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1. Verifying and Testing the EPC 10

1.1 Testing the EPC 10 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 EPC 10 amplifier. At the same time it allows you to check, whether the amplifier is functioning properly. You can 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 EPC 10 supplied in the acquisition software (Patchmaster, Pulse, TIDA)

1.1.1 The Model Circuit

The model circuit connects to the probe input via a BNC adapter and the plug goes to the black GND connector on the probe: The model circuit provides a switch with three positions simulating the following conditions

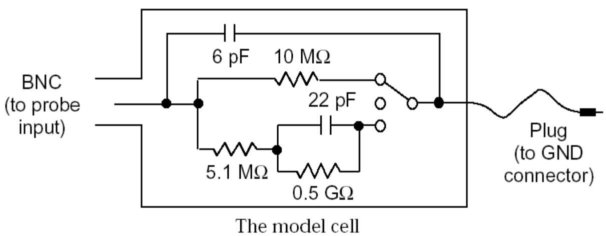

Figure 1.1: Model Circuit

typically observed during an electrophysiological experiment:

- In the top position an "open" pipette with a resistance of 10 $M\Omega$ is simulated. This mode is useful for applying a test pulse and for correcting offset potentials.
- 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.
- In the bottom position a "model cell" in the whole cell patch-clamp configuration is simulated. The "input resistance" is 5.1 $M\Omega$, the "membrane resistance" is 500 $M\Omega$ and the "membrane capacitance" is $\sim$ 22 pF. This mode allows testing the C-slow compensation and the current clamp mode. Furthermore it is useful to check stimulation patterns you design within the acquisition software.

  Note: *This model cell has a long "membrane" time constant (about 10 ms).*

The following tutorial can be best executed with PULSE. However, since PATCHMASTER and TIDA offer the same functionality with respect to the EPC 10, you could also use one of these programs instead. The figures shown were taken from PULSE.

1.1.2 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 PULSE is not running yet, start the program 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. Press the \`SPACE´ key to bring up the so called "virtual front panel". It provides a graphical representation of the EPC 10 amplifier. The panel lets you control all hardware settings of the amplifier(s) such as gain or filters. Signal display is provided by an oscilloscope-like display.

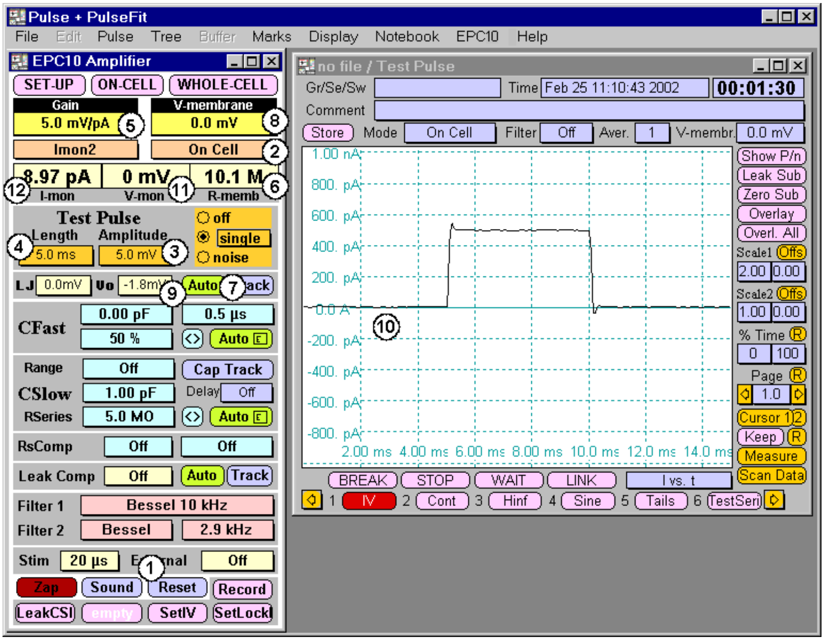

Figure 1.2: EPC 10 amplifier and oscilloscope dialog

Put the model circuit into the "10 M" setting, which simulates a 10 $M\Omega$-pipette that is open to the bath solution. Reset the amplifier (1), set PULSE to ``‘‘On Cell’’`` 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 see a rectangular current of about 500 pA in response to the test pulse. This represents the ohmic resistor you are recording from:

$$(I = U/R = 5mV/10M\Omega = 500pA)$$

PULSE will online calculate the pipette resistance and update it in the `R-memb` field (6) where you should read a value close to $10M\Omega$.

A possible voltage offset can be automatically cancelled by clicking on the `Auto-`V_0 button (7). After doing so, the command potential will be set to 0 mV (8) and the V_0 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 V_0 control (9) and dragging the mouse up and down until the first segment in the oscilloscope and the `I-mon` display (12) match zero.

In PATCHMASTER and PULSE the steps listed above can be automatically executed by clicking on 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, sets the gain of the amplifier to 5 mV/pA, creates a rectangular test pulse, and then performs an automatic compensation of the voltage offsets:

```
1 : SET-UP
 E  Reset:              ; reset the amplifier
 E  Mode:       1       ; On Cell
 E  Gain:      10       ; set gain to 5.0 mV/pA
 E  PulseAmp:   5.0mV   ; set test pulse amplitude
 E  PulseDur:   5.0ms   ; set test pulse duration
 E  PulseOn:    TRUE    ; Switch on test pulse
 E  AutoZero:           ; compensate voltage offsets
```

> ***Note:*** PULSE *has 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 *to set the gain popup in the EPC 10 window to the 10th value (5 mV/pA). The predefined macros are stored in a text file called `DefaultEpc10.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* PATCHMASTER *or manual* PULSE *manual for a detailed description on how to record and modify macros.*

1.1.3 "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 hitting 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, when you select `Show Keys` from the `Help` menu.*

In the oscilloscope you will see two fast capacitive transients (shown as a blue line in the figure above) 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. While you are approaching a value close to 6 pF you should see the spikes become smaller. You may 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 (shown as a red line). This should be the case at a around 6 pF (2).

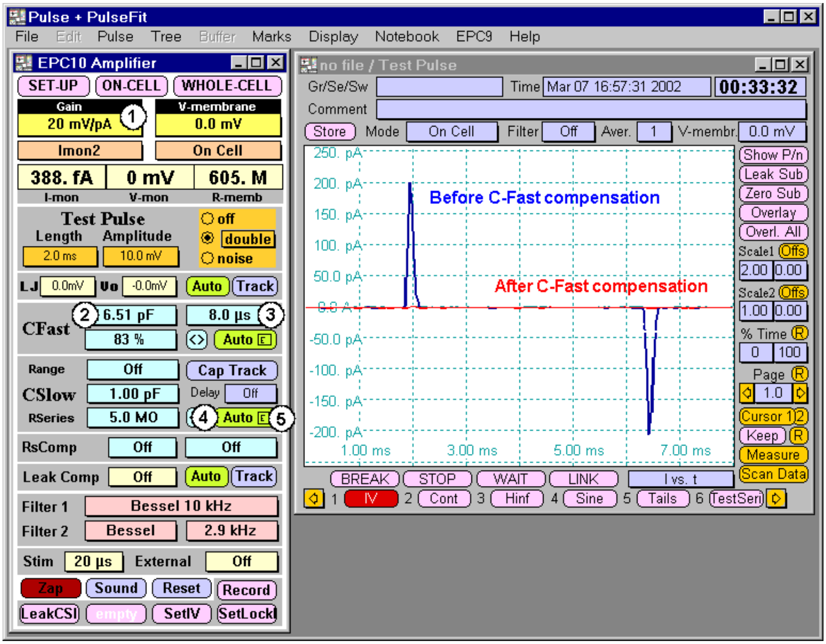

Figure 1.3: C-fast compensation

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, until it succeeds and the `E`-field becomes normal again. The steps listed above can be automatically executed by clicking on 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
```

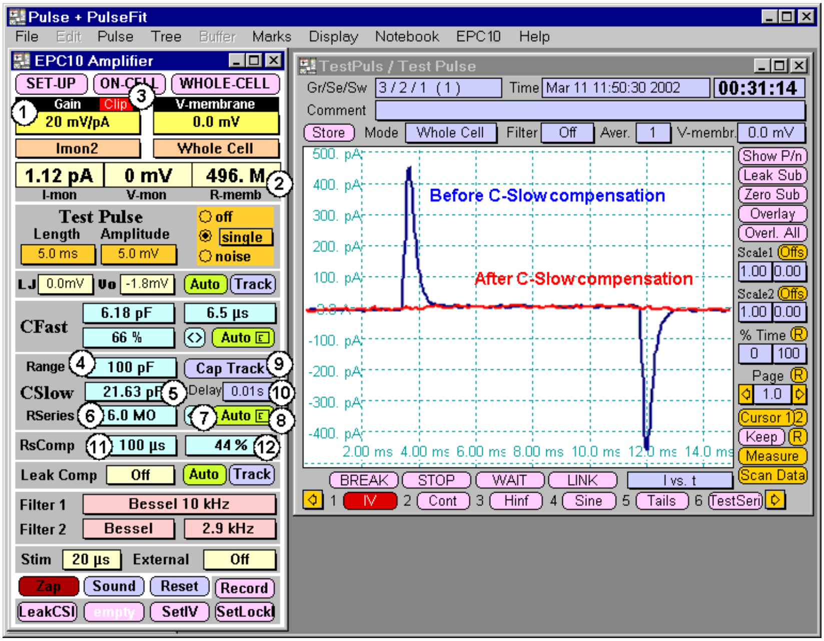

Figure 1.4: C-slow compensation

```
E  AutoCFast:            ; repeat compensation
E  Bell                  ; beep
```

1.1.4 "Whole-Cell" Voltage-Clamp Recording

After compensating C-fast well, you can now switch into the "0.5 GOhm" position of the model circuit. This will simulate a "model cell" with 22 pF "membrane capacitance", 500 $M\Omega$ "membrane resistance" and 5.1 $M\Omega$ "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 `R-memb field` should reflect the changed "membrane" resistance and display a value close to 500$M\Omega$

(2). You should see in the oscilloscope two capacitive transients (blue line) caused by 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

$$\tau = R_s \cdot C_m = 5.1\ M\Omega \cdot 22\ pF = 112\ \mu s$$

The peak current can be calculated from

$$I_{max} = C_m \cdot U/\tau = 22\ pF \cdot 5\ mV/112\ \mu s = 982\ pA$$

With the actual gain setting of 20 mV/pA this would generate a voltage of 19.6 V 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 PULSE (3).

Activate the C-slow compensation by selecting the 100 pF range from the `Range` field (4). Now start the compensation by increasing the C-slow (5) and the R-series (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 C-slow 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. When this happens, you should repeat the auto-compensation, until it succeeds and the `E`-field becomes normal again.

> ***Note:*** *The speed and success of automatic C-slow compensation depends on the actual values of C-slow and R-series. These two values should be reasonably near to the real values. Therefore, you should always check, whether the values are reasonable before executing the automatic compensation. It is much better to have too large estimates than too small ones.*

Clicking the `Cap Track` button (9) does this automatic compensation repetitively after a delay specified 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 option Log Tracking from the EPC 10 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 EPC 10 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 reasonable values:

```
3 : WHOLE-CELL
 E  Gain:     12         ; set gain to 20 mV/pA
 E  CSlow:    30.00pF    ; set C-slow value to 30 pF
 E  RSeries:  10.0MO     ; set R-series value to 10 MOhm
 E  AutoCSlow:           ; automatic C-slow compensation
 E  AutoCSlow:           ; repeat compensation
 E  Bell                 ; beep
```

1.1.5 "Whole-Cell" Current-Clamp Recording

If C-slow has been compensated so far, switch into the current-clamp mode by selecting `C-Clamp` from the `Mode` popup. This should automatically select the voltage monitor Vmon to become your active channel displayed in the oscilloscope. If this is not the case, e.g. with older versions of PULSE, change the active channel to `Vmon`. Note, that the unit of the test pulse amplitude changes from "mV" to "pA" as soon as you switch from voltage clamp (VC) into current clamp (CC) mode. PULSE uses 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. The current injection will charge the "membrane" of the "model cell" at a time constant

$$\tau = R_m \cdot \sum C_m = 500\ M\Omega \cdot 22\ pF = 11\ ms$$

to a final value of

$$V_{max} = R_m \cdot \sum I = 500\ M\Omega \cdot 100\ pA = 50\ mV$$

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 a more appropriate value of 100 ms.

> ***Note:*** *In contrast to voltage clamp conditions, were t 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 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.

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

1.1.6 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. Pulse 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, e.g., by optimizing the grounding of the setup. First, remove anything from the probe and shield its input with the metallic cap. It is recommended to click the `Reset` button in the amplifier dialog in order to reset the EPC 10 to its initial default configuration. Now click the `Noise` button (1) to start the noise test. In the noise test mode no stimulation will occur. Instead, Pulse 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 intrinsic noise by switching into a gain of 50 mV/pA or higher (3).

> ***Note:*** *The three different gain ranges of the EPC 10 are separated by lines in the gain popup menu. 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.*

> ***Note:*** *Because of poor dielectric properties in the internal switch, the model circuit introduces excess random noise above the level that can be obtained with a gigaseal.*

The action of the internal filters on the background noise level and the temporal response can be observed by changing Filter 1 and Filter 2. An improved signal-to-noise ratio should be apparent when the gain is increased to 50 mV/pA or greater (which selects the 50 $G\Omega$ measuring resistor). With Filter 2 set to 2.9 kHz (4) and nothing attached to the probe you should read a noise value below 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 Pulse 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 about 100 fA of additional noise.

1.2 Making a Full Test

If you ever encounter any hardware problems that can not be solved by simply re-calibrating the amplifier (see Calibrating the EPC 10 on page **??**) you can run the Full Test in PATCHMASTER or PULSE. This feature is a diagnostic tool that allows us at HEKA Elektronik to make some conclusions about possible defects of the amplifier. Otherwise, this function and its output should only be of little interest for you.

> ***Note:*** *The Full Test sometimes reports errors although the amplifier is absolutely fine. This may have multiple reasons. First, we opted to have stringent test specifications such that possible problems are not missed. Second, errors may be caused by procedural errors, or even because of defective BNC cables. If you get the message that your hardware might be "not ok", please contact HEKA first and supply us the report before sending the amplifier in. You might save yourself valuable time!*

Before starting the `Full Test` you should make sure to have nearby a shield for the probe (e.g., the metallic cap delivered with the EPC 10), a 10 $M\Omega$ resistor (e.g., the model circuit) and 5 short BNC cables. In case you have an EPC 10 Double or Triple, select the amplifier you want to test in the EPC 10 amplifier dialog: 1. Amplifier, 2. Amplifier or 3. Amplifier.

Now select the menu item `Full Test` from the EPC 10 menu. You will be told to remove everything from the probe and shield its input. You can use the metallic cap that came with your amplifier and put it on the BNC connector at the probe to shield it. Please make sure that really nothing except the metallic cap is connected to the probe and that especially the black pin jacks is free. You should also make sure that no BNC cables are connected to the main unit of the amplifier.

After a short while you will be prompted to connect 3-5 BNC cables, depending on the amplifier you are testing. If the connection test fails, you will get an error message and the chance to repeat the test an additional time. After the connection test you will have to remove all BNC cables and connect a 10 $M\Omega$ resistor to the probe input. You can use the model circuit and switch it into the "10 M" position. If the resistance is out of range (e.g. due to a wrong position of the switch) you will see an error message and will be asked to repeat the resistor test. After removing all BNC cables, you can proceed with the test. It will continue for a while and you will get a final message reporting the status of the amplifier, the probe and the connections. If any one of these fails you will see an alert similar to the following one and get the chance to print out the error protocol.

> ***Important note:*** *Please, remind: if you get a message like this, please, contact HEKA first and supply us the error protocol before sending the amplifier in. You might save yourself valuable time and effort!*

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