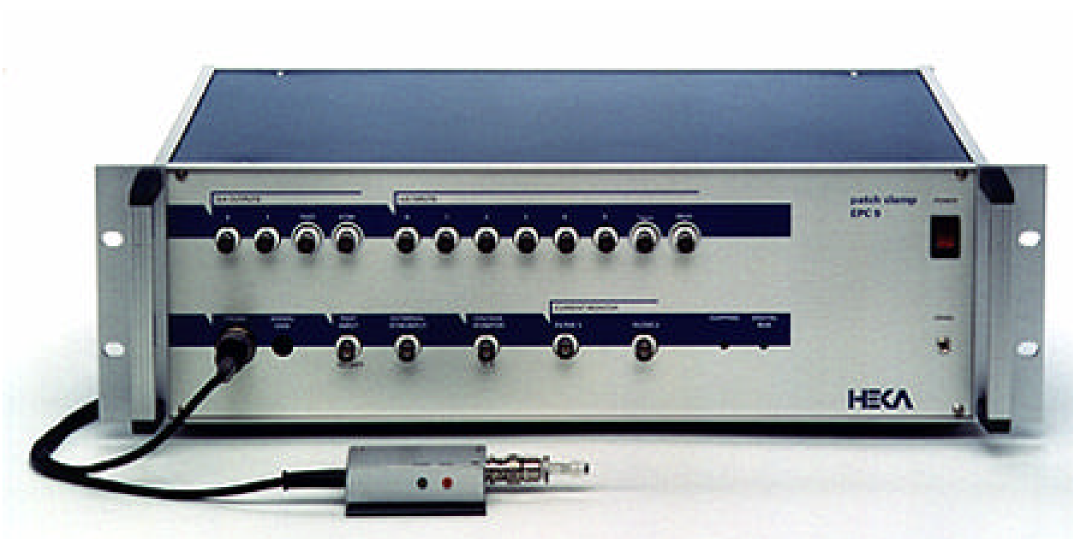
8.52
EPC 9 Manual



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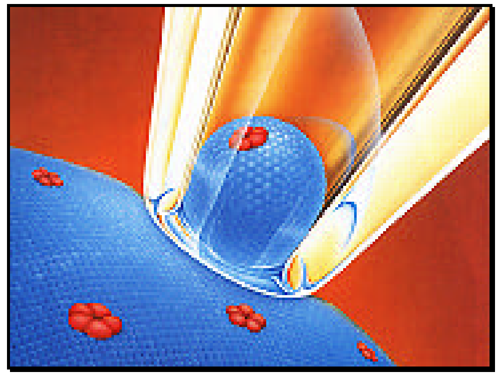
1. Introduction



The patch-clamp technique was introduced by Neher and Sakmann (1976) for recording the currents in a small patch of membrane under voltage-clamp conditions. In the intervening years a number of changes have occurred, most notably the development of the “gigaseal” by E. Neher (1981). Various recording configurations allow intracellular recordings to be made with the same type of recording setup as used for patch recording from the cell surface or cell-free membrane patches (Hamill et al., 1981).

Introducing the EPC 9

The EPC9 represents roughly the ninth in the series of patch-clamp designs in use in the Göttingen laboratories. It is a logical successor to the EPC7, retaining all of its features but adding a number of capabilities, the most important being implementation of full digital control of the various functions. Thus, the new digitally controlled EPC9 patch-clamp amplifier has no knobs, switches or dials. The *Pulse* software replaces the analog controls of conventional amplifiers by using Macintosh computers and a built-in ITC-16 interface. The convenient graphics display and mouse operations provide unsurpassed versatility and ease of operation.

In addition to the controls for the amplifier and the built-in filters, the *Pulse* software contains a powerful data acquisition system (sampling and storage in pulse, ramp and continuous modes), a fully programmable pulse generator, and a digital oscilloscope. Thus, the EPC9 offers all the features of a complete workstation for controlling experiments and acquiring data. Furthermore, there is the *PulseFit* software package as well as *TAC* (Threshold Analysis for single Channels) available, which allow data analysis, data export, and graphics output.

The EPC9 also accepts a stimulus input and provides current monitor outputs just like conventional amplifiers to operate in combination with a host computer running custom and commercial software from other sources. The versatility of the EPC9 can best be appreciated by the variety of experiments that can be carried out with it. Besides high-resolution recordings of single channels, it can be used in studies of whole-cell voltage and current clamp, exocytosis (by monitoring changes in cell membrane capacitance), and recordings from artificial membranes or loose-patch experiments. Technically, the EPC9 is noteworthy for three special features, the range-changing capability of the head stage, the extremely wide bandwidth available from the current monitor circuitry, and the integrated transient cancellation (automatically if desired) and series-resistance compensation functions. Together these features mean that a single head stage suffices for both single-channel and whole-cell recordings, and that both kinds of recordings can be made with high time resolution and low noise.

This manual is designed to provide a general guide for setting up and using the EPC9 for experiments. It covers general information about the hardware, the *E9Screen* program, and basic principles o f the EPC9's functions and patch-clamp techniques.

It is assumed that the reader has some familiarity with patch-clamp techniques; should you be a newcomer to the field perhaps the best place to start would be the paper by Hamill et al., where the basic gigaseal techniques are described and the first three chapters of *Single Channel Recording* (B. Sakmann & E. Neher, eds., Plenum Press, New York, 1995). Certainly, it will be worthwhile to read this manual carefully. Many users will want to read some of the more advanced and complete discussions of individual topics which can be be found in original articles and in the books *Single Channel Recording* (B. Sakmann & E. Neher, eds., Plenum Press, New York, 1995) and *Methods in Enzymology*, vol. 207 (Academic Press, New York, 1992).

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Original Articles

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Book Chapters

Penner, R. (1995) Chapter 1: *A practical guide to patch clamping*. In: *Single-Channel Recording* (B. Sakmann & E. Neher, eds.) Plenum Press, New York.

Marty, A. & Neher, E. (1995) Chapter 2: *Tight-seal whole-cell recording*. In: *Single-Channel Recording* (B. Sakmann & E. Neher, eds.) Plenum Press, New York.

Heinemann, S. H. (1995) Chapter 3: *Guide to data acquisition and analysis*. In: *Single-Channel Recording* (B. Sakmann & E. Neher, eds.) Plenum Press, New York.

Sigworth, F. J. (1995) Chapter 4: *Electronic design of the patch clamp*. In: *Single Channel-Recording* (B. Sakmann & E. Neher, eds.) Plenum Press, New York.

Neher, E. (1995) Chapter 6: *Voltage offsets in patch-clamp experiments*. In: *Single Channel-Recording* (B. Sakmann & E. Neher, eds.) Plenum Press, New York.

Colquhoun, D. & Sigworth, F. J. (1995) Chapter 19: *Fitting and statistical analysis of single-channel records*. In: *Single-Channel Recording* (B. Sakmann & E. Neher, eds.) Plenum Press, New York.

Neher, E. (1992) *Correction for liquid junction potentials in patch clamp experiments.* In: *Methods in Enzymology* **207**, 123-131, Academic Press, New York.

Naming Conventions

EPC 9, EPC 9 Double, and EPC 9 Triple

Throughout the present manual we will address all three amplifier types as “EPC9”. We will explicitly mention the particular amplifiers, where it is required.

Windows versions

The EPC9 is supported on Windows 3.1, Windows 95, Windows 98, Windows NT 3.51, Windows NT 4.0, and Windows 2000.

Throughout the present manual we will address all the above Windows versions as “Windows”. We will explicitly mention the particular Windows versions, whenever it is required.

Support Hotline

If you have any question, suggestion, or improvement, please contact HEKA's support team. The best way is to send us an e-mail or fax specifying:

- Your postal and e-mail address (or fax number)
- The program name:
  E9SCREEN, PULSE, PULSEFIT, etc.
- The program version number:
  v8.31, v8.50
- Your operating system and its version:
  MacOS 7.6.1, MacOS 8.5,
  Windows 98, Windows NT 4.0, etc.
- Your type of computer:
  Mac PPC 8500, Pentium II 300 MHz, etc.
- Your acquisition hardware, if applicable:
  EPC9, ITC–16, ITC–18
- Your amplifier, if applicable:
  EPC9, EPC9 Double, Axon 200B, etc.
- The serial number and version of your EPC9, if applicable:
  EPC9 single, version "920552 D".
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs

We will address the problem as soon as possible.

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2. Description of the Hardware

The hardware components of the EPC9 patch-clamp system consist of the head stage (or probe), the amplifier main unit with the integrated ITC-16 interface board, and the computer system. Specific information about the hardware installation is given below in Chapter **3. Installation** on **page 16**.

Probe

The probe, or “head stage” of the EPC9 is contained in a small enclosure designed to be mounted on a micromanipulator and directly attached to the recording micropipette. It contains the sensitive amplifier that constitutes the current-to-voltage converter, as well as components for injecting test signals into that amplifier. On the probe are the following connectors:

Input Connector: This is a Teflon-insulated BNC connector. The standard pipette holder plugs directly into this connector; the center pin is the amplifier input, and the shield is driven with the command potential *Vp*.

Note: Avoid touching the probe's input terminal, since the input circuitry of the probe can be damaged by static electricity. When it is necessary to touch the input (e.g., while inserting a pipette into the holder), ground yourself first by touching a grounded metal surface.

Ref Output: The red 0.04" pin jack carries the command potential *Vp*.

Note: The metal case of the probe is also connected to this signal, and therefore must be insulated from ground.

Gnd Connector: The black pin jack carries a high quality ground signal which is useful for grounding the bath electrode and nearby shields without potential errors that could arise from ground loops. This ground is connected directly to the signal ground on the controller through the probe's cable. More details on grounding practices will be provided in Chapter **8. Patch-Clamp Setup** on **page 67**.

Main Unit

The main unit of the EPC9 contains the power supply, the signal processing electronics, the AD and DA converters and the connectors for analog and digital input/output. Essentially all of the calibration adjustments are made by digital switches in the main unit, including those which depend on the properties of components in the probe. The calibration parameters are preset by the manufacturer and contained in the software package as the files “Scale.epc” and “CFast.epc”. Unlike conventional amplifiers, hardware calibration of the EPC9 can also be performed by the user if necessary (see section **Calibrating the EPC 9** on **page 16).**

Note: Calibration parameters are unique to each amplifier and head stage combination. Thus, if you exchange the head stage, be sure to perform a new hardware calibration.

Voltage Switch: A switch on the rear panel of the main unit selects between the 110 and 220 volt operation. Make sure that the switch is in the proper position and that the correct fuse is installed.

Power Switch: In order to be initialized properly, the EPC9 must be switched on before starting the software program that drives it, e.g., *E9Screen* or *Pulse*. These programs however allow you to re-initialize the amplifier in case you forgot to turn it on first.

Note: Since the calibration settings of the amplifier have been determined for a warmed-up amplifier, switch on the amplifier ~15 min before starting an experiment. This will ensure that the amplifier has warmed up to regular working temperature and calibration parameters are most accurate.

Chassis Gnd: The chassis is connected to the ground line of the power cord, as is typical of most instruments. The *Signal Ground* is kept separate from the chassis to avoid ground loops, but is connected to it through a 10 Ω resistor.

Test Input: This input is used for the *Test* mode. An external stimulus fed into the *Test Input* is converted into a current (scaling is fixed at approximately -100 pA/V) and injected as a test signal into the probe input. The current injection circuitry has a very wide bandwidth (1 Hz to 1 MHz), allowing very precise determination of the frequency response of the current monitor circuitry. With the *Gain* set to 10 mV/pA, any signal applied to the *Test Input* connector will be reproduced with approximately the same amplitude (but inverted) at the *Current Monitor* outputs.

Note: You can use the internal stimulator to generate a test pulse for determining the accuracy of the calibration parameters. Simply connect the “Test Output” (DA 2) with the “Test Input” and stimulate with the desired voltage pulse.

External Stim. Input: Signals from an external stimulus source are applied here; they can be summed with the internal stimulus if desired. The combined stimulus signal is passed through a 2-pole filter to round off stepwise changes in voltage. This avoids nonlinearities (from slew-limiting amplifiers) in the command processing circuitry and also reduces the amplitude of the current transients from rapid charging of the pipette. Two degrees of filtering, specified as the risetimes (time from 10% to 90% of the amplitude of a step change) are available in the software: 2 µs, which is the minimum required to avoid nonlinearities in the internal circuitry, and 20 µs, which is preferable for all but the fastest measurements, to reduce the capacitive transients.

Voltage Monitor: This output signal provides a monitor of the pipette potential. It is scaled up by a factor of 10 relative to the potential applied to the pipette. The output impedance is 50 Ω. The unscaled signal may be viewed on the software oscilloscope.

Current Monitor: The output signals are filtered according to the settings in the software. Positive voltages correspond to currents flowing out of the pipette. Typically, the left-hand output (Filter 1) is fed to a data recorder (e.g., tape recorder, PCM/VCR combination, or DAT recorder) to record the signal at wide bandwidth, while the additionally-filtered signal from the right-hand output (Filter 2) is applied to an oscilloscope for monitoring the progress of the experiment. Either signal may be viewed on the software oscilloscope.

Probe: This input accepts the multi-pin connector of the head stage.

Signal GND: This banana jack is a high-quality signal ground connection that can be used to ground other parts of the experimental setup as necessary (see **Chapter 8. Patch-Clamp Setup** on **page 67**).

Clipping: This LED lights whenever an amplifier saturates in the current monitor pathway. The indicator is important in voltage-clamp experiments where capacitive artifacts will be subtracted in a computer; the subtraction will work well only as long as no saturation occurs, and this indicator serves as a simple monitor of this condition. It is particularly useful since it will indicate clipping by internal amplifiers even in cases where, because of filtering, the output voltage is not saturated.

Digital Bus: This LED lights whenever digital information is sent from the computer to the EPC9 amplifier.

AD Inputs: The built-in laboratory interface (ITC-16) provides eight AD channels (0-7). AD channels 6 and 7 are internally connected to the EPC9 and are used by the software supplied. Channel 6 is labeled “I-Mon” and carries the *Current Monitor 2* output. Channel 7 carries the output of the EPC9's internal multiplexer, which in most operating modes is set to the voltage monitor signal. You normally should not connect anything to channel 6 and 7, unless you wish to inspect the signals for diagnostic reasons. However, channels 0-5 are freely available for application programs. For example, the *Pulse* program can use these channels to monitor temperature, pressure or outputs from other sensors.

DA Outputs: Four DA channels are provided (0-3). They carry the following signals:

- **DA-0 -** Free (*C-slow* during *Cap. Track*)
- **DA-1 -** Free (*G-series* during *Cap. Track*)
- **DA-2 -** Test output (used in self-test and calibration)
- **DA-3 -** Internal stimulus output (used to monitor the stimulus)

Note: These are output connectors! Make sure that you never feed stimuli into these outputs.

DA-1 is typically used to trigger an oscilloscope or an isolation unit. In the *Capacitance-Tracking* mode of *E9Screen*, DA channels 0 and 1 can be used to provide optional special-purpose outputs. DA-2 can be used to inject test signals into the EPC9 circuitry in the *Test* mode (see Chapter **Measuring the Frequency Response** on **page 34**). DA-3 is wired internally as the internal stimulus generator.

The specific DA-channel assignments are made in the software (see *Pulse Manual, Chapter 6 - EPC9 Amplifier*). DA channel 2 (*Test*) should normally not be used. Also, DA-3 should normally not be used to monitor the stimulus output, because this may degrade the noise performance of the EPC9. The buffered *Voltage Monitor* output should be used instead. The voltage at the connector is 10 times the nominal stimulus amplitude.

Rear-panel connectors: Three 40-pin connectors and an analog trigger input allow connection of the EPC9 to other devices:

- **Computer-Interface**: This is the connection to the Mac-23, AT-16, PCI-16, or PCI-18 board in the host computer, that allows the computer to communicate with the EPC9.
- **Digital I/O**: TTL-level, digital input and output lines are available here for the control and monitoring of digital signals. See pin assignments in Appendix II: Technical Data.
- **VR10/100**: This digital input port is provided by the ITC-16 but is not used by the EPC9 and its software.
- **Trigger In**: Input for an external trigger to start data acquisition when the ITC-16 is waiting for an external trigger. This mode is set in *Pulse+PulseFit* when either *Trigger Series* or *Trigger Sweeps* is selected in the *Pulse Generator*.

EPC 9 Double and Triple

The EPC9 Double and Triple contain two and three independent EPC9-amplifiers in one case, respectively. All amplifiers share a single ITC-16 AD / DA-board.

The ITC-16 board provides 4 DA-channels which are sufficient to allow simultaneous stimulation of every individual amplifier. The 8 available AD-channels can independently read all voltage- and current-outputs of the individual amplifiers.

One amplifier is always selected as the "active" amplifier. The "active" amplifier is the one which is enabled to receive configuration commands. One can visually identify the "active" amplifier by checking the DIGITAL BUS light. The green LED-light of the "active" amplifier flickers when the digital lines are alive during command transmission. The notion "active amplifier" does not imply that the other amplifiers are not active. They remain fully functional, but not enabled to receive programming commands.

The internal, hardwired DA- and AD-channel assignments for the EPC9 Double and Triple are as follows:

	EPC9 amplifier board		
	1	2	3
Stim-DA	0	1	2
Vmon-AD	0	2	4
Imon2-AD	1	3	5
Test-DA [1,2]	3	3	3
Mux-AD [1]	7	7	7

Note 1: Mux-AD, Test-DA, and Clipping-lines are connected to the selected "active" EPC9 board only. Stim-DA, Vmon-AD, and Imon2-AD are always accessible for all individual EPC9 boards.

Note 2: The Test-DA can be connected to the stimulus input of all 3 amplifiers. This allows to simultaneously stimulate all 3 amplifiers. Separate scaling factors are available for each individual amplifier.

3. Installation

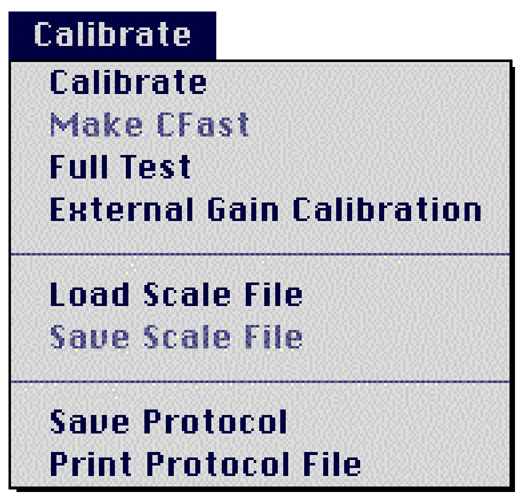
Installation Procedure

Please, installation procedure for the EPC9 and the required software is described in the separate **“Installation_8x5”** manual.

Calibrating the EPC 9

If you wish to calibrate your amplifier proceed as stated in this section. Calibration is usually not necessary with a new amplifier, since you can use the calibration files supplied by HEKA. However, it is advisable to recalibrate the EPC9 twice a year or whenever the frequency response of the amplifier is not accurate or offset currents become noticeable.

Note: The calibration file contains the settings of the digital switches and controls of the amplifier. These are unique to a given combination of amplifier and headstage (probe) and cannot be used for another EPC9. Therefore, you have to recalibrate the amplifier, when you replace the probe! This is a big advantage of the EPC9, since you can use any probe with any amplifier and replace a broken probe without having to send the amplifier in for recalibration.

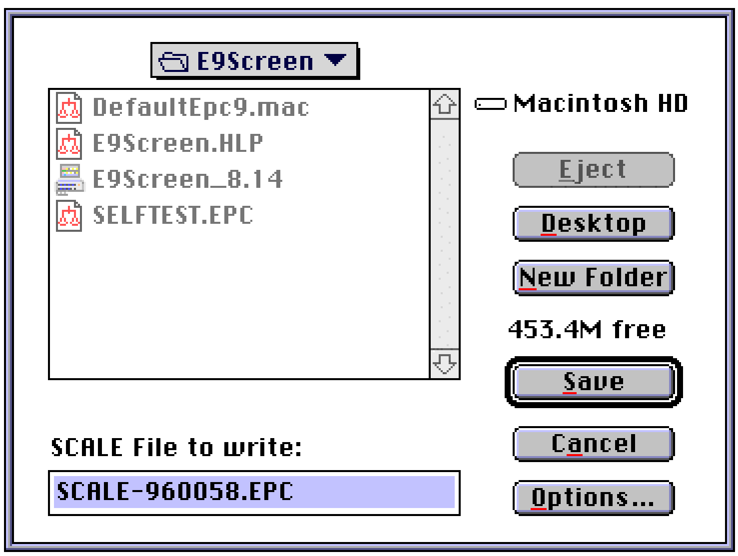


Before starting the calibration make sure that the amplifier has reached its operational temperature, since the calibration depends on temperature. We advice to let the EPC9 warm up for 60 minutes after powering the amplifier on. Start the **E9SCREEN** program. The default installation copies it into the E9Scren folder inside the HEKA folder. Windows users might alternatively use the **Start** button to launch **E9SCREEN** from **Programs** → **HEKA**. In the program, go to the **Calibrate** menu. This menu contains all the items involved in calibration generation of scale files. If there is no valid calibration, the menu item Make CFast will be disabled. If you have an **EPC9 Double** or **Triple** this menu item will also be disabled, unless each amplifier has been calibrated correctly. To perform the calibration select **Calibrate** from the **Calibrate** menu. **E9SCREEN** will warn you that this procedure may take up to 10 minutes, depending on the speed of your computer (6 minutes on a 300 MHz PII system). Go ahead by clicking the **Yes** button.

Then you are instructed to remove anything from the probe and shield its input:

You can use the metallic cap that came with your EPC9 and put it on the BNC connector of the probe to shield it. Please make sure that really **nothing** except the metallic cap is connected to the probe (the red and the black pin jack should be free) and that no BNC cables are connected to the inputs and outputs of the EPC9 !

At the end of the calibration, **E9SCREEN** will let you know, whether the calibration succeeded or failed. If it succeeded, the program will ask you, whether you want to save the new calibration file and generates the proper name for the calibration file.

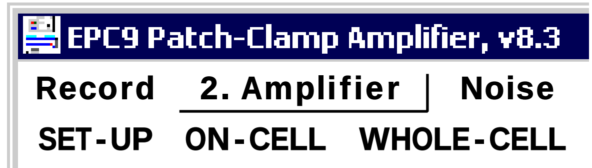


It is not advicable to save the file under a different name, because in that case you would have to manually load the scale file during every initialization.

Note: It is very advisable to store the calibration files only in the one place. The best place to put the calibration files is the E9Screen folder. Most users will run more than one program which needs the calibration files to properly control the EPC9, e.g. **PULSE** and **E9SCREEN**. Both programs will load by default the calibration files from the E9Screen folder. If the calibration files are in multiple places the programs may load different calibration files with possibly unpleasant consequences!

Finally, **E9SCREEN** re-initializes the amplifier.

If you have an **EPC9 Double** or **Triple,** you should proceed to calibrate the second and third amplifier as well. From the amplifier pop-up menu, choose **2. Amplifier** or **3. Amplifier** and repeat the steps listed above.



After having calibrated the amplifier (all amplifiers in case of an **EPC9 Double** or **Triple)**, you should also create a new C-fast lookup table for each amplifier as stated in the next section.

Creating the C-fast Lookup Table

E9SCREEN and **PULSE** try to load the C-fast lookup table from the same location as the calibration file when initializing the **EPC9**. You will get an appropriate error message, if the Cfast file was not successfully loaded. To create a new C-fast lookup table select **Make CFast** from the **Calibrate** menu in **E9SCREEN**. A confirmation dialog will be displayed and will instruct you to remove anything from the probe and shield its input. Again, please make sure that nothing is connected to the probe except for the metallic cap that came with your amplifier. Now **E9SCREEN** will create the C-fast lookup table. This usually takes a few minutes (30 seconds on a 300 MHz PII system). If you get the message that the noise of the probe is suspiciously low this may indicate that your probe is not connected to the main unit of the EPC9 – or you have a very good probe! Finally, **E9SCREEN** will ask you to save the modified C-fast lookup table to disk and suggest a reasonable path and file name corresponding to your active calibration.

Finally, **E9SCREEN** re-initializes the EPC9. If you have an **EPC9 Double** or **Triple,** you should continue and create the C-fast lookup table of the second and third amplifier. From the amplifier popup menu, choose ”2. Amplifier” or ”3. Amplifier” and repeat the steps listed above.

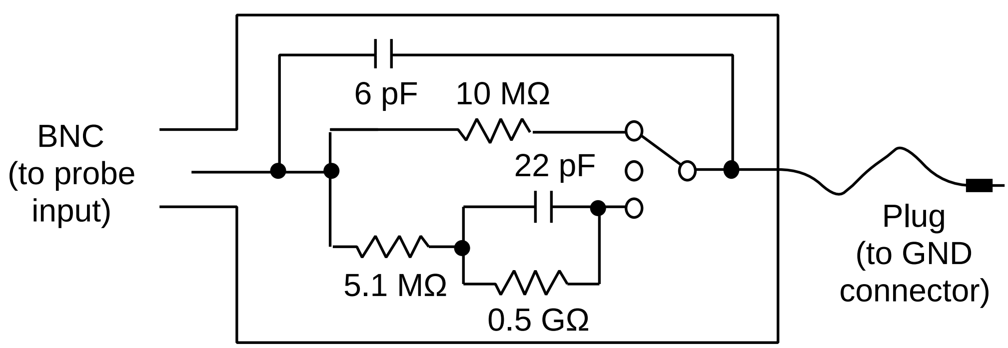
4. Verifying and Testing the EPC 9

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, whether 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 in the program **E9SCREEN**.

The Model Circuit MC 9

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 cell MC9

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 6pF 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.

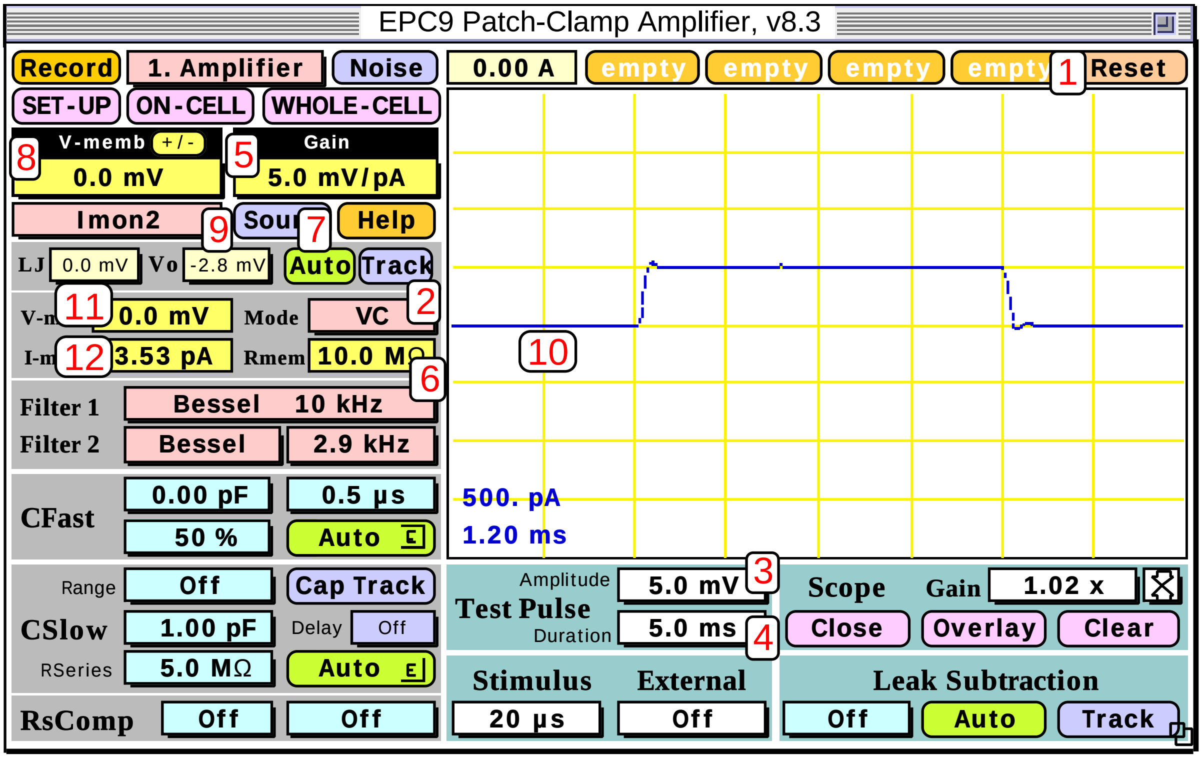
3. In the bottom position a ”model cell” in the whole cell patch-clamp configuration is simulated. The ”input resistance” is 5.1 MΩ, the ”membrane resistance” is 500 MΩ and the ”membrane capacitance” is 22pF. This mode lets you test the *C-slow* compensation and the current clamp mode. Furthermore it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

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

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

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) such as 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 MOhm" 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: $\Delta I = \Delta U \ / \ R = 5 \ mV \ / \ 10 \ M\Omega = 500 \ pA$. *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 automatically canceled by clicking on 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.

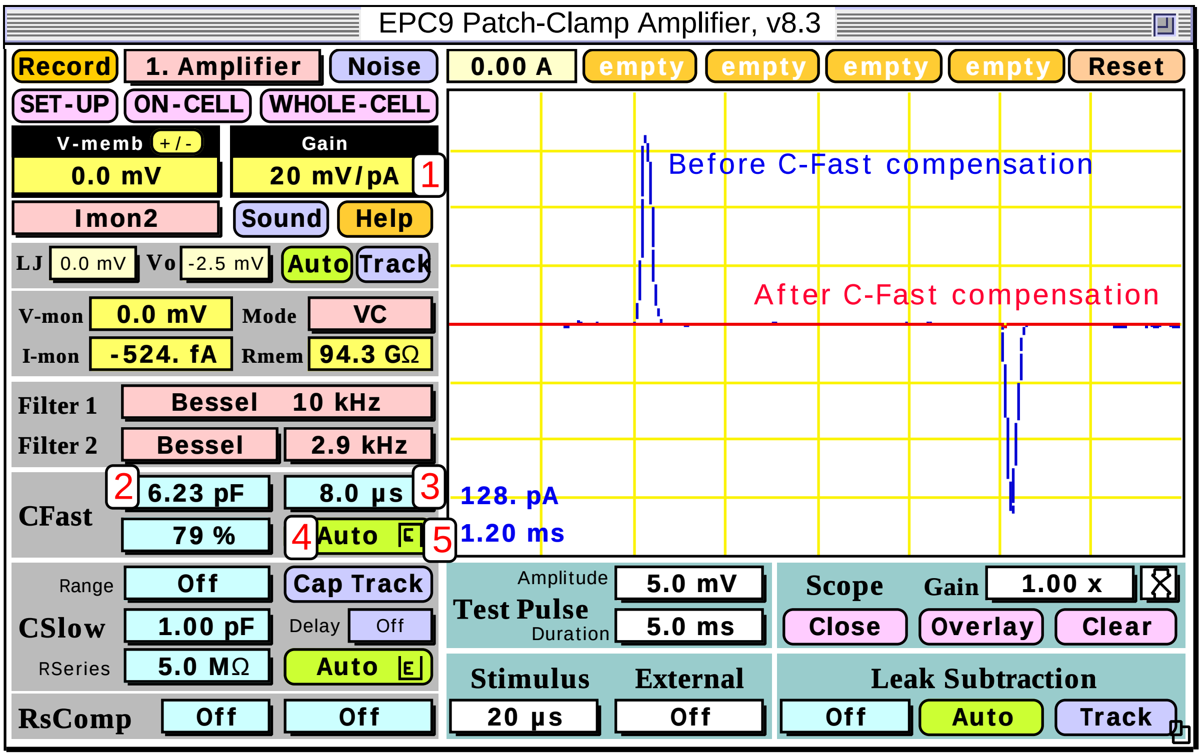
The steps listed above can be automatically executed by clicking on the **SET-UP** button or pressing the '1' key on the numerical keypad. This will execute the following built in macro that resets the amplifier, creates a rectangular test pulse, sets the gain of the amplifier to 5 mV/pA and then performs an automatic compensation of the 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** 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 and E9SCREEN to set the **gain** popup in the EPC9 window to the **10**th 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 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 (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. 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 (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, 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
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 GOhm” 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) 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 $\tau = R_s \; C_m$ = 5.1 MΩ 22pF = 112 μs. The peak current can be calculated from $I_{max} = C_m \; \Delta U / \tau$ = 22 pF 5 mV / 112 μs = 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 *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. 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 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 option **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 reasonable values:

```
3 : WHOLE-CELL
E  Invert:   TRUE    ;
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  SoundOn:  TRUE    ; beep
E  SoundOn:  FALSE   ;
```

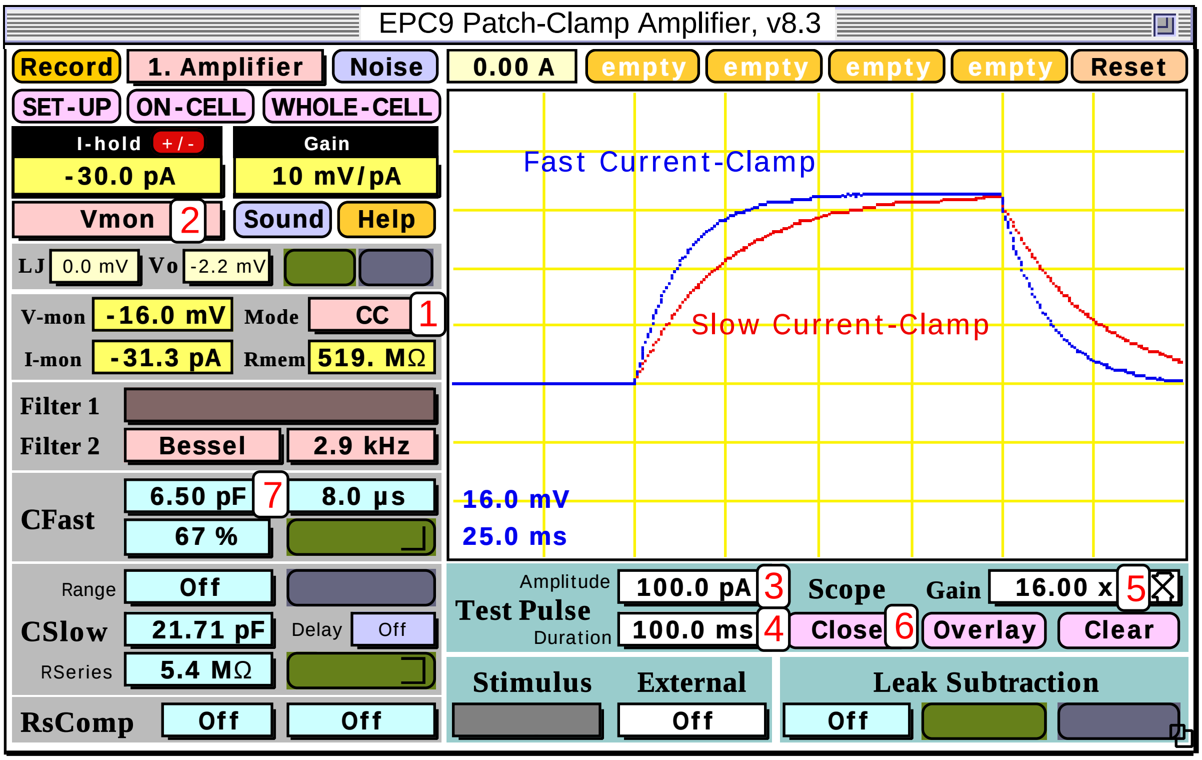
Step 4: ”Whole-Cell” Current-Clamp Recording

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 $\tau = R_m \cdot C_m$ = 500 MΩ 22pF = 11 ms to a final value of $V_{max} = R_m \cdot I$ = 500 MΩ 100 pA = 50 mV. Due to the slower time constant compared with voltage clamp conditions it takes much longer to reach V_{max}, therefore you should increase the duration of the test pulse to a more appropriate value of 100 ms (4).

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

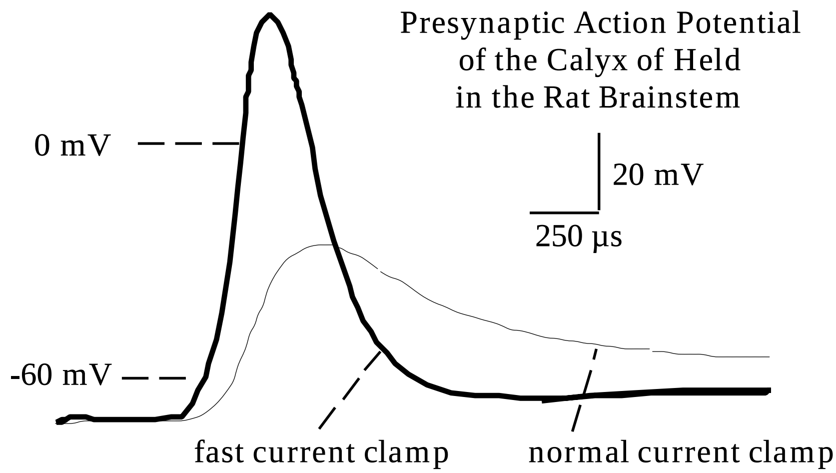
The normal setting of the oscilloscope scales the voltage monitor at 250 mV per division. You should therefore increase the gain of the oscilloscope to 16 (5) which scales the display to be 16 mV per division. Please remember that the oscilloscope gain is different from the amplifier gain and only scales 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 circuitry 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. The board version of your *EPC9* amplifier is displayed in the last menu item of 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 to misadjustment 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 around 8.1 pF and above, when the fast current clamp mode has been activated.



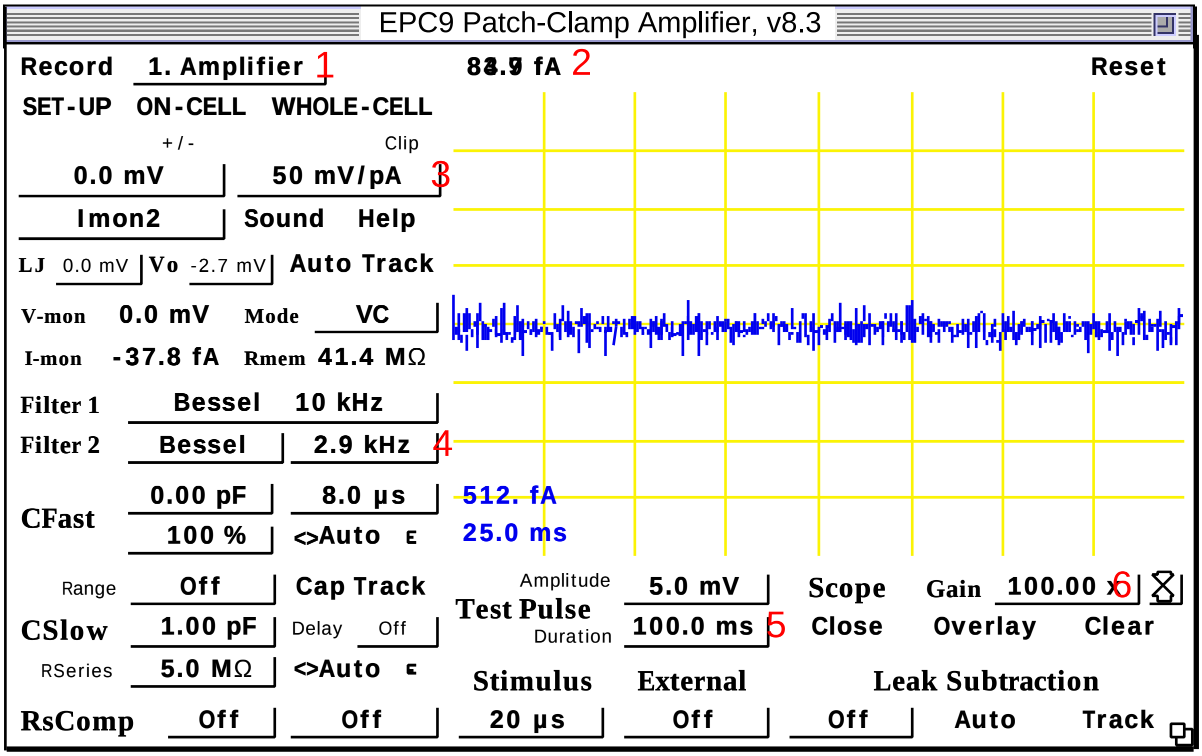
Note: The 500 MΩ setting of the model circuit is not a good method for testing the fast clamp speed of the EPC9 due to the long time constant of 11 ms which the amplifier can easily follow. If you want to have a better estimation of the amplifier's speed under current clamp conditions you should do the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

The following figure shows a recorded action potential (from H. Taschenberger & H. von Germsdorff):

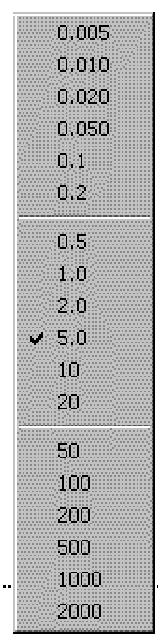


Step 5: Measuring the Noise of the Amplifier

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, e.g., by optimizing the grounding of the setup.



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 intrinsic 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 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 GΩ measuring resistor).

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).

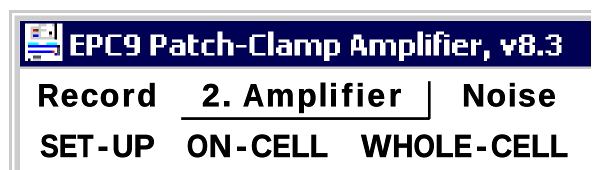
Tip: If you wish to ground your setup you should now attach the pipette holder to the probe, insert a glass pipette, bring the pipette tip into the recording position near the recording chamber and power on every piece of equipment that introduces noise (lamps, oscilloscope, camera, ...). Setting the 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 about 100 fA of additional noise.

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 9 on **page 16**) you can run the **Full Test** in **E9SCREEN.** 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.

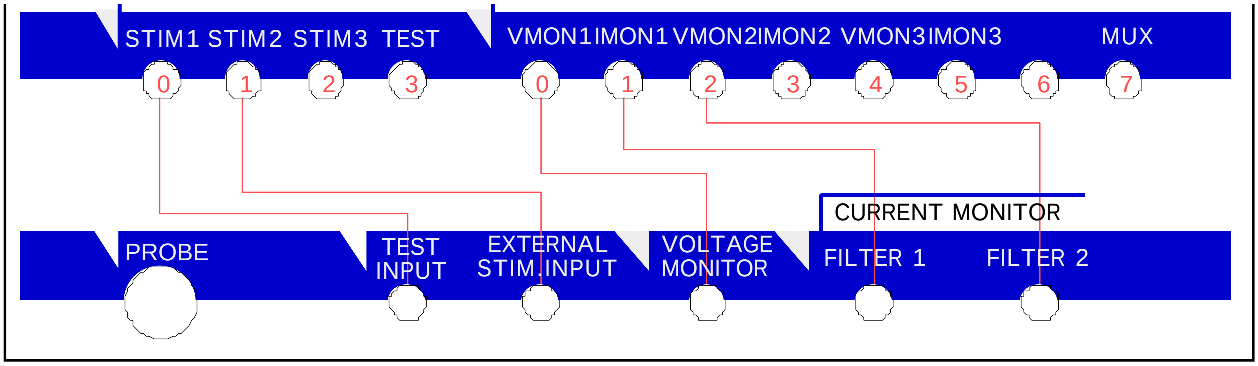
Note: The Full Test sometimes reports errors although the amplifier is absolutely fine. This may have multiple reasons. First, we opted to have stringent test specifications such that possible problems are not missed. Second, errors may be caused by procedural errors, or even because of defective BNC 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 EPC9), a 10 MΩ resistor (e.g., the model circuit) and 5 short BNC cables. Start the **E9SCREEN** program. In case you have an **EPC9 Double** or **Triple,** select the amplifier you want to test from the amplifier popup: 1. Amplifier, 2. Amplifier or 3. Amplifier.



Now select the menu item **Full Test** from the **Calibrate** 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 red and the black pin jacks are free. You should also make sure that **no BNC cables are connected** to the main unit of the amplifier.

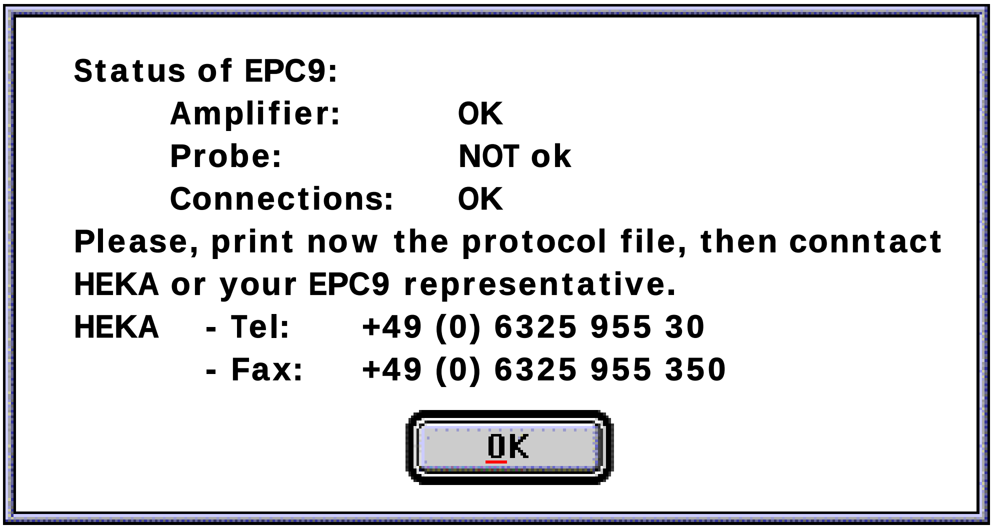
After a short while you will be prompted to connect 3-5 BNC cables, depending on the amplifier you are testing, see table below. The following figure shows you how to make the connections for the **EPC9**. Please note, that the description of the analog-to-digital converter part (top row of BNCs) is for the **EPC9 Triple** and looks different for the **EPC9** and the **EPC9 Double.** The DA- and AD-channels are numbered from 0 to 3, and 0 to 7, respectively.



The following table shows you the combinations for the different amplifiers: **EPC9, EPC9 Double,** and **Triple.** The **EPC9 Triple** has only one free AD input left (ADC-6), the remaing channels are internally connected with the corresponding channels of the amplifier. Therefore, only three connections (2 DA-channels and 1 AD-channel) have to be made for the three amplifiers.

	Amplifier Input		**Amplifier Output**		
	Test Input	External Stim. Input	Voltage Monitor	Filter 1	Filter 2
EPC9	DAC 0	DAC 1	ADC 0	ADC 1	ADC 2
EPC9 Double 1. Amplifier	DAC 2	DAC 3	ADC 4	ADC 5	ADC 6
EPC9 Double 2. Amplifier	DAC 2	DAC 3	ADC 4	ADC 5	ADC 6
EPC9 Triple 1. Amplifier	DAC 2	DAC 3	not tested	not tested	ADC 6
EPC9 Triple 2. Amplifier	DAC 2	DAC 3	not tested	not tested	ADC 6
EPC9 Triple 3. Amplifier	DAC 1	DAC 3	not tested	not tested	ADC 6

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 MΩ 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!

Measuring the Frequency Response

The **Test** mode allows one to easily check the basic current-measuring circuitry of the EPC9. Select **Test** from the **Mode** list, connect a function generator or stimulator to **Test Input,** and connect the right-hand **Current Monitor Output** (Filter 2) to an oscilloscope. Alternatively, you can connect the **Test Output** of the internal stimulator (DA 2) to the **Test Input** and look at the response. With the **Gain** set to 10 mV/pA, any signal applied to the **Test Input** connector should be reproduced with the same amplitude at the **Current Monitor Output.** An applied signal amplitude of about 1 volt is appropriate.

Note: The probe input usually needs to be shielded from low-frequency noise. A simple way to do this is to cover the input connector with a cap made from aluminum foil.

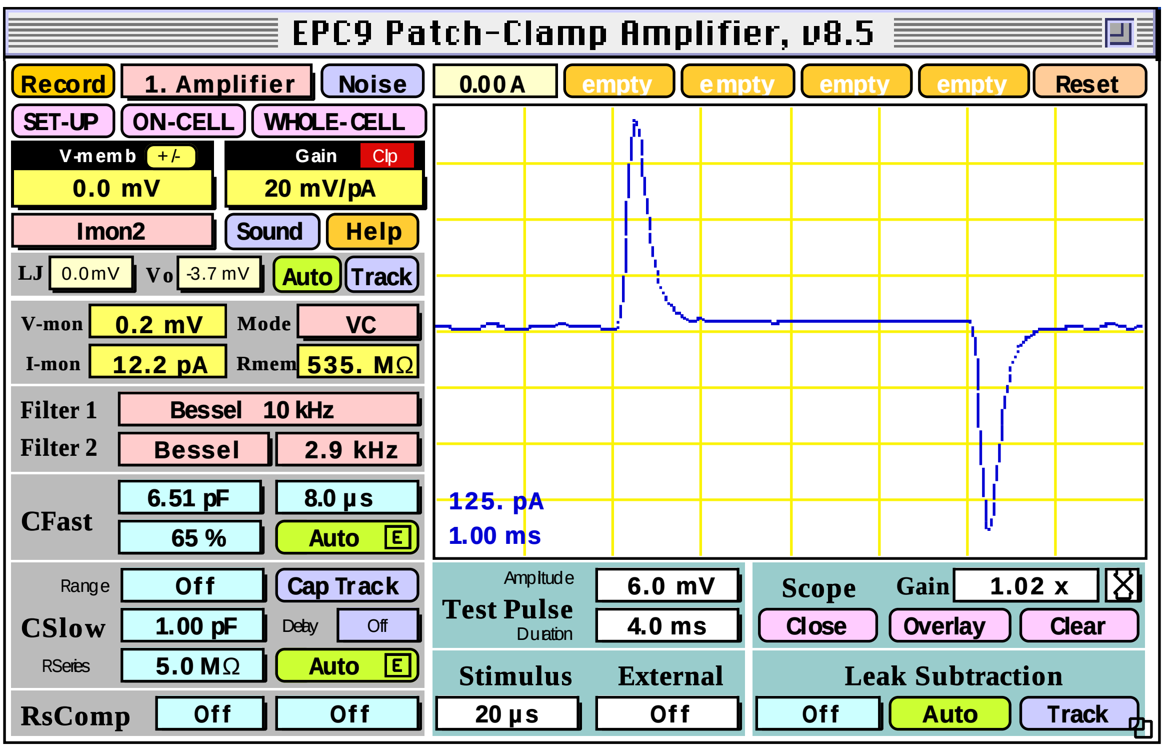
Note: The Test mode current-injection system is intrinsically AC-coupled, which means that long (>30 ms) pulses may be distorted in shape. Also, large low-frequency inputs can cause the current-injection circuitry to saturate.

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 GΩ measuring resistor). If the signal source is a sine-wave generator, the frequency response of the patch clamp can be verified directly.

5. E9SCREEN Software

The *E9Screen* software provides the control and the graphical representation of the EPC9 amplifier by a “virtual panel” with “buttons”. Signal display is provided by an oscilloscope-like display. In addition, there is a *Notebook* window and drop-down menus with options for the calibration procedures. *E9Screen* is mostly of interest to those who use the EPC9 without *Pulse*, as it provides the only other means by which the amplifier can be controlled. *Pulse* users will use *E9Screen* mainly for diagnostic or calibration purposes.

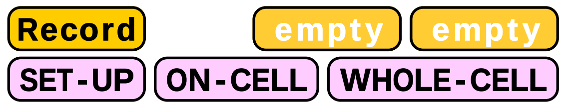
EPC9 Window



Main Controls

Amplifier Selection: On an EPC9 Double or Triple, one selects the "active" amplifier from the "amplifier" pop-up list. Only the available amplifiers can be selected in the amplifier list. When

switching, E9Screen updates all parameters to show the state of the selected amplifier. The state of each amplifier is independent of the settings of the other amplifiers.



Macros: Macro buttons allow to record and play back a sequence of actions, such as activation of buttons and parameter inputs via mouse or keyboard. The macros *Set-Up*, *On-Cell*, and *Whole-Cell* are preset. *Set-Up* resets all parameters (with the exception of *LJ* and V_0), and defines the parameters of the test pulse. *On-Cell* switches the *Gain* range to a typical setting for a cell-attached patch recording, sets initial *C-fast* estimates, and invokes an *Auto C-fast* compensation. *Whole-Cell* switches the *Gain* range to a typical setting for a whole-cell recording, sets initial *C-slow* estimates, and invokes an *Auto C-slow* compensation. These macros can be redefined and other macros be recorded using the macro record function (see below). All macro controls that have nothing stored and therefore perform no action are shown in white letters.

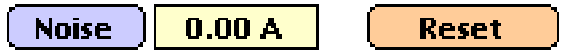
Note: The content of the macros can be written to the Notebook using the option Macros…List in the EPC9 drop-down menu.

Record: To start macro recording, click on the *Record* button. Then, perform all desired actions (the *Notebook* window will print a protocol of the macro actions). While recording a macro you have the option of actually executing all actions as they are entered or disable execution and only log the actions to the macro (see *EPC9* menu *Macro* options). To specify a parameter value, enter it as usual by dragging or typing. When clicking on a macro-button during recording, you will see a dialog with the following options:

- **Cancel**: This will disregard the macro call. You can continue recording.
- **Record call to macro itself**: This will execute the macro as part of the macro being recorded (embedded macro call).
- **Copy contents of macro**: This will copy each of the macro instructions of the selected macro into the macro being recorded. This avoids the potential problems of recursive macros (i.e., macros calling each other and causing an infinite loop).
- **Assign and name recorded macro**: This will prompt you to give a name to the macro. This name will become the button text.

Do not forget to save the macros in a file on disk (you can only save all 7 macros at once). Otherwise they will only be remembered until you leave the program. The default macro file is named “Default.Epc9_Macros” and it will be automatically loaded next time the program is started.

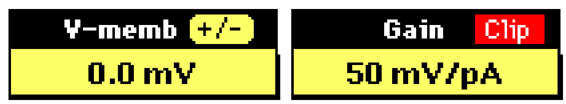
Note: There is a limit of 50 actions per macro. To abort recording of a macro, click again on “Record” and the just recorded sequence will be lost.



Noise: The rms noise is continuously measured and updated when the noise mode is selected with the button *Noise*. For the determination of the noise level, no pulses are output and current is sampled via the active AD-channel using the current filter settings (usually the *Current Monitor 2* output, so that the bandwidth is determined by Filter 2). It is sampled in sections of 10 times 256 points with a sample interval of 100 µs, i.e., a total length of 256 ms.

I-monitor: DC current monitor.

Reset: Selecting this button will reset the EPC9 to its initial default configuration. It will reset the DA channels to zero, which is useful to cancel the output of *C-slow* and *R-series* set by *Cap. Track*. Also, *Reset* is very useful to define the initial state of the EPC9, when recording a macro.



V-membrane: Displays the actually-measured pipette voltage after correcting for liquid-junction potentials and offsets (provided the zero-current potential has been set correctly). This may differ (temporarily) from the holding voltage (e.g., during long stimulation pulses) as it indicates the average sum of *V-membrane* and the scaled stimulus voltage. *V-membrane* is converted to *I-hold* in *Current Clamp* mode.

+/-: This button inverts the polarity of *V-membrane*.

Gain: Sets the scaling of the current monitor output. The range is 0.005 to 2000 mV/pA and can be set by dragging the mouse or pressing ↑ and ↓. The gain setting automatically selects one of the three available current-measuring feedback resistors in the probe (5 MΩ, 500 MΩ, and 5 GΩ), corresponding to low, medium and high gain ranges. The table below summarizes the main features and limitations of the gain ranges:

	Low	Medium	High
Feedback Resistor	5 MΩ	500 MΩ	50 GΩ
Gain	0.005-0.002	0.5-20	50-2000
I_{max}	± 2 µA	± 20 nA	± 200 pA
Bandwidth	100 kHz	100 kHz	60 kHz
C-slow Ranges	30 • 100 • 1000	30 • 100 • 1000	30 • 100
Current Clamp	no	yes	no
R_S-compensation	yes	yes	yes

The lowest range may be used for experiments (e.g., bilayers, loose-patch, or large cells) in which large currents need to be delivered (up to about 2 µA). Capacitance compensation of up to 1 nF is available and R_S-compensation can be used for R_S values down to 10 Ω in this range.

The medium and high gain ranges operate similarly to the EPC7. In the medium gain range, the background noise is larger than in the high gain, but the full 100 kHz bandwidth is available, and currents of up to about 20 nA can be recorded. This range is used mainly for whole-cell recordings, and for this purpose the special features of the 1000 pF transient cancellation range (see *C-slow Ranges*), series resistance compensation, and the current-clamp modes are made available. On the other hand, the high gain range is intended for single-channel recording. It has a very low noise level, but this is obtained at the expense of a maximum current limit of about 200 pA. The maximum available bandwidth is about 60 kHz, and the special features mentioned above do not function in this range.

Slow capacitance cancellation ranges (30-100-1000 pF) can be set to any desired value. However, in the high gain range (50 GΩ resistor) the 1000 pF range will not operate. If the 1000 pF range is selected while the gain is higher than 20 mV/pA, the gain will automatically be reduced to the highest possible setting (20 mV/pA). Similarly, since the current-clamp mode is only possible in the intermediate gain range (0.5-20 mV/pA), the gain will be reduced or increased appropriately when selecting *Current Clamp* mode from an invalid gain range.

Clipping: A blinking box labeled “Clip” in the *Gain* title indicates saturation of amplifiers in the current monitor circuitry. Like the *Clipping* LED on the EPC9 main unit, this is a warning that excess artifacts or noise may occur due to the saturation of amplifiers.

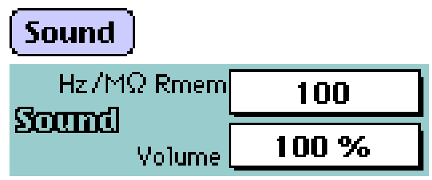
Note: This indicator may appear to be more sensitive than the LED on the EPC9. It is not; it just latches the clipping status longer than the LED light.

AD-Channel: The oscilloscope can display the following:

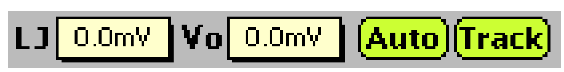
- **F2-Ext** - External Stim. Input filtered by Filter 2
- **Imon-1** - Current monitor 1
- **Imon-2** - Current monitor 2
- **Vmon** - Voltage monitor output
- **AD 0...5** - Any of the other AD channels.

The *F2-Ext* setting allows you to use Filter 2 as a general-purpose variable filter. In this setting the *External Stim Input* becomes the filter input, and the filter output is available at Filter 2 and is displayed in the *Oscilloscope* window.

Help: Displays keyboard equivalents that are assigned to EPC9 controls. In this mode you can click on any control to have the *Help* window display a short description of that control.



Sound: If this control is *On*, a sound is played with its frequency coding for *R-membrane*. The sensitivity (Hz/MΩ) and the volume can be specified. The controls that set these parameters are normally hidden, but may be accessed by closing the *Oscilloscope* window.



V_0 (*Pipette Offset*): V_0 displays the offset voltage (a voltage which is added to *V-membrane* to obtain the pipette command voltage). It can be set either by the *Auto-*V_0 operation or by manually dragging the mouse after clicking into the item. Furthermore, V_0 is changed automatically by the controlling program whenever the user changes the variable *LJ*. This is necessary for *LJ* and the *Auto-*V_0 operation to interact properly. It is not recommended that the user changes V_0 manually, because this interferes with the software features for automatic offset correction.

Auto-V_0: The *Auto-*V_0 button calls a procedure for automatic zeroing of the pipette current. Thereby, an offset voltage (V_0) to the pipette potential is systematically varied until pipette current is zero. Range of V_0 is ± 200mV. *Auto-*V_0 is typically performed before seal formation. It works properly only when a pipette is inserted into the bath.

The *Auto-*V_0 procedure interacts with the variable *LJ* to provide for online correction of liquid junction potentials and other offsets (see Chapter **7. Compensation Procedures** on **page 59**). This requires that *V-membrane* is set to the value of *LJ* (for *On Cell* and *In Out* Recording Modes) or to the opposite polarity of *LJ* (for *Whole Cell* and

Out Out Recording Modes), before the actual zeroing operation is performed. *Auto*-V_0 does this automatically and leaves *V-membrane* at that value.

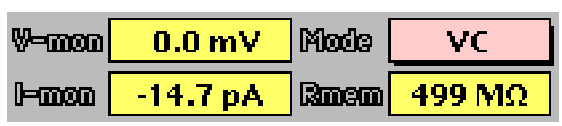
Note: V_0 is not changed by the Reset function.

Track: This option implements a *Search Mode* (see Chapter **6. Operating Modes** on **page 58**), which is essentially a repetitive *Auto*-V_0 procedure at a holding potential of 0 mV. The rate at which *Auto*-V_0 is performed is determined by the *Search Mode Delay* in the EPC9 menu.

LJ (*Liquid Junction*): *LJ* is a variable, to be set by the user, which allows to correct for liquid junction potentials and other offsets. It works in conjunction with the V_0 operation. An online correction requires an *Auto*-V_0 operation to be performed before seal formation and *LJ* to be set to an appropriate value. No correction is performed if *LJ* = 0. See Chapter **7. Compensation Procedures** on **page 59** for more information on how to determine *LJ*.

LJ can be adjusted within ± 200 mV by dragging the mouse or typing after a double-click. Please note that *LJ* is not changed by the *Reset* function, and cannot be set by macros. This restriction is imposed to avoid unintentional offset corrections.

LJ should be 0 mV when using identical pipette and bath solutions. It may be changed to any desired value within ± 200 mV in case asymmetrical solutions are used or the bath solution is changed during an experiment. For the standard liquid junction potential correction, the polarity of the entered value should be such that it represents the potential of the bath with respect to the pipette solution. For example, if the pipette solution contains glutamate or aspartate (with chloride in the bath), then the polarity of *LJ* should be positive (+10 mV). After an *Auto*-V_0 operation, *V-membrane* will be changed to -10 mV (in *Whole Cell* and *Out Out* Recording Modes) or +10 mV (for *On Cell* and *In Out* Recording Modes), which corresponds to the true zero-current potential.



V-mon: Displays the actually-measured pipette voltage after correcting for liquid-junction potentials and offsets (provided the zero-current potential has been set correctly). This may differ (temporarily) from the holding voltage (e.g., during long stimulation pulses) as it indicates the average sum of *V-membrane* and the scaled stimulus voltage.

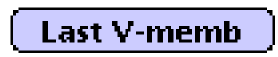
I-mon: DC current monitor.

R-membrane: The *Seal Resistance* (*R-membrane*) is determined from the current sampled during the baseline and the second half of the test pulse. *R-membrane* can be encoded into a tone using the *Sound* feature.

Mode: Sets the *Recording Mode* (in the above example it is *Voltage Clamp*). For details on the various recording modes see Chapter **6. Operating Modes** on **page 54**.

- **Test** - Sets the *Test* mode.
- **VC** - Sets the *Voltage Clamp* mode.
- **CC** - Sets the *Current Clamp* mode.

When switching from voltage-clamp to current-clamp mode, *I-hold* will be set to inject the holding current required to keep the membrane voltage constant ("gentle switch", see description of the *Gentle CC-Switch* control below, subchapter *Hidden Controls*). Thus, V-mon will be identical to the original *V-membrane* commanded under voltage clamp. In analogy, upon returning to voltage clamp, *V-membrane* will be set according to the membrane potential under current clamp and rounded to the next 2 mV.



Quite often, the membrane potential under current clamp will have changed, and when returning to voltage clamp, *V-membrane* will possibly differ from that set before switching to current-clamp mode. *Last V-membrane* can then be used to conveniently restore the original *V-membrane* value. This button is normally hidden behind the oscilloscope window; it can be seen when closing the oscilloscope. For details see Chapter **6. Operating Modes** on **page 54**.

Starting with the version "C" of the EPC9, the current-clamp circuitry has two speed settings (which can be toggled by a button that is normally hidden behind the oscilloscope window; it can be seen when closing the oscilloscope). For details see Chapter **6. Operating Modes** on **page 54**.

Filter 1: Controls an analog 3-pole filter in the current monitor pathway. The pop-up menu provides for the following settings:

- **Bessel 100 kHz**
- **Bessel 30 kHz**
- **Bessel 10 kHz**
- **HQ 30 kHz**

Under most conditions a 10 kHz bandwidth is more than ample, and the filtering reduces the high-frequency noise substantially. The HQ 30 kHz setting is selected automatically when fast R_S-compensation is in use; it is of little use otherwise.

Filter 2: Controls an analog 4-pole filter for the current monitor 2 (*I-mon 2*). Filter 2 is in series with Filter 1. Dragging the mouse or typing allows fine adjustment from 0.1-16

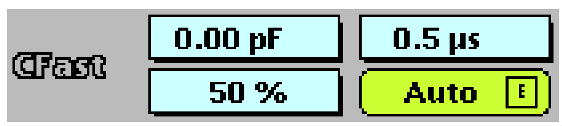
kHz in 0.1 kHz steps (guaranteed accuracy 0.5-15 kHz). Two filter characteristics can be selected from the pop-up menu:

- **Bessel**
- **Butterworth**

The -3 dB point is given in both cases. *Bessel* is the best characteristic for general use; the *Butterworth* response rolls off more rapidly with frequency and is mainly useful for power spectral analysis. The setting of the Filter 2 control is the actual effective bandwidth of the series combination of Filter 1 and Filter 2, i.e., the bandwidth of the signal actually recorded at *I-mon 2*.

If you want to use an external filter rather than Filter 2, you can proceed as follows: Connect current monitor 1 (*I-mon 1*) to the external filter input, feed the filter output back into one of the AD channels (AD 0...AD 5), and select this channel as input channel.

***Note**: Filter 2 is disabled when I-mon 1 is selected. This is to prevent the user to mistake the Filter 2 bandwidth with the active filter bandwidth used for the sampled data, i.e., Filter 1 bandwidth. To change Filter 2 in this case, select I-mon 2 first.*



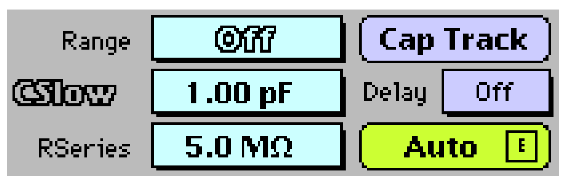
C-fast: This is used to cancel fast capacitive currents that charge the pipette and other stray capacitances (range: 0-15 pF). With nothing connected to the probe input, cancellation is typically obtained at a setting of 1-1.5 pF due to the residual input capacitance of the current-measuring amplifier. The compensation can be performed manually by dragging the mouse or typing. Actually, the setting consists of two components, a completely unfiltered one and a second one filtered by τ-*fast*. The program allows you to set the amplitude of the sum of the two components and the relative contribution of the filtered component (in %). The %-setting acts like a vernier to τ-*fast*. A useful way of adjusting the values manually would be to roughly adjust the value of *C-fast* at a setting of 50% contribution and then adjust τ-*fast*. Thereafter, one can fine-tune the %-setting to fully compensate capacitive transients. A much easier way of adjusting *C-fast* and τ-*fast* is provided by the automatic compensation feature (simply select the *Auto* button; see below).

τ-fast: This determines the time constant of *C-fast* (range: 0.5-8 µs in steps of 0.5 µs). The degree to which the filtered component contributes to the compensation is specified by the %-setting in *C-fast*. The value of τ-*fast* may be adjusted by dragging the mouse, or typing, or automatically by selecting the *Auto* function.

<>: This will select both amplitude and time constant of *C-fast*. Values for each may be changed manually depending on whether the mouse is moved horizontally or vertically.

Auto C-fast: Selection of this button performs an automatic compensation of *C-fast* and τ-*fast*. The procedure uses a routine that applies a number of small pulses (5 mV), averages the resulting currents and fits an exponential to deduce the capacitance compensation values required to cancel the current. During this procedure some parameters are changed temporarily: R_S-*comp* is turned off, the stimulation is interrupted and the *Gain* is internally set to an appropriate value (0.2, 2 or 50 mV/pA, depending on the effective gain range). The values are updated in the respective displays. If the automatic compensation routine fails to converge to ideal adjustment, the small box in the *Auto* button (*E*) will be darkened to indicate the error. Auto compensation works best with Sylgard-coated pipettes in the cell-attached configuration with *C-slow* turned off.

The software allows for two ways of making the compensation. The fastest is to use a look-up table for setting *C-fast*. In order to do this you have select the option *Make C-fast*. If the file created by this procedure ("CFast.epc") is found in the default directory ("E9Screen"), the table will be used. Otherwise, when starting *E9Screen*, a message will appear that the file could not be found and that the (slower) default method will be used (iterative compensation).



C-slow Range: Selects the range for slow capacitance compensation:

- **Off** - Turns cancellation off.
- **30 pF** - Small cells.
- **100 pF** - Small and medium-sized cells.
- **1000 pF** - Large cells (low and intermediate *Gain Range* only).

The program will prevent oscillations by limiting compensation according to the selected range and the effective capacitance values (*R-series* * *C-slow* > 5 µs). Selecting the 1000 pF range will limit the *Gain* adjustment to a maximum of 20 mV/pA.

Note: If you are using the "Cap. Track" feature to measure small capacitance changes, it is advantageous to use a higher C-slow range when possible. Counter-intuitive as it may seem, the digital control of the C-slow circuitry is more precise in higher ranges, resulting in higher resolution, consistent with the gain range and capacitance.

C-slow: This is used to cancel slow capacitive currents that charge the cell membrane in the whole-cell configuration. The 30, 100 and 1000 pF ranges actually allow capacitance values to be compensated in the ranges of 0.12-30 pF, 0.4-100 pF and 4-1000 pF, respectively. The adjustment range is also limited by the program in order to make the time constant *R-series* * *C-slow* greater than 5 µs to prevent oscillations. The *C-slow* value may be adjusted manually by dragging the mouse, or typing, or automatically by selecting the *Auto* function. Auto-compensation will adjust *C-slow* as well as *R-series*.

R-series: Adjusts the resistance in series with the slow capacitance (range: 0.1 MΩ - 10 GΩ) to determine the time constant of the *C-slow* transient and also for R_S-compensation. Adjustment is limited by the capacitance values and the range as described above. The value can be changed manually by dragging the mouse, or typing, or automatically by clicking on *Auto*.

<>: This will select both *C-slow* and *R-series*. Values for each are changed depending on whether the mouse is moved horizontally or vertically.

Auto C-slow: Selecting this function performs an automatic compensation of *C-slow* and *R-series*. These settings are used by the Rs-compensation circuitry as the measure of series resistance. The procedure uses a routine that applies short trains of square-wave pulses (number and amplitude of these pulses are specified by *C-slow Num. Cycles* and *C-slow Peak Amplitude*), averages the resulting currents and fits an exponential to deduce the compensation values required to cancel the current (see Chapter **7. Compensation Procedures** on **page 59**). During this procedure some parameters are changed temporarily: *Rs-comp* is turned off, the stimulation is interrupted and the gain is internally set to an appropriate value (0.2, 2 or 50 mV/pA, depending on the effective gain range). If the *C-slow Range* is set to *Off*, it will automatically be set to the highest possible range, i.e., the 1000 pF range in the low and medium gain ranges, and the 100 pF range in the high gain range.

Auto-compensation works best when *C-fast* is canceled beforehand in the cell-attached configuration. The compensation may be improved by alternating cycles of *Auto C-fast* and *Auto C-slow*. If the compensation fails, the small box in the *Auto* button (*E*) will be darkened to indicate the error. Continuous Auto-compensation can be performed by selecting *Cap. Track* option, see below.

Note: It is necessary that reasonable estimates for C-slow and R-series are supplied before the Auto C-slow is started, otherwise the auto-compensation may fail.

Cap. Track: Selecting this button will perform repetitive *Auto C-slow* compensation. Clicking on *Cap. Track* again will turn it off.

Note: The “Output Cap. Track” option in the EPC9 menu determines whether the capacitance and G-series values are output as voltages on the DA channels 0 and 1. If selected, the C-slow value is available at DAC 0, and the G-series value (1/R-series) is available at DAC 1. The scaling for G-series is 100 nS/V; scalings for C-slow are 0.5, 5 or 50 pF/V for 30, 100 and 1000 pF ranges, respectively. The DAC values will be reset to zero when activating “Reset”. Make sure that the trigger DAC is neither DAC 0 nor DAC 1 when you want to activate "Output Cap. Track".

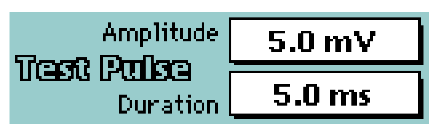
Delay: This determines how many seconds to wait before the next auto-compensation is started. With short delays (e.g., 1 ms), membrane capacitance will be tracked at rates of >10 Hz (depending on the Macintosh model).

Note: You can increase the rate of Cap. Track (about two-fold) when closing the Oscilloscope.

R_S-comp: The series resistance compensation corrects for membrane voltage errors under conditions of high access resistance between pipette and cell interior (see see Chapter **7. Compensation Procedures** on **page 59**). The amount of compensation can be changed by dragging the mouse or typing (range 0-95%). The compensation is based on the value of R-series and will be effective only when *R_S-comp* is not *Off*, i.e., set to a speed value. The following settings determine the speed of feedback compensation:

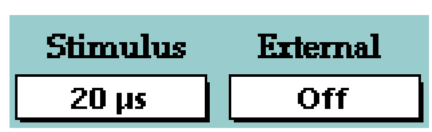
- **Off** - Turns compensation off.
- **100 µs** - Slow compensation.
- **10 µs** - Fast compensation.
- **2 µs** - Very fast compensation.

The choice of speed depends on the recording time constant and the degree of compensation desired, as described in Chapter **7. Compensation Procedures** on **page 59.** Fast R_S-compensation requires more critical adjustment of the controls but provides the maximum voltage-clamp speed. In fast modes, Filter 1 will be automatically switched to its HQ-30 kHz setting. In *Current Clamp* mode, *R_S-comp* will act as a bridge compensation.



Test Pulses: Test pulses are added to the holding potential and applied to the pipette; the current responses are sampled and displayed. Test pulses are applied at maximal rates depending on the durations specified.

Amplitude / Duration: Duration and amplitude of built-in test pulses can be specified in the dialog. The minimum pulse duration is 1 ms with 100 points sampled per pulse, i.e., the sampling interval is 1/100 times the pulse duration.



Stimulus: The stimulus can be filtered (2-pole Bessel) to reduce the amplitude of fast capacitance transients when the speed of potential changes is not critical. Two settings are available:

- **2 µs**
- **20 µs**

Usually a setting of 20 µs is sufficient, unless very fast currents such as Na^+ currents are studied.

External: The external *Stim. Input* is scaled by an editable factor (range: ±0-10x), to allow for different external stimulators. It is strongly recommended to set *External* to *Off* (i.e., equal to zero), if no external stimulator is connected to the *Stim. Input*. This will prevent pick-up of external noise. Please note that the internal *V-membrane* is not affected by changing the external scale factor. If the user sets the holding potential externally (e.g., with a stimulator or another computer), then the scaling will affect the holding potential.

Gain: Sets the gain of the oscilloscope display. Clicking on the arrow icon will cause the sweeps to be centered in the oscilloscope.

Close: Closes the oscilloscope display. With the oscilloscope closed the controls for sound encoding of *R-membrane* and *Current-Clamp Speed* (EPC9 version "C" and later) become accessible. There will be no test pulse while the oscilloscope remains closed.

Overlay: Acquired sweeps will be overlayed if this switch is selected.

Clear: Wipes the oscilloscope display.

Leak Sub.: This controls a hardware leak compensation which uses the inverted and scaled stimulus signal and adds it to the current monitor outputs. *Leak* can be adjusted manually by dragging the mouse, or typing, or automatically by clicking on *Auto*. The maximal values attainable depend on the *Gain Range*. They are 2 nS, 200 nS and 20 µS for high, medium, and low ranges, respectively.

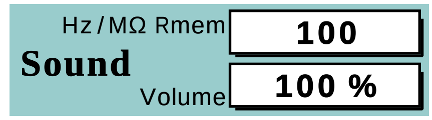
Auto: Determines and corrects for the leak conductance automatically.

Track: Continuously updates the leak compensation, thus tracking changes in membrane conductance.

Hidden Controls

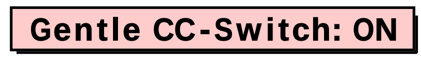
Some rarely used controls are hidden behind the oscilloscope display. They can be accessed by closing the oscilloscope display:

Sound Settings: Sensitivity (Hz/MΩ) and volume (in %) of the sound encoding of R-membrane can be specified here. To enable the sound option press the *Sound* button.



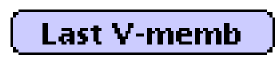
CC Fast Speed: Clicking on this button enables the fast current-clamp mode (only version "C" and later versions of the EPC9). For details see Chapter **6. Operating Modes** on **page 54**).

Gentle CC-Switch: This control selects between the two alternate modes of switching between voltage and current clamp and back.



When selecting *Gentle CC-Switch:* ***On***, *I-hold* will be set to inject the holding current required to keep the membrane voltage constant while switching to current clamp mode. Thus, V-mon will be identical to the original *V-membrane* commanded under voltage clamp. In analogy, upon returning to voltage clamp, *V-membrane* will be set according to the membrane potential under current clamp and rounded to the next 2 mV.
When selecting *Gentle CC-Switch:* ***Off***, *I-hold* will be set to 0 pA when switching to current clamp mode.

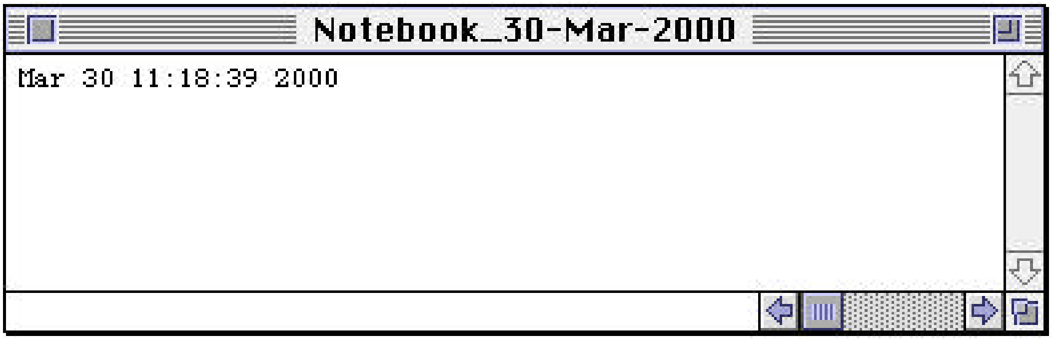
Last V-membrane: Quite often, the membrane potential under current clamp will have changed, and when returning to voltage clamp, *V-membrane* will possibly differ from that set before switching to current-clamp mode. *Last V-membrane* can then be used to conveniently restore the original *V-membrane* value.



CC-range: Two current clamp scaling ranges can be selected: 1 and 10 pA/mV (with the exception of the EPC9 version "A" and "C", which have a fixed CC-scaling of 1 pA/mV). When the CC-scaling is set to 1 pA/mV, a command pulse of 1 mV gives rise to 1 pA in*Current Clamp* mode. This relationship of 1 and 10 pA/mV holds regardless of the *Gain* setting. The maximal output current in*Current Clamp* mode is 1 and 10 nA, respectively.

Relative Value: This button is useful when recording macros. A change of any value will be recorded as a relative change, e.g. if you hyperpolarize the holding potential from -60 to -70 mV while recording a macro, a step of -10 mV will be recorded (see description of *Macros* at the beginning of the subchapter *Main Controls*).

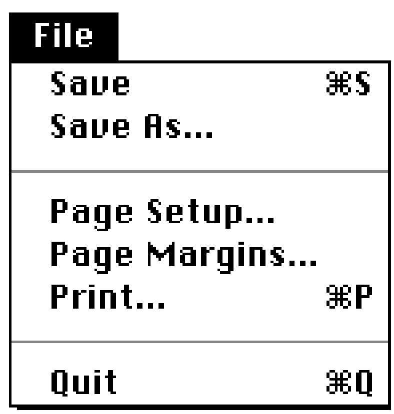
Notebook Window



The *Notebook* window is used to display printed output of the program, such as error messages or information about calibration and diagnostic data, for example. The content of the *Notebook* can be stored in a memory file; its maximal size can be to be specified in the drop-down menu *Notebook*. One can modify text in the *Notebook* just as in any other text file. One can add further information to the text file or delete messages that should not be stored. While in the *Notebook* window you can copy to and paste from the clipboard. Thus, you can exchange text with other applications, such as spreadsheets.

Drop-Down Menus

File Menu



Save: Saves the *Notebook* content to disk.

Save As…: Asks for a filename before saving.

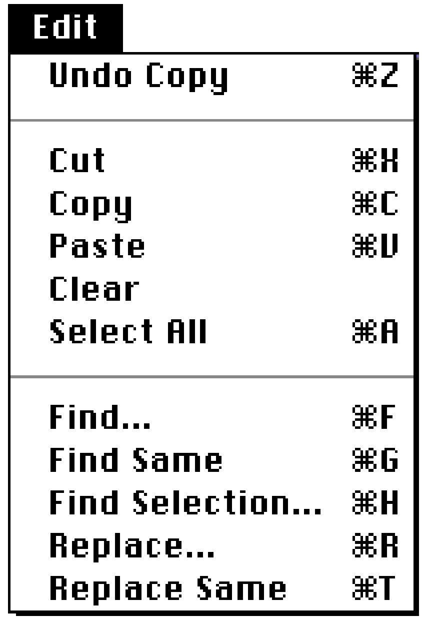
Page Setup…: Prompts for printer page format.

Page Margins…: Sets the page margins for printing.

Print…: Prints the *Notebook* content. If some text is selected (i.e., highlighted), only that text selection is printed.

Quit: Exits *E9Screen*.

Edit Menu

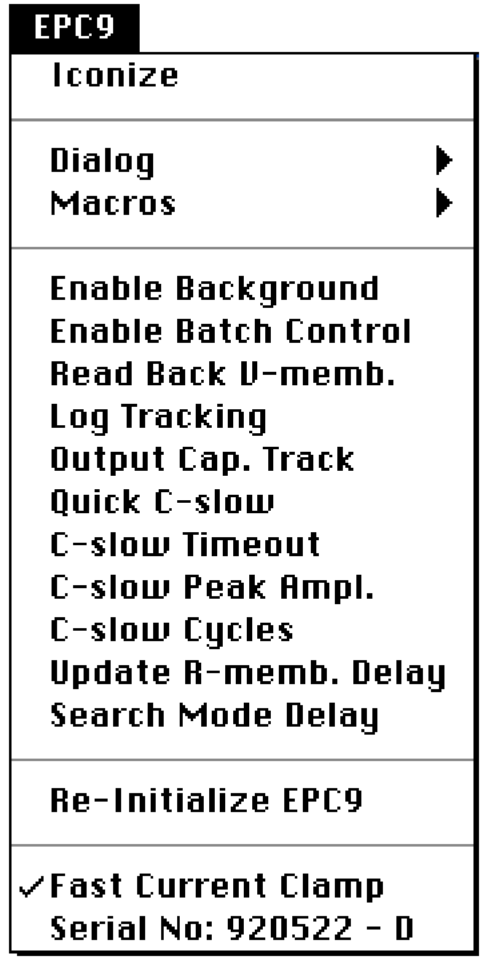


The *Edit* menu is not used by *E9Screen* except for text manipulation of the *Notebook* content. All entries are normally disabled unless the *Notebook* is selected. The menu entries conform to the standard Macintosh functions.

The *Cut*, *Copy*, and *Paste* commands copy the text selection to and from the clipboard. *Clear* removes the text.

Find…, *Find Same*, *Find selection…*, *Replace…*, and *Replace same* perform the obvious functions.

EPC9 Menu



Iconize: Collapses the window and leaves the title bar of the window on the desktop.

Dialog: Selects a submenu for the *Amplifier* dialog window. The following functions are available:

- **Load**…: Loads a saved dialog window.
- **Save**…: Saves a modified dialog window.
- **Save as PICT**…: Saves window as Pict file.
- **Reset**: Resets the dialog window to default.
- **Enable Dragging**: Enables the dragging feature for most amplifier controls.

Macros: Selects a submenu for macro functions.

- **Load**…: Loads a saved macro file.
- **Save**…: Saves a macro file.
- **List**: Prints the current macros in the *Notebook*.
- **Start Recording**: Starts macro recording.
- **Execute while recording**: If checked, the actions will be executed. Otherwise, the actions will be logged to the macro but not change any settings.

EPC9 Help: Enters the online *Help* mode for EPC9 functions.

Enable Background: Enable *E9Screen* to run in the background, i.e., when it is not the front application. Thus, *E9Screen* will continue to perform *Cap. Track* and to scan the clipboard for batch file instructions (see *Appendix I*).

Enable Batch Control: Enables *E9Screen* to communicate with other applications via batch files (see *Appendix I*).

Read Back V-memb.: Required when using *E9Screen* in combination with another acquisition program. When active, *E9Screen* will read the momentary *V-membrane* when the user switches from another application to *E9Screen*. This allows *E9Screen* to know the *V-membrane* this other application has set.

Log Tracking: Writes the updated values of *C-slow, G-series,* and *G-leak* to the *Notebook* when capacitance tracking is on.

Output Cap. Track: Outputs the values of *C-slow* and *G-series* as analog voltages to DA 0 and DA 1, respectively.

Quick C-slow: Selects an alternate procedure for *Auto C-slow* compensation. It speeds up repetitive compensations, as it does not switch the gain, filter settings, etc. This option does not check as thoroughly for proper convergence and will sometimes adjust *C-slow* and *R-series* when the normal *C-slow* compensation would have returned an error. Use this option with caution.

C-slow Timeout: The time after which *E9Screen* will terminate an *Auto C-slow*.

C-slow Peak Amplitude: The amplitude of the voltage pulses used to compute *C-slow* and *G-series*.

C-slow Cycles: The number of voltage pulses used to compute *C-slow* and *G-series*.

Update R-membrane Delay: This option sets the refresh rate of *R-membrane* in the EPC9 window. To suppress *R-membrane* calculation and update, enter a large value.

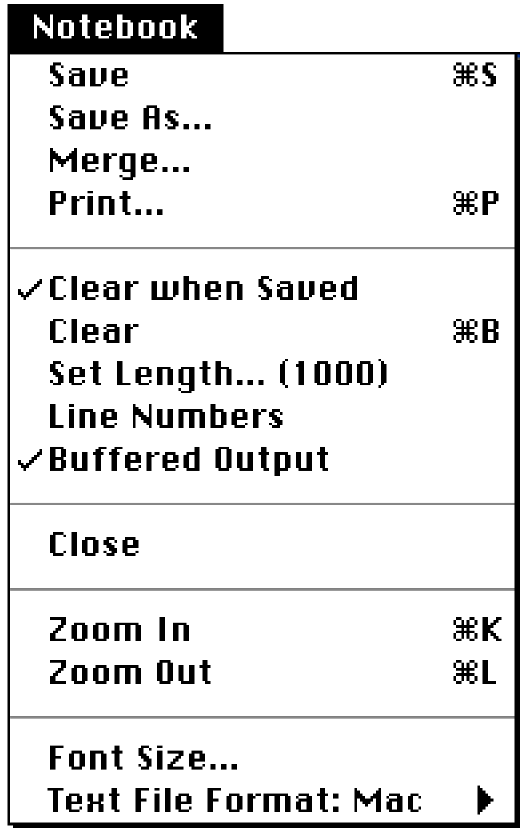
Search Mode Delay: The *Search Mode* (see **Chapter 6. Operating Modes on page 58**) is essentially a repetitive *Auto-*V_0 procedure at a holding potential of 0 mV. The delay specifies at what rate *Auto-*V_0 is performed.

Re-Initialize EPC9: This is used to restart the AD/DA interface; e.g., when *E9Screen* was started with the interface being turned off.

Fast Current Clamp: Selects the fast current-clamp mode (only version “C” and later versions of the EPC9). For details see Chapter **6. Operating Modes** on **page 54**).

Serial No: [Serial #] - [EPC9 version]: Displays the serial number and version of the connected (and initialized) EPC9 amplifier.

Notebook Menu



Save: Saves the *Notebook* under its default name: “Notebook_Date”.

Save as…: Asks for a filename before saving.

Merge...: Appends the present *Notebook* to an existing one.

Print…: Output content of *Notebook* to a printer. If some text is selected (i.e. highlighted), only that text selection is printed.

Clear when Saved: Automatically clears the *Notebook* after the present content is saved to disk.

Clear: Clears *Notebook*.

Set Length…: Specifies maximal number of text lines in the *Notebook*. The maximal number of lines is given in parentheses.

Note: Large notebook buffers require a lot of CPU time and RAM. If execution time during acquisition is an issue, the buffer size should be kept small or the “Buffered Output” should be turned off. The RAM requirement is 260 bytes per line. Thus, 1000 lines consume 260 kbytes.

Line Numbers: Shows line and column numbers in the *Notebook*.

Buffered Output: Keeps all text written to the *Notebook* in the buffer. If deselected, information printed to the *Notebook* window will not be saved.

Open/Close: Opens or closes a *Notebook* window.

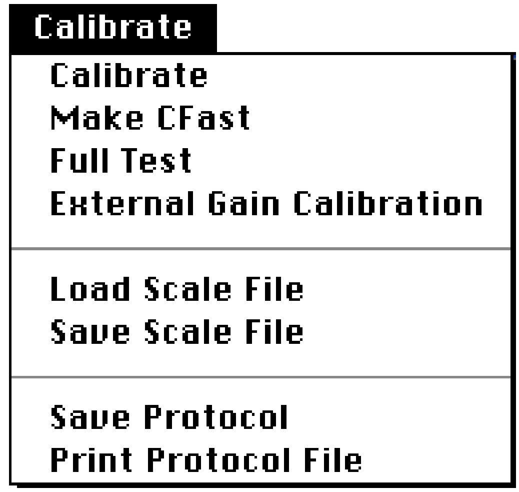
Zoom In/Out: Expands and shrinks the *Notebook* window.

Font Size…: Specifies the font size of the *Notebook* text.

Text File Format: Specifies in which format the *Notebook* text is saved:

- **Mac** - Line Feed
- **DOS** - Line Feed + Carriage Return

Calibrate Menu



Calibrate: Performs a full calibration of the EPC9. The result of this calibration may be stored as a "SCALE.EPC" file. This file contains the settings of the digital switches and controls of the amplifier. These are unique to the amplifier and cannot be used for another EPC9. A calibration should be performed in intervals of about 6 months or whenever the frequency response of the amplifier is not accurate or offset currents become noticeable. Depending on the speed of the computer, the calibration process takes 5-10 minutes.

Note: Calibrations should be performed after amplifier and probe have reached normal operation temperature (i.e., powered up for about 15 min.utes)

Make C-fast: Generates a look-up table for *Auto C-fast* compensation. The file is loaded automatically if it has the name "CFAST.EPC" and is found in the *E9Screen* folder.

Full Test: Performs a hardware test. This generates a list of diagnostic values. This may be used to inform the manufacturer (HEKA) about the condition of the EPC9. If you have any doubts about your EPC9 test, please contact HEKA to find out whether or not it needs to be serviced.

External Gain Calibration: This option allows to use an external precision resistor to improve the internal, automatic gain calibration. Usually there is no need to perform an external gain correction, but some users may chose to do so. A gain correction factor can be given for each gain range, i.e., low, medium, and high gain. Thus, this enables a "user specified" gain correction factor for matching the computed the R-membrane value with a calibration resistor.

Load / Save Scale File: Loads or saves calibration files.

Save Protocol: Saves the protocol file of a *Full Test.*

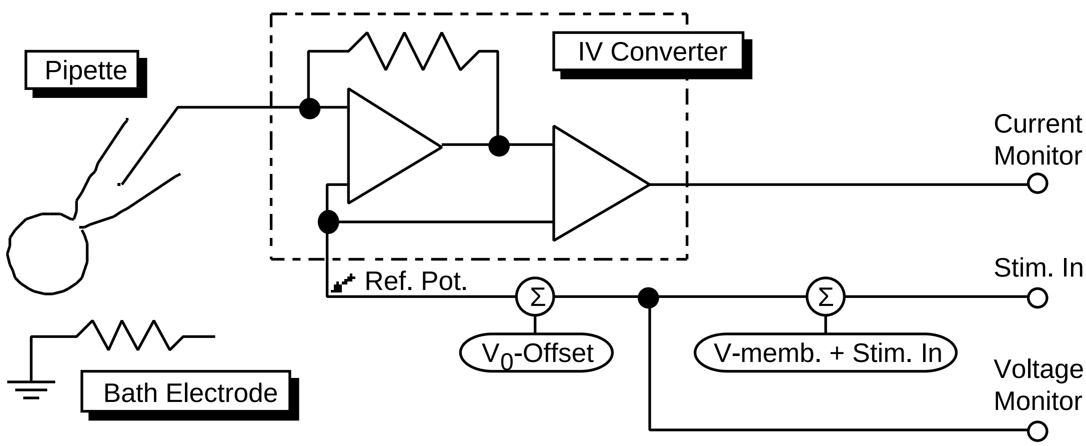
Print Protocol File: Prints the protocol file of a previously saved *Full Test.*

6. Operating Modes

The EPC9 is fundamentally an instrument for measuring small electrical currents. It uses a current-to-voltage (I-V) converter circuit to convert the currents to an analog voltage, which is then made available at the current monitor outputs for display or recording. At the same time that pipette currents are being recorded, the potential must be specified, and the various operating modes of the EPC9 correspond mainly to different ways of controlling that potential.

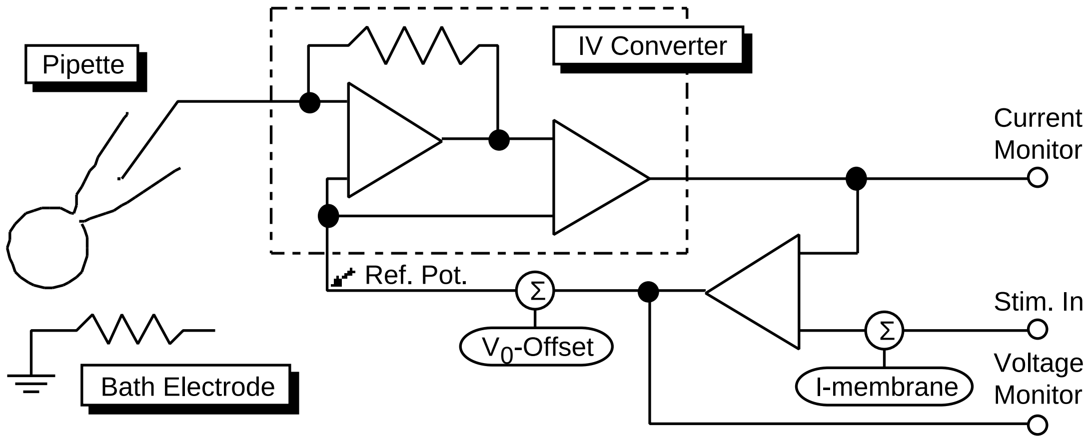
Voltage-Clamp Mode

This is the basic patch-clamp mode, and is implemented by the circuitry shown in the figure below. The pipette potential is derived from the signal applied to *Stim. In*, with a variable offset added from the *V-membrane* control. The sum of these two sources is displayed and monitored as the *V-mon* signal. Before being applied to the pipette a further variable offset is added from V_0 (which includes the value of *LJ*, see Chapter **7. Compensation Procedures** on **page 59)**.



Current-Clamp Mode

In the *Current Clamp* mode, the feedback is employed between the current monitor signal and the pipette potential (see figure below). The feedback acts rapidly (with a time constant of about 30 µs) to keep the current at zero by varying the pipette potential appropriately; in this way, a high-impedance voltage follower is created, with the output voltage available at the *V-mon* output. The original *V-membrane* viewport now becomes an *I-membrane* display and may be used to inject a steady command current through the action of the feedback amplifier. In *E9Screen*, the membrane potential is shown in the *I-mon* display; in *Pulse*, the *I-mon* display is converted to *V-mon*).



You can use the *Current Clamp* mode to measure the resting potential or spontaneous action potentials in a whole-cell recording, and the membrane potential will be shown on the *V-mon* display. A commanded current can be injected while the pipette potential is measured. The commanded current is determined by the sum of voltages from the *Stim. In* signal and the *I-membrane* control.

Two current clamp scaling ranges can be selected: 1 and 10 pA/mV (with the exception of the EPC9 versions “A” and “C” which have a fixed CC-scaling of 1 pA/mV). When the CC-scaling is set to 1 pA/mV, a command from the *Pulse Generator* or from the *I-membrane* control that would give rise to 1 mV in the *Voltage Clamp* mode instead gives rise to 1 pA in the *Current Clamp* mode. This relationship of 1 and 10 pA/mV holds regardless of the *Gain* setting. The polarity is the usual one, in which positive stimuli result in currents flowing out of the pipette.

Note: The “Gain” settings are restricted to the intermediate range, i.e. 0.5-20 mV/pA, in current-clamp mode.

When switching from voltage-clamp to current-clamp mode, *I-membrane* will be set to whatever is needed in order to keep the membrane voltage at the value that was commanded in voltage clamp. Likewise, upon returning to voltage clamp, *V-membrane* will be maintained. Thus, switching modes will be as gentle as possible, since the membrane potential will always remain constant. If you wish to switch modes under different conditions, it is convenient to program a macro to, for example, set a holding current value immediately after switching to current clamp. The EPC9 allows the functions of *C-fast* and R_S*-comp* to be on when entering current-clamp mode. *C-fast* then becomes a “negative capacitance” adjustment and R_S*-comp* becomes a “bridge” adjustment.

The main problem with current-clamp settings is stability, as the feedback loop will oscillate when *C-fast* is set to its true value; the loop expects at least a small amount of uncompensated *C-fast* for a phase lead at high frequencies. The EPC9 software will assume that the user has compensated *C-fast* correctly, and in the process of switching to current-clamp mode, it will set its value slightly lower (0.5 pF) to optimize the speed of recording. The *Current Monitor* output can be used to monitor the currents being passed, with the *Gain* control determining the sensitivity.

The new version "C" of the EPC9 hardware (and later verisons):

In the new EPC9 versions the current-clamp circuitry has been improved to better follow rapid changes in membrane potential, such as in neuronal action potentials. In the current-clamp mode a feedback circuit causes the effective input resistance of the EPC9 headstage to be greater than 10e11 Ω. The speed of the feedback loop determines the effective input capacitance of the amplifier; a high capacitance can cause the time course of the action potential to be slowed. The capacitance of the electrode, and to some extent the amplifier, can be neutralized by the *C-fast* setting, which acts as a capacitance neutralization adjustment in the current-clamp mode. However, like capacitance neutralization settings on conventional microelectrode amplifiers, excessive capacitance neutralization can result in oscillation and potentially the destruction of the cell membrane. The best way to use the *C-fast* control is to first adjust it in the voltage-clamp mode, e.g., by using the *Auto* button; it is then automatically adjusted to neutralize all but the amplifier input capacitance when you switch to current-clamp mode.

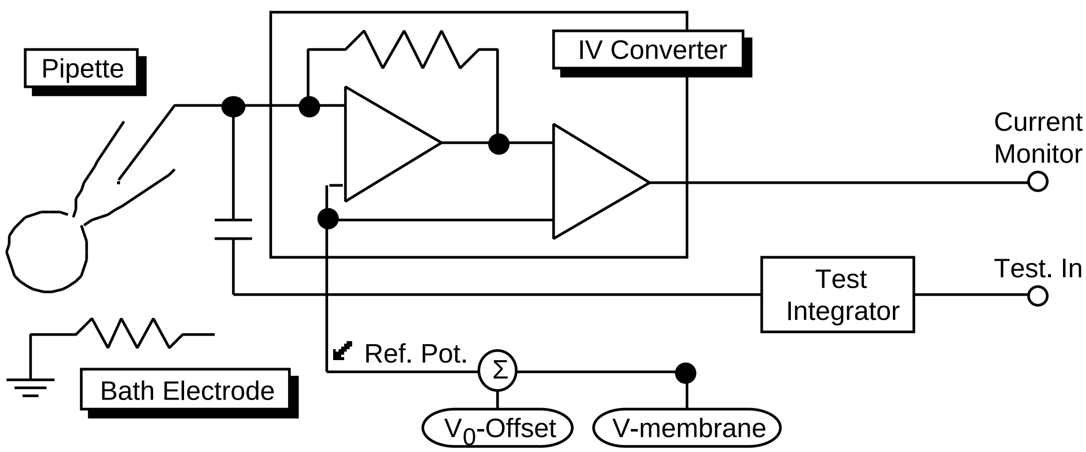
The current-clamp circuitry now has two speed settings (which can be toggled by a button that is normally hidden behind the oscilloscope window; it can be seen when closing the oscilloscope). At the *fast* setting, the effective input capacitance of the headstage is 2 pF. This amount of capacitance will cause little loading of most cells. If desired you can neutralize a fraction of this capacitance as well by increasing the *C-fast* setting by up to 2 pF from its initial value. Overshoot or ringing of the response will occur as you approach complete compensation.

The *fast* current-clamp setting must be used with some caution because if *C-fast* is grossly misadjusted, either by being too high (i.e., overcompensation) or too low, oscillation may occur. The *fast* current-clamp mode may also oscillate in situations where the impedance of the electrode and the cell are low, below about 5 MΩ at a frequency of 10 kHz. Thus oscillation may occur when the pipette has a resistance below 5 MΩ and is open to the bath solution, or in recording from a cell with substantial membrane capacitance when the series resistance is less than 5 MΩ.

In the *slow* setting, the current-clamp system is more stable, but an effective input capacitance of 24 pF is produced. Because this value is larger than the range of the *C-fast* controls, misadjustment of *C-fast* will not cause oscillation; the amplifier is also stable with pipette and series resistance down to 1 MΩ. The large capacitance will however produce an extra load on small cells, slowing the time course of membrane potential changes.

Test Mode

This mode is only available in *E9Sreen*. It is very much like the voltage-clamp mode (see figure below) except that the *Test Integrator* signal is used to inject small test currents into the I-V converter input. Internal stimulation can be used for this purpose by connecting DA 2 (*Test Output*) to the *Test Input*.



The scaling of the current that is injected is fixed at approximately -100 pA per volt applied at the *Test Input*. This scaling is independent of the *Stim. scaling* setting. The exact scaling is measured in the calibration program and is stored in the "Scale.epc" file. The signal is not DC-coupled, but square waves of >20 Hz, pulses >30 ms in duration, and sine waves at >1 Hz are reproduced faithfully.

Note: The test integrator inverts the externally-supplied signal. For accurate frequency response and to avoid degrading the sensitivity of the I-V converter, the test currents are injected through a capacitor (rather than a resistor) into the probe's input terminal. The applied signal is integrated by an auto-zeroing integrator before being applied to the capacitor. The integrator may require several seconds of recovery time when a signal with a substantial DC component is applied to the input.

The *Test* mode is particularly useful for measuring the step response of the entire recording system, which is a necessary step in high-resolution channel analysis, as described by Colquhoun and Sigworth (1995). The current-injection circuitry has a useful bandwidth greater than 100 kHz in the high range of gain settings and can be used for quantitative measurement of the step response.

Search Mode

In the EPC9, this mode has no special analog circuitry. Rather, it is a software emulation of the traditional search mode found in the EPC7 amplifier. The EPC7 accomplished the search mode by analog circuitry in order to ease the adjustment of the zero-current potential V_0. The EPC9 does not require special analog circuitry, as the software provides for an easy way of doing this. The *Search* mode of the EPC9 simply forces *V-hold* to 0 mV and invokes the *Auto*-V_0 procedure (at the rate specified by *Search Mode Delay* in the *EPC9* menu), which will adjust the offset potential automatically in order to zero the current. Thus, the search mode is simply a repetitive update of V_0.

7. Compensation Procedures

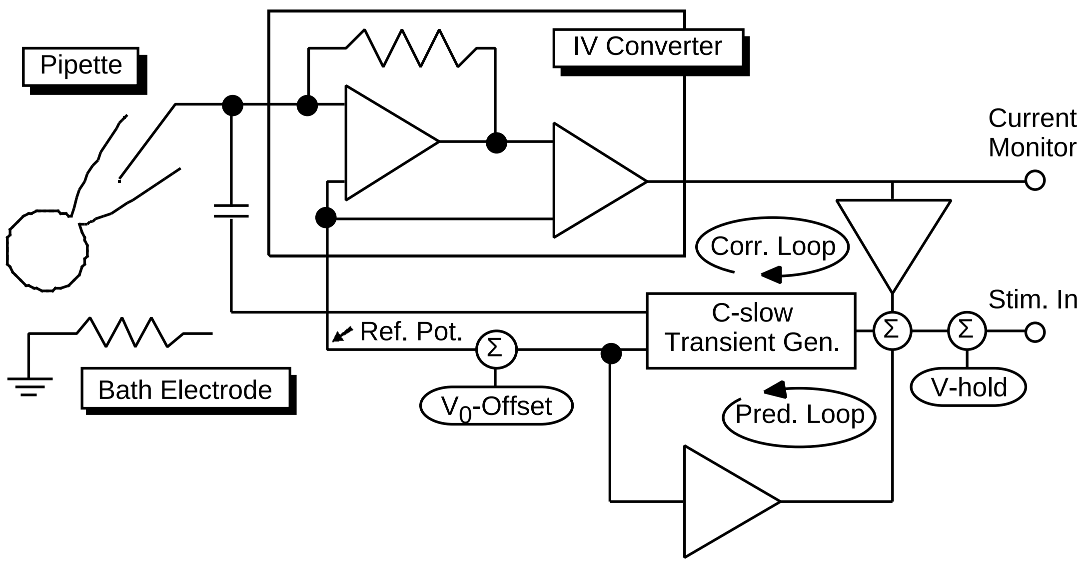
Series Resistance Compensation

In whole-cell voltage-clamp recording, the membrane potential of the cell is controlled by the potential applied to the pipette electrode. This control of potential is not complete, but depends on the size of the access resistance between the pipette and the cell interior, and on the size of the currents that must flow through this resistance. This access resistance is called the series resistance (R_s) because it constitutes a resistance in series with the pipette electrode. Part of the series resistance arises from the pipette itself, but normally the major part arises from the residual resistance of the broken patch membrane, which provides the electrical access to the cell interior. In practice, we find that the series resistance usually cannot be reduced below a value about two times the resistance of the pipette alone.

Series resistance has two detrimental effects in practical recording situations. First, it slows the charging of the cell membrane capacitance because it impedes the flow of the capacitive charging currents when a voltage step is applied to the pipette electrode. The time constant of charging is given by $\tau_u = R_s \times C_m$, where C_m is the membrane capacitance. For typical values of R_s = 5 MΩ and C_m = 20 pF, the time constant is 100 µs. This time constant is excessively long for studying rapid, voltage-activated currents such as Na^+ currents in neurons, especially since several time constants are required for the membrane potential to settle at its new value after a step change. The second detrimental effect of series resistance is that it yields errors in membrane potential when large membrane currents flow. In the case of R_s = 5 MΩ, a current of 2 nA will give rise to a voltage error of 10 mV, which is a fairly large error; for studying voltage-activated currents, errors need to be kept to ~2 mV at most.

Electronic compensation for series resistance in voltage-clamp systems has been in common use since the days of Hodgkin and Huxley. The principle of the compensation in the case of a patch clamp is that a fraction of the current monitor signal is scaled and added to the command potential (correction pathway, see figure below). When a large current flows in the pipette, the pipette potential is altered in a way that compensates for the potential drop in the series resistance. This arrangement constitutes positive feedback, and can become unstable when overcompensation occurs.

The EPC9 incorporates additional circuitry to allow capacitance transient cancellation to occur while R_S-compensation is in use (see Sigworth, Chapter 4 in *Single Channel Recording*). This is shown as the prediction pathway in the figure below, and it accelerates the charging of the membrane capacitance by imposing large, transient voltages on the pipette when step changes are commanded (this is sometimes called “supercharging”). These voltages would occur due to the action of the correction pathway alone as the large capacitive charging currents elicit pipette voltage changes; however, when these currents are cancelled by the transient cancellation, their effect must be predicted by the cancellation circuitry: hence the prediction pathway.



Together, the two parts of the EPC9 R_S-compensation circuitry cancel the effects of a fraction of the series resistance. This means that the charging of the membrane capacitance is accelerated, with a time constant under compensation of

$$\tau_c = (1 - \alpha)\,\tau_u$$

where τ_u is the uncompensated time constant. Similarly, the voltage errors due to membrane currents are also reduced by the factor (1-α). The fractional compensation α is determined by the setting of the *%-comp* control on the EPC9 software. For proper compensation, however, the circuitry needs to have an estimate of the total series resistance (for the correction pathway), and both the series resistance and membrane capacitance must be known for the capacitance transient cancellation (*C-slow*) circuitry. In the EPC9, the estimation of series resistance has been combined with the transient cancellation, in that the R_S control has a dual effect. Its setting affects both the kinetics of the transient cancellation and the scaling of the correction feedback signal. This means that in practice the estimation of the series resistance consists of adjusting *C-slow* and *R-series* to cancel the transient currents due to the cell membrane capacitance. Once this has been done, the relative amount of R_S-compensation can then be selected with the *%-comp* control.

Theoretically, it is desirable to compensate as much of the series resistance as possible. In practice, however, a degree of compensation above 90% can involve considerable technical problems, and in some recording situations a value below 90% is preferable. To illustrate one technical problem, consider the case when a 100 mV potential change is commanded and 90% compensation is in use. This degree of compensation means that the cell membrane capacitance will be charged 10 times faster than normally. The rapid charging is accomplished in the compensation circuitry by forcing the pipette potential to (very transiently) reach a potential of 1V. The resulting large current causes the membrane capacitance to charge quickly to its final value of 100 mV. In general, when a voltage step of size ΔV is commanded, the pipette potential actually receives an initial transient of size ΔV / (1-α) due to the compensation effect. The technical problem comes from the fact that the maximum pipette potential excursion in the EPC9 is about ±1.2 V, implying that 90% compensation can be used for steps only up to about 120 mV in amplitude. Overload of amplifiers (obvious in practical use due to the loss of proper transient cancellation) will occur if larger pulses are applied, unless the *%-comp* setting is reduced.

The degree of R_S-compensation is also limited by stability considerations. Stable R_S-compensation requires that the *C-fast* control is properly set to cancel the fast capacitance transients; when the series resistance is high, say above 10 MΩ, misadjustment of *C-fast* can easily cause oscillation. In cases where R_S is this size or larger, it is often advisable to use the slower settings of the R_S switch which, in slowing down the speed of the compensation feedback, makes it less susceptible to high-frequency oscillations. In cases where R_S is relatively small, on the other hand, it is sometimes not possible to use full 90% compensation because of the limited speed of the compensation feedback, even in the fastest, 2 µs setting of the switch. This problem arises when the time constant τ_u is smaller than about 100 µs, and comes from the fact that compensated membrane time constant τ_C cannot be made smaller than a value that depends on the speed of the R_S-compensation feedback. If you turn up the *%-comp* control to try to obtain a smaller τ_C, you will observe overshoot or ringing in the current monitor signal, due to an overshoot in the membrane potential. The minimum value for τ_C is given approximately by

$$\tau_{c(\min)} = \sqrt{\tau_u - \tau_f}$$

where τ_f is the effective time constant of the feedback loop. The corresponding maximum α values are given by

$$\alpha_{(\max)} = 1 - \sqrt{\tau_f / \tau_u}$$

τ_u (µs)	α	τ_c (µs)
90	0.85	13
50	0.80	10
30	0.75	8
22	0.70	7
13	0.60	5
8	0.50	4

The table gives maximum α values (i.e., *%-comp* settings) and the resulting τ_c values in the 2 µs setting for some values of the uncompensated time constant τ_u. At the 10 µs setting, full 90% compensation may be used without overshoot for time constants τ_u greater than about 1 ms; the 100 µs setting is appropriate for τ_u values on the order of 10 ms or longer. In practice, you can estimate τ_u from the ratio of the settings of *C-slow* and *R-series*. For example, if *C-slow* is 10 pF and R_s is 10 MΩ, the time constant is 10 pF · 10 MΩ = 100 µs.

The use of the R_s-compensation circuitry can be summarized as follows: When you set the capacitance transient cancellation (*C-slow*, *R-series*, *C-fast*, *τ-fast*) to minimize the size of the transients when voltage pulses are applied, you have also properly set them for series resistance compensation. Then you enable *R-series* and turn up the *%-comp* control to the desired value. Any misadjustment of the transient cancellation will be apparent and can be compensated. The EPC9 makes the procedure very easy: *C-slow* and *R-series* values can be obtained automatically by clicking on *Auto C-slow*. Beyond that, the pulse generator provides an option where *Auto C-slow* is performed before each command pulse, achieving an accurate update of *R-series*. The procedure will be described in more detail later (Chapter **10. Using the Patch Clamp** on **page 75**).

Capacitance Compensation

The EPC9 provides automatic procedures for both fast and slow capacitance subtraction. In both cases, the ongoing pulse protocols are suspended and short trains of square-wave pulses are applied (number and amplitude of these pulses are specified by *C-slow Num. Cycles* and *C-slow Peak Amplitude*). The resulting capacitive transients are averaged, leak-subtracted, and then used to calculate the required corrections to the components of the compensation network. A detailed description of the procedures to estimate capacitance is found in Sigworth *et. al* (1995). The procedure for *C-fast* compensation (pipette and stray capacitance) is concerned mainly with the very initial portion of the transient (the first 10-30 µs), whereas the procedure for *C-slow* compensation (whole-cell capacitance) regards a somewhat later time window. The width of this window is based on an initial guess of what the time constant of the slow transient might be. Therefore, it is a good idea to set typical values for *C-fast*, *τ-fast*, *C-slow* and *R-series* in advance (they may be saved in the macro for whole-cell recording, for example). This will provide the fitting routine with reasonable starting values. Alternatively, the *Auto* procedures can be performed alternatingly to arrive at settings for which the remaining transient is minimal. Settings for *C-fast* are left at their respective values whenever an *Auto C-slow* is performed and vice versa. This way, a combined fit can be obtained by alternatingly executing *Auto C-slow* and *Auto C-fast*. It is better, however, to perform *Auto C-fast* in the cell-attached recording configuration and not to touch it any more when proceeding to whole-cell.

During *Auto C-fast* and *Auto C-slow*, certain settings are changed temporarily and restored upon completion. These are:

- R_S-compensation is switched off
- The gain is set to 0.2, 10 or 50 mV / pA, depending on the gain range in use
- External stimuli are disabled
- Filter 1 is set to 10 or 30 kHz, depending on the required bandwidth

These changes are not displayed on the screen, since they usually are only effective for fractions of a second. In *Cap. Track*, however, the changed values are effective for longer times, since the *Auto C-slow* procedure is called periodically. It should be kept in mind that under this condition the parameters on the screen may not represent the hardware settings.

The relationship between the values of the compensation network (*C-slow* and *R-series*) and those of the pipette-cell assembly (C_m and R_s) is straightforward, if the membrane conductance is negligible. In this case, perfect compensation will leave no residual current and *C-slow* will be equal to C_m. If, however, there is a finite membrane conductance, then some ambiguity exists, because a three-component network is being approximated by a two-component compensation network. Details of the compensation procedure will then determine the residual current (some filtered version of the command waveform) and C_m is likely to be underestimated. The *Auto C-slow* procedure is designed to provide unbiased estimates of the actual membrane capacitance and series resistance (Sigworth, Neher & Affolter, 1995).

Offset Compensation

In all patch-clamp configurations a number of offsets have to be taken into account. These include amplifier offsets (± 30 mV), electrode potentials (± 200 mV, depending on Cl^- concentration of pipette and reference electrode), liquid junction potentials, and potentials of membrane(s) in series with the membrane under study. Some of these offsets are fixed during an experiment (like amplifier and electrode offsets), some are variable.

It is standard practice to take care of the fixed offsets by performing a reference measurement at the beginning of an experiment. Thereby an adjustable amplifier offset is set for zero pipette current. Thereafter the command potential of the amplifier (displayed as *V-membrane*) will be equal in magnitude to the membrane potential if no changes in offset potentials occur. The polarity of the command potential will be that of the membrane for whole-cell and outside-out configurations but will be inverted in the cell-attached and inside-out configurations. In cell-attached configuration an additional offset is present due to the resting potential of the cell under study. Liquid-junction potentials may appear or disappear during the measurement when solution changes are performed or in the case that the pipette solution is different from the bath solution (Barry & Lynch, 1991; Neher, 1992; Neher, 1995).

Conventionally, these problems are handled by applying the appropriate corrections and sign inversions during offline analysis. The EPC9 software allows this to be done at the time of data acquisition, considering the relevant *Recording Mode*, which may or may not result in inversion of holding and commanded voltages. In addition, it offers three features which interact in a way to provide for simple online handling of offsets, such that *V-membrane* reads the correct membrane potential (correct both in polarity and magnitude) at all times. These are:

V_0: An adjustable hardware offset-voltage. The actual potential applied to the pipette is the sum of *V-membrane* and V_0.

Auto-V_0: An automatic operation which systematically varies V_0 for zero pipette current. Before doing so, the procedure sets *V-membrane* to an appropriate value (see below).

LJ: A software variable which can be set by the user. It represents the sum of all "variable offsets" applicable at a given time (*Offset Sum*).

In order for the variable *LJ* to actually represent the various offsets, and to correct for these, three conditions have to be met:

- The user has to calculate *LJ* correctly, according to the rules outlined below. This value should then be entered in the *LJ* control.
- A *Auto-V_0* operation has to be performed at the start of an experiment. The software will then set *V-membrane* to *-LJ* (for *Out Out* and *Whole Cell* mode) or *+LJ* (for *On Cell* and *In Out* modes) at the start of an *Auto-V_0* operation.
- During experiments, V_0 has to be changed in parallel with user-induced changes in *LJ*. This is done automatically by *Pulse* and *E9Screen*.

An analysis of the underlying offset problem and justification for the procedures can be found in Neher (1995).

The rule for calculating the *Offset Sum* (*LJ*) is to form the sum of all changes in offsets which occur between the reference measurement and the test measurement. The polarity of a given offset voltage should be taken as viewed from the amplifier input (positive, if positive side of the voltage source is closer to the input). A sign inversion has to be applied if the offset under consideration disappears.

A procedure how to measure liquid junction potentials is described in Neher (1992). Ion mobilities for calculation of liquid junction potentials can be found in Barry & Lynch (1991). The table below lists the values for some typical solutions.

Solution	LJ
145 K-glutamate, 8 NaCl, 1 $MgCl_2$, 0.5 ATP, 10 NaOH-HEPES	10 mV
145 KCl, 8 NaCl, 1 $MgCl_2$, 0.5 ATP, 10 NaOH-HEPES	3 mV
60 Cs-citrate, 10 CsCl, 8 NaCl, 1 $MgCl_2$, 0.5 ATP, 20 CsOH-HEPES	12 mV
32 NaCl, 108 Tris-Cl, 2.8 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 NaOH-HEPES	–3 mV
70 Na_2SO4, 70 sorbitol, 2.8 KCl, 2 $MgCl_2$, 1 $CaCl_2$, 10 NaOH-HEPES	6 mV

In each case, a liquid junction potential between the given solution and physiological saline (main salt: 140 mM NaCl) is listed. Polarity is that of physiological saline with respect to the given solution (according to the convention of Barry & Lynch).

Note: When applying the above rules for calculating the correction LJ, two sign inversions of the liquid junction potential are effective for the standard liquid junction potential correction. First, the liquid junction potential that was present during the reference measurement disappears during the experiment (after seal formation). Second, according to Barry & Lynch, the potentials are defined with opposite polarity as those for patch-clamp experiments (bath vs. electrode instead of electrode vs. bath). Thus, values in the table can be taken as they are and entered as such in the LJ control. If however, a liquid junction potential appears during a measurement (e.g., during solution changes), then only one sign inversion applies. In that case, the sign of the value in the table must be inverted before adding it to the “Correction Sum”.

In the following, some specific examples together with explanations will be given. In all these cases it is assumed that the reference measurement is performed in standard saline.

Example 1: An outside-out or whole-cell measurement with normal saline in the pipette. In this case, *LJ* should be set to zero. This is one of the few measurements which do not require any correction. It is quite unphysiological, however.

Example 2: An outside-out or whole-cell measurement with KCl-based internal solution in the pipette. *LJ* should be set to 3 mV (see table) in order to correct for the disappearance of a liquid junction potential between the KCl containing pipette and the NaCl-based bath solution.

Example 3: An episode with low-chloride bath solution during the experiment of example 2. It is assumed that the reference electrode in the bath includes a salt bridge such that the change in Cl^- concentration is not "seen" by the Ag-$AgCl^-$-wire. Nevertheless, a liquid junction potential will develop at the bath/salt-bridge interface, unless a "bleeding" KCl-bridge is used (see Neher, 1992). Similarly, a liquid junction potential will develop during local microperfusion. Thus, the correction during the episode in low-chloride medium will be the sum of this liquid junction potential and the correction of *Example 2* (3 mV). Taking the value for a low Cl^- solution (e.g., sulfate Ringer; see table), we arrive at a value of *LJ* = 3 + (-6) = -3 mV, which should be set during that part of the experiment.

Note: The sulfate Ringer in this case is -6 mV (the inverse of the value in the table), because this potential "appears" during the measurement with inverted polarity to the convention of Barry & Lynch.

Example 4: An outside-out or whole-cell measurement with Cs-citrate-based internal solution. In this case, *LJ* should be set to 12 mV (see table above).

Example 5: A cell-attached measurement with sulfate-Ringer in the pipette. Two corrections apply: 1. the correction for the liquid junction potential during the reference measurement (6 mV, see table above) and 2. the resting potential of the cell. We assume the latter to be -60 mV and therefore set *LJ* to -54 mV. In the cell-attached mode polarities of the amplifier readout are inverted, thus the amplifier will display the "physiological" patch potential.

8. Patch-Clamp Setup

Mounting the Probe

For low-noise recording, the pipette holder must be attached directly to the EPC9 probe. Although the probe amplifier can tolerate the additional capacitance of a short connecting cable without instability or oscillations, we find that the dielectric and electrostrictive properties of coaxial cables introduce excessive noise. In typical setups, the probe is therefore mounted directly on a 3-axis micromanipulator. The EPC9 probes are supplied with a plastic mounting plate for mounting on a flat surface. Holes can be drilled through the protruding surfaces for attachment to a matching plate or other surface. Please remember, that the metal case of the probe must remain insulated from ground.

Because of the extreme sensitivity of the EPC9, special care must be taken in grounding all surfaces that will be near the probe input in order to minimize line-frequency interference. Even one millivolt of AC on a nearby surface, which can easily arise from a ground loop, can result in significant 50 or 60 Hz noise. A high-quality ground is available at the *Gnd* terminal of the probe; this is internally connected through the probe's cable directly to the *Signal Gnd* in the main unit. The *Gnd* terminal on the probe is best used for the bath electrode, and perhaps for grounding nearby objects such as the microscope.

Ground Wires

It is a good idea to run a separate ground wire from the *Signal Ground* jack on the main unit to ground large objects such as the table, Faraday cage, etc. It is best to have the high quality ground wire run parallel to the probe's cable in order to avoid magnetic pickup and ground loop effects. Besides 50 or 60 Hz magnetic pickup, there may be some 35 kHz pickup from the magnetic deflection of the computer monitor. This pickup becomes visible only when the EPC9 filters are set to high frequencies; it can usually be nulled by changing the orientation or spacing of the ground wire from the probe cable.

Grounding the Microscope

In most cases, the patch clamp is used in conjunction with a microscope; it and its stage typically constitute the conducting surfaces nearest the pipette and holder. In a well-grounded setup, the microscope can provide most of the shielding. Make sure there is electrical continuity between the various parts of the microscope, especially between the microscope frame and the stage and condenser, which are usually the large parts nearest the pipette. Electrically floating surfaces can act as “antennas”, picking up line-frequency signals and coupling them to the pipette. Make sure the lamp housing is also grounded. It is usually not necessary to supply DC power to the lamp, provided that the cable to the lamp is shielded and that this shield is grounded at the microscope.

External Shielding

Especially when an unshielded pipette holder is used, some electrostatic shielding of the experimental setup is necessary to avoid line-frequency pickup from lights and power lines in the room. Most experimenters use a table-top Faraday cage with a closable front, and lead all of the cables (e.g., from the microscope lamp, probe, cooling system, ground lines) through a hole in the cage to an equipment rack mounted outside. If the pipette holder is somewhat exposed, or if the Faraday cage has an open front, a small grounded screen placed near the pipette holder may help.

Connections to other Instruments

Depending on the needs of the particular user, the EPC9 software can substitute (to a great extent) for many analog instruments (e.g., variable filter, oscilloscope, pulse generator, data storage). However, for test purposes, it is still a good idea to have an oscilloscope connected to the EPC9. You may connect a PCM/VCR combination or DAT tape recorder to *Current Monitor 1*, in order to record single-channel activity continuously at high bandwidth. Also, it is often convenient to observe the voltage and current monitor signals at the recorder's outputs, rather than directly from the patch clamp. In most data recorders, the input signals are passed through to the outputs, and observing the signals there can increase the experimenter's confidence that the data are being recorded correctly.

Pipette Holder and Electrode

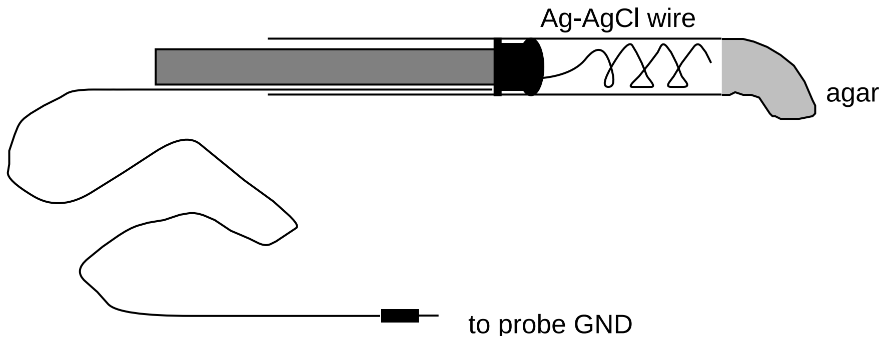
A shielded version of the pipette holder is available; this holder and a properly grounded microscope can provide sufficient shielding from 50-60 Hz interference even without the use of a Faraday cage. The shielded holder, however, introduces much more random noise than the unshielded one. This random noise arises from the non-ideal dielectric properties of the plastic in the holder and from thermal voltage fluctuations in films of aqueous solutions; the metal shield allows more of this noise to be coupled capacitively into the amplifier input. For single-channel recordings, the unshielded holder is strongly recommended. The difference in background noise level between the two holder types is roughly a factor of two.

The unshielded holder is made from polycarbonate, having low dielectric loss. If you make your own holder, you should give some thought to the choice of materials. The insulating parts of the holder should be of a low-loss material, and should have a hydrophobic surface to prevent the formation of conducting water films. Polycarbonate fulfills these criteria better than any material we have tried. You can test the noise level of a holder by mounting it (with the electrode wire installed, but dry) on the probe input, and measuring the noise using the *Noise Test* facility. The probe should be in a shielded enclosure, so that no line-frequency pickup is visible on an oscilloscope connected to the current monitor output at a bandwidth of 3 kHz or less. A good holder increases the rms noise only by about 10%, e.g., from 95 to 105 fA. Noise sources are discussed in more detail later (Chapter **10. Using the Patch Clamp** on **page 75**).

The pipette electrode is simply a thin silver wire that is soldered onto the pin that plugs into the probe's BNC connector. The chloride coating on the wire gets scratched when exchanging pipettes, but we find that this does not degrade the stability very much; the wire does need to be re-chlorided occasionally, perhaps once per month. A wire for the standard electrode holder should be about 4.5 cm long; after it is chlorided an O-ring is slipped onto it and the wire is inserted into the holder. Chloriding can be done by passing current (e.g., 1 mA) between the wire and another silver or platinum wire in a Cl-containing solution (e.g., 100 mM KCl, or physiological saline). Current is passed in the direction which attracts Cl-ions to the electrode wire; this produces a gray coating.

Bath Electrode

The main requirements for a bath electrode are that it have a stable electrode potential and that it does not disturb the composition of the bathing solution. A bare, chlorided silver wire makes a good bath electrode; however Ag-ions are tolerated only by som†of cells, such as muscle cells. A good alternative is an electrode incorporating an agar salt bridge, as illustrated below.



The body of the electrode is a 1 ml plastic syringe body that has been heated and pulled to form a small, bent tip. The electrode proper is a chlorided Ag wire that is first inserted with the plunger into the fluid-filled body; then hot agar is sucked into the tip by withdrawing the plunger partially. The filling solution can be a typical bath solution or something similar, such as 150 mM NaCl. More concentrated salt solutions are not necessary, and they can leak out, changing the composition of the bath solution.

9. Patch-Pipettes

Glass Capillaries

Procedures for fabricating pipettes are presented in some detail in the paper by Hamill et al. We will summarize the procedure and present some tips that we have found helpful. The main steps in pipette fabrication are to form a smooth tip on the pipette (to allow seals to be formed without damaging the cell membrane) and to coat the pipette with a suitable insulating coating to reduce the background noise.

Pipettes can be made from many different types of glass. Our impression is that different types of glass work better on different cell types. Glass capillaries are available from soft (soda glass, flint glass) or hard glasses (borosilicate, aluminosilicate). Some sources of glass pipettes:

Soft Glass (*Supplier*)	**OD**
Non-heparinized hematocrit tubing *any scientific supplier*	1.3 mm
Drummond Microcaps *Drummond Scientific, Bloomall, PA, U.S.A.*	1.4 mm

Hard Glass (*Supplier*)	**OD**
Kimax 51 *Kimble Products, Vineland, NJ, U.S.A.*	1.7 mm
Boralec 100 *Rochester Scientific, Rochester, NY, U.S.A.*	1.7 mm
Corning Sealing Glass (#7052, #7040) *Dow Corning, Midland, MI, U.S.A.*	1.6 mm
GCASS 150-4 (aluminum glass) *A-M Systems, Everett, WA, U.S.A.*	1.5 mm

Soft-glass pipettes have a lower melting point (800 ˚C vs. 1200 ˚C), are easily polished, and can be pulled to have a resistance of 1-2 MΩ. They are often used for whole-cell recording, where series resistance rather than noise is the limiting criterion. The large dielectric relaxation in soft glass sometimes results in additional capacitive-transient components that interfere with good capacitance compensation.

Hard-glass pipettes often have a narrow shank after pulling and consequently a higher resistance. Hard glasses tend to have better noise and relaxation properties, however: the important parameter here is the dielectric loss parameter, which describes the AC conductivity of the glass. Although the DC conductivity of most glasses is very low, soft glasses in particular have a conductivity around 1 kHz; that is sufficiently high to become the major source of thermal noise in a patch-clamp recording. We find that *Kimax* glass is a good compromise for whole-cell recording.

Borosilicate and, especially, aluminosilicate glasses (Rae and Levis, 1984) have low dielectric loss and are desirable for the lowest-noise recordings. They do not necessarily form the best seals, however; this might be due to evaporation of metal onto the glass surface during the high-temperature pulling and polishing steps.

Pulling

Pipettes are pulled in two stages: the first to thin the glass to 200-400 µm at the narrowest point over a 7-10 mm region, and the second to pull the two halves apart, leaving clean, symmetrical breaks. Both halves can be used. The length of the first pull and the heat of the second pull are the main determinants of the tip diameter of the final pipette.

A number of commercial pullers can be used to make pipettes. For reproducibility, however, a regulated current supply to the heater coil is best. A mechanical stop to set the length of the first pull is also important for reproducibility.

Coating

The capacitance between the pipette interior and the bath, and also the noise from dielectric loss in the glass, can be reduced by coating the pipette with an insulating agent such as *Sylgard* (Dow Corning Corp., Midland, MI, U.S.A.). *Sylgard* is pre-cured by mixing the resin and catalyst oil and allowing it to sit at room temperature for several hours (or in an oven at 50 ˚C for 20 min) until it begins to thicken. It can then be stored at -18 ˚C for many weeks until use. The *Sylgard* is applied around the lower few mm of the electrode to within 10-20 µm of the tip and then rapidly cured by a hot-air jet or coil. Coating should be done before the final heat-polishing of the pipette, so that the heat can evaporate or burn off any residue left from the coating process.

Heat Polishing

Heat polishing is used to smooth the edges of the pipette tip and remove any contaminants left on the tip from coating. It is done in a microforge or similar setup in which the pipette tip can be observed at a magnification of 400-800x. The heat source is typically a platinum or platinum-iridium wire; to avoid metal evaporation onto the pipette, the filament is coated with glass at the point where the pipette will approach it. To produce a steep temperature gradient near the filament (which helps make the pipette tip sharply convergent), an airstream can be directed at the filament. The amount of current to pass through the filament must be determined empirically for each type of glass, but a good place to start is with sufficient current to get the filament barely glowing. The typical practice is to turn on the filament current and move the filament toward the pipette (which, being stationary, should remain in focus). Since the opening in the pipette tip is usually at the limit of resolution of viewing, you might not see the change in shape at the tip, but instead only a darkening of the tip. You can tell whether you have melted the tip closed, and also get an idea of the tip diameter, by blowing air bubbles in methanol with air pressure supplied by a small syringe.

Use of Pipettes

Pipettes should be used within 2-3 hours after fabrication, even if stored in a covered container; small dust particles from the air stick readily to the glass and can prevent sealing. However, with some easy-sealing cells we have made the experience that pipettes may even be used the next day. It is very important to filter the filling solutions (e.g., using a 0.2 µm syringe filter). Pipettes can be filled by sucking up a small amount of solution through the tip. This can be done by capillary force (simply dipping the tip for a few seconds in a beaker containing the pipette solution), or by applying negative pressure at the back of the pipette (e.g., using a 5 ml syringe). Thereafter, the pipette is back-filled; the pipette should only be partially filled, just far enough to make reasonable contact with the electrode wire (the pipette holder is not filled with solution, but is left dry). Overfilling the pipette has disastrous consequences for background noise because the solution can spill into the holder, wetting its internal surfaces with films that introduce thermal noise. Bubbles left in the pipette from filling can be removed by tapping the side of the pipette.

For low-noise recording, the electrode holder should be cleaned before each experiment with a methanol flush, followed by drying with a nitrogen jet. Before you insert a pipette into the holder, it is a good idea to touch a hand to a metal surface of the setup to discharge any static electricity that you may have picked up. Be sure to tighten the holder firmly enough that the pipette does not move (on a scale of 1 µm) when you give suction. Then, when you change pipettes during an experiment, check the noise level of the empty holder using the *Noise Test* function; if it increases, solution has probably spilled inside the holder; in this case the holder should be cleaned again and dried thoroughly.

10. Using the Patch Clamp

We give here a brief description of the techniques for establishing a seal and recording from a membrane patch or from an entire small cell.

Forming a Seal

Initial Setup

The object is to apply voltage pulses to the pipette and observe the *Current Monitor* signal on an oscilloscope to monitor the pipette resistance. A convenient way to set the desired adjustments of the amplifier is by activating the *Set-Up* mode. The approach to the cell membrane and the formation of a giga-seal cause the resistance to increase, reducing the currents. A convenient pulse amplitude is 2 mV, which can be obtained from the built-in pulse generator. The 2 mV pulses will cause 1 nA to flow in a 2 MΩ pipette.

For observation of the current pulses, it is convenient to pick a *Gain* setting and oscilloscope sensitivity such that the current through the open pipette is reasonably sized. Other recommended settings are also preset in the *Set-Up* mode: *Voltage Clamp* and *V-membrane* = 0; *Filter 1* is set to 10 kHz and *Filter 2* to 3 kHz, R_S*-comp* to *Off*, *C-slow Range* to *Off* and *Stimulus Rise Time* to 20 µs. Before the pipette is inserted into the bath, the current trace should be flat, except for very small capacitive pulses due to the stray capacitance of the pipette and holder.

Entering the Bath

The surface of the solution is relatively “dirty”, even if (as we strongly recommend) you aspirate some solution from the surface to suck off dust and contaminants. For this reason it is important to apply a small amount of positive pressure to the pipette before you move its tip into the bath, and also to avoid going through the air-water interface more than once before forming a seal. When you do move the pipette tip into the bath, the current trace may go off-scale (check clipping); in that case, click on the *Auto-*V_0 button or reduce the gain until the trace reappears. From the size of the current response to the test pulses, the pipette resistance can be calculated (the pipette resistance is displayed in the EPC9 window as *R-membrane*). If there should be no change in the trace upon entering the bath, check for an open circuit, for example: 1. a bubble in the pipette; 2. faulty connection to the probe input; 3. bath electrode not connected.

There is invariably a small offset potential between the pipette and bath electrodes. The V_0 control is designed to provide a bucking potential to cancel this offset. With the pipette in the bath, select the *Auto-*V_0 button.

Forming a Gigaseal

When the pipette is pushed against a cell, the current pulses will become slightly smaller to reflect the increasing seal resistance; when the positive pressure is released, the resistance usually increases further. Some cell types require more "push" from the pipette than others, but an increase in resistance of 1.5 (i.e., a reduction in the current pulses by this factor) is typical.

Application of gentle suction should increase the resistance further, and result (sometimes gradually, over maybe 30 s; sometimes suddenly) in the formation of a gigaseal, which is characterized by the current trace becoming essentially flat again (hyperpolarizing the pipette to -40 to -60 mV often helps to speed the seal formation). To verify gigaseal formation, increase the *Gain* to perhaps 50 mV/pA; the trace should still appear essentially flat except for capacitive spikes at the start and end of the voltage pulse.

Cell-Attached Recording

You can now use the *On Cell* mode, which will automatically adjust *C-fast* and *τ-fast* controls to minimize the size of these spikes (some improvement can sometimes be obtained by manual adjustment). Transient cancellation will be essential if you will be giving voltage pulses in your experiment. It is a good idea to start out with *V-membrane* set to zero, as we specified above; an alternative is to start with *V-membrane* set to the holding potential you desire (e.g., -70 mV). If no voltage jumps are required, turn the stimulus off to avoid introducing artifacts. If voltage jumps are to be applied, switch the *Gain* and *Filters* to the values you will be using (the settings may be programmed under *On Cell*).

Be sure to use *Gain* settings of 50 mV/pA or above for lower noise in single-channel recordings. Keep the *Filter 1* switch set at 10 kHz unless you actually will need the full 60 kHz bandwidth for some reason; otherwise you might drive the current monitor output or your recorder's input amplifiers into saturation with the very large amount of high-frequency noise. Should you use the full bandwidth, you should avoid gain settings above 100 mV/pA for the same reason.

If you are applying voltage pulses to the patch membrane, you probably will want to subtract control traces from the traces containing the channels of interest in order to remove the capacitive transients. Nevertheless, it is important to try to cancel the capacitive transients as well as you can in order to avoid saturating any amplifiers, the recording medium or the AD converter. It is a good idea to set the *C-fast* and τ-*fast* controls while you observe the signal without any filtering beyond the internal 10 kHz filter. Then, during the recording, watch to see if the *Clipping* light blinks. When it does, it means that internal amplifiers in the EPC9 are about to saturate, and/or that the *Current Monitor* output voltage is going above 13 V peak, on the peaks of the transients, and you should readjust the transient cancellation controls. Otherwise, it is likely that the recording will be non-linear and subtraction will not work correctly.

The fast transient cancellation is not sufficient to cancel all of the capacitive transients in a patch recording. This is partly because the pipette capacitance is distributed along the length of the pipette; therefore, each element of capacitance has a different amount of resistance in series with it, so that a single value of τ-*fast* will not provide perfect cancellation. The time course of the transients also reflects dielectric relaxation in the plastic of the pipette holder and in the pipette glass. These relaxations are not simple exponentials, but occur on time scales of about 1 ms. If you use pipette glass with low dielectric loss (e.g., aluminosilicate glass) or if you are careful to coat the pipette with a thick coating and near to the tip, the relaxations will be smaller. You can cancel part of these slow relaxations by using the *C-slow* controls, with the *C-slow Range* set to 3 pF.

Note: For cell-attached or inside-out patch configuration, positive pipette voltages correspond to a hyperpolarization of the patch membrane, and inward membrane currents appear as positive signals at the Current Monitor outputs. The E9Screen and Pulse programs compensate for this by inverting digital stimulus and sampled values in these recording configurations such that the stimulation protocols, holding voltages, and displays of current records in the oscilloscope all follow the standard electrophysiological convention. In this convention, outward currents are positive and positive voltages are depolarized. However, the analog current and voltage monitor outputs are not inverted in these recording modes.

Whole-Cell Recording

Breaking the Patch

After a gigaseal is formed, the patch membrane can be broken by additional suction or, in some cells, by high voltage pulses (600-800 mV, see *Zap* function). Electrical access to the cell's interior is indicated by a sudden increase in the capacitive transients from the test pulse and, depending on the cell's input resistance, a shift in the current level. Additional suction sometimes lowers the access resistance, causing the capacitive transients to become larger in amplitude but shorter. Low values of the access (series) resistance are desirable and, when R_S-compensation is in use, it is important that the resistance be stable as well. A high level of Ca^{2+} buffering capacity in the pipette solution (e.g., with 10 mM EGTA) helps prevent spontaneous increases in the access resistance due to partial resealing of the patch membrane.

Capacitive Transient Cancellation

If the fast capacitance cancellation was adjusted (as described above) before breaking the patch, then all of the additional capacitance transient will be due to the cell capacitance. Cancelling this transient using the *C-slow* and *R-series* controls will then give estimates of the membrane capacitance and the series resistance. The easiest way of cancellation is provided by the *Auto C-slow* function which may be activated by selecting the *Auto* button or the *Whole Cell* mode (if included in the macro function). In cases where the *C-slow* transient has a short time constant (100 µs or smaller) , some improvement of the overall compensation may be achieved by alternating cycles of *Auto C-fast* and *Auto C-slow* (if this doesn't work satisfactorily you may fine-tune the compensation controls manually). After an iteration or two, it should be possible to reduce the transient to only a few percent of its original amplitude. However, if the cell has an unfavorable shape (for example, a long cylindrical cell or one with long processes), the cell capacitance transient will not be a single exponential, and the cancellation will not be as complete.

Series Resistance Compensation

Series resistance (R_S) compensation is important when the membrane capacitance is large or when the ionic currents are large enough to introduce voltage errors. To use R_S-compensation, you first adjust the transient-cancellation controls (including *C-fast* and *τ-fast* if necessary) to provide the best cancellation. Then you select *R_S-comp* by turning up the *%-comp* control to provide the desired degree of compensation.

***Note**: The "R-series" control determines (along with the "%-comp" control) the amount of positive feedback being applied for compensation. It should be adjusted with some care, since too high a setting causes overcompensation (the EPC9 will think that R_S is larger than it is); this can cause oscillation and possible damage to the cell under observation.*

How you should set the R_S-compensation controls depends on the approximate value of the uncompensated membrane-charging time constant τ_u, which you can calculate as the product of the *C-slow* and *R-series* settings (for example, suppose *C-slow* is 20 pF and *R-series* is 10 MΩ; τ_u is then 20 pF · 10 MΩ = 200 µs). If τ_u is smaller than about 500 µs, you should use the 2 µs setting of the R_S-compensation switch to provide the necessary rapid compensation; however, the slower settings will provide compensation that is less prone to high-frequency oscillations from misadjustment of the controls. How much compensation you can apply is also determined by tu. If τ_u is larger than about 100 µs, you can use any degree up to the maximum of 90% compensation without serious overshoot or ringing in the voltage-clamp response. For smaller values of τ_u, the *%-comp* setting should be kept below the point where ringing appears in the current trace.

As in the case of patch recording, there is rarely need to use the full bandwidth of the Filter 1 in whole-cell recording. This is because typical membrane charging time constants (even after R_S-compensation) are considerably longer than 16 µs, which is the time constant corresponding to a 10 kHz bandwidth. Thus, the current monitor signal is expected to contain no useful information beyond this bandwidth.

In whole-cell recording, the voltage and current monitor signals follow the usual convention, with outward currents being positive. This is because the pipette has electrical access to the cell interior.

Current-Clamp Recording

To switch from voltage-clamp to current-clamp recording, just select the *Current Clamp* mode. This provides a recording of the cell membrane potential, which can be monitored at the *V-mon* output of the EPC9.

When you switch to the *Current Clamp* mode, the following things happen inside the EPC9 main unit: The current monitor circuitry is forced into the intermediate *Gain Range* (if it was not before), *I-membrane* is set to a value that will keep the membrane voltage constant, internal stimulation is stopped, the 10 kHz Bessel characteristic for *Filter 1* is selected, *C-slow Ranges* is set to *Off*, and the value of *C-fast* is reduced by 0.5 pF to avoid oscillations. R_S-compensation will now act as a “bridge balance”. For the user, these changes are of little consequence and are mainly designed to make current-clamp recording simple and reliable. The holding current will be displayed in the original *V-membrane* display (now converted to *I-membrane*) and the membrane potential is shown in the *V-mon* display of *E9Screen*. In *Pulse*, the *I-mon* display is converted to *V-mon*.

Once you have switched to *Current Clamp*, you can use *I-membrane* to set a holding current, and you can apply stimulus pulses via *Stim. In*. The rule for the scaling of current stimuli is easy to remember: Any combination of applied stimulus and *Stim. Scale* settings that would provide 1 mV of stimulus in the *Voltage Clamp* mode provides 1 pA of stimulus in the *Current Clamp* mode.

11. Low-Noise Recording

The EPC9 amplifier has a particularly low background noise level. The noise level is in fact low enough that in most experimental situations it can be neglected in view of other background noise sources that make larger contributions to the total. As we consider these other sources, first let us make it clear that in this section we are concerned with random noise, which is fundamentally due to the thermal motion of electrons and ions; we assume that any user who is interested in low-noise recording has shielded and grounded his setup sufficiently well to take care of any synchronous noise due to line-frequency pickup, computer power supplies, TV cameras, etc. Synchronous noise can be readily identified as stationary features on an oscilloscope trace when the oscilloscope is triggered by the appropriate signal source, for example, line-frequency triggering. Grounding and shielding is discussed in Chapter **10. Using the Patch Clamp** on **page 75**.

The *Noise* feature of the EPC9 makes it easy for the user to identify important noise sources. When the *Noise* button is selected, the *I-mon* display shows the rms noise current present in the *Current Monitor* signal in the frequency band selected by *Filter 2* (3 kHz is recommended). For noise measurements, the standard setting of the *Gain* control is 50 mV/pA. With the probe placed in a shielded enclosure and with nothing connected to the input, the *Noise* reading is usually 80-100 fA. If you get a reading higher than this, try varying the *C-fast* control. If you have a noisy stimulus source connected to *Stim. In*, the induced current noise will vary with *C-fast*, with a minimum occurring with the control set to 1-2 pF, i.e., for best cancellation.

Starting from the intrinsic noise reading of 80-100 fA, one observes increments in the noise level when the holder and pipette are installed and when an actual recording is made. By analyzing these increments, you can see where there is the most room for improvement in your technique. Under the best conditions (i.e., with a clean holder, an aluminosilicate pipette, etc.), we have observed the noise reading increase to about 130 fA when the holder and pipette are present, and 160 fA when the pipette tip is in the bath, sealed on a cell. These are rms current values, which means that they are equal to the standard deviation of the fluctuating current.

Since the noise sources in the patch clamp amplifier, pipette holder, pipette and patch membrane are statistically independent, their contributions to the total noise do not add linearly; instead, their variances (the squares of the standard deviations) add. This means that the rms reading on the EPC9's display represents the square root of the sum of the squares of the rms currents from each source. Taking this into account, one can calculate the relative contributions from the amplifier, pipette holder, and the combination of pipette immersion and patch noise. The table below shows the relative contributions calculated in this way for the “optimum” situation just described.

Noise Source	Contribution	rms Current
Amplifier	35 %	95 fA
Holder	21 %	73 fA
Pipette + Patch	44 %	105 fA

The contributions to the variance from the three sources are seen to be comparable in size, and improvements in the amplifier noise level will not help very much, unless corresponding improvements are made in the other noise sources. As it is, rms noise values as low as those quoted here are obtained only with considerable care. Some of the important considerations are outlined below.

As we mentioned in Chapter **8. Patch-Clamp Setup** on **page 67**, the unshielded holder is greatly superior to the shielded one for low background noise. For low noise, the holder must be made from a low-loss, hydrophobic plastic; polycarbonate is one of the best, and plexiglas one of the worst materials. (For our purposes, low-loss materials are those that show little dielectric relaxation in the frequency range of a few kHz. Dielectric relaxation involves the reorientation of dipoles within the material; since any dipoles will be in thermal motion, thermal reorientations in this frequency range will result in current fluctuations coupled capacitively into the pipette.)

It is very important that the pipette holder be kept clean and dry. Noise can be coupled into the pipette from the thermal motion of ions in films of aqueous solution, especially on the inside of the pipette. A good practice for low-noise work, is to connect a valve to the pipette-suction line, and arrange for dry air or nitrogen to flow into the suction line during the time while you change pipettes. This will dry out any such aqueous films and keep the noise level low.

Films of aqueous solutions and dielectric relaxation are also serious problems with pipette glass. Coating with Sylgard helps because it is hydrophobic and because it has good dielectric properties. Also, its thickness helps to reduce the capacitance between the pipette interior and the bath. This is mainly important because it reduces the coupling of the glass's dielectric noise into the pipette interior. Clearly, making thicker coatings (especially in the tip region) and coating closer to the tip will reduce the pipette noise. The best glass type we know of is aluminosilicate; this glass requires fairly high temperatures in pulling, and does not necessarily give the best gigaseals; but its dielectric relaxation appears to be about a order of magnitude smaller than soft glass.

Some improvement is probably to be gained by taking steps to prevent formation of aqueous films on the back end of the pipette. It is a good idea to wipe the outside of the pipette to remove any spilled solution, fingerprints, etc. before inserting it into the holder. It might also help to treat the inside of the pipette to prevent the formation of a film, for example, by shooting some dimethyl-dichlorosilane vapor (caution: nasty stuff!) into the back of the pipette before or after filling it.

Lower noise is obtained by immersing the pipette a shorter distance into the bath: this reduces the coupling of noise currents arising in the pipette glass. A significant amount of noise seems to arise in the sealed membrane itself and is probably lower in higher-resistance seals. This noise is generally more than one would calculate from the resistance of the gigaseal.

The usual goal of low-noise recording is better time resolution: if the noise level is lower, you can use a wider filter bandwidth to observe single-channel events of a given amplitude. Judicious use of filtering can improve the time resolution of your analysis. For example, if you are using the 50%-threshold-crossing analysis technique to analyze channel open and closed times, the best filter bandwidth is the one that makes the rms background noise about 1/10 of the channel amplitude. Since one rarely wants to go through the process of choosing the optimum bandwidth during an experiment, the best procedure is to record the data at a wide bandwidth and perform any necessary filtering (analog or digital) later, during analysis of the data.

In typical voltage-clamp, whole-cell recordings the predominant noise source arises from the combination of the access resistance R_s and the cell membrane capacitance C_m. Above 1 kHz or so, the current variance from this source increases with this resistance and capacitance as

$$\sigma^2 = \alpha \; R_s C_m$$

so that it is clearly desirable to keep R_s as small as possible, and, even more important, to select small cells, if one is interested in low noise. See the chapter by Marty and Neher (1983) for a more complete description of this and other fine points of whole-cell recording.

Appendix I: Controlling E9SCREEN

Communication between E9Screen and other Programs

E9Screen has two functions which enable a second acquisition program to share the AD/DA-board. This allows the E9Screen to control the EPC9, while the second program can use the AD/DA-board for acquisition:

1. E9Screen uses the two functions in the drivers of the AD/DA-board to reserve and release the AD/DA-board for exclusive use.
2. E9Screen writes a file containing gain and holding potential applied to the EPC9, and the user program can extract those information from it.

Reserving the AD/DA-board for exclusive use

E9Screen reserves the AD/DA-board for exclusive use, while it performs acquisition. E9Screen can release the AD/DA-board, when it stops acquiring. This occurs when the user switches to another application and the option "Enable Background" in the EPC9 drop-down menu is de-selected. E9Screen then resumes acquisition, when the user switches back and the AD/DA-board driver is not reserved by another process. Thus, both programs can use the AD/DA-board at well defined times. Please, read in the ITC-16 driver manual ("Driver.pdf") the description of the functions ITC16_Reserve and ITC16_Release:

- E9Screen calls ITC16_Release, when it is switched to the background and option "Enable Background" is de-selected.
- E9Screen calls ITC16_Reserve, when it is switched back to the foreground. E9Screen will continue to call ITC16_Reserve until the function messages that the AD/DA-board has been released by the other application. Only then E9Screen will resume acquisition.

The “EPC9out.EPC” file

E9Screen writes the file **“EPC9out.EPC”** in the “E9Screen” folder every time when switching from *E9Screen* to another application. This feature can be used by other acquisition programs to obtain the gain and holding potential applied to the EPC9. The information in this file is as follows:

- **In voltage clamp mode** (VC-mode):

```
VHOLD:     -0.080;  IGAIN:  1.0000E+09
0 [V]  1.0000E+01
1 [-]  1.0000E+00
2 [-]  1.0000E+00
3 [-]  1.0000E+00
4 [-]  1.0000E+00
5 [-]  1.0000E+00
6 [A]  1.0000E+09
7 [A]  1.0000E+09
```

- **In current clamp mode** (CC-mode):

```
IHOLD:  1.0000E-10;  IGAIN:  1.0000E+09
0 [V]  1.0000E+01
1 [-]  1.0000E+00
2 [-]  1.0000E+00
3 [-]  1.0000E+00
4 [-]  1.0000E+00
5 [-]  1.0000E+00
6 [A]  1.0000E+09
7 [A]  1.0000E+09
```

The meaning of the parameters in the first text line are:

- The first character (“V” in VHOLD and “I” in IHOLD, respectively) indicate the amplifier mode (voltage and current clamp).
- VHOLD gives the applied holding potential in volts. IHOLD gives the applied holding current in amperes.
- IGAIN gives the amplifier gain in ohms, i.e. V / A. To compute the real current from a measured voltage, divide it by this value.

The meaning of the parameters in the following lines are:

- The first number is the index of the described AD-channel.
- The letter in the square brackets gives the logical unit of that channel, i.e., "V" for the voltage monitor, and "A" for the current monitors. A hyphen marks AD-channels without a logical EPC9-function.
- The last number in the line is the gain of that AD-channel. You can derive the voltage acquired from that AD-channel by the given number to get the correct value, e.g. the control voltage and the pipette current.

Notes: The line marked "[V]" gives the value of the "Voltage Monitor" BNC-connector on the EPC9 front panel.
The lines marked "[A]" give the values of the "Current Monitor" BNC-connectors on the EPC9 front panel.

Make sure that the option *Enable Background* is de-selected, when you want to acquire data with the EPC9 acquisition board. When *E9Screen* is in the background, the AD-channel assignments are as follows:

- AD-6 is the *I-mon 2* input, i.e., the current input after filter 1 and filter 2.

- AD-7 is the *I-mon 1* input, i.e., the current input after filter 1.

To read *V-mon*, one has to connect the corresponding *V-mon* output of the EPC9 to any suitable AD-input channel (but not 6 or 7). The user can now switch between two programs. In this configuration, one can use the user program for data acquisition and processing, while *E9Screen* is used to control the EPC9 settings, such as gain and compensations. The holding potential is best set in *E9Screen*. If the holding potential should be set from the user program, one has to control DA-3, which directly applies the voltage to the EPC9 (scaling is 0.1, i.e., an input of 100 mV will translate to 10 mV). Alternatively, voltages can be applied to the EPC9 by setting DA-0, DA-1, or DA-2, and connecting that DAC to the external stimulus input of the EPC9. In this case, activate the *External Stimulus Input* option in *E9Screen*, i.e., setting an appropriate external stimulus scaling factor.

Controlling the EPC9 from another Program

Sending Commands to E9Screen

E9Screen can be controlled from another program by a simple "batch file control" protocol. This "batch file control" protocol is simple, fast, and platform independent. The new control protocol allows to control the EPC9 over a network, even one with different platforms, such as Windows, Macintosh, Unix, OS/2, or workstations. Thus, it is now possible to control the EPC9 from computers running a multitasking operating system which can create and read shared files (e.g., Windows 95, Windows NT, MacOS, etc.).

Controlling *E9Screen* from another program is possible by communicating via two ASCII-files. The user writes the commands to one file (the "command" file) and *E9Screen* communicates back by writing to a second file (the "response" file). The user program has write permission (plus sharing permission) on the "command" file it will write to. *E9Screen* will access that file with read and shared permission only. The reverse is used on the second file, the "response" file: *E9Screen* will have write and sharing permission, and the user program read permission only (plus sharing permission, of course).

The first line in the "command" file must contain one positive number (as ASCII, e.g., "+1234"). This "command index" is interpreted by *E9Screen* as follows:

- If this number is zero or negative, *E9Screen* does not execute the commands in the "command" file.
- If the number is larger than zero, *E9Screen* will execute the instructions immediately. *E9Screen* will write that number to the "response" file to flag execution once all commands have been executed.
- To prevent *E9Screen* to execute the instructions more than once, *E9Screen* will not execute any further commands until the "command index" value is changed by the user program.

- Every command plus the required parameters must be in one text line, i.e., terminated by a “CR” character code (any following linefeed character will be ignored).
- *E9Screen* writes the responses to the “response” file. In the first line of that file, *E9Screen* writes the “command index”. The following lines will contain the responses, if any, one response per line.
- The name of the “command” file must be “**E9Batch.In**”, the one of the “response” file “**E9Batch.Out**”. Both files will be inside the default folder of *E9Screen*, usually the folder “E9Screen”.

Thus, communication would proceed as follows:

❶ The user program is started first:
It has to create a file in the “E9Screen” folder named “E9Batch.In”. It has to keep this file open with “write” and “shared” access permission.

❷ Then *E9Screen* is started:
Enable the “Enable Batch Control” option. *E9Screen* will open the file “E9Batch.In” with “read” and “shared” access permission. Also, E9Screen will now create the "E9Batch.Out" file with “write” and “shared” access permission.

❸ Now, E9Screen will immediately execute the commands in the command file, provided that the “command index” is larger than zero. *E9Screen* writes the “command index” and eventually any error and requested answer to the “response” file.

❹ Next, the user switches back to the user program.

❺ Any time the user program writes to the “command” file, *E9Screen* will scan the command file and execute the commands, if the “command index” changed.

Windows 95 and Windows NT: On computers running Windows 95 or Windows NT, *E9Screen* will immediately execute the commands.

Macintosh: On computers running MacOS, *E9Screen* will immediately execute the commands, if it is the front application. If *E9Screen* is not the front application, then *E9Screen* will get active when the front application calls the “Toolbox” routine “GetNextEvent” or “WaitNextEvent”.

❻ The user program can now read the “response” file. The first line should mirror the “command index”. Subsequent text lines may contain responses and error messages (see list below). There are the following possibilities:

- There is no further line in the response file. This means, that no error occurred during execution and that no response was requested.
- There is additional text in the file. The user can easily recognize error messages, because the first character in an error message is a lower case letter. All other responses start with an upper case letter.

❼ When the user program wants to issue new commands, it writes a new "command index" and the new commands to the "command" file, then continues with step 5.

Here is an example of such a command file:

```
1. line: "1234"
2. line: "SetVHold -0.080"
3. line: "GetCurrentRange"
4. line: "MakeAnError"
```

And this would be the content of the "response" file:

```
1. line: "1234"
2. line: "CurrentGain 1.000E+09"
3. line: "unknown"
```

Error Messages

Errors begin with lower case letters:

```
'syntax' :        parameter missing or misspelled
'range'  :        parameter is out of allowed range
'unknown':        command is unknown
'ioerror':        error during an I/O-operation
'unknown error':  an unidentified error occurred
```

Implemented Commands and Messages

Messages send by E9Screen. They do not contain a response string.

```
Started           E9Screen started communication link
Terminated        E9Screen terminated communication
InFront           E9Screen got the front application
Running           E9Screen was switched to the background
                     (see "Query" command below).
ADReserved        E9Screen reserved the AD/DA-board for
                      exclusive use and resumed acquisition
ADRelease         E9Screen released the AD/DA-board and
                      and stopped acquisition
```

Operations without parameters. They do not return a response.

```
AutoCFast
AutoCSlow
AutoGLeak
AutoVpOffset
```

"Get" operations: They have no parameters and return one parameter. The response always repeats the command string without the "Get" sub-string.

```
GetCCFastSpeed
GetCCIHold
GetCCStimScale
GetCFast1
GetCFast2
```

```
GetCFastError
GetCFastTau
GetClipping
GetCSlow
GetCSlowError
GetCSlowRange
GetCurrentGain       get gain in Ohms (i.e., V/A)
GetE9SBoard
GetE9SerialNo
GetF2Butterworth
GetF2Frequency
GetFilter1
GetGLeak
GetGSeries
GetIpipette
GetLastVHold
GetMode
GetRmembrane
GetRsFraction
GetRsMode
GetRsValue
GetStimFilterOn
GetVCStimScale
GetVersion
GetVHold
GetVLiquidJunction
GetVmonitor
GetVpOffset
```

"Set" operations: They have no or one parameter and do not return a response.

```
SetAdcChannel        integer: 0 to 12 -> FExt, Vmon, Imon1, Imon2,
                                         none, ADC0 .. ADC7
SetCCFastSpeed       boolean: 0 or 1
SetCCIHold           real:    current in Amperes
SetCCRange           boolean: 0 or 1
SetCFast2            real:    capacitance in Farad
SetCFastTau          real:    tau in seconds
SetCSlow             real:    capacitance in Farad
SetCSlowRange        integer: 0,1,2,3 -> off,30,100,1000pF range
SetCurrentGain       set gain in Ohms (i.e., V/A)
SetE9Board           integer: 1,2,3
SetF2Frequency       real:    bandwidth in Hertz
SetF2Butterworth     boolean: 0 or 1
SetFilter1           integer: 0,1,2,3 -> 100,30,10kHz,HQ30kHz
SetGLeak             real:    in Siemens (i.e., 1/Ohms)
SetGSeries           real:    in Siemens (i.e., 1/Ohms)
SetLastVHold         no parameters
SetMode              0 to 3, corresponding to Test, VC, and CC.
SetRsFraction        real:    0.0 to 0.95
SetRsMode            integer: 0,1,2,3 -> off,100,10,2 µs
SetRsValue           real:    in Ohms
SetStimFilterOn      boolean: 0 or 1
SetStimScale         real:    -10.0 to +10.0 (0.0 means off)
SetTestPulse         boolean: 0 or 1
SetVHold             real:    -1.0 to +1.0 Volt
SetVLiquidJunction   real:    -1.0 to +1.0 Volt minus VHold
SetVpOffset          real:    -0.2 to +0.2 Volt
```

Miscellaneous Operations

```
acknowledged (re-synchronize command index)
   syntax: "acknowledged"
      no parameters
      no response

ADRead
   syntax: "ADRead  AD-channel"
      AD-channel: integer 0 to 7
      returns a real (read AD-value in Volts)

ADRelease
   syntax: "ADRelease"
      no parameters
      returns string "ADReleased"

ADReserve
   syntax: "ADReserve"
      no parameters
      returns:
         if it could reserve the AD-board: string "ADReserved"
         otherwise:                        string "ADReleased"

DAWrite
   syntax: "DAWrite  AD-channel  Volts"
      DA-channel: integer, 0 to 3
      Volts:      real,    +/-10.24 volt
      no response

ExecMacro
   syntax: "ExecMacro MacroIndex"
      MacroIndex: integer, 0 to 6
      no response

MeasureNoise
   syntax: "MeasureNoise"
      no parameters
      returns a real

Query
   syntax: "Query"
      no parameters
      returns the string "Running"

Reset
   syntax: "Reset"
      no parameters
      no response

Terminate
   syntax: "Terminate"
      no parameters
      returns the string "Terminated"

Key
   syntax: "Key  ASCII-code  Key-Code"

   Key-Codes:
      2 -> Normal character input
```

```
cursor keys:
   3 -> KeyCursorUp
   4 -> KeyCursorDown
   5 -> KeyCursorLeft
   6 -> KeyCursorRight

numeric keypad:
   7 -> 0 (zero) on numeric keypad
   8 -> 1 on numeric keypad
   9 -> 2 on numeric keypad
  10 -> 3 on numeric keypad
  11 -> 4 on numeric keypad
  12 -> 5 on numeric keypad
  13 -> 6 on numeric keypad
  14 -> 7 on numeric keypad
  15 -> 8 on numeric keypad
  16 -> 9 on numeric keypad
  17 -> Enter
  18 -> minus  on numeric keypad
  19 -> plus   on numeric keypad
  20 -> period on numeric keypad
  21 -> clear  on numeric keypad (above '7')
  22 -> '='    on numeric keypad (above '8')
  23 -> '/'    on numeric keypad (above '9')
  24 -> '*'    on numeric keypad

function keys (F1 to F15):
  24 + function key number, e.g., 25 for F1

key block above cursor keys:
  40 -> HOME
  41 -> END
  42 -> PageUp
  43 -> PageDown
  44 -> HELP
  45 -> Delete Left
  46 -> Delete Right
```

Notes to Programmers

If one uses the EPC9 acquisition board to acquire data in a user program, one has to be very aware that the EPC9 is controlled through its AD/DA-board as well. Thus, every time E9Screen has to talk to the EPC9, it will unconditionally make use of the AD/DA-board. Yet, when E9Screen is not the front application (Windows programmers call it "focus") and the "Enable Background" option is not selected, then E9Screen gets active only when it receives a command through the "batch control" handshaking. Therefore, the "Enable Background" option in the EPC9 drop-down menu must be off.

One other problem spot is to define when to open the message file. This file is created by E9Screen. Therefore it cannot and should not be opened before E9Screen is running. Thus, the user program has to delete any message file it finds upon starting. That file may still be left around from a previous session. A good option is to create an empty command file in the directory where E9Screen is located and to wait for the message file to appear in the E9Screen directory. At that moment one can start E9Screen and enable the option "Enable Batch Control" in the EPC9 drop-down menu. The message file will then be created and the user program can now open it for reading and sharing.

The first response E9Screen writes to the message file is "Started". Thereafter the user can proceed to send commands to E9Screen and read back the response.

It is advisable to write to the command file in an analogous way as it is done in the example code below. In principle, one should proceed as follows:

1. Write a minus sign ("-") to the first byte of the command file. This prevents E9Screen from interpreting anything in the file while the user programs writes to it. Please, recall that some operating systems are multitasking. This means that both programs, E9Screen as well as the user program, run concurrently. Thus, E9Screen may attempt to read from the message file while the user program is writing to it!

2. Write the remaining of the first text line. The first text line must be the signature number. The sample code below writes the negative value of the signature to achieve:
   - a negative sign is placed in the first byte of the file;
   - the signature value is written; and
   - the final, positive signature can be obtained by replacing the negative sign with a plus ("+").

3. Write to following text lines the required instructions, one instruction per text line.

4. Finally, replace the negative sign in the first byte of the file with a plus ("+"). This will signal to E9Screen to proceed to read and interpret the command file.

5. Monitor the content of the message file. Do not interpret the content of the message file, as long as the first byte is a minus sign ("-") or the signature value in the first line is the one used in writing the last command.

6. E9Screen writes responses to the message files in the following situations:
   - On starting "batch" processing, it messages "Started".
   - Responding to a "Get", "Query", or "Terminate" instruction.
   - When an error occurred while scanning the message file.

Sample program

The listing below is an excerpt from the program used to test the E9Screen communication protocol. It demonstrates the functions needed to write an own program to control the EPC9 through the E9Screen program. The code is written in Modula-2. The code can be easily understood also by readers familiar with BASIC, PASCAL, C, C++, or FORTRAN.

```
MODULE UserCommands;


CONST
   CommandName    = 'EPC9In.EPC';
   MessageName    = 'EPC9Out.EPC';

VAR
   CommandFile    : IOFiles.FileHandleType;
   MessageFile    : IOFiles.FileHandleType;
   Signature      : INTEGER;
   LastSignature  : INTEGER;


PROCEDURE WriteError( Text : ARRAY OF CHAR );
VAR
   Work  : ARRAY[0..79] OF CHAR;
BEGIN
   IOFiles.Message( IOFiles.GetError(), Work );
   TermIO.WriteString( Text );
   TermIO.WriteString( ' failed: ' );
   TermIO.WriteLine( Work );
   Alert.Beep;
END WriteError;


PROCEDURE WriteToCommandFile( Text : ARRAY OF CHAR ): BOOLEAN;
VAR
   Work  : ARRAY[0..79] OF CHAR;
   Count : INTEGER;
BEGIN

   IF NOT CommandFile.IsOpen THEN RETURN FALSE; END;

   IF Text[0] = 0C THEN RETURN TRUE; END;

   Work[0]  := 0C;
   Decode.Integer( - ABS( Signature ), Work, 0 );
   Count    := Strings.Length( Work );

   (* First, we write a negative signature to the first text line.
      This prevents to the target E9Screen to read and interpret
      the content of this file. We use the actual signature already
      now, so that we can just change the sign from negative to
      positive by overwriting one character!
    *)

   IF
```

```
      ( NOT IOBytes.SetLength( CommandFile, 0 ) ) OR
      ( NOT IOText.Write( CommandFile, Count, ADR( Work ) ) )
   THEN
      WriteError( 'Writing negative signature' );
      RETURN FALSE;
   END; (* IF *)

   Count := Strings.Length( Text );

   (* Second, we write the command text to the second (and following)
      line.
    *)

   IF NOT IOText.Write( CommandFile, Count, ADR( Text ) ) THEN
      WriteError( 'Writing to command file' );
      RETURN FALSE;
   END; (* IF *)

   (* Third, if the signature should be positive, we now overwrite the
      negative sign (i.e., "-") of the signature in the first line with
      a plus (i.e., "+"), thus changing the negative signature to positive.
      See above for the explanation.
    *)

   IF Signature >= 0 THEN
      Work[0] := '+';
      Count   := 1;

      IF
         ( NOT IOBytes.SetPosition( CommandFile, IOFiles.FromStart, 0 ) ) OR
         ( NOT IOBytes.Write( CommandFile, Count, ADR( Work ) ) )
      THEN
         WriteError( 'Writing positive signature' );
         RETURN FALSE;
      END; (* IF *)
   END; (* IF *)

   TermIO.WriteLn;
   TermIO.WriteString( 'command : [' );
   TermIO.WriteInt( Signature, 0 );
   TermIO.WriteString( '] ' );
   TermIO.WriteLine( Text );

   INC( Signature );

   IF Signature < 1 THEN Signature := 1; END;

   RETURN TRUE;

END WriteToCommandFile;


PROCEDURE OpenMessageFile(): BOOLEAN;
BEGIN

   IF NOT CommandFile.IsOpen THEN RETURN FALSE; END;

   IF MessageFile.IsOpen THEN
      Alert.String( 'Message file already open! ' );
      RETURN FALSE;
```

```
   END; (* IF *)

   IF NOT
      IOText.Open(
         MessageName,
         IOFiles.Read + IOFiles.Shared,
         MessageFile )
   THEN
      IF IOFiles.GetError() <> IOFiles.FileNotFound THEN
         WriteError( 'Opening message file' );
      END; (* IF *)
      RETURN FALSE;
   END; (* IF *)

   LastSignature := -1;

   RETURN TRUE;

END OpenMessageFile;


PROCEDURE PollForCommands;
VAR
   InputString     : ARRAY[0..127] OF CHAR;
   NewSignature    : INTEGER;
   Count           : INTEGER;
   Dummy           : BOOLEAN;

BEGIN

   IF NOT CommandFile.IsOpen THEN
      RETURN;
   END; (* IF *)

   IF ( NOT MessageFile.IsOpen ) AND ( NOT OpenMessageFile() ) THEN
      RETURN;
   END; (* IF *)

   (* Read the first text line. If reading fails, or converting to a number
      fails, or the resulting number is negative, then the answer from the
      target E9Screen is not ready.
    *)

   NewSignature    := -2;
   Count           := HIGH( InputString );

   IF
      ( NOT IOText.SetPosition( MessageFile, IOFiles.FromStart, 0 ) ) OR
      ( NOT IOText.Read( MessageFile, Count, ADR( InputString ) ) )
   THEN
      RETURN;
   END; (* IF *)

   IF ( InputString[0] = 0C ) OR ( InputString[0] = '-' ) THEN
      RETURN;
   END; (* IF *)

   Count := Encode.Integer( InputString, NewSignature );

   IF ( NewSignature < 0 ) OR ( LastSignature = NewSignature ) THEN
```

```
      RETURN;
   END; (* IF *)

   TermIO.WriteString( 'response: [' );
   TermIO.WriteInt( NewSignature, 0 );
   TermIO.WriteString( '] ' );

   Count := HIGH( InputString );

   IF IOText.Read( MessageFile, Count, ADR( InputString ) ) THEN
      TermIO.WriteLine( InputString );
   ELSE
      (*
         If the "response" text is empty, then the command has been
         sucessfully processed!
       *)
      TermIO.WriteLine( 'done.' );
   END; (* IF *)

   LastSignature := NewSignature;

   IF ( NewSignature > Signature ) OR ( NewSignature = 0 ) THEN
      Signature := NewSignature + 1;
      Dummy     := WriteToCommandFile( 'acknowledged' );
   END; (* IF *)

END PollForCommands;


PROCEDURE Startup(): BOOLEAN;
VAR
   Temp  : IOFiles.FileNameType;
   Path  : IOFiles.FileNameType;
BEGIN

   IF CommandFile.IsOpen THEN
      Alert.String( 'Command file already open! ' );
      RETURN FALSE;
   END; (* IF *)

   Strings.Assign( Temp, 'E9Screen.exe' );
   Strings.Clear( Path );

   IF NOT
      FileSelect.Select(
         Temp,
         Path,
         '*.*',
         FileSelect.ExistingFile + FileSelect.ChangeDirectory,
         'Select any file in target E9Screen folder:' )
   THEN
      RETURN FALSE;
   END; (* IF *)
```

```
   IF NOT
      IOText.Open(
         CommandName,
         IOFiles.Create + IOFiles.Write + IOFiles.Shared,
         CommandFile )
   THEN
      WriteError( 'Opening command file' );
      RETURN FALSE;
   END; (* IF *)

   Signature := 1;

   RETURN TRUE;

END Startup;


PROCEDURE InitModule;
BEGIN
   CommandFile.IsOpen := FALSE;
   MessageFile.IsOpen := FALSE;
   Signature          := 1;
   LastSignature      := -1;
END InitModule;


PROCEDURE Shutdown;
VAR
   Dummy : BOOLEAN;
BEGIN
   Dummy := WriteToCommandFile( 'Terminate' );
   Dummy := IOText.Close( CommandFile );
   Dummy := IOText.Close( MessageFile );

   InitModule;
END Shutdown;


END UserCommands.
```

Appendix II: Technical Data

Digital I/O Connector

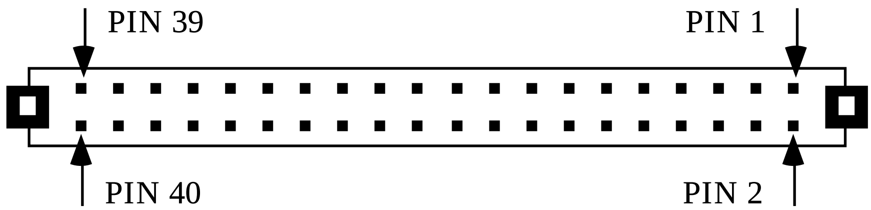
EPC9

The pin assignment of the 40-pin connector on the EPC9 is:

Pin number	EPC9	Pin number	EPC9
1	IN-0	2	OUT-14
3	Not connected	4	OUT-15
5	IN-2	6	IN-10
7	IN-3	8	IN-11
9	IN-4	10	IN-12
11	IN-5	12	IN-13
13	IN-6	14	IN-14
15	IN-7	16	IN-9
17	IN-1	18	CLIPPING
19	GND	20	GND
21	GND	22	GND
23	Not connected	24	STROBE-out
25	OUT-0	26	OUT-8
27	OUT-1	28	OUT-9
29	OUT-2	30	OUT-10
31	OUT-3	32	OUT-11
33	OUT-4	34	OUT-12
35	OUT-5	36	OUT-13
37	OUT-6	38	IN-8
39	OUT-7	40	Not connected

The digital IN and OUT lines carry TTL-compatible signals.

PIN 1 of the 40-pin connector is labled with a small arrow:

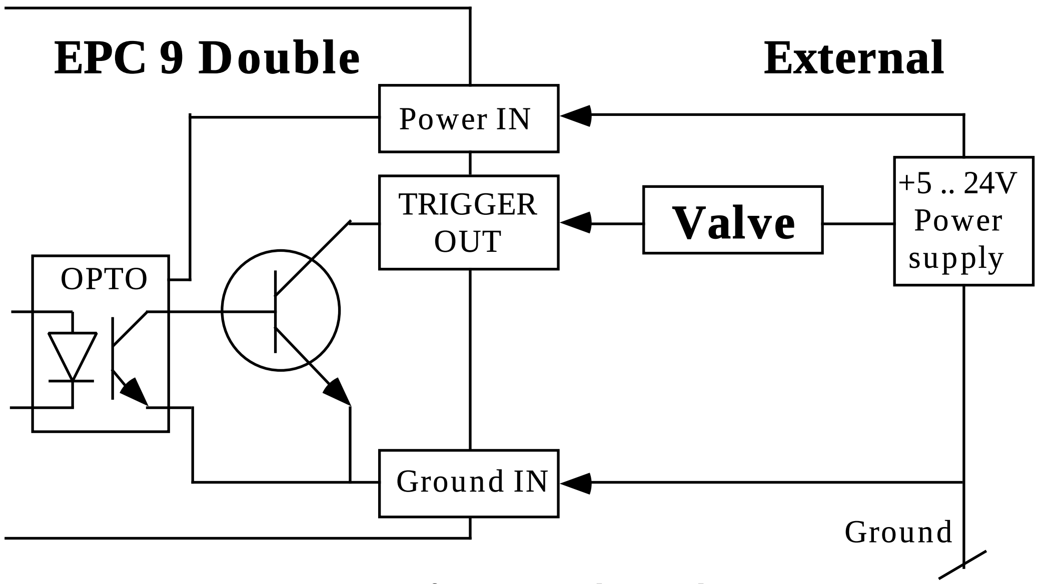


EPC9 Double and Triple

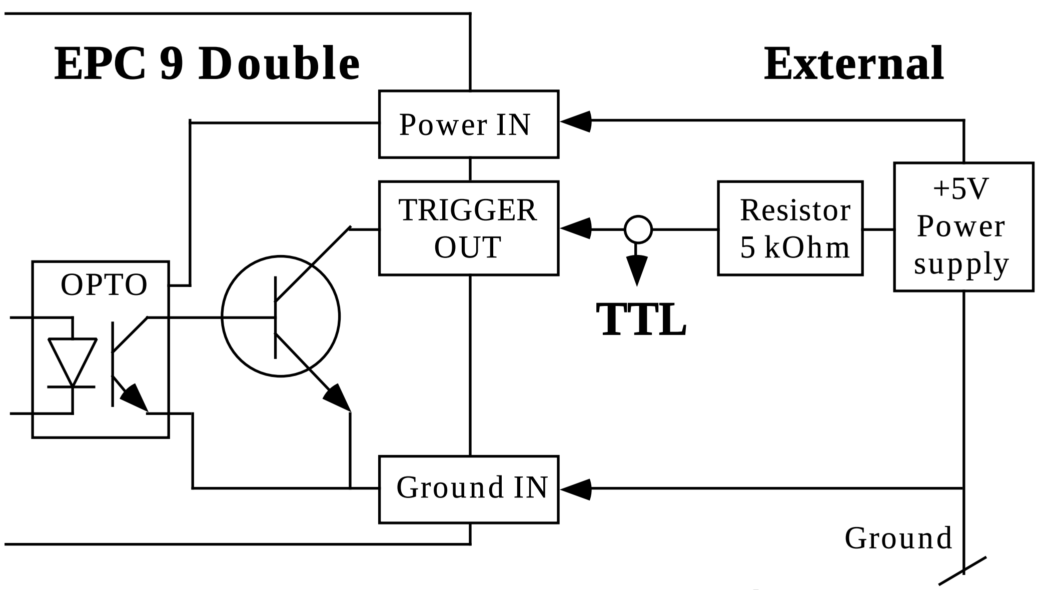
The pin assignment of the 40-pin connector on the EPC9 Double and Triple is:

Pin number	EPC9	Pin number	EPC9
1	TRIGGER-0	2	TRIGGER-1
3	TRIGGER-2	4	TRIGGER-3
5	TRIGGER-4	6	TRIGGER-5
7	TRIGGER-6	8	TRIGGER-7
9		10	
11		12	
13		14	
15		16	
17	Ground IN	18	Power IN
19	Ground IN	20	Power IN
21		22	
23		24	
25		26	
27		28	
29		30	
31		32	
33		34	
35		36	
37		38	
39		40	

The TRIGGER OUT 0 to 7 are opto-coupled open collector outputs (max. 1A). One can use these outputs to control external devices, e.g. solenoid driven valves. One can also generate TTL signals. Below it is described how to generate both types of connections. The current drivers can sink up to 1 A current. The maximal power supply voltage should not exceed 24V.



Arrangement for current driven devices



Arrangement to generate TTL signals

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