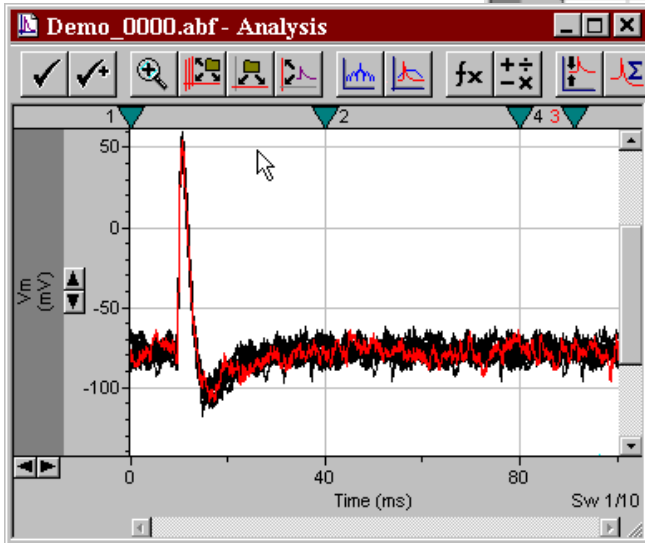
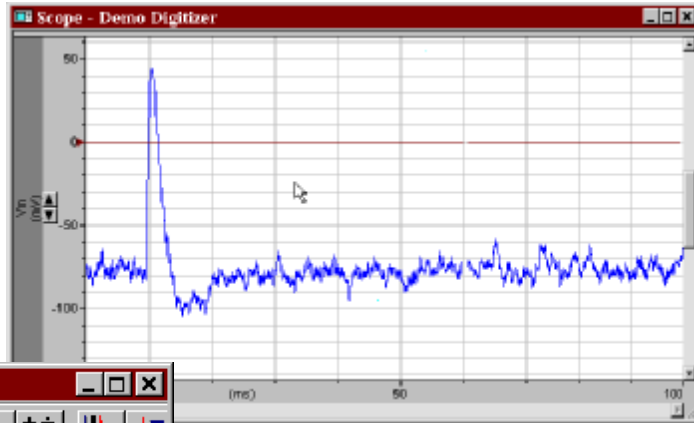
The signal created in the Lab Bench for Analog input Channel #0 was chosen in the protocol as the signal of interest for this experiment.



Since the protocol is in the oscilloscope mode, the number of sample points before a triggering threshold can be set on the Trigger page. In this case, 200 sample points were chosen as the Pre-trigger length (10 ms.)

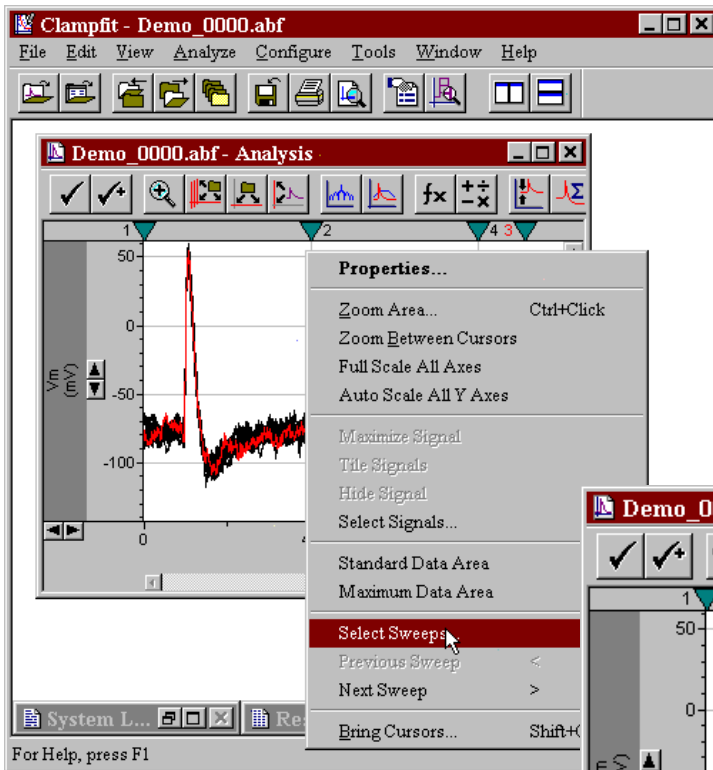


When the protocol is run in the view only mode (Shift + F2 or button), the Trigger level can be adjusted such that the events are synchronous, which is important since the 10 sweeps collected will be averaged. One trial was recorded and the data file was named "Demo\_0000.abf"

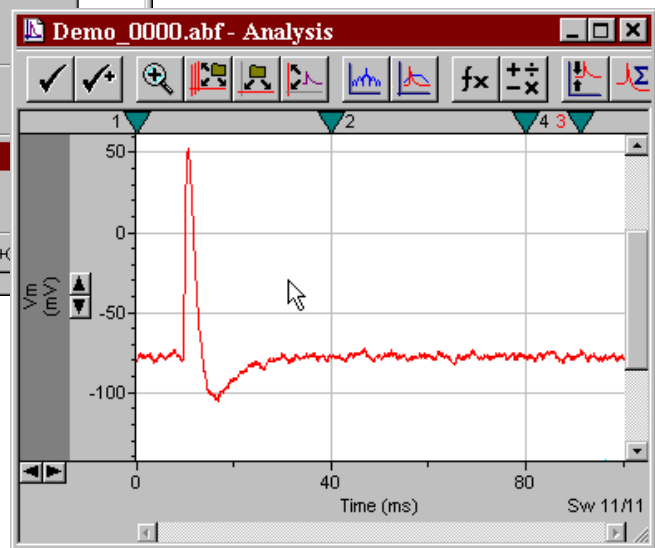


The data file, "Demo\_000.abf", was opened in Clampfit. To reduce the noise associated with the waveform, the 10 sweeps were averaged by selecting Analyze/Average Traces from the drop down menu.

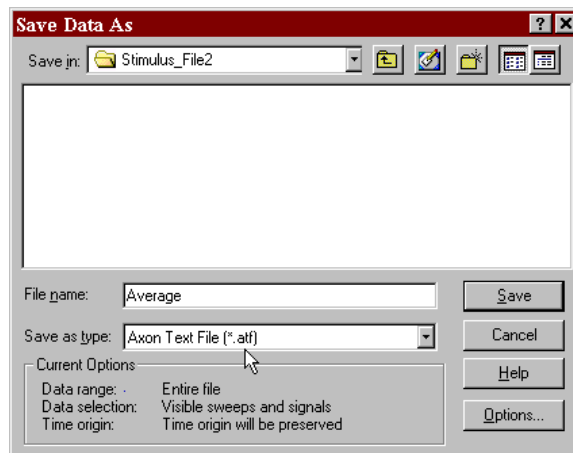




The 10 sweeps were averaged generating an 11<sup>th</sup> sweep in this data file. To view this sweep independently of the other sweeps, place the cursor in the white space of the Analysis window and then right-click. From the right-click menu, select “Select Sweeps”. From the Select Sweeps dialog, highlight sweep #11.

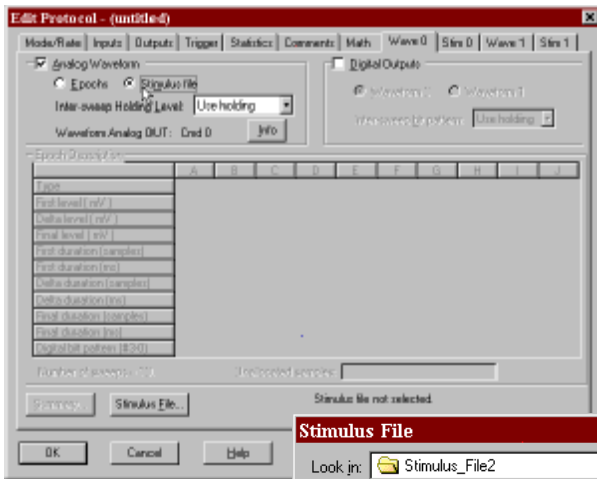
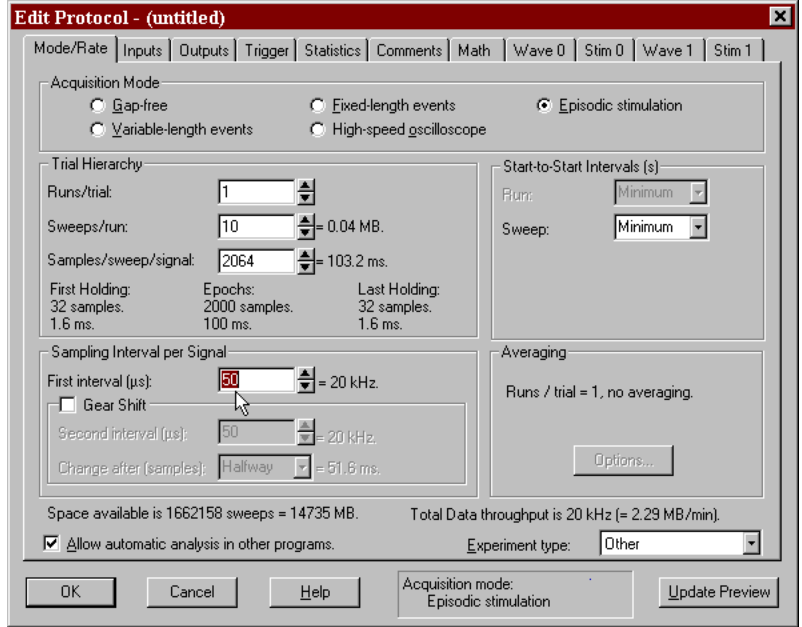


Any file in the axon binary format (\*.abf) or the axon text format (\*.atf) may be used as a stimulus file. Since this file will be manually edited later in Excel, it was saved in the axon text format. Only sweep #11 was visible at the time that file was saved, therefore only sweep #11 will be in the new file named “Average.atf”.



To use this averaged data file as a stimulus waveform, it is necessary to create a new, episodic protocol.

To maintain the time base of the original waveform, the sampling interval was set to match the sampling interval originally used, 20 kHz. *Nota Bene*, the sampling interval of the episodic protocol and the number of samples per sweep will determine the time base of the stimulus waveform, **not** the time base of the original file. Since the file “Average.atf” contains only one sweep, the Sweeps per run should be set to 1.

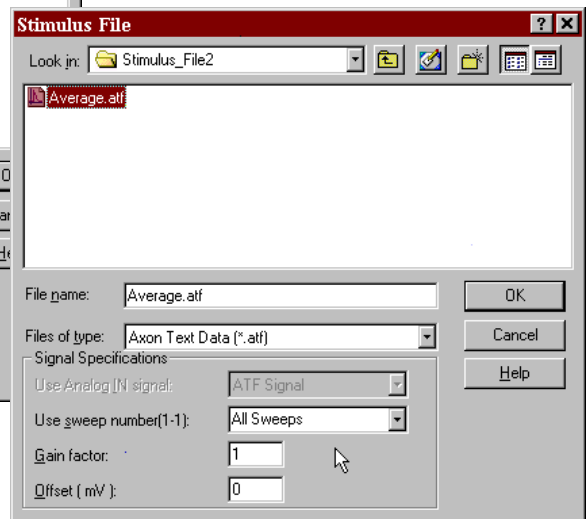
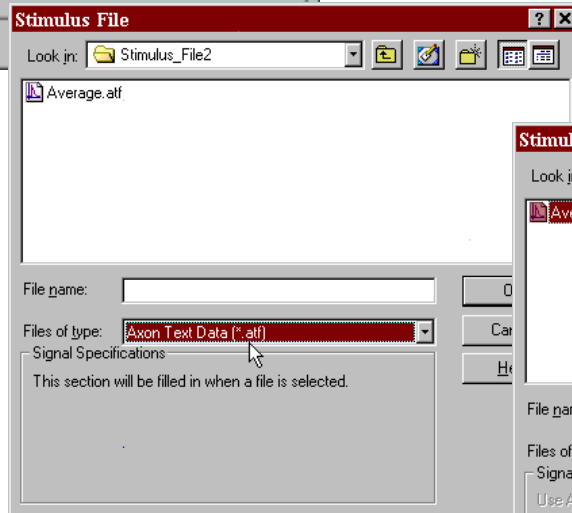


On the Wave 0 page of this new protocol, the Analog Waveform was set to use a stimulus file instead of Epochs defined in the table. When the Stimulus file is selected as the waveform, the Stimulus File button becomes active.



Clicking the Stimulus File button brings up the Stimulus File dialog. Once the file of interest is selected, the signal specification fields are populated.

Note that the file Average.atf has only one sweep.

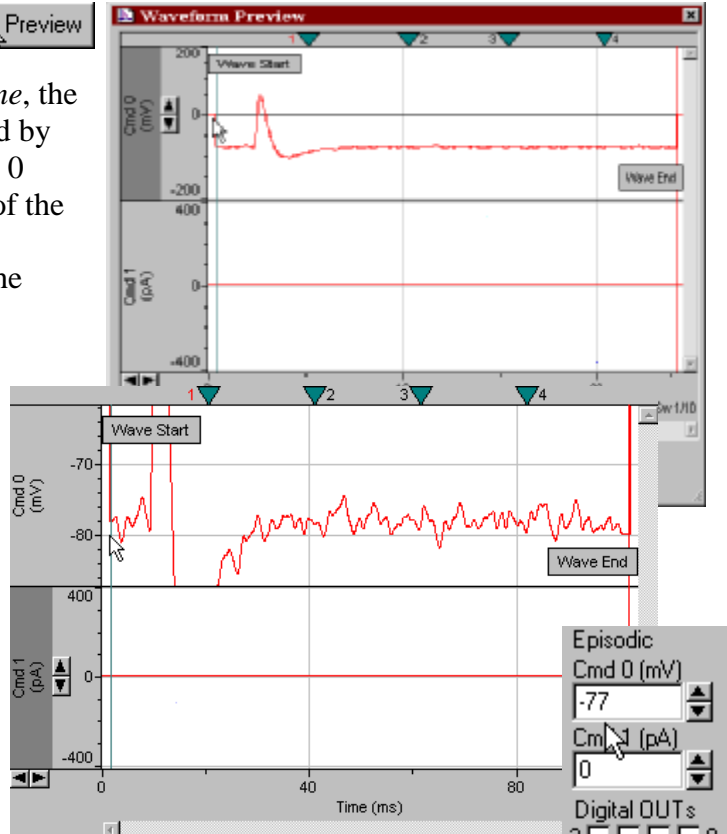


To see what the waveform will look like when executed, click on the Update Preview button. *Nota Bene*, the amplitude of the waveform is determined by data in the stimulus file, **not** by the Cmd 0 scale factor, however the interpretation of the command waveform by the amplifier is affected by the Cmd 0 scale factor and the status of the amplifier.

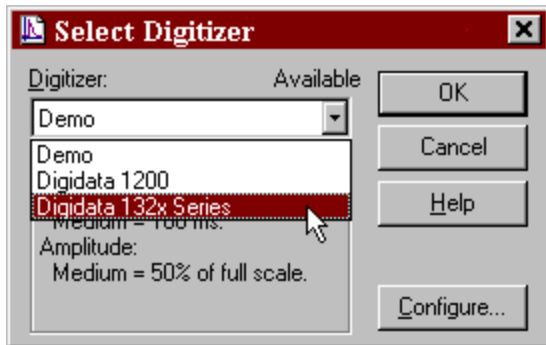
In this case the holding command is set to 0 mV and, in the episodic mode, Clampex collects a few data points at the initial and final holding level. Therefore in the waveform preview there is a step from 0 mV.

To set the holding level closer to the baseline level of the event, use the real time control panel (RTC) to set the holding level to -77 mV or so.

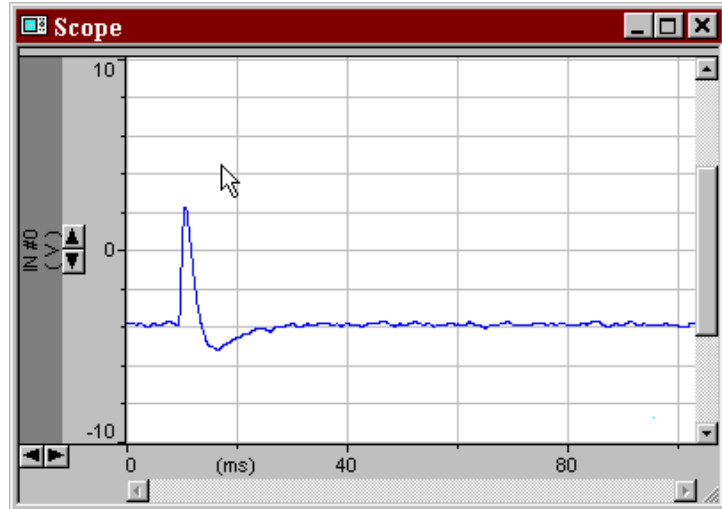
Update Preview



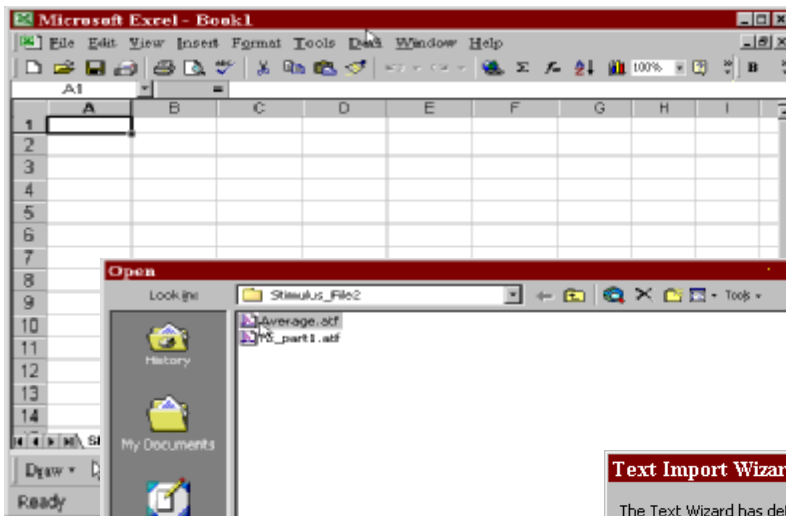
To determine what the waveform will actually look like as a command signal going to the amplifier, connect a BNC cable from Analog Out #0 to Analog In #0. In Clampex, Configure the Digitizer. In this configuration, the data collected when a protocol is executed is simply the command waveform.



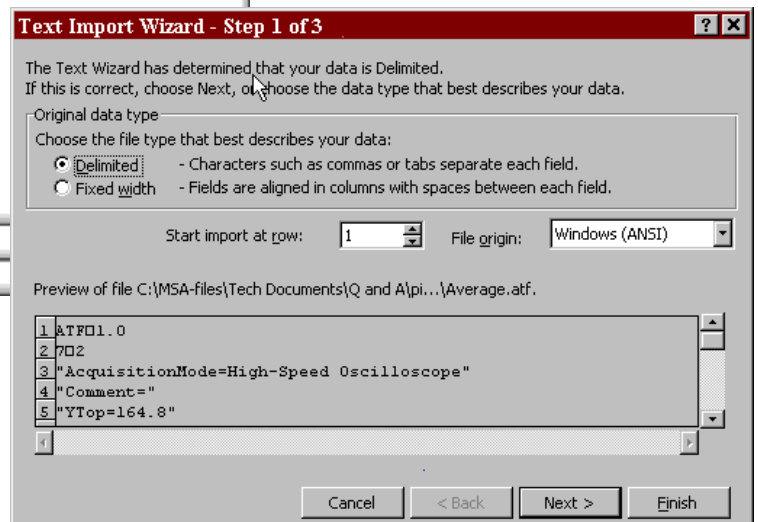
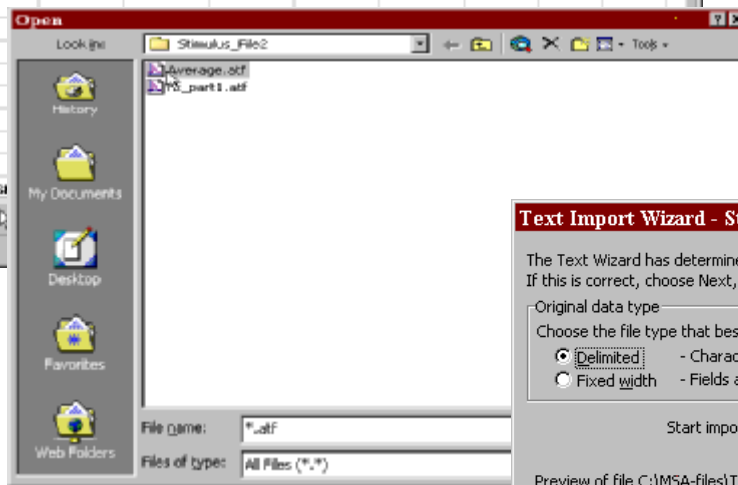
When the digitizer is configured to feedback on itself (OUT #0 to IN #0), the data collected is simply the command waveform. In this case the scaling for the signal "IN #0" was set to 1V/V. Since the Cmd 0 output signal has a scale factor of 20mV/V, the holding level of -77 mV is in fact a command of -3.85V, which is what is seen at IN 0.



Note that even though this command waveform is the average of 10 events, the data is still a bit noisy. Clampfit could have been used to further filter the data, however care needs to be exercised since filtration may alter the critical part of the event (the AP). Since the data file was saved in the axon text format, one can use MS Excel to manually smooth out the holding levels.



When an ATF file is selected as the file to open in Excel, the Text Import Wizard will open. The default settings are usually sufficient.



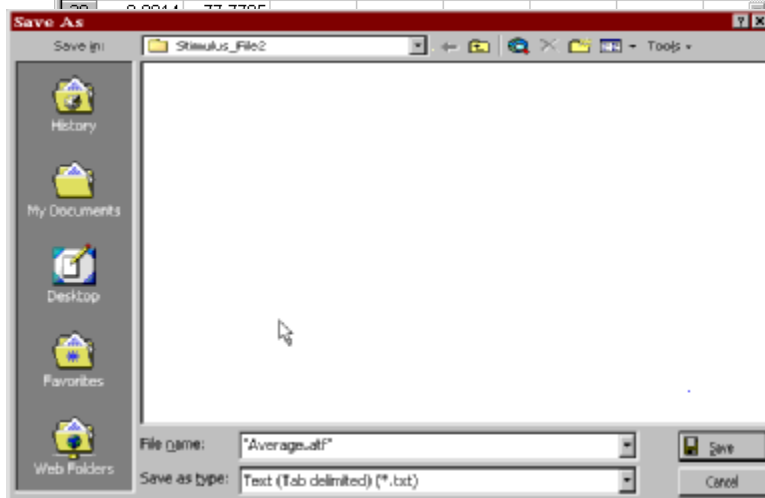
	A	B	C	D	E	F	G
1	ATF	1					
2	7	2					
3	AcquisitionMode=High-Speed Oscilloscope						
4	Comment=						
5	YTop=164.8						
6	YBottom=-244.8						
7	SweepStartTimesMS=1102.900						
8	SignalsExported=Vm						
9	Signals= Vm						
10	Time (s)	Trace #1 (mV)					
11	0	-79.9648					
12	5.00E-05	-79.882					
13	1.00E-04	-79.7773					
14	1.50E-04	-79.6468					
15	2.00E-04	-79.4878					
16	2.50E-04	-79.2992					
17	3.00E-04	-79.0825					
18	3.50E-04	-78.8427					
19	4.00E-04	-78.5875					
20	4.50E-04	-78.3275					
21	5.00E-04	-78.0744					
22	5.50E-04	-77.8395					
23	6.00E-04	-77.633					
24	6.50E-04	-77.4625					
25	7.00E-04	-77.3332					
26	7.50E-04	-77.2437					
27	8.00E-04	-77.1993					
28	8.50E-04	-77.1931					
29	9.00E-04	-77.2191					
30	9.50E-04	-77.2692					
31	0.001	-77.3347					
32	0.00105	-77.4069					
33	0.0011	-77.4785					
34	0.00115	-77.5441					
35	0.0012	-77.6012					
36	0.00125	-77.65					
37	0.0013	-77.6933					
38	0.00135	-77.7348					

The first few lines of an ATF file contain header information. *Nota Bene*, when a data file is saved in the ATF format, a significant portion of the original header is lost.

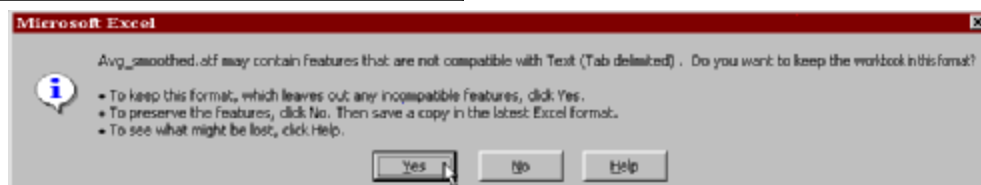
The first column contains the time base of the data. When the High speed oscilloscope protocol was created the first interval (i.e. the time between any two data points) was set to 50  $\mu$ s (0.00005 s), alternatively put the sampling rate was 20 kHz (20,000 data points per second). Each sweep contained 2000 data points (i.e. 100 ms).

The second column of data is the signal of interest. This is the data that one wants to ‘smooth out’.

From 0 to 0.0094 s (row 10 – 200) the level was set to a flat  $-76$ , likewise the post-event level. The after hyperpolarization was smoothed out in Clampfit using a 3<sup>rd</sup> order exponential fit.



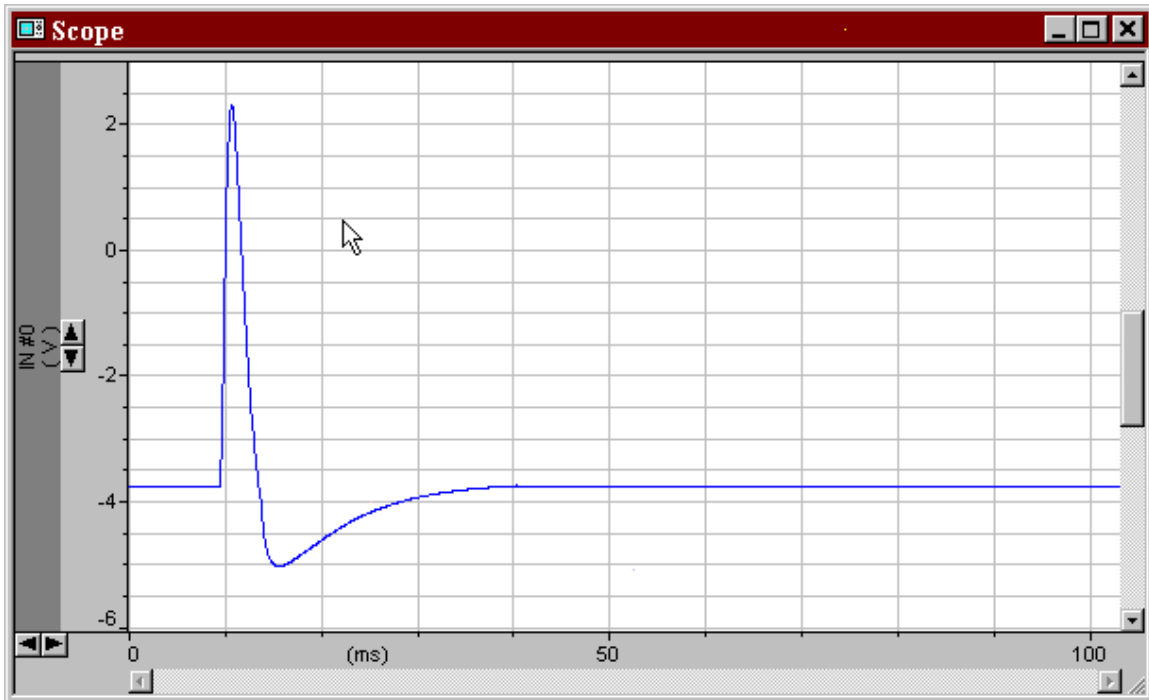
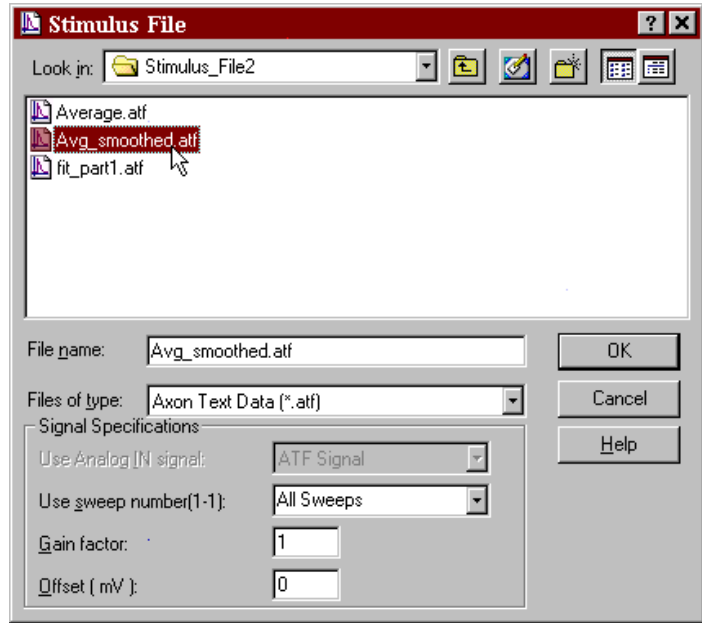
When saving an ATF file after editing in Excel, remove the quotation marks. In this case, “Average.atf” was replaced with Avg\_smoothed.atf. After clicking the Save button, a warning message will appear, click Yes. A similar warning may appear when you exit Excel.





A new, episodic protocol was created using the file which was edited in Excel, Avg\_smoothed.atf, as the stimulus file.

When the command waveform is 'previewed' by sending the Analog Out to the Analog In, the signal is now much 'cleaner'.



Clampex version 8.2.0.228  
Microsoft Excel 2000, build 9.0.3821 SR-1.