



Training Exercise 1:

Introduction to the Computer Software: DataPro

1. Don't Panic. When you run DataPro, you will see a large number of windows, buttons, and boxes. In fact, it isn't as complicated as it first appears, and you will not even need to use many of the features of the program.

2. What is it? DataPro is a series of software routines developed by Dr. Nelson Spruston of Northwestern University. It is designed to work within the IGORPro programming environment. IGOR Pro has its own high-level programming language, which bears some resemblance to the C programming language. The strength of DataPro is that it has been designed expressly to do the kind of experiments that you will be performing in the laboratory. *You do not need to know anything about programming, but you will need to learn the basics of operating DataPro.*

3. What does it do? DataPro records the signals that are measured from recorded cells with your Dagan amplifier. One can also inject currents into the cell to excite it, and the user has control over the amplitude, duration, and even the shape of the current injection. You can also trigger other pieces of equipment to be synchronized to the start of a period of data collection. You will eventually use this feature to trigger a stimulator to apply small shocks to synaptic inputs.

4. DataPro Panels

DataPro Main (located at the upper left of the screen). When you switch between voltage and current-clamp modes, one must check the appropriate box. This does nothing to the recording. It merely changes some settings that the computer program uses to keep track of, for example, how much a signal is being amplified by the amplifier (the amount of amplification is different in voltage and current clamp modes...they involve different electronic circuits).

ADC/DAC Control (located at the bottom of the screen). This panel keeps track of various settings on the amplifier so that the program can assign the right values to the recorded electrical signals. *You do not have to change anything in this panel. Mess with it, and you will surely be sorry that you did.*

Test Pulse Panel (located at the upper right). This panel is used to monitor electrode resistance in the bath, and during seal formation during patch recordings. A pulse is

delivered to the recorded cell repetitively at high frequency. Its amplitude can be controlled either by toggling between two preset values (boxes marked “low” and “high”), or specific values can be entered into the windows. The duration of the pulse can be controlled in the same manner with the “short” and “long” check boxes and windows. The recorded traces are continually displayed in the window below, but are not saved to the computer. The program calculates the resistance of the electrode using Ohm’s Law and displays the value in the window.

Data Acquisition Panel (located middle-left). This panel allows is divided into 2 parts: StepPulse_DAC, and SynPulse_TTL.

StepPulse_DAC allows simple steps of current or voltage to be injected into the recorded cell. The amplitude and duration of the pulse is controlled in the same way as in the Test Pulse Panel. For the curious, DAC stands for digital-to-analog converter. A digital waveform is being converted into a continuously variable analog signal, and injected into the cell.

SynPulse_TTL triggers a stimulating electrode to deliver an extracellular stimulus, or shock, to axons synapsing onto the recorded cells. the time and duration of the *trigger pulse* is set by the software. Note, however, that the amplitude and duration of the *shock* is set by the controls on the AMPI stimulus isolation unit.

Step pulses and synaptic pulses may be delivered at the same time. *One starts the stimulus delivery and data acquisition by clicking the “Get Data” button.* Any procedure that is checked (step injection or synaptic stimulation) will be performed.

DataPro Tutorial, Using the Software

Goal:

To familiarize you with the operation of the electrophysiological equipment and the data acquisition software (IGOR and DataPro). By the end of the tutorial you will be able to make electrophysiological measurements, perform simple analyses, and construct meaningful graphs and layouts of the results.

Experimental Setup

1. In the equipment rack, turn on the amplifier, letting it warm up.
2. Start DataPro by double clicking the “startup” icon on the desktop. This should bring up the many windows that allow you to “communicate” with your recording.
3. Immediately save the experiment as an “unpacked experiment”, giving it a unique and informative name, such as “lab1” or Sept5_07. The save options are under the *File* heading in the bar on top of the screen. Save the file in the folder marked “Student Data”.
4. Attach your model cell to the headstage of the amplifier.

The blue box attached to the manipulator is the headstage of the amplifier, which contains electronics that provide the first stage of amplification of the small electrical signals you will eventually be recording. Your model cell is merely a collection of

resistors and capacitors wound together and soldered to the connector on the model cell (also in a separate blue box).

Exercise 1:

1. Make sure the amplifier is in “Current-clamp” mode. This is a circuit that will allow you inject current (in pA), and measure voltages (in units of mV). On the Dagan amplifier, there is a simple toggle switch.

2. Ensure that the “Bridge Balance” and “Capacitance Compensation” dials are turned all the way off (counterclockwise), then set the “Monitor Source” switch to “Vm”. Finally, adjust the “Offset” dial until the meter reads 0 mV.

3. In DataPro, on the computer screen: click on the “Current clamp” box in the *DataPro Main* panel, which engages the settings appropriate for this recording mode.

4. Start the testpulse (TestPulse Panel). This injects pulses of current into the model cell at a high rate of repetition. The program measures the voltage change at the end of the pulse, and, using the amplitude of the current injection, calculates the resistance using Ohm’s law. Note that the resistance reading is displayed in gigaohms, which is 1000 MΩ. Now play with the TestPulse controls. Switch between the two preset amplitude and duration controls. Write in values of your own. Does the resistance reading change? Why or why not?

5. Stop the testpulse by pressing the spacebar, and holding it down until the pulse stops. Note no data is saved to the computer when the testpulse is used. The waveform now displayed in the testpulse window will be overwritten when you next deliver the testpulse. This feature is useful because it allows you to calculate and monitor your electrode resistance without filling your hard disk up with mind-numbing amounts of useless waveforms.

6. Now collect real data for storage on your computer.

Going to the *Data Acquisition Panel* in DataPro, write in the following settings:

Amplitude (“Pulse”), (high): 100 (pA)

Duration (long): 100 (ms)

Click the button, “Get Data”. Observe that a wave, also called a trace or sweep, appears in the *Data Acquisition Display* window. The number of the wave collected is displayed in the small box called “Sweep”.

****IMPORTANT NOTE*****

In DataPro, all units of current are always in picoamps ($1\text{ pA}=10^{-12}\text{ A}$), and units of duration are in milliseconds (ms). Units of voltage are millivolts (mV). For reasons of space, the units are not listed next to the numerical values in DataPro panels.

7. Make a new graph with your new data in it. Click on the “Windows” menu item at the top of DataPro and hold down the mouse to see a submenu of commands. Slide your pointer (without letting go of the mouse button) to select the first item down in the list, which is “New Graph...”, and let go. In the window that pops up, go to the list of “Y waves”, and scroll to the bottom. Click on the last item in the list, which should be “ad1_1”. Get help if the name at the bottom is different. The wave ad1_1 is the first wave collected on analog-to-digital channel 1, and will form the Y axis on your new graph. The “X wave” does not have to be selected explicitly. The program default for the X axis is time (in ms), which is what you want here. Now click the “Do it” button to make the graph.

8. Play with the graph display. Double click on top of the wave to bring up a window that will allow you to change its appearance (adjust line size, change color, etc...). Now double click on either axis to bring up a bewildering array of options to modify how the graph looks. Don't worry, you will generally ignore all of them except two: *axis label*, and *axis range*. Note you can apply changes to x and y axes independently.

9. Add notes or drawing items to the graph. On every graph you can access a drawing layer, where lines, arrows, text and simple shapes can be drawn superimposed on the graph. *Nothing drawn in this layer physically affects the data*, and it can be easily removed. It's like putting a marked-up transparency over a data-containing piece of paper. Type Apple and “T” keys simultaneously to enter or exit the drawing layer.

10. Make simple measurements. Press simultaneously Apple and “I” to enter and exit “information mode”. At the bottom of the graph on the left side there are two shapes, a circle and a square. You can drag these cursors with your mouse anywhere onto your wave, and the x and y values of that particular point will be displayed. If both cursors are placed on the graph, the information at the bottom of the graph will also display the difference between the two points selected. These cursors can be used to make measurements quickly and easily. *Measure the amplitude of the response of the model cell to the 100 pA current pulse you just injected.* Do this by placing Cursor A at the baseline of the voltage response, and placing Cursor B on the graph where the voltage response reaches steady-state. Using Ohm's law, calculate the resistance of the model cell, and record the resistance in your notes, along with the identifying number of your model cell (the white label on the blue box).

11. Fit an exponential to your data to determine the time constant of the model cell's voltage response. You will need to consult the appropriate tip sheet from the course web page. Once you have a figure that displays your recorded sweep and exponential fit line, export this figure as a PNG file (you will use this graphic file in your blog entry for this week). Again, you will need to consult the appropriate tip sheet from the course web page.

12. Take additional sweeps, using pulse amplitudes of 200, 300, 400, and 500 pA, and measure the amplitude of each voltage response (make a note of the values). You

should have five waves now stored. Make graphs of these responses and measure their amplitude using the cursors (Apple “I” to make them appear on the graph).

13. Plot all five waves on the same graph, label the graph’s axes, and indicate with text the stimulus current associated with each trace. Consult with your instructor or TA for ways to improve your graph.

14. Export this graph as a PNG graphics file. Consult the appropriate “Tip Sheet” on the course website. You will use this file for this week’s blog entry.

15. Make a table by selecting the menu item “Window”, then “New Table...”. Do not make any further selections. Just click “Do it” in the pop-up window to create a new empty table.

16. Enter data into the table. In the first column, enter the values of the 5 current pulses you just delivered to the model cell (100-500 pA, in 100 pA steps). IGOR will assign the name wave0 to this one-dimensional array of points. Now click on the first line of the empty column of points in the next column to the right, and enter in the values of the corresponding voltage changes you just measured.

17. Make a graph of wave1 vs wave0. Select wave 1 as the Y wave (which should be at the bottom of the list of waves), and select wave0 was the X wave (also near the bottom of the list). Click “Do it”.

You should see a red line that increases linearly. Get help if you do not. You may add labels to the axes by double-clicking on the desired axis (“voltage” for the y axis, “current” for the x axis).

18. Get some data from another lab group. Consult with one of the other rigs to get their data for the voltage response of their model cell to stimulus intensities of 100, 200, 300, 400, and 500 pA. Enter the measured responses in your data table in a new column (it will be assigned the name Wave 2). Calculate the resistance of the new model cell, and make a note of which model cell these data came from.

19. From your table, plot Waves 1 and 2 vs. Wave 0 on the same graph. Label the axes, and label each line with the number of the Model Cell that produced those data. Consult with your instructor or TA for ways to improve your graph.

20. Export this graph as a PNG file.

21. You should now have three graphs stored as PNG files. One contains a single sweep with an exponential fit line, another contains the five sweeps of data from your first model cell, and the second contains a plot of voltage versus current for two different model cells you used.

22. Everyone in your group should now log in to Blackboard and join the appropriate group for your rig. Select “Communication” from the main navigation menu, then select

“Groups”. You can now view the sign up sheet and join the group corresponding to your Rig number.

23. Upload your three graphics files into the “File Exchange” area for your group in BlackBoard. You will insert these files into your first blog entry.

24. Congratulate yourselves. You have already made a lot of progress in learning how to use your electrophysiology rig and software!