Testing the EPC9 with the Model Circuit

The following tutorial will guide you through most of the basic and some of the unique and more sophisticated features of the EPC9 amplifier. At the same time it allows you to check if the amplifier is functioning properly. You will use the model circuit you got together with the amplifier as a substitute for a real patch-clamp recording and explore the virtual ”front panel” of the EPC9 supplied as the program E9SCREEN.
The model circuit provides a switch with three positions simulating the following conditions typically observed during an electrophysiological experiment:

1. In the top position an ”open” pipette with a resistance of 10 MΩ is simulated. This mode is useful for applying a test pulse and for correcting offset potentials.

2. The middle position simulates a pipette attached to the cell membrane after the Giga-Ohm seal formation. In this setting only a capacitance of 6 pF is left over corresponding to the ”fast” capacitance of a pipette sealed to the cell membrane. This mode allows you to test the C-fast compensation.

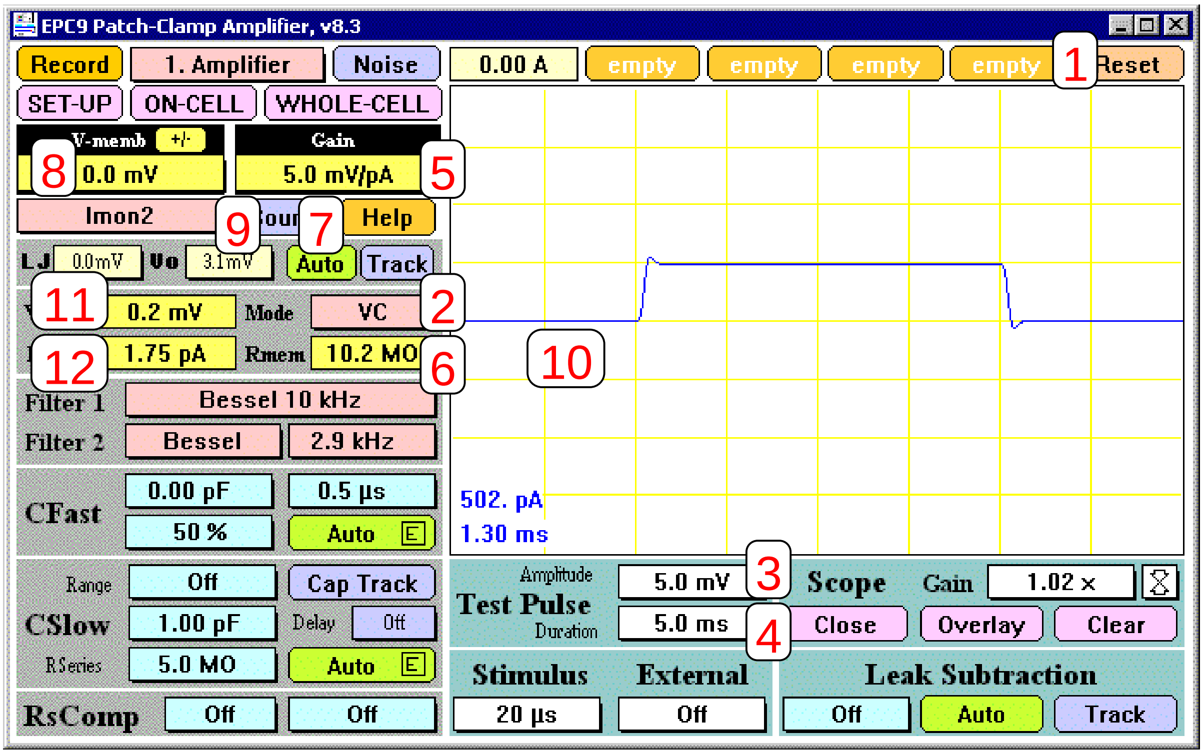
3. In the bottom position a ”model cell” in the whole cell patch-clamp configuration is simulated. The ”input resistance” is 5.1 MΩ, the ”membrane resistance” is 500 MΩ and the ”membrane capacitance” is 22 pF. This mode lets you test the C-slow compensation and the current clamp mode. Furthermore it is useful to check stimulation patterns you design within PULSE.

The following tutorial can be best executed with E9SCREEN. However, since PULSE offers the same functionality with respect to the EPC9 you can also use that program instead. The hardcopies shown were taken from E9SCREEN on the PC.

Step 1: Applying the Test Pulse

First, connect the model circuit to the probe input via a BNC adapter and plug the black cable to the black ground connector on the probe. If E9SCREEN is not running yet start the program which is located in the E9Screen folder inside the HEKA folder. Windows users might alternatively use the Start button to launch E9SCREEN from Programs → HEKA. The left side of the E9SCREEN window, the so called ”virtual front panel”, provides a graphical representation of the EPC9 amplifier. The panel lets you control all hardware settings of the amplifier(s) like gain or filters. Signal display is provided by an oscilloscope-like display in the right part of the window.
Put the model circuit into the ”10 M” setting, which simulates a 10 MΩ-pipette that is open to the bath solution. Reset the amplifier (1), set E9SCREEN to

VC ("voltage clamp") mode (2) and apply a test pulse of 5 mV amplitude (3) and 5 ms duration (4). The current response will be displayed on the digital oscilloscope. If your gain range is appropriate, i.e. 5 mV/pA (5) you should a see a rectangular current of about 500 pA in response to the test pulse. This represents the Ohmic resistor you are recording from: $\Delta I = \Delta U / R = 5$ mV / 10 MΩ = 500 pA. E9SCREEN will online calculate the pipette resistance and update it in the Rmem field (6) where you should read a value close to 10 MΩ.



A possible voltage offset can be canceled automatically by clicking the Auto-V0 button (7). After doing so, the command potential will be set to 0 mV (8) and the V0 control (9) displays the offset potential. The baseline of the current response (10), the voltage monitor V-mon (11) and the current monitor I-mon (12) should be close to zero. You could also do the offset potential cancellation in a more classical way by clicking into the V0 control (9) and dragging the mouse up and down until the first segment in the oscilloscope and the I-mon display (12) match zero.

The steps listed above can be automatically executed by clicking the SET-UP button or pressing the '1' key on the numerical keypad. This will execute the following built in macro that resets the amplifier, creates a rectangular test pulse, sets the gain of the amplifier to 5 mV/pA and then does an automatic compensation of voltage offsets.

```
1 : SET-UP
E  Reset:           ; reset the amplifier
```

E PulseAmp: 5.0mV ; set test pulse amplitude
E PulseDur: 5.0ms ; set test pulse duration
E Gain: 10 ; set gain to 5.0 mV/pA
E AutoZero: ; compensate voltage offsets
E SoundOn: TRUE ; beep
E SoundOn: FALSE ;

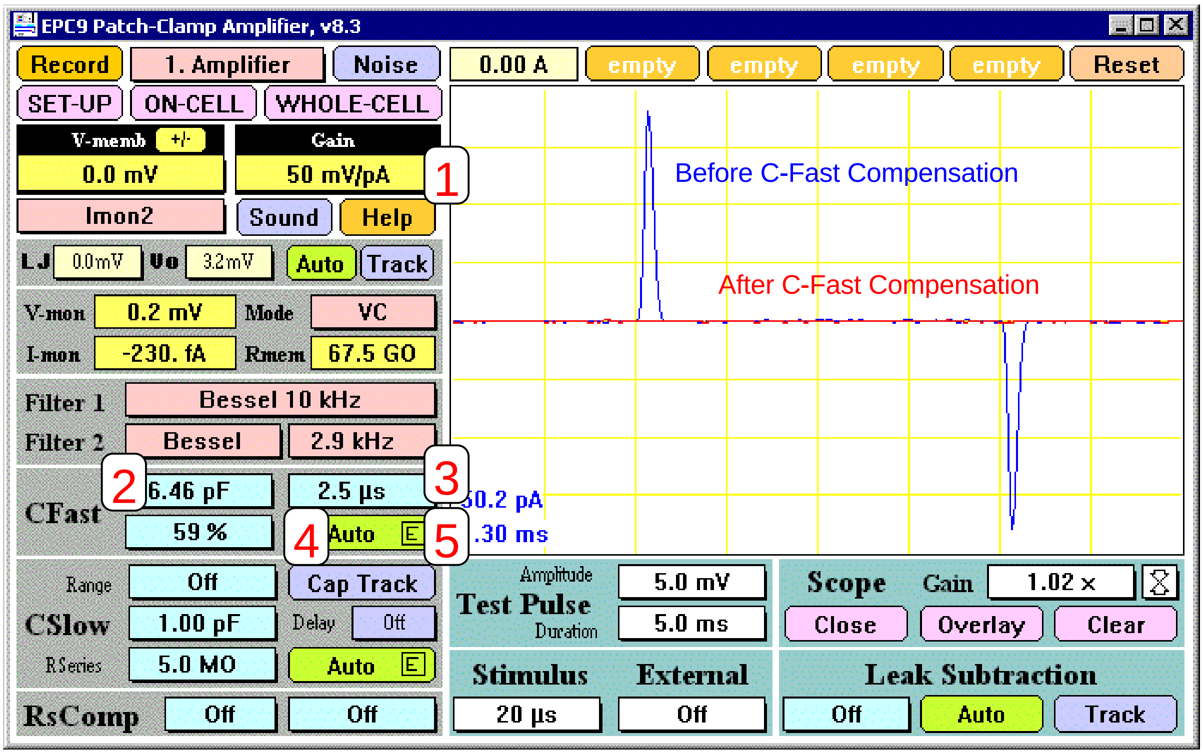
Note: E9SCREEN and PULSE have a built in macro interpreter that executes command lines of the form ”Window Control[: parameter; comment]”. E.g., the line ”E Gain: 10” would instruct PULSE and E9SCREEN to set the gain popup in the EPC9 window to the 10th value (5 mV/pA). The predefined macros are stored in a text file called ”DefaultEpc9.mac” and can be edited with any text editor. For this tutorial it is not necessary to know all possible commands and their syntax. Therefore, please, refer to the PULSE manual for a detailed description on how to record and modify macros.

Step 2: ”On-Cell” Voltage-Clamp Recording

Now move the switch of the model circuit to the center position which leaves only a capacitance of about 6 pF connected. This simulates a Giga-Ohm seal and the C-fast controls can be used to cancel the capacitive spikes resulting from the stimulus test pulse.

In order to see the small currents resulting from the high resistance of the model circuit set the gain to 50 mV/pA by either using the gain popup menu (1) or by typing 3 times the up arrow key.

Note: Alternatively to using the mouse, most of the controls can also be changed directly by the keyboard. You can see the actual keyboard assignments if you select Show Keys from the Help menu.



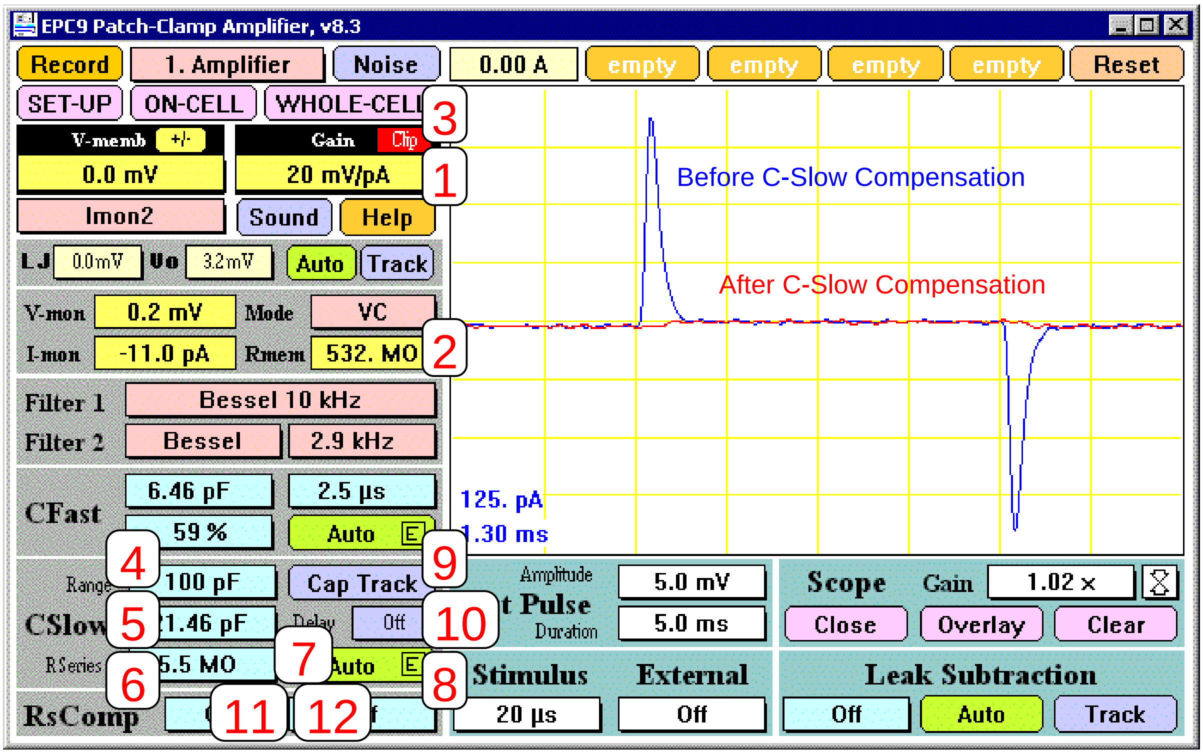
In the oscilloscope you will see two fast capacitive transients (blue line) coming from the 6 pF capacitor in the model circuit. Activate the C-fast compensation by clicking into the C-fast field (2) and dragging the mouse upwards. As long you are approaching a value close to 6 pF you should see the spikes become smaller. Perhaps you have to adjust τ-fast (3) in the same way. As soon as you are overcompensating you will see the spikes going into the opposite direction, this indicates that you should decrease C-fast – using the model circuit it is not very critical to misadjust τ-fast. Continue adjusting C-fast and τ-fast unless you see an almost flat line in the oscilloscope (red line). This should be the case at a around 6 pF (2).

Instead of compensating C-fast "by hand" you can also press the Auto button (4) in the CFast section of the amplifier control panel for an automatic compensation of C-fast and τ-fast. If the compensation fails, the E-field (5) in the Auto button becomes black. If this happens, you should repeat the auto-compensation unless it succeeds and the E-field becomes normal again. The steps listed above can be automatically executed by clicking the ON-CELL button or pressing the '2' key on the numerical keypad. This will execute the following predefined macro that increases the gain and then performs twice an auto-compensation – considering a possible failure in the first attempt.

2 : ON-CELL
E Gain: 14 ; set gain to 50 mV/pA
E AutoCFast: ; automatic C-fast compensation
E AutoCFast: ; repeat compensation
E SoundOn: TRUE ; beep
E SoundOn: FALSE ;

Step 3: ”Whole-Cell” Voltage-Clamp Recording

After compensating C-fast well you can now switch into the ”0.5 G” position of the model circuit. This will simulate a ”model cell” with 22 pF ”membrane capacitance”, 500 MΩ ”membrane resistance” and 5.1 MΩ “input resistance” in the whole-cell configuration. This mode can be used to verify the C-slow controls, the action of series resistance compensation with C-slow enabled and the current clamp mode (see below).
After reducing the gain to 20 mV/pA (1) the RMem field should reflect the changed “membrane” resistance and display a value close to 500 MΩ (2). You should see in the oscilloscope two capacitive transients (blue line) coming from the 22 pF capacitor in the model circuit. The ”slower” time constant of the model cell – compared to the ”fast” time constant from the middle position – is $\tau = Rs \cdot Cm = 5.1\ M\Omega \cdot 22pF = 112\ \mu s$. The peak current can be calculated from $Imax = Cm \cdot \Delta U / \tau = 22\ pF \cdot 5\ mV / 112\ \mu s = 982\ pA$. With the actual gain setting of 20 mV/pA this would generate a voltage of 19.6 mV at the current-to-voltage converter output which exceeds the amplifier’s voltage range. This is signalized by the red Clipping indicator at the amplifier and in the ”virtual panel” in E9SCREEN (3).
Activate the C-slow compensation by selecting the 100-pF range from the Range field (4). Now start the compensation by increasing the CSlow (5) and the RSeries (6) values – again by clicking and dragging the mouse upwards. Since there are two variables to adjust this is more difficult than the C-fast compensation, however, with some praxis you will get a better feeling for these parameters and how they effect the recording. With increasing quality of the compensation you should approach the real values of the model circuit and the transients should disappear (red line). Instead of compensating C-slow ”by hand” you can also press the Auto button (7) in the CSlow section of the amplifier control panel for an automatic compensation of C-slow and R-series. If the compensation fails, the E-field (8) in the Auto button becomes black. If this happens, you should repeat the auto-compensation unless it succeeds and the E-field becomes normal again.



Note: The automatic C-slow compensation depends strongly on the actual values of C-slow and R-series. The closer these two values match the reality the better the estimation will become. Therefore, you should always check if the values are reasonable before executing the automatic compensation.

Clicking the Cap Track button (9) does this automatic compensation repetitively after a delay you specify in the Delay field (10). With a delay of 1 ms and a contemporary computer (Pentium II, 300 MHz) this feature allows you to measure the membrane capacitance at a rate of 15 Hz. You can output the results of the Cap-Track mode into the notebook window if you activate the feature Log Tracking from the EPC9 menu.

Note: If you are a novice to patch-clamping it is useful to perform the C-fast and C-slow compensation at least a couple of times manually before getting used too much to the convenience of the automatic routines. Doing so you will get a better feeling for the quality of a recording and how it is affected by the various parameters, especially the input resistance R-series.

In a similar way as you explored the C-slow compensation you could now have a closer look into the Rs-Compensation. Turn the compensation on by setting an appropriate compensation speed, 2, 10 or 100 µs (11) and gradually increase the percentage of compensation from 0 to 95% by clicking and dragging the mouse upwards (12). As soon as you are overcompensating the series resistance typical oscillations will occur in the oscilloscope. Series Resistance Compensation is a more complicated topic and is therefore treated in more detail in the EPC9 Manual.

The steps listed above can be automatically executed by clicking the WHOLE-CELL button or pressing the '3' key on the numerical keypad. This will execute the following macro that sets the right gain, does a C-Slow compensation with useful :

```
3 : WHOLE-CELL
E  Invert:   TRUE    ;
E  Gain:     12      ; set gain to 20 mV/pA
E  CSlow:    20.00pF ; set C-Slow value to 20 pF
E  RSeries:  10.0MO  ; set R-Series value to 20 MOhm
E  AutoCSlow:        ; automatic C-slow compensation
E  AutoCSlow:        ; repeat compensation
E  SoundOn:  TRUE    ; beep
E  SoundOn:  FALSE   ;
```

Step 4: "Whole-Cell" Current-Clamp Recording

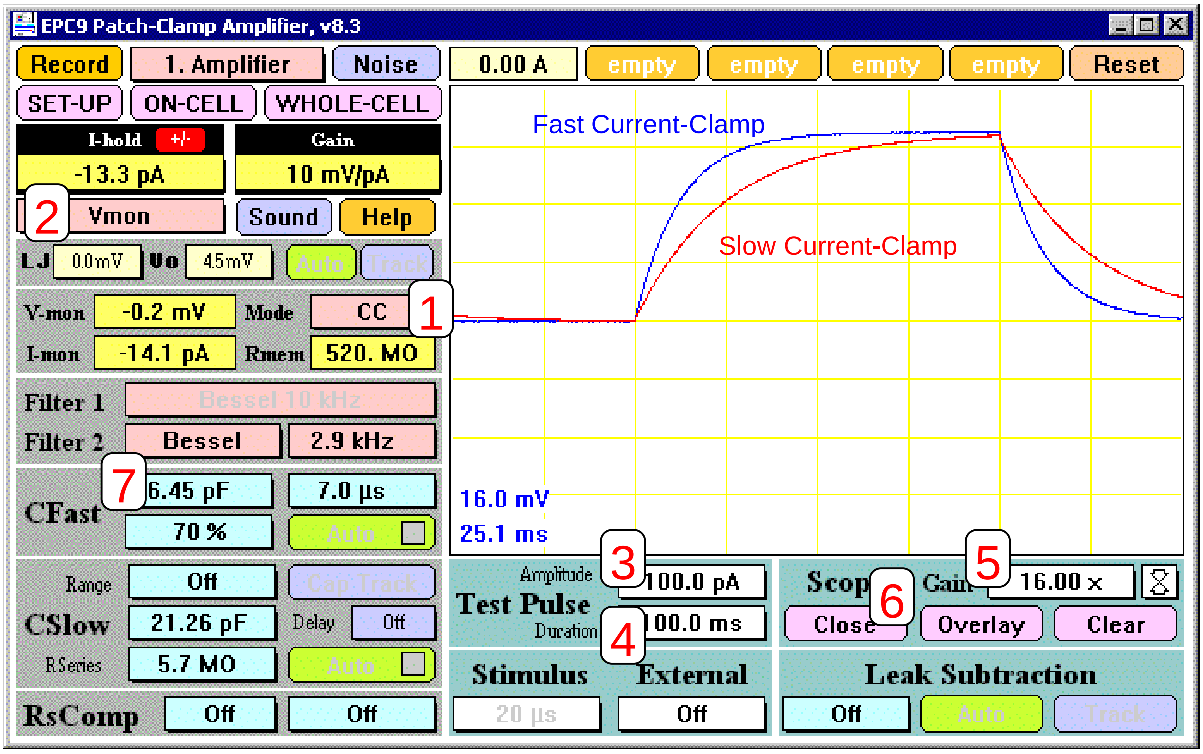
If C-Slow has been compensated so far, switch into the current-clamp mode by selecting CC from the Mode popup (1). This should automatically select the voltage monitor Vmon (2) to become your active channel displayed in the oscilloscope. If this is not the case, e.g. with older versions of E9SCREEN or PULSE, change the active channel to Vmon (2). Note, that the unit of the test pulse amplitude changes from "mV" to "pA" as soon as you switch from voltage into current clamp mode (3). E9SCREEN and PULSE use two different amplitudes for VC and CC modes, therefore the test pulse is set to "0 pA" initially. Now you need to inject current into the circuitry, 100 pA should be a reasonable value (3). The current injection will charge the "membrane" of the "model cell" at a time constant τ = Rm · Cm = 500 MΩ · 22pF = 11 ms to a final value of Vmax = Rm · I = 500 MΩ · 100 pA = 50 mV. Due to the slower time constant compared with voltage clamp conditions it takes much longer to reach Vmax, therefore you should increase the duration of the test pulse to an appropriate value of 100 ms (4).

Note: In contrast to voltage clamp conditions were τ is proportional to the access- or series resistance (Rs) of the pipette, in current clamp experiments τ depends on the membrane resistance (Rm).

The normal setting of the oscilloscope scales the voltage monitor at 250 mV per division. You should therefore increase the gain of the oscilloscope to 16 (5) which scales the display to be 16 mV per division. Please remember that the oscilloscope gain is different from the amplifier gain and only scales the display, not the acquisition of data. Using a very high oscilloscope gain together with a low amplifier gain allows you to determine the digital resolution of the analog-to-digital converter.

The EPC9 has two feedback circuitries for current clamp recordings. The so called "fast" current clamp mode was introduced with the "C" version of the hardware in 1995 and is also available in the EPC8. The EPC7 and older

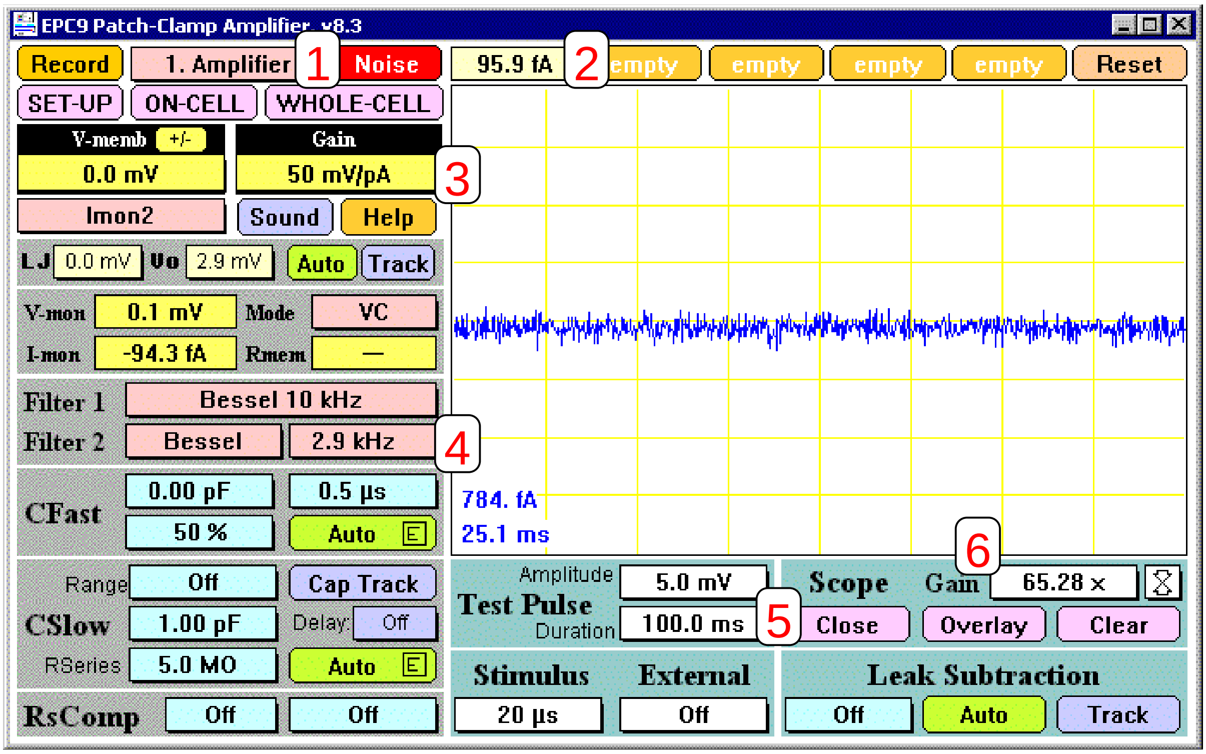
EPC9 amplifiers ("A" and "B" version) lack the fast current clamp mode, therefore these amplifiers can not be compared with the new EPC9 or the EPC8 with this respect. The board version of your EPC9 amplifier can be determined by the last menu item in the EPC9 menu. If your amplifier supports the fast current clamp speed it will be activated by default (blue line in the oscilloscope). To turn this mode off close the oscilloscope (6), click the red button labeled CC Fast Speed and then open the oscilloscope again (6). Now, you will see a much slower signal (red line in the oscilloscope). Please note, that the fast current clamp mode is very sensitive against misadjustments of the C-fast setting. Especially overcompensation causes the signal to oscillate. You can test this quickly by slightly increasing C-fast from its value of about 6.5 pF (7). With the settings from this tutorial you should see oscillations occur at a 8.1 pF and above if the fast current clamp mode has been activated. Please, read chapter 5 of the Read Me EPC9 Manual if you need further information about the fast current clamp mode.



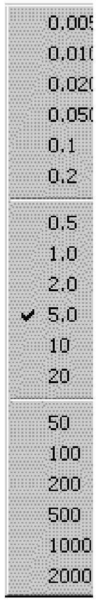
Note: The 500 MΩ setting of the model circuit is not a good method for testing the fast clamp speed of the EPC9 due to the long time constant of 11 ms the amplifier can easily follow. If you want to have a better estimation of the amplifier's qualities under current clamp conditions you should do the same test as above with the 10 MΩ setting. This results in a much shorter "membrane" time constant of only 60 µs.

Step 5: Measuring the Noise of the Amplifier

Now let us come to the final section of the tutorial and check the intrinsic noise of the amplifier. E9SCREEN has a built in feature that allows you to easily and quickly check the noise of your amplifier and to minimize your setup's noise by grounding. First, remove anything from the probe and shield its input with the metallic cap. Now click the Noise button (1) to start the noise test. In the noise-test mode no stimulation will occur. Instead, E9SCREEN will calculate the noise of the current monitor 2 (Imon2) and display it in (2). Select the highest feedback resistor of the preamplifier which has the lowest noise by switching into a gain of 50 mV/pA or higher (3).



Note: The three different gain ranges of the EPC9 are separated by lines in the gain popup. The low-gain range goes from 0.005 to 0.2, the medium-gain range from 0.5 to 20 and the high-gain range from 50 to 2000 mV/pA.



With filter 2 set to 2.9 kHz (4) and nothing attached to the probe you should read a noise value between 90 and 110 fA (2).

Tip: If you wish to ground your setup you should now attach the pipette holder to the probe, insert a glass pipette, bring the pipette tip into the recording position near the recording chamber and power on every piece of equipment that introduces noise (lamps, oscilloscope, camera, ...). Setting the duration of the test pulse to 100 ms (5) and the gain of the E9SCREEN oscilloscope to a high value (6) will make the noise and the 50/60 Hz pickup very obvious. In a well grounded setup all these components should introduce no more than 100 fA of additional noise.