PULSE Tutorial: The first Experiment

This chapter will guide you briefly through the main features of the *PULSE* program and should take you a maximum of about 2 hours to read it. It briefly describs how a very simple first experiment with *PULSE* could look like. Of course, you will not have to do a *real* experiment, instead you shoud use the model circuit to *simulate* the conditions of a patch-clamp recording. The reader should not worry about options that are unclear, because more detailed descriptions of all of the mentioned steps are to follow. This section is thought for users that can't wait to get something done with *PULSE*. The basic requirements for starting the program and for doing a simple experiment are outlined. For more detailed descriptions of the features, refer to the later sections in this manual.

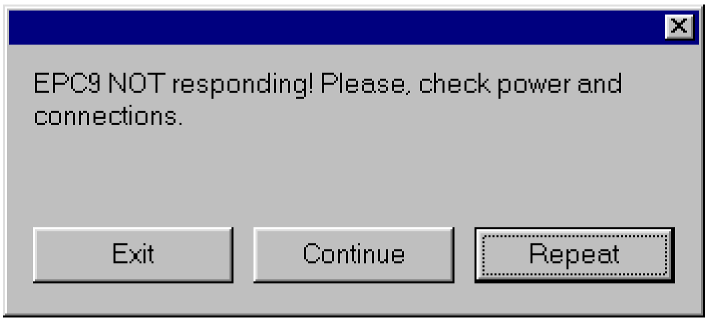
Note: In the following it is assumed that the hard- and software has already been set up correctly. Please refer to the chapter "Software Installation" for the installation of PULSE+PULSEFIT. If you plan to use the EPC9 Double or Triple you should also first read the Chapter "Setting up the EPC9" to get an idea of the basic amplifier operation.

Step 1: Starting *PULSE*

If you have a model circuit (*MC8* or *MC9*), connect it to the probe input of your amplifier via a BNC adapter and plug the black cable to the black ground connector on the probe. Turn on the amplifier, the computer and - if you don't have an *EPC9* amplifier - the AD/DA interface (*ITC-16*, *ITC-18,* or *Digidata 1200*). To start *PULSE* double-click on **Pulse.exe** (Windows) or Pulse+PulseFit (MacOS) which is located in the Pulse folder inside the HEKA folder. Windows users might alternatively use the **Start** button to launch *PULSE* from **Programs** → **HEKA**. Since *PULSE* and *PULSEFIT* are combined into one application, you will be asked to select which part of the program you wish to load. PULSE is used for data acquisition and PulseFit for data analysis.

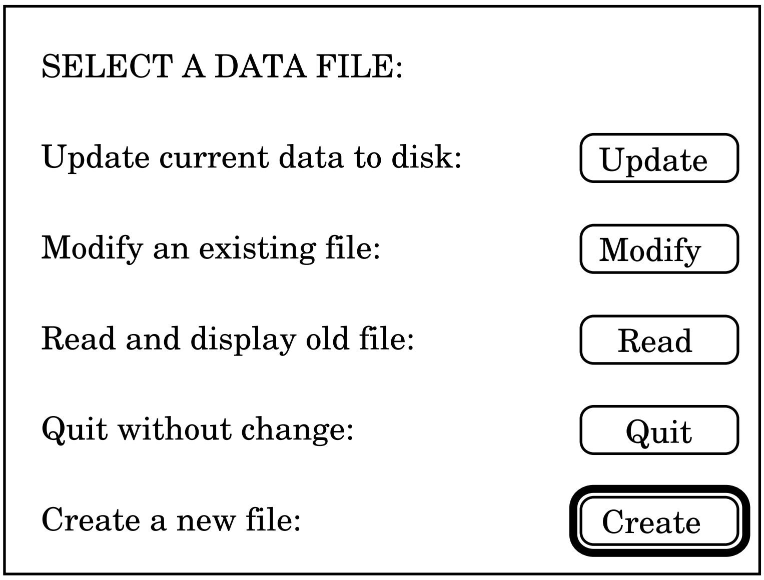
When *PULSE* starts it will first look for its *Configuration File* that contains all of your individual program settings. This file is called DefaultPulse.set and resides in the same path as *PULSE*. If this file does not exist, *PULSE* will come up with an error message and will give you the opportunity to either locate the file (Find File) or to use default settings (Use Defaults). If you do not have a configuration file or wish to reset to the factory settings, select Use Defaults. In this case, *PULSE* will try to detect your hardware and generate reasonable settings.

PULSE will recognize, whether you have not connected any AD/DA hardware, and will ask you to abort (Exit), to continue (Continue), or to try again (Repeat). If you just forgot to turn on the power on the *EPC9* or the AD/DA converter, do so and select Repeat. If you don't have either of them, you still can select Continue, i.e., *PULSE* will continue to run in the so called *"Demo Mode"* that allows you to test the software and analyze the data. Of course, data acquisition will be disabled in *Demo Mode* but opening, saving and printing files, as well as creating and modifying pulse protocols will be possible.



Based on your configuration file, *PULSE* will now look for file paths and a default *Pulse Generator File (PGF)*, that contains your stimulus protocols. The factory default is the file DefPGF.pgf in the Data folder inside the HEKA folder. If *PULSE* cannot find your PGF file, it will write a message into the *Notebook* window and will create a default file with only one entry called *"Test"*. There may be other paths missing and *PULSE* will put up an alert to that effect. You can safely ignore that error message, we will setup these paths next in the *Configuration* window (see below).

After loading its configuration and pulse generator file, *PULSE* will ask, whether you wish to create a new experiment or just want to analyze some data:



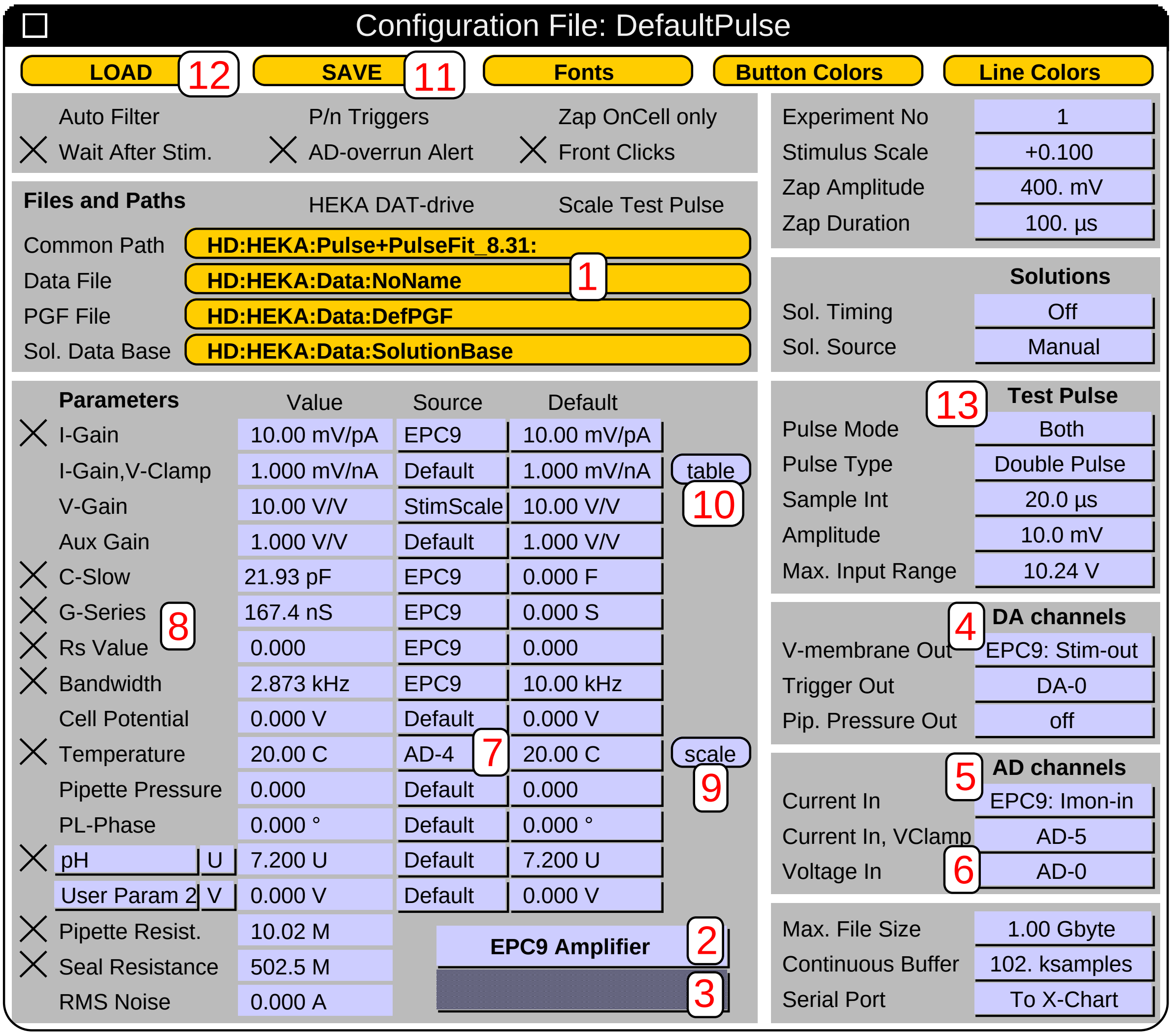
There are five possibilities:

- Modify opens an existing experiment for modification, i.e. you can delete or add further experimental data to a file.
- Read opens an existing experiment. The file will be write protected, so that modification (or loss) of the data is prevented.
- Quit cancels the dialog (or quits the program if you were starting *PULSEFIT*).
- Create allows you to create a new experiment file.

Select the Create option to start with a new experiment. You can call the file whatever you like, e.g. Tutorial.dat.

Note: A PULSE experiment consists of at least 3 files, the raw data (file name extension: *.dat), the pulse protocols used (*.pgf) and a file that contains the amplifier settings and structure of your experiment (*.pul). You don't have to create all files by yourself and can also ignore the file extensions. If you create a new experiment, simply type the name of the experiment, e.g. "Tutorial".

Step 2: Configuring the Hardware

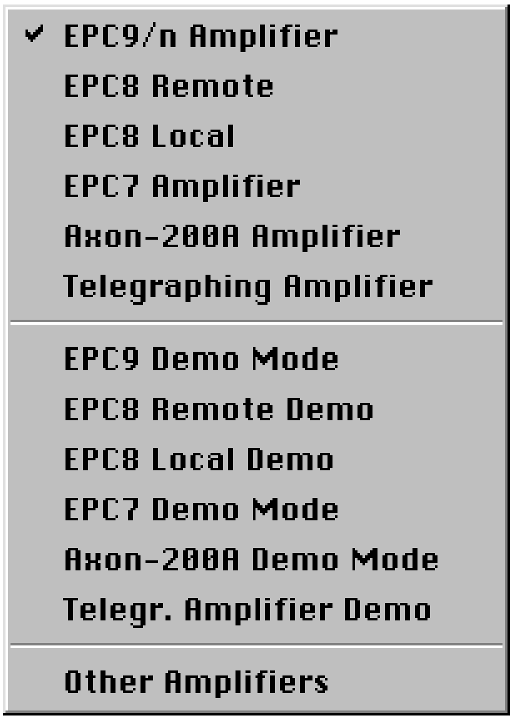


PULSE will open some windows: the most obvious ones are the *Amplifier* and the *Oscilloscope* window. We will deal with these windows soon; however, first we have to make sure that the hardware is connected properly and that the software settings meet the requirements. The most important hardware settings are defined in the *Configuration* window. Select Configuration from the drop-down menu PULSE or type F11 (MacOS) or F8 (Windows).

Paths

The Configuration window provides a variety of parameters that can be adjusted. First of all, in order to tell *PULSE* where to look for the relevant files and where to store your data, you need to set up the paths. This is done by selecting the Common Path, Data File, PGF File, and Sol. Data Base button in the Files and Paths section of the *Configuration* window (1).

AD/DA Channels



PULSE has to know whether to use an *EPC9* patch-clamp amplifier or another amplifier: use the control in the lower part of the screen (2). If your particular amplifier is not in the list, select the *EPC7*, if you have no amplifier at all, use one of the *Demo* modes instead. If your amplifier is not an *EPC9* (nor *EPC9/2* or *EPC9/3*) you will also have to define the AD/DA converter you use (*ITC16*, *ITC18,* or *Digidata 1200*) in the lower popup control (3). In this example you cannot select an AD/DA-board (the selections are disabled), since the *EPC9* uses its built in AD/DA converter.

The next step will be to define the AD and DA channels to be used for stimulation and acquisition of data in the sections DA channels and AD channels. For users of the *EPC9*, some of these channels are predefined. With the *EPC9* i.e. the voltage stimulus is always expected to go via DA-3 (V-membrane Out, 4). The current input is sampled via AD-6 (Current In, 5) and the voltage is sampled from AD-0 (Voltage In, 6). Therefore, if you have an *EPC9*, you should connect the *VOLTAGE-MONITOR* output of the *EPC9* to the channel you defined with the Voltage In (6) popup by a BNC cable. In the *EPC9 Double* and the *Triple* the above connections are already hardwired inside the amplifier and therefore can not be changed.

Note: The EPC9 has 4 DA output channels (0…3) and 8 AD input channels (0…7). For the EPC9, AD channel 7 is reserved and should not be used for anything else. In addition, DA-0, DA-1, and AD 0...3 are not freely available for the EPC9 Double and DA 0...2 and AD 0...5 are not freely available for the EPC9 Triple since they are internally hardwired to the current- and voltage outputs of the respective amplifiers.

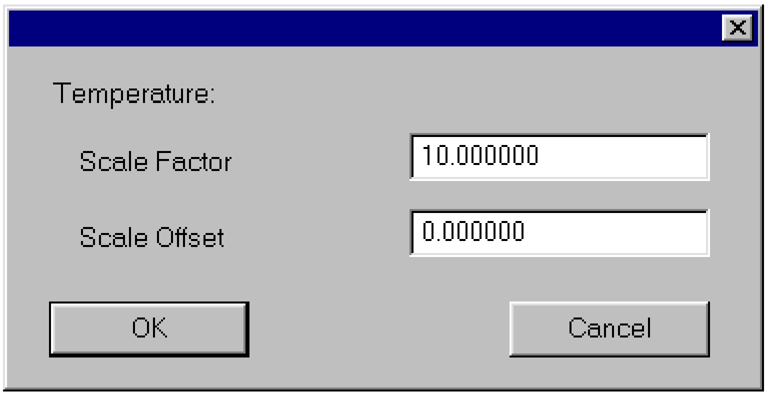
Parameter Input

So far we specified the most important settings. On the left-hand side of the *Configuration* window - the Parameters section - there is a list of further values that are acquired and stored together with the experiment. These *Parameters* can be input via different means: they can either be sampled through a free AD channel (Source = *AD-0 ... AD-7*, 7), they can be derived directly from the *EPC9* (Source = *EPC9*) like the settings of the *C-Slow* compensation (C-Slow and G-Series, 8) or they can be typed in by the user during the running experiment (Source = *Default*).

The checkboxes in the parameter list left to each parameter determine, whether the parameter is displayed in the *Parameters* window (see *Chapter Parameters*).

Note: The checkboxes to the left of each parameter only define, if the corresponding setting will be visible within the PULSE session. Despite of this setting, PULSE will always store every parameter with every block of data acquired. A separate block of data is called "sweep" in the PULSE terminology.

Go through all of these parameters and select the input source and maybe new *default* values. If an AD channel is used as input source, then the scaling can be specified. Let us suppose the *Temperature* is read from a temperature control unit via AD channel 4 (7). Let's further assume that the control unit delivers an analog signal of 100 mV/°C and 0 V at 0 °C. Then the scaling factor to be entered is *10* (1V corresponds to **10** °C) and the offset is *0*. Click the scale button (9) next to the *Temperature Parameter* and fill in these settings.



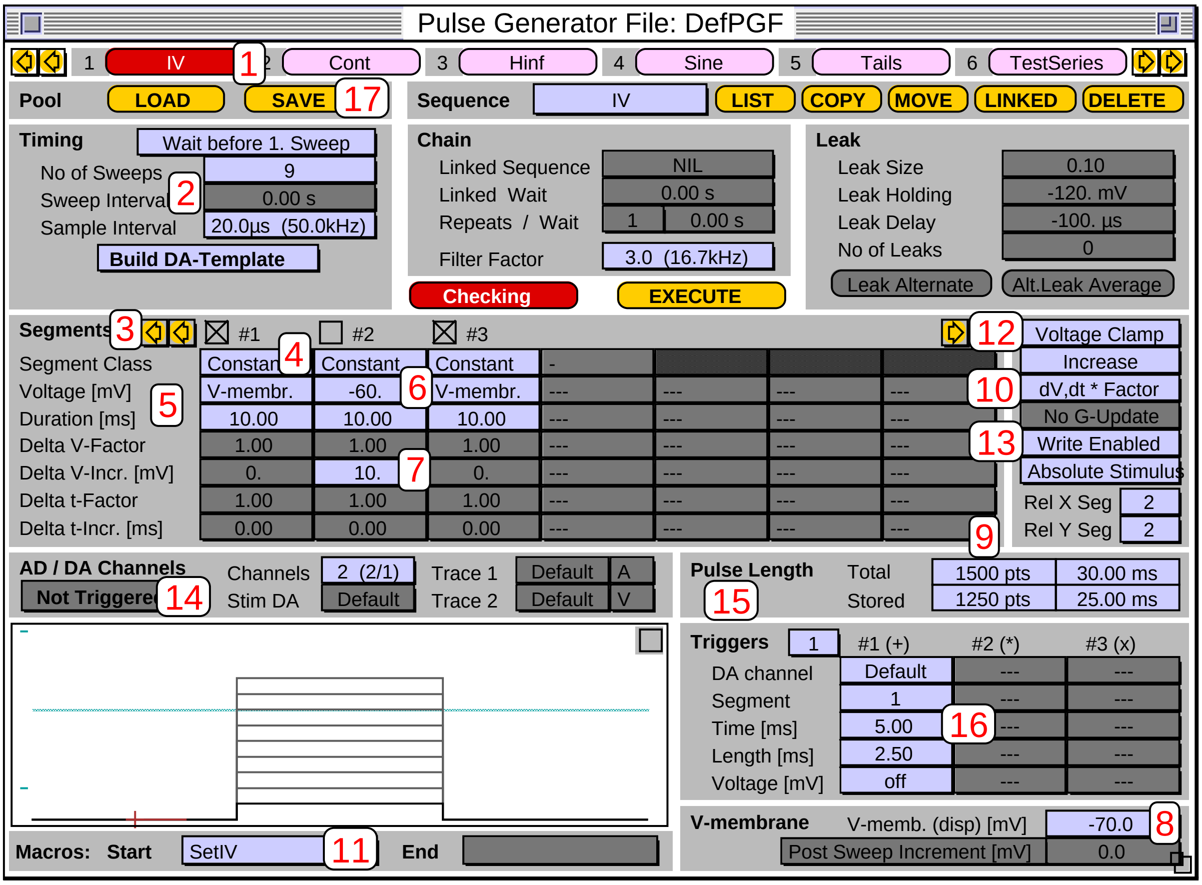
If you are sampling from a so called *"telegraphing"* amplifier, the determination of the encoded amplifier gain and filter bandwidth settings is much more involved and is done via lookup tables that are provided as ASCII files (see *Appendix IV*). You can select the corresponding gain or bandwidth table with the button table (10).

Test Pulse

The *Test Pulse* is applied to the pipette whenever you activate the amplifier by bringing the *Amplifier* window to the front. You can define most of its settings in the Test Pulse section (13) of the *Configuration* window. The amplitude, duration and pulse type are also available from the *Amplifier* window, however, the Pulse Mode can be only defined herein. Current means, that the current trace is displayed in the *Oscilloscope* every time the test pulse runs, while Both displays both, the current and the voltage trace. Use this later *Pulse Mode,* if you frequently want to apply test pulses in the *Current Clamp* configuration.

Finally, you can save the configuration: click the SAVE button (11) and provide a file name. *PULSE* allows you to store the configuration under any file name, however, when the program comes up, it will look for a file called DefaultPulse.set. You can load a different configuration at any time using the LOAD (12) button, but you should keep in mind, that many changes, e.g. those involving the redefinition of the digitizer hardware, require a restart of the program.

Step 3: Generating a Simple Pulse Protocol



The *PULSE* software allows you to stimulate your cell with pulse pattern from a basic rectangular pulse to highly complicated stimulation patterns. Stimulus templates are edited in the *Pulse Generator* window. To open it select Pulse Generator from the Pulse drop-down menu or type ‘F9’ (Windows) or ‘F12’ (MacOS). A pulse pattern (called *Sweep*) consists of an arbitrary number of pulse *Segments* that have a constant, ramp, or sinusoidal voltage. In the default *Pulse Generator File* only one sequence is created, however, the file DefPGF.pgf distributed with the software release and usually installed into the Data folder inside the HEKA folder contains a lot of useful pulse protocols which are a good starting point to create your own ones.

Creating a new sequence

Click a free position in the PGF pool (1). If there is no free position, click the right arrow unless you reach the end of the pool. *PULSE* will ask you for a new Entry-Name: type *IversusV*. The first six protocols will be directly available from the *Oscilloscope* window. If you want to bring this new protocol to a more handy position click the MOVE button and select *1* to *6*as the new position.

Timing

We want to create a protocol that gives us a *Current-Voltage* relationship. The response to 9 depolarizing pulses in steps of 10 mV given at an interval of 1 s has to be studied. In the Timing section (2) set No of Sweeps to *9* and the Sweep Interval to *1*. To edit the fields shift-click the corresponding field, i.e. click it with the mouse while keeping the 'SHIFT' key pressed. Usually *PULSE* will wait the time defined as the Sweep Interval before starting the pulse sequence. If you however want the sequence to start immediately after activating it, select No wait before 1. Sweep from the popup next to Timing.

Defining the Segments

The section Segments (3) of the *Pulse Generator* defines the actual pulse protocol to be applied. It will consist of three parts: the cell will be held at the actual holding potential in the beginning and the end of the protocol, the middle part has the depolarizing step. The individual parts of the pulse protocol are called *Segments*. At the beginning the protocol only has one segment of 5 ms duration. To add the further 2 necessary segments, click on the button labeled Constant (4) and select Insert from the popup. Repeat this step for the third segment.

Although you can edit the segments in any order, it is often advisable to start by defining the length of the individual segments. Segment 1 and 3 are to be 10 ms long, while the depolarizing pulse has a duration of 20 ms. Fill out the corresponding Duration [ms] fields appropriately (5).

The first and last segment should be at holding potential, so keep the corresponding checkboxes on the top set. Deselect the middle checkbox to disable it. The value in Voltage [mV] changes from *V-membr.* (i.e. the actual pipette holding potential at the time of executing the protocol) to *0* to reflect the fact, that *PULSE* has to set an absolute voltage (depending on the setting in 13). Change this value to *-60* (6) and set the Delta V-incr. [mV] field to *10* (7). this will instruct *PULSE* to jump to *-60 mV* when it first executes the protocol and then depolarize this segment by *10 mV* every other time of the 8 repeats (-50, -40, -30, ..., +20 mV).

Watch the preview in the lower left part of the window! If a segment is set to *V-membr. PULSE* will show it using the actual pipette potential (usually 0 mV if you are defining the protocol *off-line*). If you later want to apply the protocol from a hyperpolarized potential you should change the value V-memb. (disp) [mV] accordingly, e.g. to *-70 mV* (8). This will change the way *V-membr.* segments are displayed in the preview. Note, this value does not affect your measurement, it is only used for previewing the sweep!

Maybe you wondered why the first segment is drawn in red color in the preview while the rest is black. *PULSE* can perform an *Online Analysis* whenever you run or replay an experiment (see below). This is done by analyzing one segment (Rel Y Seg, 9), e.g. determining its peak- or mean current, and plotting it against another parameter like the duration or potential of any other (or the same) segment (Rel X Seg, 9). You can define which segment has to be analyzed by setting the so called *Relevant Segment* (9). This can be done separately for the segment that delivers the x- and the y-value. Set both values to *2*. Your later analysis will of course not be restricted to the segments you define here. In the *Online Analysis* window you can set a positive or negative *Segment Offset* that will be added to the relevant segment, thus allowing you to analyze also the other segments (e.g. the pre-trigger).

Other Settings in the Pulse Generator

C-Slow Update: If you are using the *EPC9* amplifier you can associate some of the automatic compensation procedures with the pulse protocol. If the option Sweep C-Slow (10) is activated *PULSE* will perform an estimation of the input resistance *R-Series* and membrane capacitance *C-Membrane* before every single sweep thus canceling capacitive artifacts.

Macros: Start: Before running the protocol, *PULSE* is instructed to execute a macro (SetIVMean, 11) that we will define in the next section. Running this macro will make sure that the *Online Analysis* window is always correctly calculating the mean current of the depolarized segment against its holding potential, when executing the protocol. At this point you should not care about the error message that tells you that the macro is not defined.

Various Settings: There are a few more options in the right and bottom part of the *Pulse Generator* window that had not to be changed in our case. Nevertheless, it is still important to know what they do: the setting Voltage Clamp (12) will restrict the execution of the pulse protocol to the *Voltage-Clamp* modes only. Thus, *PULSE* will refuse to start this sequence if you are in the *Current-Clamp* mode and instead will produce an error message.

Note: A given pulse protocol only makes sense for Voltage- or Current-Clamp conditions, never for both modes. The option VC+CC Modes in the Pulse Generator window is only there for compatibility with older versions of PULSE. If you want to be able to run a Current-Clamp sequence while you are in a Voltage-Clamp mode, you should create a macro that switches to the CC mode and associate it with the pulse protocol (Macros: Start:).

The option Write Enabled (13) will make sure, that the pulse sequence can be stored to disk. For some protocols it might not be required to save the data (such as during a pre-conditioning waiting period), so you can disable this feature in these cases.

The section AD / DA Channels (14) defines which channel to stimulate and from which channel(s) to acquire the data. Stim DA is set to *Default* which means that you are stimulating through the *EPC9* stimulus channel. The number of input Channels is set to 1 (*PULSE* allows you to acquire two channels simultaneously), making a total of one input and one output channel (the bracketed number next to Channels). The channel to be sampled (Trace 1) is also defined as *Default*, i.e. *PULSE* will acquire the *Current Monitor 2* in *Voltage-Clamp* and the *Voltage-Monitor* in *Current-Clamp* modes.

The section Pulse Length gives you some important information about the pulse protocol (15). The value Total is the total length of stimulation. Each sweep has a duration of 10 + 20 + 10 = *40 ms* sampled at an interval of *50 µs* or a frequency of *20 kHz* (2). This makes a total number of *800* data points (15). From these data the first 5 ms will not be stored, because *PULSE* only saves the data **after the first trigger** (16). This makes a Stored Pulse Length of *700* data points or *35 ms* (15). If you want to store the whole sweep to disk set the first trigger to *0 ms* or set the number of triggers to *0*. This feature is mainly thought to allow you to limit storage of pre-trigger segments.

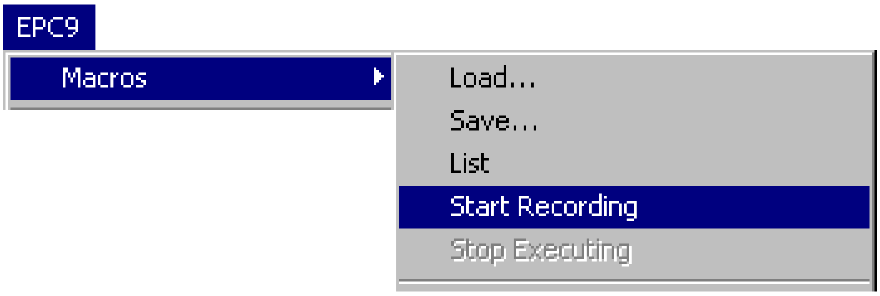
Note: PULSE allows you per default a maximum of 16384 points per sweep. If you are sampling two input channels this number reduces to half (8192 points). If you want to be able to acquire more points in **one** sweep, refer to the Chapter “Pulse advanced”.

This new, modified *Pulse Generator File* should now be stored to disk by clicking on SAVE (17). It can have any name; *PULSE* automatically adds the file extension *“.pgf”*. If you want *PULSE* to come up with this *PGF-file* already loaded at the next launch, simply save the *Configuration File* before exit.

Step 4: Recording a Macro

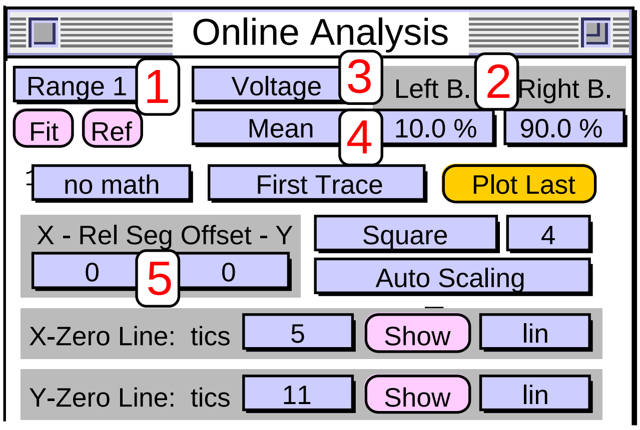
If you run the above IV-protocol, you might want to see not only the raw current response of the cell, but also the resulting IV-curve, where the mean- or peak- current is plotted against the underlying potential. This is a typical task for the built in *Online Analysis*. To do the right kind of calculation on the right data, we will have to setup the *Online Analysis* first. Since we will often need this type of analysis it is a good idea to create a macro that does this setting up automatically in future. This macro can then be associated with the pulse protocol from above.

Macros can be generated in *PULSE* by letting the program record your commands, while you are executing them. Afterwards *PULSE* will convert these actions into a text file that you can easily edit with any ASCII editor (e.g. the Windows **NotePad** or Apple's SimpleText) in order to "fine tune" it. You can start the macro recorder by selecting Start Recording from the EPC9 -> Macros sub-menu.



Bring the *Online Analysis* window to the top. You can do this by clicking on the window or selecting Online Analysis from the Pulse menu. Alternatively, you can type the keyboard equivalent 'F7' (Windows) or 'F8' (MacOS).

PULSE can perform two calculations at a time (Range 1 and Range 2). In order to define the first analysis we have to activate *Range 1* from the Range popup (1). Set Left Boundary to 10% and Right Boundary to 90% (2). This will restrict the analysis to the range of 10-90% of the requested relevant segment (or 2-18 ms of the depolarizing pulse). From the Abscissa popup (3) select *Voltage* and from the Mode popup (4) select *Mean*. These settings tell *PULSE* to calculate the mean current of the analyzed sweep and plot it against its holding potential. From the Relevant Segment Offset popup choose *0* for both settings to analyze the relevant segment we defined in the *Pulse Generator*.



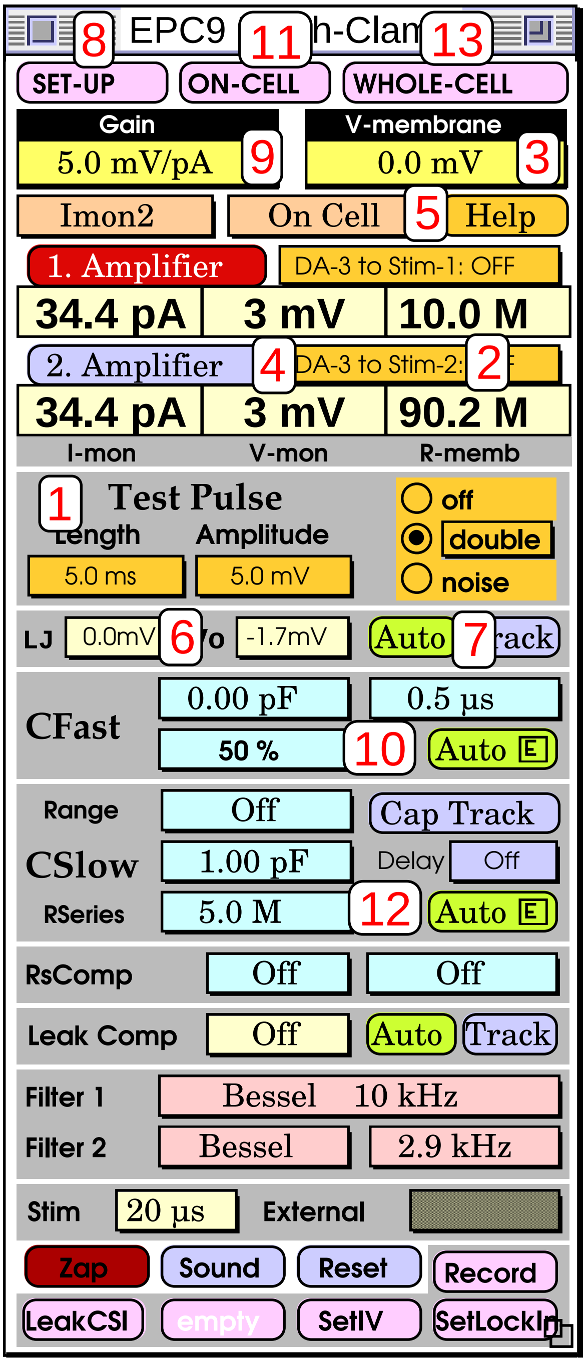
The macro is finished by now, so select Stop Recording from the EPC9/Macros submenu. You will be asked for an index and a name for the macro. You should not select the indices 1 through 3 because these are the predefined macros SET-UP, ON-

CELL, and WHOLE-CELL. Use the index 4 instead. The name for the macro should be the one that you supplied within the *Pulse Generator* (*"SetIVMean"*), since macros are internally recognized upon their names rather than their index. Finally, let us have a look at the macro by selecting List from the EPC9 -> Macros sub-menu. The fourth macro should give you in the *Notebook* window something like the following:

```
4 : SetIVMean
  A  Range:             0; Range 1
  A  LeftB:            10.0%
  A  RightB:           90.0%
  A  Abscissa:          0; Voltage
  A  Mode:              5; Mean
  A  RelXSeg:           0
  A  RelYSeg:           0
  A  Scale:             1; Auto Scaling
```

Note: PULSE has a built in macro interpreter that executes command lines of the form "Window Control[: parameter; comment]". E.g., the line "A LeftB: 10%" would instruct PULSE to set the **left** boundary in the Online Analysis window to **10%**. For this tutorial it is not necessary to know all possible commands and their syntax. Please, refer to the Chapter "Macros" for further details about macros.

Step 5: Amplifier Control



The *EPC9 Patch Clamp* window provides the amplifier control functions, when an *EPC9* amplifier is used (the picture is for an *EPC9 Double)*. A more detailed tutorial describing the most common functions of the *EPC9* and its control window is given in the *Chapter "Setting up the EPC9"*. If you are using an *EPC9* you are highly recommended to read that tutorial first. An extensive description of the amplifier windows is given in the two chapters "EPC9 Amplifiers" and "EPC7, EPC8, and other Amplifiers".

If you have an amplifier other than the *EPC9* you have to make sure that:

- the command potential at the amplifier is set to zero,
- the stimulus scaling is set correctly (see *Chapter Configuration*),
- the DA channel for *Stimulus Out* is connected to the amplifier's stimulus input,
- the amplifier's current monitor is connected to the *Current In* AD channel,
- if the amplifier has telegraphing capabilities for gain and/or bandwidth, that the corresponding analog outputs are connected to the assigned AD channels. For telegraphing amplifiers, gain and bandwidth lookup tables in ASCII format can be used that translate the voltage output to the setting of the corresponding switch (see *Chapter Configuration*).

Make a Seal

Now it's time for the experiment. Switch the model circuit into the *"10 Mohm"* setting to simulate a 10 MΩ-pipette that is open to the bath solution. Hit the space bar in the main dialog to activate the *Amplifier* window - if the *Oscilloscope* is not in front, hit the space bar twice, the space bar toggles between the *Oscilloscope* and the *Amplifier* window. As long as the *Amplifier* window is on top the program will generate test pulses according to the settings in the Test Pulse section (1): a *"double"* pulse of *5 mV* amplitude and a duration of *5 ms* per pulse will be output. The sampled current responses will be shown in the *Oscilloscope* window. The resistance of the pipette is calculated from the responses and displayed in (2).

Besides the fast test pulses (single or double) you can select the third entry in the Test Pulse pop-up list, which requires to specify a sequence from the *Pulse Generator File*. Instead of the fast test pulses, this sequence is then repeated continuously providing an alternative and quite flexible *Test Pulse* mode.

Note: The currently measured resistance of the pipette is always called "R-membrane" because the program cannot distinguish between an open and a sealed pipette. As long as the pipette is open to the bath, "R-membrane" corresponds to the pipette resistance.

The command potential is controlled by the program via the control V-membrane (3) and displayed in (4). This variable always displays the *physiological* membrane potential, i.e., the *Recording Mode* (5) is already taken into account reverting the polarity of the applied potential in *On Cell* and *Inside Out* modes.

Note: Most functions, such as canceling the offset current, setting the amplifier gain, or holding potential, etc. should be obvious, but make sure that the "Recording Mode" is always set properly, because this setting will automatically determine the actual polarity of the voltage at the patch pipette!

You can correct pipette offset potentials by adjusting the Vo value (6) or you can alternatively click on the Auto Vo button (7) to let *PULSE* do this correction automatically for you. The same is done by calling the macro SET-UP (8), in this case, *PULSE* will also adjust the amplifier gain (9) and the test pulse. When the pipette potential is adjusted and you are ready to form a seal, store the value of the *Pipette Resistance* - which is the actual R-memb value that will be overwritten after forming the seal. This is done by typing "W" (write). This value is not changed any further, unless you type "W" again.

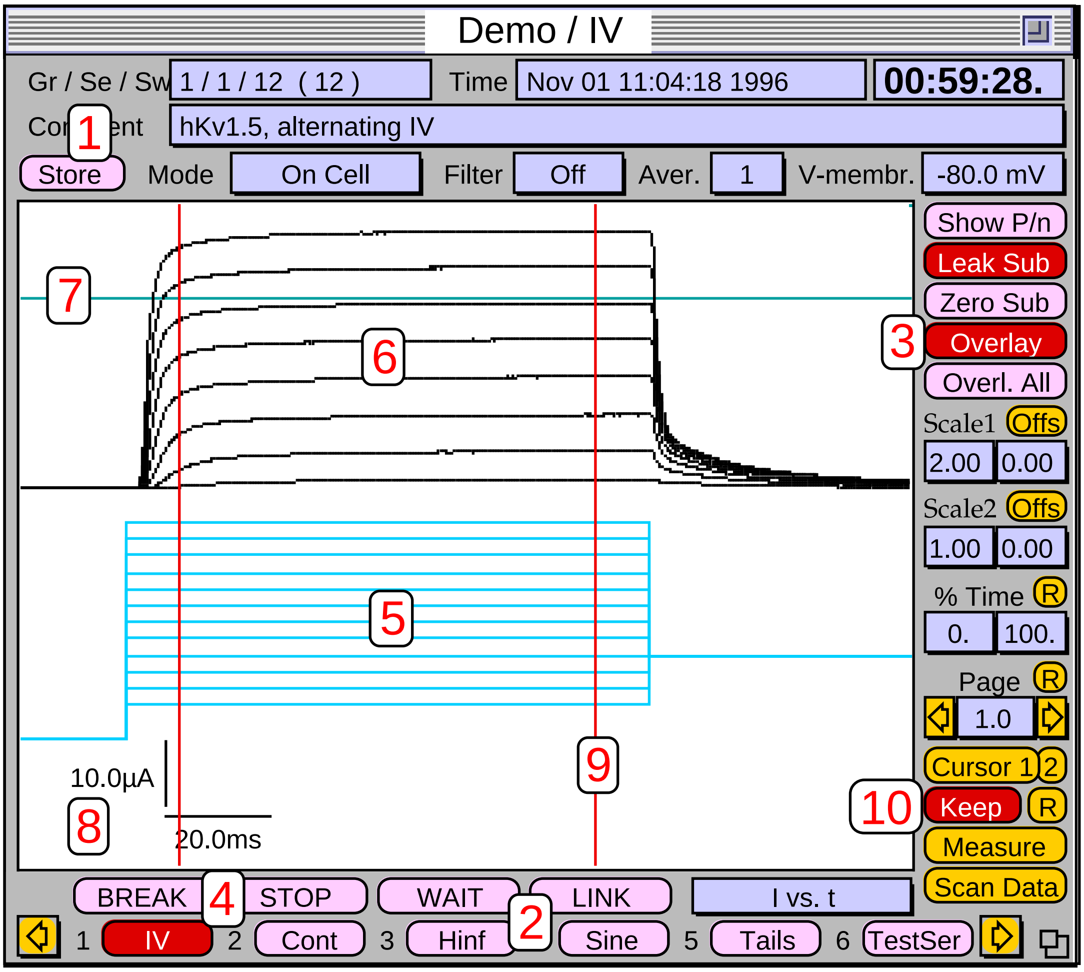
Note: R-memb is updated as long as the test pulses are active, i.e. every time the Amplifier window is in front, and stored as variable "Seal Resistance" with every acquired sweep. The Pipette Resistance will be stored together with every acquired series of sweeps. This value is updated every time you type the "W" key.

Now simulate a pipette sealed to the membrane by switching the model circuit into the middle position. If you have an *EPC9* make an automatic fast capacitance cancellation by clicking on the Auto CFast (10) or SET-UP macro (11) button. Otherwise compensate your amplifier for the pipette capacitance of about 6 pF.

To break into the cell, set the switch of the model circuit to its bottom or *"0.5 GOhm"* position. If you have an *EPC9* make an automatic slow capacitance cancellation by clicking on the Auto CSlow (12) or WHOLE-CELL macro (13) button. Otherwise compensate your amplifier for the "cell" capacitance of about 22 pF. Watch the R-memb display that now shows "*500 M"* instead of *"10 M"*. With the V-membrane control (3) change the pipette holding potential to *-70 mV*, now we are ready to run the pulse protocol we defined before.

Step 6: Run the Pulse Sequence

Bring the *Oscilloscope* window to the front. If the button Store (1) is not highlighted click on it to make it active. Otherwise *PULSE* will show the data but not write them to disk. If you did not create a file yet, *PULSE* will ask you to do this now.



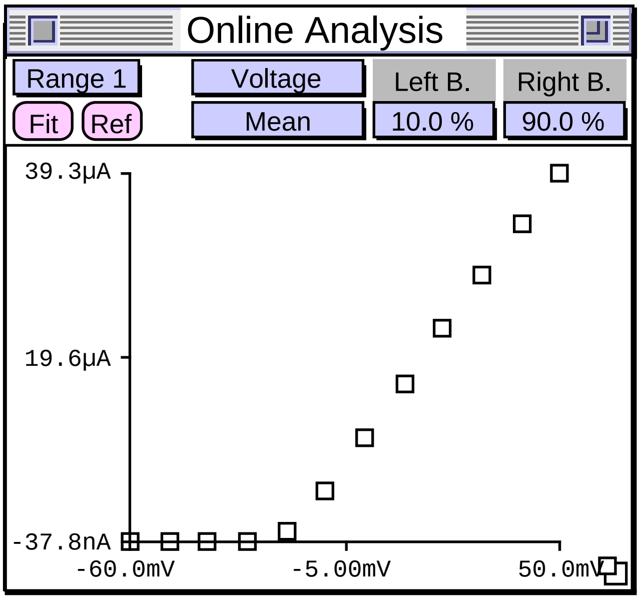
The bottom of the *Oscilloscope* window shows the currently available *Pulse Generator Pool* (2). An individual *Sequence* can be executed by either clicking on one of the controls e.g. the IV button, by entering the corresponding number ('1' ... '9') or by pressing "E" (for *Execute*) for the highlighted *Sequence*. A *Sequence* with an index higher than 9 can be executed from the keyboard by typing the pound key "#" followed by the number of the sequence.

Before we execute the IV sequence (or*"Series"* in *PULSE* terminology, which describes a number of individual sweeps based upon the same *Pulse Generator Protocol*) we will set up the display: in the Display menu activate Show Zero Line, Show Potential, Dimmed Overlay, Labelling → Labels Only and Display Mode → I vs. t. To see all sweeps from the series activate the Overlay button (3), otherwise, the *Oscilloscope* will be erased before every sweep. Now click on the IV button (4) or type "1", if *IV* is the sequence in the first position: The pulse pattern we defined above is output via the specified DA channel (blue color, 5) and the response is shown on the screen. The last sweep of the series is shown in black color, the other sweeps are gray (6) since we activated Dimmed Overlay.

The zero current line is drawn in green color (7) and scaling bars are given in the lower left side of the *Oscilloscope* (8). The two red lines (9) mark the range of the *Online Analysis* (see next section). You can make this range visible by turning on the Keep button (10).

Online Analysis

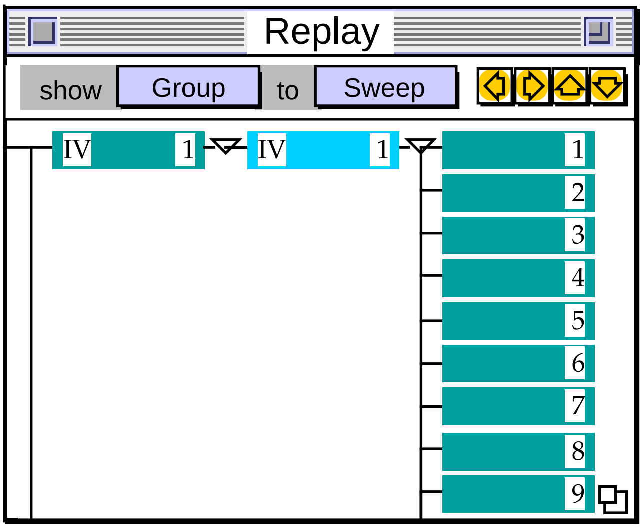
Based on the relevant segments (the depolarizing pulse) as specified in the *Pulse Generator*, a quick online analysis of the acquired (or replayed) data is performed immediately after the series has been collected entirely. The criteria for this analysis (i.e., type of analysis, time range, format of display) were specified before in the *Online Analysis* window, when we recorded the *SetIVMean* macro; now the result - an I versus V curve - is plotted in the graph inside the *Online Analysis* window. The results are also sent to the *Notebook* as a table. If you bring the *Notebook* window to the front (e.g. by selecting Pulse → Notebook), you should see something like the following:




```
Execute: IV
#   V(2)[mV]   t[ms]     i[A]
 1     -60.0   100.0  -38.289n
 2     -50.0   100.0  -36.313n
 3     -40.0   100.0  -34.633n
 4     -30.0   100.0   28.789n
 5     -20.0   100.0   1.0937µ
 6     -10.0   100.0   4.9068µ
 7       0.0   100.0   10.167µ
 8      10.0   100.0   15.724µ
 9      20.0   100.0   21.289µ
10      30.0   100.0   26.742µ
11      40.0   100.0   32.118µ
12      50.0   100.0   37.413µ
```

Storing Data

The structure of the stored data - if the Store button was active - will be shown in the *Data Tree* of the *Replay* window. To open it select Pulse → Replay or type ‘F5’ (Windows) or ‘F13’ (MacOS). Double-click the *“IV 1”* entry to replay the just recorded sequence; double-click a single sweep to watch it in the *Oscilloscope* window. You might use the cursor keys ('UP', 'DOWN', 'LEFT' and 'RIGHT') to walk through the data tree. If you press 'RETURN' the currently active group, series or sweep will be displayed in the *Oscilloscope* and the *Online Analysis* will be calculated.



Using the options from the Tree menu it is possible to modify the entries. E.g. a single sweep, a series, or a whole *Group* of series can be removed by activating the item and then selecting Tree → Delete.

To write the recorded data to disk select File → Update File or close the experiment (File → Close), this will automatically store all files associated with the experiment. To create a new file for data acquisition select File → New... , *PULSE* will close the running experiment and then come up with an empty new one.

Exit

If *PULSE* is quit (File → Quit), you are asked, whether you want to save the *Configuration File*. At least the first few times of running *PULSE*, after tuning the system, you should do that, since this file contains all of the settings that were adjusted as outlined above. Once you have a stable system that you don't want to modify anymore, you can safely ignore this question. Finally, *PULSE* will automatically store the recorded data on the hard disk and close all open files.