EPC 10 USB
Computer-controlled Patch Clamp Amplifier
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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).

1.1 Introducing the EPC 10 USB

The EPC 10 USB represents roughly the tenth in the series of patch clamp designs in use in the Göttingen laboratories. It is a logical successor to the classical EPC 7, retaining all of its features but adding a number of capabilities. Like the EPC 9, the EPC 10 USB implements full digital control of the various functions. Thus, the digitally controlled EPC 10 USB patch clamp amplifier has no knobs, switches or dials. The acquisition software (Patchmaster) replaces the analog controls of conventional amplifiers by using a computer and a built-in AD/DA converter. 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, our acquisition software contains a powerful data acquisition system (sampling and storage in pulse, ramp and continuous modes), a fully programmable Pulse Generator, a digital Oscilloscope and the Protocol Editor for automation processes. Thus, the EPC 10 USB offers all the features of a complete workstation for controlling experiments and acquiring data. Furthermore, there are several analysis software packages available, which allow data analysis, data export, and graphics output.

The EPC 10 USB also accepts a stimulus input and provides analogue current and voltage 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 EPC 10 USB 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 EPC 10 USB is noteworthy for three special features, the range-changing capability of the probe, the extremely wide bandwidth available from the current monitor circuitry, and the integrated transient cancellation (automatically if desired) and series-resistance compensation functions. In current clamp mode, the EPC 10 USB pre-amplifier acts as an “voltage-follower”, similar to classical microelectrode amplifiers, which guarantees very fast and accurate membrane potential recordings (Magistretti et al., 1996). Together, these features mean that a single probe 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 EPC 10 USB for experiments. It covers general information about the hardware, basic principles of the EPC 10 USB’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 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).

1.2 References

Original Articles


Book Chapters


1.3 Naming Conventions

EPC 10 USB Single, EPC 10 USB Double, EPC 10 USB Triple and EPC 10 USB Quadro: Throughout the present manual we will address all EPC 10 USB amplifier types as “EPC 10 USB”. We will explicitly mention the particular amplifiers, where it is required.

MS Windows versions

The EPC 10 USB is supported by Windows XP, Windows 7, Windows 8 and Windows 10. Throughout the present manual we will address all the above Windows versions as “MS Windows”. We will explicitly mention the particular MS Windows versions, whenever it is required. A free USB 2.0 port is required.

Macintosh

The EPC 10 USB USB is supported by Macintosh computers running Mac OS X 10.4 or newer. A free USB 2.0 port is required.
2. Description of the Hardware

The hardware components of the EPC 10 USB series patch clamp system consist of the headstage (or probe), the amplifier main unit with an integrated AD/DA converter board. The EPC 10 USB amplifier is connected to the computer via a USB 2.0 cable. Specific information about the hardware installation is given below in chapter 3 on page 11.

2.1 Headstage

The EPC 10 USB can be used with different headstages. The default headstage delivered with the EPC 10 USB is the Red Star headstage. Other exchangable headstages are available:

- S-Probe (see chapter 14 on page 69)
- 3 Electrode Headstage
- Amperometry Headstage

If you are interested in another headstage and their appropriate purpose please contact us (sales@heka.com).

2.2 Red Star Headstage

![EPC 10 USB Red Star Headstage](image)

Figure 2.1: EPC 10 USB Red Star Headstage

The probe of the EPC 10 USB 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 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).

*Important 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.

**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 on page 49.
2.3 Main Unit

The main unit of the EPC 10 USB contains the power supply, the signal processing electronics, the A/D and D/A 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-xxxxxx.epc" and "Cfast-xxxxxx.epc". Unlike conventional amplifiers, hardware calibration of the EPC 10 USB can also be performed by the user if necessary (see chapter 3.7 on page 14).

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

2.3.1 Front-panel

Power Switch: In order to be initialized properly, the EPC 10 USB must be switched on before starting the software program that drives it, e.g., Patchmaster. 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 ~ 30 min before starting an experiment. This will ensure that the amplifier has warmed up to regular working temperature and calibration parameters are most accurate.

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

D/A Outputs: The built-in interface (LIH 8+8) provides four D/A output channels (DA-0 - DA-3). Regarding the different versions of the EPC 10 USB the following D/A output channels are freely available:

- EPC 10 USB USB Single: DA-0, DA-1, DA-2
- EPC 10 USB USB Double: DA-0, DA-1
- EPC 10 USB USB Triple: DA-0
- EPC 10 USB USB Quadro: none

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

A/D Inputs: The built-in interface (LIH 8+8) provides eight A/D input channels (AD-0 - AD-7). Regarding the different versions of the EPC 10 USB the following A/D output channels are freely available:

http://www.heka.com
2.3 Main Unit

- EPC 10 USB USB Single: AD-0, AD-1, AD-2, AD-3, AD-4
- EPC 10 USB USB Double: AD-0, AD-1, AD-2
- EPC 10 USB USB Triple: AD-0
- EPC 10 USB USB Quadro: none

For example, the PatchMaster program can use these channels to monitor temperature, pressure or outputs from other sensors.

**Trigger Input:** Input for an external trigger to start data acquisition when the amplifier is waiting for an external trigger. This mode is set in PatchMaster when either Trigger Series or Trigger Sweeps is selected in the Pulse Generator of PatchMaster.

**Trigger Output:** Three TTL trigger outputs for synchronization of your patch clamp experiments with other devices.

**Probe:** This input accepts the multi-pin connector of the probe.

**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 on page 49).

**External Stim. Input CC:** Signals from an external stimulus source are applied here; they can be summed with the internal stimulus if desired.

**External Stim. Input VC:** 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 rise times (time from 10% to 90% of the amplitude of a step change) are available in the software: 2 $\mu$s, which is the minimum required to avoid nonlinearities in the internal circuitry, and 20 $\mu$s, 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 $\Omega$. 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 Filter 1 is fed to a data recorder (e.g., tape recorder, PCM/VCR combination, DAT recorder, amplification unit) to record the signal at wide bandwidth, while the additionally-filtered signal from Filter 2 is applied to an oscilloscope for monitoring the progress of the experiment. Either signal may be viewed on the software Oscilloscope window.

**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 the host 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 EPC 10 USB amplifier.

*Note:* Prior to the initialization of the amplifier the status of the Clipping and the Digital Bus LEDs is irrelevant.
2.3.2 Rear-panel

![EPC 10 USB Rear Panel](Image)

**Power**: Connect the enclosed power cord to this connector. It is not necessary to switch between different voltage ranges manually. The built-in power supply detects and covers the two major voltage ranges of 90-120 V and 210-250 V at 50-60 Hz.

**Digital Out / Digital In**: TTL-level, digital input and output lines are available here for the control and monitoring of digital signals (see chapter 12.2 on page 62).

**Digital I/O**: This connector can be used to connect an EPC 8 patch clamp amplifier or a TIB 14 trigger interface box to the EPC 10 USB amplifier.

**Trigger**: This port does not exist any more. Use Trigger In on the front panel of the EPC 10 USB.

**USB**: This port is the connection to the USB 2.0 port in the host computer, that allows the computer to communicate with the EPC 10 USB.

**Slave Sync / Master Sync**: 2 CAT5 connectors for synchronization of a second amplifier (EPC 10 USB only) or interface (LIH 8+8 only) system.

**AUX DAC / Telegraphing Inputs**: These ports are for inspection purposes only.

**Sound**: A 3.5 mm jack for connecting phones or speakers. The volume and resistance/frequency ratio can be adjusted by the PATCHMASTER software.

2.4 EPC 10 USB DOUBLE, TRIPLE, and QUADRO

The EPC 10 USB Double, Triple, and Quadro contain two, three and four independent EPC 10 USB amplifiers in one case, respectively. All amplifiers share a single AD/DA board. The AD/DA 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.

The number of freely available DA and AD channels for the different EPC 10 USB patch clamp amplifiers are listed in the table below.

<table>
<thead>
<tr>
<th>Amplifier</th>
<th>DA channel</th>
<th>AD channel</th>
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<tbody>
<tr>
<td>EPC 10 USB SINGLE</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>EPC 10 USB DOUBLE</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>EPC 10 USB TRIPLE</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EPC 10 USB QUADRO</td>
<td>-</td>
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Table 2.1: Number of free DA/AD channels

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.

2.5 Special Features of the EPC 10 USB

2.5.1 Timing of the AD-/DA-channels

Double Staggered Acquisition (EPC 10 USB using the LIH 8+8): The EPC 10 USB uses the double staggered acquisition mode. To support cophasic acquisition of the two most relevant signals, e.g. the current and voltage trace of the amplifier, two AD converters are used in parallel. That design results in supporting cophasic acquisition for 2 AD channels. Also, there is no time delay between two DA channels firing and the time of acquisition of two cophasic AD channels. In the design of the LIH 8+8, time delays of 5 microseconds per pair of AD channels will occur, when acquiring more than 2 AD channels.
3. Installation

There are several possible installation procedures:

1. Software only, e.g. Patchmaster, Potmaster, Chartmaster or Fitmaster
2. EPC 10 USB and software
3. rTEV 90 USB and software
4. PG 310 USB, PG 340 USB or PG 390 USB and software
5. LIH 8+8 and software

3.1 Installing Software only

Installing software typically requires two steps:

1. Installing the protection key (“dongle”, see chapter 3.5 on the following page)
2. Installing the required software (see chapter 3.6 on page 13)

3.2 Installing Hardware and Software

Installing the hardware and the software requires four steps:

1. Making the physical connection between the hardware and the computer (see chapter 3.4 on the following page)
2. Installing the protection key (“dongle”, see chapter 3.5 on the next page)
3. Installing the required software (see chapter 3.6 on page 13)
4. Calibrate the hardware

Our software provides the controls and the graphical representation of the amplifier/potentiostat by a “virtual panel" with “buttons". Signal display is provided by an oscilloscope-like display. The software provides the capability of data acquisition and analysis, but can also be used for calibration and self-testing the hardware.

3.3 Conventions

Software: All software packages of HEKA use a common installation procedure. Therefore, throughout the present manual, we will address the program to be installed as SOFTWARE, as a place holder for the program you want to install.

Hardware: Throughout the present manual we will address all amplifiers/potentiostats types as HARDWARE. We will explicitly mention the particular amplifiers/potentiostats, where it is required.

MS Windows versions: The SOFTWARE is supported on Windows 7 (32/64-bit), Windows 8 (32/64-bit) and Windows 10 (32/64-bit). Throughout the present manual we will address all the above Windows versions as “MS Windows”. We will explicitly mention the particular MS Windows versions, whenever it is required.

MacOS versions: The SOFTWARE is supported on MacOS 10.6 or more recent. Throughout the present manual we will address all the above MacOS versions as “MacOS”. We will explicitly mention the particular MacOS versions, whenever it is required.
3.4 Connecting the Hardware

1. The Hardware can be installed into a standard nineteen-inch instrument rack or used as a desktop unit. If installing on a rack, please do not use the Hardware as a shelf to support any other instrument. The Hardware case was not designed to do this and damage to the front panel will result. To minimize noise, it is advisable to mount the Hardware away from devices that emit high-frequency signals (i.e monitors, power supplies, etc).

2. Connect the power cord to the Hardware. The internal power supply used in the Hardware is an auto-switching multi-voltage supply that will operate from 90 Volts to 250 Volts. Make sure that the Hardware power cord is plugged into a properly grounded AC receptacle. Improper grounding of the Hardware could result in an electrical shock hazard. It is advisable to plug all equipment into a common outlet strip. This will minimize power line induced noise in the system.

   Note: Please note that the status display of the Clipping and the Digital Bus LED is irrelevant until the acquisition software has initialized the Hardware.

3. Install the USB cable from the USB connector on the rear panel of the Hardware, labeled “USB”, to an available USB 2.0 Hi-Speed port on the computer.

   Important note: This connection should be made directly to the computers USB 2.0 port and not to an USB HUB.

4. As soon as the Hardware is detected by the host operating system the appropriate system files will be initialized and the Hardware will be ready for use.

   Note: The host operating system treats the Hardware USB device as it would any Flash memory device. Therefore, only standard operating system files are required. This provides ease of installation and flexibility for moving the Hardware from one computer system to another.

5. It is possible to “Join” two units (only for EPC 10 USB and LIH 8+8) to extend the number of analog channels controlled by one instance of the Patchmaster software. For proper synchronization of the acquisition clocks, a connection between the Master clock output of one unit to the Slave clock input of the other (connectors located on the rear panel) must be made using standard CAT5 patch cables.

   Important note: Please note that the sync clock is a high-frequency signal. The shortest possible length patch cable should be used.

   Note: For “Joining” two units you need to use the Patchmaster software from HEKA.

The Hardware is now connected and ready to go.

   Note: There will be no calibration files included on a floppy disk or a USB stick. The calibration files will be stored by default on the mainboard of the device. During the calibration process the old calibrations files will be substituted by the new ones (see chapter 3.7 on page 14).

3.5 Protection Key ('Dongle') Installation

To be able to use our Software a protection key (“dongle”) is required. We provide dongles for the USB port which can be used on MS Windows and MacOS operating systems.

Please download the latest dongle driver software from our website. There you will always find the latest version of all drivers and software packages.

3.5.1 USB Dongle Installation

   Important note: Please install the USB dongle drivers before connecting the USB dongle. After successfully installing the driver, the USB dongle can be connected.

After the successful installation of the dongle drivers the red LED of the USB dongle should light up continuously.

http://www.heka.com
3.6 Software Installation

3.5.1.1 MS Windows
1. Unzip and execute the downloaded file (HASPUserSetup.exe)
2. Follow the installation instructions

3.5.1.2 MacOS
1. Unzip and open the downloaded file (Sentinel_Runtime.dmg)
2. Execute Install Sentinel Runtime Environment
3. Follow the installation instructions

3.6 Software Installation

Now, you can install and run the SOFTWARE. Please pay attention to the supported OS and the according Release Notes. If you have questions or doubts regarding the hardware/software compatibility please refer to the description of the product or contact the HEKA support hotline.

Please download the latest software version from our website. There you will always find the latest version of all drivers and software packages.

3.6.1 MS Windows

Before installing the SOFTWARE on computers running MS Windows 7 you should make the following precautions:

1. Log in as an administrator
2. Lower the threshold of the "User Account Control Settings" (Control Panel - User Accounts - Change User Account Control Settings – > Never Notify)
3. Reboot the system
4. Unzip and execute the downloaded file (e.g. Software_Installer.exe)
5. Follow the installation instructions
6. Execute the SOFTWARE once
7. Undo the settings made in the "User Account Control Settings".

If you do not use a MS Windows 7 OS you only have to perform the following steps:

1. Unzip and execute the downloaded file (e.g. Software_Installer.exe)
2. Follow the installation instructions

3.6.2 MacOS

1. Unzip and open the downloaded file (Software_Installer.dmg)
2. Execute Install_Software
3. Follow the installation instructions

Important note: SOFTWARE version 2x73.5 is the last version supporting processor based on the PowerPC architecture!

http://www.heka.com
3.7 Calibrating the Hardware

It is advisable to re-calibrate the Hardware once or 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 probe and cannot be used for another amplifier/potentiostat. Therefore, you have to re-calibrate the amplifier/potentiostat, when you replace the probe! This is a big advantage of our Hardware, since you can use any probe with any amplifier/potentiostat and replace a broken probe without having to send the amplifier/potentiostat in for re-calibration.

Before starting the Calibration make sure that the Hardware has reached its operational temperature, since the Calibration depends on temperature. We advice to let the Hardware warm up for 30-60 minutes after powering it on. The Calibration procedure can be performed with any Software. In the program, go to the Hardware menu (e.g. EPC 10 USB or PG 340 USB) → Test and Calibrate → Calibrate.

The following Calibration procedure is exemplary for the EPC 10:

The Software will warn you that this procedure may take up to 10 minutes, depending on the speed of your computer. 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 Hardware 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 black GND pin jack should be free) and that no BNC cables are connected to the inputs and outputs of the Hardware!

At the end of the Calibration, the Software will let you know, whether the Calibration succeeded or failed. If it succeeded, the program will automatically generate a new calibration (scale) file (SCALE_xxxxx.EPC) and the C-fast Lookup Table (CFAST_xxxxx.EPC). The files will be stored by default on the mainboard of the Hardware (EEPROM).

**Note:** To store these file on your hard drive of your PC please go to the Hardware menu → Test and Calibrate and change the default setting from Scale from/to EEPROM to Scale from/to Disk.

**Note:** It is not advisable to change the name of the files, because in that case you would have to manually load the files during every initialization. It is very advisable to store the files only in the one place.

http://www.heka.com
Finally, the software re-initializes the amplifier.

3.8 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 specifying:

- Your postal address and e-mail address
- The program name: e.g. Patchmaster, Fitmaster
- The program version number: e.g. v2x65, v2x73.1
- Your operating system and its version: e.g. Mac OS 10.6, Windows 7 64-bit
- Your type of computer: Intel Core 2 Duo, Intel Core i5 2.5 GHz, Mac G4
- Your acquisition hardware, if applicable: EPC 10 USB, LIH 8+8
- Your amplifier, if applicable: EPC 10 USB, PG 310 USB
- The series number and version of your Hardware, if applicable: e.g. EPC 10 USB Single, serial number ‘520552’, revision letter ‘R’.
- The questions, problems, or suggestions you have
- Under which conditions and how often the problem occurs
- Screenshots demonstrating the problem

We will address the problem as soon as possible.

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4. Verifying and Testing the EPC 10 USB

4.1 Testing the EPC 10 USB with the Model Circuit

The following tutorial will guide you through most of the basic and some of the unique and more sophisticated features of the EPC 10 USB amplifier. At the same time it allows you to check, whether the amplifier is functioning properly. You can use the model circuit you got together with the amplifier as a substitute for a real patch clamp recording and explore the virtual "front panel" of the EPC 10 USB supplied in the acquisition software (Patchmaster).

4.1.1 The Model Circuit

The model circuit connects to the probe input via a BNC adapter and the plug goes to the black GND connector on the probe:

The model circuit provides a switch with three positions simulating the following conditions typically observed during an electrophysiological experiment:

- 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.
- The middle position simulates a pipette attached to the cell membrane after the GΩ seal formation. In this setting only a capacitance of 6 pF is left over, corresponding to the "fast" capacitance of a pipette sealed to the cell membrane. This mode allows you to test the C-fast compensation.
- In the bottom position a "model cell" in the whole cell patch clamp configuration is simulated. The "input resistance" is 5.1 MΩ, the "membrane resistance" is 500 MΩ and the "membrane capacitance" is ~ 22 pF. This mode allows testing the C-slow compensation and the current clamp mode. Furthermore it is useful to check stimulation patterns you design within the acquisition software.

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

The following tutorial can be best executed with Patchmaster.

4.1.2 "SETUP" - 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 of the probe. If Patchmaster is not running yet, start the program which is located in the Patchmaster folder inside the HEKA folder. MS Window users might alternatively use the Start button to launch Patchmaster from Programs → HEKA. Press the SPACE key to bring up the so called "virtual front panel" (Amplifier Window). It provides a graphical representation of the EPC 10 USB amplifier. The panel lets

![Figure 4.1: Model Circuit](image-url)
you control all hardware settings of the amplifier(s) such as Gain or Filters. Signal display is provided by the Oscilloscope window.

Figure 4.2: EPC 10 USB Amplifier and Oscilloscope dialog

Put the model circuit into the "10 M" setting, which simulates a 10 MΩ-pipette that is open to the bath solution. Reset the amplifier (1) and apply a test pulse of 5 mV amplitude (2) and 5 ms duration (3). The current response will be displayed on the digital Oscilloscope. If your Gain range is appropriate, i.e. 5 mV/pA (4) you should see a rectangular current of about 0.5 nA in response to the test pulse. This represents the ohmic resistor you are recording from:

\[ I = \frac{U}{R} = \frac{5 \text{ mV}}{10 \text{ MΩ}} = 0.5 \text{ nA} \]

Patchmaster will online calculate the pipette resistance and update it in the R-memb field (5) where you should read a value close to 10 MΩ.

A possible voltage offset can be automatically canceled by clicking on the Auto-\(V_0\) button (6). After doing so, the \(V_0\) control (7) displays the offset potential. The baseline of the current response (8), the voltage monitor (\(V_{-\text{mon}}\)) (9) and the current monitor (\(I_{-\text{mon}}\)) (10) should be close to zero. You could also do the offset potential cancellation in a more classical way by clicking into the \(V_0\) control (7) and dragging the mouse up and down until the first segment in the Oscilloscope and the \(I_{-\text{mon}}\) display (10) match zero.

In Patchmaster similar steps as listed above can be automatically executed by clicking on the SETUP button or pressing the 1 key on the numerical keypad (Amplifier Window has to be active). This will execute the following built-in protocol (see Protocol Editor window) that resets the amplifier, sets the Gain of the amplifier, creates a rectangular Test Pulse, and then performs an automatic compensation of the voltage offsets:

http://www.heka.com
4.1 Testing the EPC 10 USB with the Model Circuit

1: SETUP
   E Reset ;
   E Mode 3 ; Whole Cell
   E PulseAmp 5.0mV ;
   E PulseDur 5.0ms ;
   E CSlow 50.00pF ;
   E RSeries 20.0MOhm ;
   E Gain 10 ; 5.0 mV/pA -> medium range
   E AutoZero ;
   E PulseOn TRUE ; Switch on test pulse
   E SaveRpip ;
   E Beep ;

**Note:** Patchmaster 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 Patchmaster to set the gain pop up in the EPC 10 USB window to the 10th value (5 mV/pA). A list of all so-called Macro Items can be output into the Notebook via the Help menu of Patchmaster.

The predefined protocols are stored in a text file called *.pro and can be edited with any text editor. For this tutorial it is not necessary to know all possible commands and their syntax. Therefore, please, refer to the Patchmaster manual for a detailed description on how to record and modify protocols in the Protocol Editor.

4.1.3 ”SEAL” - 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 GΩ seal and the C-fast controls can be used to cancel the capacitive spikes resulting from the stimulus Test Pulse.

![Figure 4.3: Before C-fast compensation](http://www.heka.com)
In order to see the small currents resulting from the high resistance of the model circuit, set the Gain to an appropriate value by either using the Gain pop up menu (1) or by hitting 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 (deflection in the black current trace) 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 maladjustment of τ-fast. Continue adjusting C-fast and τ-fast unless you see an almost flat line in the Oscilloscope window. 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 C-fast section of the amplifier control panel for an automatic compensation of C-fast and τ-fast. If the compensation fails, the background of the E-field in the *Auto* button becomes red. If this happens, you should repeat the auto-compensation, until it succeeds and the E-field becomes normal again.

![Figure 4.4: After C-fast compensation](http://www.heka.com)
4.1 Testing the EPC 10 USB with the Model Circuit

The steps listed above can be automatically executed by clicking on the SEAL button or pressing the key “2” on the numerical keypad (Amplifier Window has to be active). This will execute the following predefined protocol lines that increases the gain and then performs twice an auto-compensation - considering a possible failure in the first attempt.

2 : SEAL
E Mode 3 ; Whole Cell
E Gain 12 ; 20 mV/pA
E AutoCFast ;
E AutoCFast ;
E Beep ;

4.1.4 ”Whole-Cell” - Voltage Clamp Recording

After compensating C-fast well, you can now switch into the ”0.5 GΩ” 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).

Figure 4.5: Before C-slow compensation

After reducing the gain to 20 mV/pA (1), the R-memb field should reflect the changed ”membrane” resistance and display a value close to 500 MΩ (2). You should see in the Oscilloscope three capacitive transients (black line) caused by the 22 pF capacitor in the model circuit. The ”slower” time constant of the model cell - compared to the ”fast” time constant from the middle position - is

\[ \tau = R_s \cdot C_m = 5.1 \, M\Omega \cdot 22 \, pF = 112 \, \mu s \]
The peak current can be calculated from

\[ I_{\text{max}} = C_m \times U/\tau = 22 \text{ pF} \times 5 \text{ mV}/112 \text{ µs} = 982 \text{ pA} \]

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 Patchmaster (3).

Activate the C-slow compensation by selecting the 100 pF range from the Range field (4). Now start the compensation by increasing the C-slow (5) and the R-series (6) values either separately or by using the using the drag button (7) for both parameters (drag the mouse in all four directions). With some exercise 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 (black line). Instead of compensating C-slow "by hand" you can also press the Auto button (8) in the C-slow section of the amplifier control panel for an automatic compensation of C-slow and R-series. If the compensation fails, the E-field in the Auto button becomes red. 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.

---

![Figure 4.6: After C-slow compensation](http://www.heka.com)

**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 (9), and gradually increase the percentage of compensation from 0 to 95% by clicking and dragging the mouse upwards (10). 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 chapter 7.1 on page 43.

The steps listed above can be automatically executed by clicking the Whole-Cell button or pressing the key ”3” on the numerical keypad (Amplifier Window has to be active). This will execute the following protocol (see Protocol Editor) that sets the right Gain and does a C-slow compensation with reasonable values:

```
3 : WHOLE-CELL
E Mode 3 ; Whole Cell
E Gain: 11 ; 10 mV/pA
E CSlow: 50.00pF ;
E RSeries: 20.OMOhm ;
E AutoCSlow:
E AutoRSeries:
E Beep ;
```

4.1.5 ”Whole-Cell” - Current Clamp Recording

If C-slow has been compensated so far, switch into the current clamp mode by selecting C-Clamp from the Recording Mode pop up. This automatically selects the voltage monitor (Vmon) as the active channel displayed in the Oscilloscope. Note, that the unit of the Test Pulse amplitude changes from ”mV” to ”pA” as soon as you switch from voltage clamp (VC) into current clamp (CC) mode. Patchmaster uses two different amplitudes for VC and CC modes, therefore the Test Pulse is set to ”0 pA” initially. Now you need to inject current into the circuitry, 100 pA should be a reasonable value. The current injection will charge the ”membrane” of the ”model cell” at a time constant

\[
\tau = R_m \sum C_m = 500 \times 22 \times 10^{-12} \times 10^{-6} = 11 \text{ ms}
\]

to a final value of

\[
V_{\text{max}} = R_m \sum I = 500 \times 0.01 \times 100 \times 10^{-12} = 50 \text{ mV}
\]

Due to the slower time constant compared with voltage clamp conditions it takes much longer to reach Vmax, therefore you should increase the duration of the Test Pulse to a more appropriate value of 100 ms.

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

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

**Note:** The 500 MΩ setting of the model circuit is not a good method for testing the fast clamp speed of the EPC 10 USB 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.
4.1.6 Measuring the Noise of the Amplifier

Now let us come to the final section of the tutorial and check the intrinsic noise of the amplifier. Patchmaster has a built in feature that allows you to easily and quickly check the noise of your amplifier and to minimize your setup’s noise, e.g., by optimizing the grounding of the setup. First, remove anything from the probe and shield its input with the metallic cap. It is recommended to click the Reset button in the Amplifier window in order to reset the EPC 10 USB to its initial default configuration. Now click the Noise button (1) to start the noise test. In the Noise test mode no stimulation will occur. Instead, PATCHMASTER will calculate the noise of the current monitor 2 (Imon2) and display it in (2). Select the highest feedback resistor of the pre-amplifier, which has the lowest intrinsic noise by switching into a gain of 50 mV/pA or higher (3).

**Note:** The three different Gain ranges of the EPC 10 USB are separated by lines in the Gain pop up 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 (4) and Filter 2 (5). 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 (5) and nothing attached to the probe you should read a noise value below 110 fA (2).

![Figure 4.7: Noise measurement](http://www.heka.com)

**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 (6) and the Gain (3) of the PATCHMASTER Oscilloscope to a high value 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.
4.2 Making a Full Test

If you ever encounter any hardware problems that can not be solved by simply re-calibrating the amplifier (see chapter 3.7 on page 14) you can run the Full Test in PATCHMASTER. 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 and money!

Before starting the Full Test you should make sure to have nearby a shield for the probe (you can use the metallic cap that came with your amplifier and put it on the BNC connector at the probe to shield it), a 10 MΩ resistor (e.g., the model circuit) and 5 short BNC cables. In case you have an EPC 10 USB Double or Triple, select the amplifier you want to test in the EPC 10 USB amplifier dialog: 1. Amplifier, 2. Amplifier or 3. Amplifier.

Now select the menu item Full Test from the EPC 10 USB menu. You will be told to remove everything from the probe and shield its input. Please make sure that really nothing except the metallic cap is connected to the probe and that especially the black pin jack is free. You should also make sure that no BNC cables are connected to the main unit of the amplifier.

After a short while you will be prompted to connect 3-5 BNC cables, depending on the amplifier you are testing. If the connection test fails, you will get an error message and the chance to repeat the test an additional time. After the connection test you will have to remove all BNC cables and connect a 10 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, money and effort!
5. The control software

Our acquisition software PATCHMASTER provides the control and the graphical representation of the EPC 10 USB amplifier by a "virtual panel" with "buttons". Signal display is provided by an oscilloscope-like display. In addition, in PATCHMASTER there is a Notebook window and drop-down menus with options for the calibration procedures.

5.1 EPC 10 USB Window

5.1.1 Main Controls

The EPC 10 USB window (Amplifier window) is very similar in all our acquisition programs. It provides the amplifier control functions, such as Gain and Filter settings and it gives access to the automatic compensation routines of the EPC 10 USB.

![EPC 10 USB Amplifier dialog](image)

Figure 5.1: EPC 10 USB Amplifier dialog

If an EPC 10 USB Double, Triple or Quadro is used, one selects the "active" amplifier from the "amplifier" pop up list. Only the available amplifiers can be selected in the amplifier list. When switching, PATCHMASTER 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.
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 by pressing the up- and down-keys on the keyboard. The Gain setting automatically selects one of the three available current-measuring feedback resistors in the probe (5 MΩ, 500 MΩ, and 50 GΩ), corresponding to low, medium and high Gain ranges. The table below summarizes the main features and limitations of the Gain ranges:

<table>
<thead>
<tr>
<th>Feedback Resistor</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gain</td>
<td>0.005-0.2 mV/pA</td>
<td>0.5-20 mV/pA</td>
<td>50-2000 mV/pA</td>
</tr>
<tr>
<td>I_max VC</td>
<td>± 2 µA</td>
<td>± 20 nA</td>
<td>± 200 pA</td>
</tr>
<tr>
<td>I_max CC (out)</td>
<td>± 1 (5*) µA</td>
<td>± 10 (50*) nA</td>
<td>-</td>
</tr>
<tr>
<td>C-slow Ranges</td>
<td>30/100/1000 pF</td>
<td>30/100/1000 pF</td>
<td>30/100 pF</td>
</tr>
<tr>
<td>Current Clamp</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>R_s compensation</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>

Note: * When using the Extended Stimulus Range.

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 Rs-compensation can be used for Rs values down to 10 Ω in this range.

In the medium gain range, the background noise is larger than in the high Gain range, but the full 100 kHz bandwidth is available. 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.

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.

Clipping Indicator: A blinking box labeled "Clip" in the Gain title indicates saturation of amplifiers in the current monitor circuitry. Like the Clipping LED on the EPC 10 USB 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 EPC 10 USB. It is not; it just latches the clipping status longer than the LED light.

V-membrane: The V-membrane control can be used to set a holding potential in voltage clamp mode or a holding current in current clamp mode. The software corrects for liquid junction potentials and offsets (provided the zero-
current potential has been set correctly). The "V-membrane" label is converted to "I-membrane" in current clamp mode.

**I-mon:** Displays the actual measured pipette current.

![Image](http://www.heka.com)

**V-mon:** Displays the actual 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.

**R-memb:** 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 (see below).

**Input ADC:** The Oscilloscope can display the following signals (EPC 10 USB Single):

- Imon1: Current monitor 1 (after Filter 1)
- Imon2: Current monitor 2 (after Filter 2)
- Vmon: Voltage monitor output
- AD 0...4: Any of the other AD channels.

**Recording Mode:** Sets the Recording Mode. For details on the various recording modes see chapter 6 on page 39.

**Test Pulse:** Test Pulses are added to the holding potential and applied to the pipette; the current responses are sampled and displayed. Two built-in Test Pulse types are available: single or double pulse. Additionally any user-defined pulse pattern can be used as a Test Pulse. Test Pulses are applied at maximal rates depending on the duration specified.

**Amplitude / Length:** Duration and amplitude of built-in Test Pulses can be specified in the dialog. The minimum pulse duration is 0.1 ms with 1000 points sampled per pulse.
Noise: The Noise button can be used to measure the internal noise of the amplifier (with shielded probe input) or the noise of the environment (with open probe input). When the Noise mode is selected, the rms noise is continuously measured and updated. For the determination of the noise level there are no pulse outputs and the 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. The noise level depends on the Gain range and on the current filter setting. Reasonable Noise values are given in chapter 4 on page 17.

Liquid junction (LJ): 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 V₀ operation. An online correction requires an Auto V₀ 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 on page 43 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.

Note: LJ is not changed by the RESET function, and cannot be set by protocols. This restriction is imposed to avoid unintentional offset corrections.

![LJ 0.0mV V₀ 0.0mV (Auto)Track](http://www.heka.com)

Figure 5.8: Liquid Junction

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 V₀ 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.

V₀ (Pipette Offset): V₀ 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 V₀ operation or by manually dragging the mouse after clicking into the item. Furthermore, V₀ is changed automatically by the controlling program whenever the user changes the variable LJ. This is necessary for LJ and the Auto V₀ operation to interact properly.

Note: It is not recommended that the user changes V₀ manually, because this interferes with the software features for automatic offset correction.

Auto V₀: The Auto V₀ button calls a procedure for automatic zeroing of the pipette current. Thereby, an offset voltage (V₀) to the pipette potential is systematically varied until pipette current is zero. Range of V₀ is ±200 mV. Auto V₀ is typically performed before seal formation. It works properly only when a pipette is inserted into the bath. The Auto V₀ procedure interacts with the variable LJ to provide for online correction of liquid junction potentials and other offsets (see chapter 7 on page 43). 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 V₀ does this automatically and leaves V-membrane at that value.

Note: V₀ is not changed by the Reset function.

Track: This option implements a search mode (see chapter 6 on page 39), which is essentially a repetitive Auto V₀ procedure at a holding potential of 0 mV. The rate at which Auto V₀ is performed is determined by the Search Mode Delay in the EPC 10 USB menu.

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.

http://www.heka.com
In the upper box, the total C-fast value is displayed. $\tau$-fast determines the time constant of C-fast (up to 8 $\mu$s). The value of $\tau$-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.

![C-fast Compensation](http://www.heka.com)

**Auto C-fast:** Selection of this button performs an automatic compensation of C-fast and $\tau$-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: $Rs$ 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). 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 (default setting) is to use a lookup table for setting C-fast (EPC 10 menu → C-fast Mode → Use Lookup Table). Here, the lookup table created during the last calibration will be used. The slower method is Computed (same menu) where an iterative compensation is performed.

**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 $\mu$s 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.

**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).

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 Gain is set to value higher than 20 mV/pA, while the 1000 pF range is selected, the capacitance cancellation range will automatically be reduced to the highest possible setting (100 pF). Similarly, since the current clamp mode is not possible in the high Gain range (50-2000 mV/pA), the Gain will be reduced appropriately when selecting C-Clamp mode from high Gain range.

The program will prevent oscillations by limiting compensation according to the selected range and the effective capacitance values ($R$-series * C-slow >5 $\mu$s). 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 (Windows -> Configuration -> Display -> Show Cap. Track) 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 Compensation](http://www.heka.com)
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 Rs-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 the EPC 10 USB
menu entry Auto C-slow Settings), averages the resulting currents and fits an exponential to deduce the compen-
sation values required to cancel the current (see chapter 7 on page 43). 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 compensa-
tion 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 (red) to indicate the error. Continuous Auto-compensation can be
performed by selecting the Cap. Track option (Windows -> Configuration -> Display -> Show Cap. Track).

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.

Rs-Comp: The series resistance compensation corrects for membrane voltage errors under conditions of high
access resistance between pipette and cell interior (see chapter 7 on page 43). 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 Rs 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 on page 43. Fast Rs-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, Rs Comp will act as a bridge compensation.

Filter 1: Controls an analog 6-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 Rs-compensation is in use; it is of little use otherwise.

**Filter 2:** Controls an analog 4-pole filter for the current monitor 2 (Imon2) and the voltage monitor (Vmon). *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). Five filter characteristics can be selected from the pop up menu:

- I, Bessel
- I, Butterworth
- Bypass (with amplifiers of revision 'N' or later)
- V, Bessel
- V, 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 Imon2. If you want to use an external filter rather than *Filter 2*, you can proceed as follows: Connect current monitor 1 (Imon1) to the external filter input, feed the filter output back into one of the AD channels (AD 0...AD 4), and select this channel as input channel.

**Note:** *Filter 2* is disabled when Imon1 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 Imon2 first.

**Leak Comp.:** 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* setting. The *Leak Compensation* ranges are 2 nS, 200 nS and 20 µS for high, medium, and low *Gain* ranges, respectively.

![Leak Comp.](http://www.heka.com)

**Figure 5.13: Leak compensation**

**Auto:** Determines and corrects for the leak conductance automatically.

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

**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

![Stim](http://www.heka.com)

**Figure 5.14: External Stimulus**
Usually a setting of 20 $\mu$s is sufficient, unless very fast currents such as $Na^+$ currents are studied.

**External:** The external VC Stim.Input is scaled by an editable factor (range: ±0-1.0x), 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 "EXT STIM.INPUT VC" on the front panel of the EPC 10 USB. 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.

**2 Electrode Mode:** The default of the EPC 10 USB amplifier is the 2 Electrode Mode which is sufficient for most applications. But for some applications a third electrode (3 Electrode Mode) might be reasonable (see Patchmaster manual for further information).

![Figure 5.15: "Other" controls](image)

**Zap:** A high voltage pulse is applied to the pipette in order to rupture the patch membrane. The parameters of the Zap pulse (duration and amplitude) can be specified in the Configuration window of the acquisition software. There it can also be specified whether Zap is always enabled or whether it is restricted to the On Cell recording mode.

**Sound:** If this control is "On", a sound is played with its frequency coding for $R_{membrane}$.

**Reset:** Selecting this button will reset the EPC 10 USB 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.

### 5.1.2 Hidden Controls

Some rarely used controls are hidden. They can be accessed either by increasing the size of the Amplifier dialog to the right or by activating the "Show All" tab:

**LFVC $V_{memb}$:** The low frequency voltage clamp mode is a modified current clamp mode, which allows for the measurement of potential deflections, such as action potentials or synaptic potentials, while the average potential is kept constant at a value chosen by the user (LFVC $V_{memb}$). The circuit thus works like a current clamp for fast signals and like a voltage clamp for low frequency signals. To achieve this, the measured membrane potential is low-pass filtered and compared to the LFVC $V_{memb}$ potential. Then a current is injected into the cell to keep the membrane potential at the chosen LFVC potential. Since the cell does not distinguish currents entering through the pipette from currents crossing the membrane, the low frequency voltage clamp circuit can be considered an additional membrane conductance. Various time constants for the low frequency voltage clamp can be selected (1, 3, 10, 30, 100). These values describe the speed of regulation: '1' is the fastest regulation, '100' is 100 x slower.

![Figure 5.16: Low Frequency Voltage Clamp](image)

*Note: This feature is supported only by our Patchmaster software.*

**$R_{memb} \rightarrow R_{pip}$:** Copies the value of $R_{memb}$ into $R_{pip}$. With this function the pipette resistance is stored with the data before forming a seal.

![Figure 5.17: Gentle Switch](image)

http://www.heka.com
CC-Gain: Four current clamp scaling ranges can be selected:

- 0.1 pA/mV
- 1 pA/mV
- 10 pA/mV
- 100 pA/mV

These correspond to maximum command currents of 1 nA, 10 nA, 100 nA and 1 µA, respectively. This scaling has to be taken into account, if one intends to use the "External Stim.Input CC" for stimulation. When the CC-scaling is set to 1 pA/mV, a 100 mV command, feed into "External Stim.Input CC", would result in 100 pA command current.

**Note:** The maximum command currents can be increased by a factor of "5" when activating the Extended Stimulus Range in the EPC 10 USB menu.

**Note:** The Gain settings are restricted to the selected range, i.e. with a CC scaling of 1.0 pA/mV, only the intermediate Gain range (0.5-20 mV/pA) can be used in current clamp mode.

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

V-mon Gain: The measured voltage can be amplified by a factor of 10 or 100 before digitization. In the Vmon*100 setting the voltage range is 100 mV instead of 1 V, therefore the resolution is 10x better. This is particularly useful in field potential measurements, where small voltage deflections are to be resolved.

**Note:** This feature is available only with EPC 10 USB revision F (or newer) amplifiers.

Last V-memb: 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-memb can then be used to conveniently restore the original V-membrane value.
**Overlay:** The Test Pulse traces will be overlaid in the Oscilloscope window.

**List State:** Sends the actual amplifier state information to the Notebook window.

**One Pulse:** Executes one Test Pulse. This is useful when Test Pulses are off and simply one Test Pulse is to be output.

**I-scale/V-scale:** I-scale and V-scale can be used to determine the display scaling for the Test Pulse. The value of 1 (no display gain) corresponds to full scale (±10.24 V) of the built-in AD/DA converter. Thus, without display gain, one can easily see when the input signal saturates the AD converter. If, however, amplification is needed you should enable the setting Scale Test Pulse in the Configuration window.

![I-scale V-scale table](image)

> Figure 5.22: Test Pulse scaling

**Sound:** 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.

![Sound volume](image)

> Figure 5.23: Sound Feature
5.2 EPC 10 USB Menu

5.2.1 Test and Calibrate Menu

Calibrate: Performs a full calibration of the EPC 10 USB. The result of this calibration may be stored as a SCALE-xxxxxx.epc and CFAST-xxxxxx.epc files. The SCALE-xxxxxx.epc 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 EPC 10 USB. 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. The CFAST-xxxxxx.epc file contains the C-fast Lookup Table. Depending on the speed of the computer, the calibration process takes 5-10 minutes.

Note: Calibrations should be performed after the amplifier and probe have reached normal operation temperature (i.e., powered up for at least 30-60 minutes)

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 EPC 10 USB. If you have any doubts about your EPC 10 USB 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 choose 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 R-membrane value with a calibration resistor.

External Zero Calibration: The same as for External Gain Calibration but an offset correction factor for the three Gain ranges.

Scale from/to EEPROM: If selected the calibration file (scale file) is loaded from the EEPROM of the amplifier. The calibration procedure also writes the scale file to the EEPROM. This option only exists for newer EPC 10 USB amplifiers (since revision R for USB amplifiers and revision S for PCI amplifiers).

Scale from/to Disk: If selected the calibration file (scale file) is saved/loaded to/from a file stored on the hard-disk of the host computer. The calibration procedure also writes the scale file to a file on disk.

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

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

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6. Operating Modes

The EPC 10 USB 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 EPC 10 USB correspond mainly to the different ways of controlling that potential.

6.1 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-DA, 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 on page 43).

6.2 Current Clamp Mode

The current clamp mode can be used to measure the resting potential or spontaneous action potentials in a whole cell recording. The measured membrane potential will be shown on the V-mon display in the acquisition software. The same signal is available at the Voltage Monitor output at the front panel of the EPC 10 USB. For stimulation, a commanded current can be injected while the pipette potential is measured. The commanded current is determined by the sum of the voltages from the "External Stim.Input CC" and the I-membrane control in the software.

In current clamp mode, the input of the probe acts as an high-impedance voltage follower circuit. The feedback resistor is used for stimulation in current clamp mode. This can be done by applying a defined voltage to the feedback resistor.
Four current clamp scaling ranges can be selected: 0.1, 1, 10 and 100 pA/mV. These correspond to maximum command currents of 1 nA, 10 nA, 100 nA and 1 µA, respectively. This scaling has to be taken into account, if one intends to use the “External Stim.Input CC” for stimulation. When the CC-scaling is set to 1 pA/mV a 100 mV command, feed into “External Stim.Input CC”, would result in 100 pA command current.

**Note:** The Gain settings are restricted to the selected range, i.e. with a CC scaling of 1.0 pA/mV, only the intermediate Gain range (0.5-20 mV/pA) can be used 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 protocol to, for example, set a holding current value immediately after switching to current clamp. The EPC 10 USB allows the functions of C-fast and Rs Comp to be on when entering current-clamp mode. C-fast then becomes a “negative capacitance” adjustment and Rs Comp becomes a “bridge” adjustment.

**Note:** Although the new current clamp circuit is very stable, you should be aware, that a huge maladjustment of C-fast may cause the feedback loop to oscillate.

In the EPC 10 USB, the current clamp circuitry has been improved to better follow rapid changes in membrane potential, such as in neuronal action potentials (APs). Conventional patch clamp amplifiers, equipped with current-to-voltage converter circuits, are not able to follow rapid events, such as APs. (see Magistrett et al. (1996) TINS 19: 530-534). The current clamp circuit of the EPC 10 USB acts as a voltage-follower, thereby increasing not only speed but also stability of the circuit. This circuit causes the effective input resistance of the EPC 10 USB probe to be greater than $10^{11}$ Ω.

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; C-fast is then automatically adjusted to neutralize all but the amplifier input capacitance when you switch to current clamp mode.

### 6.3 Low Frequency Voltage Clamp Mode

The low frequency voltage clamp mode is a modified current clamp mode that allows for the measurement of potential deflections, such as action potentials or synaptic potentials, while the average potential is kept constant at a value chosen by the user ($LFVC \ V_{mem}$). The circuit thus works like a current clamp for fast signals and like a voltage clamp for low frequency signals. To achieve this, the measured membrane potential is low-pass filtered and compared to the $LFVC \ V_{mem}$ potential. Then a current is injected into the cell to keep the membrane potential at the chosen LFVC potential. Since the cell does not distinguish currents entering through the pipette from currents crossing the membrane, the low frequency voltage clamp circuit can be considered an additional membrane conductance. Various time constants for the low frequency voltage clamp can be selected (1, 3, 10, 30, 100). These time constants describe the speed of regulation. The effective feedback speed for the five possible response settings depends on the Gain Range. In the medium Gain Range, 1-100 is approximately the time in seconds, whereas in the high Gain Range it is a hundred times faster. Note that the LFVC value of 100 means slow compensation, whereas the LFVC value of 1 means fast compensation!

### 6.4 Search Mode

In the EPC 10 USB, this mode has no special analog circuitry. Rather, it is a software emulation of the traditional search mode found in the EPC 7 amplifier. The EPC 7 accomplished the search mode by analog circuitry in order to ease the adjustment of the zero-current potential $V_0$. The EPC 10 USB does not require special analog circuitry, as the software provides for an easy way of doing this. The Search mode of the EPC 10 USB simply forces V-hold to 0 mV and invokes the Auto $V_0$ procedure (at the rate specified by Search Mode Delay in the EPC 10 USB menu.

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of Patchmaster), 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

7.1 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 (Rs) 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 \( R_s = 5 \, \text{M}\Omega \) and \( C_m = 20 \, \text{pF} \), the time constant is 100 \( \mu \text{s} \). This time constant is excessively long for studying rapid, voltage-activated currents such as \( \text{Na}^+ \) 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 \( R_s = 5 \, \text{M}\Omega \), 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 \( \sim 2 \, \text{mV} \) 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 EPC 10 USB incorporates additional circuitry to allow capacitance transient cancellation to occur while Rs-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 canceled by the transient cancellation, their effect must be predicted by the cancellation circuitry: hence the prediction pathway.

![Series Resistance compensation circuit](image-url)
Together, the two parts of the EPC 10 USB Rs-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

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

where $\tau_u$ is the uncompensated time constant. Similarly, the voltage errors due to membrane currents are also reduced by the factor $(1 - \alpha)$. The fractional compensation $\alpha$ is determined by the setting of the %-comp control on the EPC 10 USB 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 EPC 10 USB, the estimation of series resistance has been combined with the transient cancellation, in that the Rs 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 Rs-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 1 V. 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 $\Delta V$ is commanded, the pipette potential actually receives an initial transient of size $\Delta V/(1 - \alpha)$ due to the compensation effect. The technical problem comes from the fact that the maximum pipette potential excursion in the EPC 10 USB is about $\pm 1.2$ 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 Rs-compensation is also limited by stability considerations. Stable Rs-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, maladjustment of C-fast can easily cause oscillation. In cases where Rs is this size or larger, it is often advisable to use the slower settings of the Rs switch which, in slowing down the speed of the compensation feedback, makes it less susceptible to high-frequency oscillations. In cases where Rs 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 $\mu$s setting of the switch. This problem arises when the time constant $\tau$ is smaller than about 100 $\mu$s, and comes from the fact that compensated membrane time constant $\tau_c$ cannot be made smaller than a value that depends on the speed of the Rs-compensation feedback. If you turn up the %-comp control to try to obtain a smaller $\tau_c$, you will observe overshoot or ringing in the current monitor signal, due to an overshoot in the membrane potential. The minimum value for $\tau_c$ is given approximately by

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

where $\tau_f$ is the effective time constant of the feedback loop. The corresponding maximum $\alpha$ values are given by

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

<table>
<thead>
<tr>
<th>$\tau_u$ ($\mu$s)</th>
<th>$\alpha$</th>
<th>$\tau_c$ ($\mu$s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>0.85</td>
<td>13</td>
</tr>
<tr>
<td>50</td>
<td>0.90</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>0.75</td>
<td>8</td>
</tr>
<tr>
<td>22</td>
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<tr>
<td>13</td>
<td>0.90</td>
<td>5</td>
</tr>
<tr>
<td>8</td>
<td>0.50</td>
<td>4</td>
</tr>
</tbody>
</table>

The table gives maximum $\alpha$ values (i.e., %-comp settings) and the resulting $\tau_c$ values in the 2 $\mu$s setting for some values of the uncompensated time constant $\tau_u$. At the 10 $\mu$s setting, full 90% compensation may be used without

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overshoot for time constants \( \tau_u \) greater than about 1 ms; the 100 \( \mu s \) setting is appropriate for \( \tau_u \) values on the order of 10 ms or longer. In practice, you can estimate \( \tau_u \) from the ratio of the settings of \( C_{\text{slow}} \) and \( R_{\text{series}} \). For example, if \( C_{\text{slow}} \) is 10 \( \text{pF} \) and \( R_s \) is 10 \( M\Omega \), the time constant is 10 \( \text{pF} \times 10 \ M\Omega = 100 \ \mu s \). The use of the \( R_s \)-compensation circuitry can be summarized as follows: When you set the capacitance transient cancellation (\( C_{\text{slow}}, R_{\text{series}}, \tau_{\text{fast}} \)) 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 \( R_{\text{series}} \) and turn up the \%-comp control to the desired value. Any maladjustment of the transient cancellation will be apparent and can be compensated. The EPC 10 USB makes the procedure very easy: \( C_{\text{slow}} \) and \( R_{\text{series}} \) values can be obtained automatically by clicking on Auto \( C_{\text{slow}} \). Beyond that, the Pulse Generator of PATCHMASTER provides an option where Auto \( C_{\text{slow}} \) is performed before each command pulse, achieving an accurate update of \( R_{\text{series}} \). The procedure will be described in more detail later (see chapter 10.3.3 on page 57).

### 7.2 Capacitance Compensation

The EPC 10 USB 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 Auto \( C_{\text{slow}} \) Settings in the EPC 10 menu). 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 \( C_{\text{fast}} \) compensation (pipette and stray capacitance) is concerned mainly with the very initial portion of the transient (the first 10 – 30 \( \mu s \)), whereas the procedure for \( C_{\text{slow}} \) 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 \( C_{\text{fast}}, \tau_{\text{fast}}, C_{\text{slow}} \) and \( R_{\text{series}} \) in advance (they may be saved in the protocol for whole-cell recording, for example). This will provide the fitting routine with reasonable starting values. Alternatively, the Auto procedures can be performed alternately to arrive at settings for which the remaining transient is minimal. Settings for \( C_{\text{fast}} \) are left at their respective values whenever an Auto \( C_{\text{slow}} \) is performed and vice versa. This way, a combined fit can be obtained by alternately executing \( C_{\text{slow}} \) and Auto \( C_{\text{fast}} \). It is better, however, to perform Auto \( C_{\text{fast}} \) in the cell-attached recording configuration and not to touch it any more when proceeding to whole cell.

During Auto \( C_{\text{fast}} \) and Auto \( C_{\text{slow}} \), certain settings are changed temporarily and restored upon completion. These are:

- Rs-compensation is switched "Off"
- The Gain is set to 0.2, 10 or 50 \( \text{mV/pA} \), depending on the Gain range in use
- External stimuli are disabled
- Filter 1 is set to 10 or 30 \( \text{kHz} \), 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 \( C_{\text{slow}} \) 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 (\( C_{\text{slow}} \) and \( R_{\text{series}} \)) 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 \( C_{\text{slow}} \) 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 \( C_{\text{slow}} \) procedure is designed to provide unbiased estimates of the actual membrane capacitance and series resistance (Sigworth, Neher & Affolter, 1995).
7.3 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 Patchmaster 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.
- An 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 Patchmaster.

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 mobility for calculation of liquid junction potentials can be found in Barry & Lynch (1991). The table below lists the values for some typical solutions.

<table>
<thead>
<tr>
<th>Solution</th>
<th>LJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>145 K-glutamate, 8 NaCl, 1 MgCl2, 0.5 ATP, 10 NaOH-HEPES</td>
<td>10 mV</td>
</tr>
<tr>
<td>145 KCl, 8 NaCl, 1 MgCl2, 0.5 ATP, 10 NaOH-HEPES</td>
<td>3 mV</td>
</tr>
<tr>
<td>60 Cs-citrate, 10 CsCl, 8 NaCl, 1 MgCl2, 0.5 ATP, 20 CsOH-HEPES</td>
<td>12 mV</td>
</tr>
<tr>
<td>32 NaCl, 108 Tris-Cl, 2.8 KCl, 2 MgCl2, 1 CaCl2, 10 NaOH-HEPES</td>
<td>-3 mV</td>
</tr>
<tr>
<td>70 Na2SO4, 70 sorbitol, 2.8 KCl, 2 MgCl2, 1 CaCl2, 10 NaOH-HEPES</td>
<td>6 mV</td>
</tr>
</tbody>
</table>

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).

http://www.heka.com
Note: When applying the above rules for calculating the correction \( L_I \), 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 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, \( L_I \) 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. \( L_I \) 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 \( L_I = 3 + (-6) = -3 \) 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, \( L_I \) 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)
2. the resting potential of the cell. We assume the latter to be -60 mV and therefore set \( L_I \) 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

8.1 Mounting the Probe

For low noise recording, the pipette holder must be attached directly to the EPC 10 USB 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 EPC 10 USB 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. Because of the extreme sensitivity of the EPC 10 USB, 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 on 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. Nearby objects should be grounded to the Signal GND of the EPC 10 USB.

*Important note:* Please remember, that the metal case of the probe must remain insulated from ground.

8.2 Ground Wires

It is a good idea to run a separate ground wire from the Signal GND 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. This pickup becomes visible only when the EPC 10 USB 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.

8.3 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.

8.4 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 tabletop 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.
8.5 Connections to other Instruments

Depending on the needs of the particular user, the EPC 10 USB 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 EPC 10 USB. You may connect e.g. additional filter units or amplification units to Filter 1 of the current monitor. Also, it might be 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.

8.6 Pipette Holder and Electrode

A properly grounded microscope can provide sufficient shielding from 50-60 Hz interference even without the use of a Faraday cage. We do not recommend to use a shielded pipette holder, since a shielded holder 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 capacitive into the amplifier input. Especially for single-channel recordings, the unshielded holder is strongly recommended. The difference in background noise level between the shielded and unshielded holder type 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 (see chapter 10.3.3 on page 57).

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-chlorinated occasionally, perhaps once per month. A wire for the standard electrode holder should be about 4.5 cm long. After it is chlorinated an O-ring is slipped onto it and the wire is inserted into the holder. Chlorination 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.

8.7 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, chlorinated silver wire makes a good bath electrode; however AgCl\(^+\)-ions are tolerated only by some types of cells, such as muscle cells. A good alternative is an electrode incorporating an agar salt bridge, as illustrated below.

![Figure 8.1: Bath electrode](http://www.heka.com)
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 chlorinated 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

9.1 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.

![Figure 9.1: Sources of glass pipettes](image)

<table>
<thead>
<tr>
<th>Soft Glass (Supplier)</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-heparinized heparinocrit tubing</td>
<td>1.3 mm</td>
</tr>
<tr>
<td>Drummond Micropipets Drummond Scientific, Bloomsil, PA, U.S.A.</td>
<td>1.4 mm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hard Glass (Supplier)</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kimax 51 Kimble Products, Vineland, N.J., U.S.A.</td>
<td>1.7 mm</td>
</tr>
<tr>
<td>Borosilicate 100 Rochester Scientific, Rochester, NY, U.S.A.</td>
<td>1.7 mm</td>
</tr>
<tr>
<td>Corning Sealing Glass #7052; #7040</td>
<td>Dow Corning, Midland, MI, U.S.A.</td>
</tr>
<tr>
<td>GCASS 158.4 (aluminum-glass) A.M. Systems, Everett, WA, U.S.A.</td>
<td>1.5 mm</td>
</tr>
</tbody>
</table>

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).

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.

9.2 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.

*Note: HEKA is offering a gravitational force two-stage vertical puller, the PIP 6.*
9.3 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.

9.4 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.

9.5 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.

10.1 Forming a Seal

10.1.1 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 SETUP mode. The approach to the cell membrane and the formation of a giga-seal cause the resistance to increase, thereby reducing the currents. A convenient pulse amplitude is 5 mV, which will cause 1 nA to flow in a 5 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 SETUP mode: The measure mode is set to Whole Cell (voltage clamp) and V-Membrane = 0; Filter 1 is set to 10 kHz and Filter 2 to 2.9 kHz, Rs Comp to "Off", C-slow Range to "Off". 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.

10.1.2 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 EPC 10 USB window as R-memb). 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.

10.1.3 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.

10.2 Cell-Attached Recording

You can now use the SEAL button, 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 SEAL). 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 100 kHz full 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 EPC 10 USB are about to saturate, and/or that the current monitor output voltage is going above 10 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 Patchmaster program compensates 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.

10.3 Whole-Cell Recording

10.3.1 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 Rs-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.

10.3.2 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. Canceling 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 button (if included in the protocol 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.

http://www.heka.com
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.

10.3.3 Series Resistance Compensation

Series resistance (Rs) compensation is important when the membrane capacitance is large or when the ionic currents are large enough to introduce voltage errors. To use Rs-compensation, you first adjust the transient-cancellation controls (including $C_{\text{fast}}$ and $\tau_{\text{fast}}$ if necessary) to provide the best cancellation. Then you select Rs Comp by turning up the $\%_{\text{comp}}$ control to provide the desired degree of compensation.

**Note:** The "R-series" control determines (along with the $\%_{\text{comp}}$ 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 EPC 10 USB will think that Rs is larger than it is); this can cause oscillation and possible damage to the cell under observation.

How you should set the Rs-compensation controls depends on the approximate value of the uncompensated membrane-charging time constant $\tau_u$, which you can calculate as the product of the $C_{\text{slow}}$ and $R_{\text{series}}$ settings (for example, suppose $C_{\text{slow}}$ is 20 pF and $R_{\text{series}}$ is 10 MΩ: $\tau_u$ is then 20 pF $\times$ 10 MΩ = 200 $\mu$s). If $\tau_u$ is smaller than about 500 $\mu$s, you should use the 2 $\mu$s setting of the Rs-compensation switch to provide the necessary rapid compensation; however, the slower settings will provide compensation that is less prone to high-frequency oscillations from maladjustment of the controls. How much compensation you can apply is also determined by $\tau_u$. If $\tau_u$ is larger than about 100 $\mu$s, 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 $\tau_u$, the $\%_{\text{comp}}$ 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 Rs-compensation) are considerably longer than 16 $\mu$s, 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.

10.4 Current Clamp Recording

To switch from voltage clamp to current clamp recording, just select the C-Clamp mode in the amplifier dialog. This provides a recording of the cell membrane potential, which can be monitored at the V-mon output of the EPC 10 USB.

When you switch to the current clamp mode, the following things happen inside the EPC 10 USB main unit: $I_{\text{membrane}}$ 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, $C_{\text{Slow Range}}$ is set to "Off", and the value of $C_{\text{fast}}$ is reduced by 0.5 pF to avoid oscillations. Rs-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.

Once you have switched to current clamp, you can use $I_{\text{membrane}}$ to set a holding current, and you can apply stimulus pulses via "External Stim.Input CC". The scaling of the "External Stim.Input CC" can be set with the CC-Gain control of the EPC 10 USB amplifier dialog. With a CC-Gain setting of 0.1 pA/mV, the maximum current for stimulation is ±1 nA. With a setting of 1 pA/mV, 10 pA/mV or 100 pA/mV the maximum current is ±10 nA, ±100 nA or 1 $\mu$A, respectively. A CC-Gain of 0.1 and 1 pA/mV can only be used in the intermediate Gain range of the EPC 10 USB and a setting of 10 pA/mV is limited to the low Gain range.
11. Low Noise Recording

The EPC 10 USB 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, 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 8 on page 49.

The noise feature of the EPC 10 USB makes it easy for the user to identify important noise sources. When the Noise radio button is selected in the EPC 10 USB dialog (Amplifier window) of Patchmaster, the Noise display shows the rms noise current present in the current monitor signal in the frequency band selected by Filter 2 (2.9 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 70-100 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-DA, 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 EPC 10 USB’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.

<table>
<thead>
<tr>
<th>Noise Source</th>
<th>Contribution</th>
<th>rms Current</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplifier</td>
<td>35%</td>
<td>95 fA</td>
</tr>
<tr>
<td>Holder</td>
<td>21%</td>
<td>73 fA</td>
</tr>
<tr>
<td>Pipette + Patch</td>
<td>44%</td>
<td>105 fA</td>
</tr>
</tbody>
</table>

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 on page 49, an 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 reorientation 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.
12. Appendix I: Digital Inputs/Outputs

12.1 Digital I/O Connector

The digital IN and OUT lines of this connector carry TTL-compatible signals. The connector is intended to connect other HEKA devices, such as EPC 8 or TIB 14 to the EPC 10 USB amplifier. For other purposes the Digital In connector and the Digital Out connector should be used.

<table>
<thead>
<tr>
<th>PIN #</th>
<th>Signal</th>
<th>PIN #</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IN-0</td>
<td>2</td>
<td>OUT-14</td>
</tr>
<tr>
<td>3</td>
<td>NC</td>
<td>4</td>
<td>OUT-15</td>
</tr>
<tr>
<td>5</td>
<td>IN-2</td>
<td>6</td>
<td>IN-10</td>
</tr>
<tr>
<td>7</td>
<td>IN-3</td>
<td>8</td>
<td>IN-11</td>
</tr>
<tr>
<td>9</td>
<td>IN-4</td>
<td>10</td>
<td>IN-12</td>
</tr>
<tr>
<td>11</td>
<td>IN-5</td>
<td>12</td>
<td>IN-13</td>
</tr>
<tr>
<td>13</td>
<td>IN-6</td>
<td>14</td>
<td>IN-14</td>
</tr>
<tr>
<td>15</td>
<td>IN-7</td>
<td>16</td>
<td>IN-9</td>
</tr>
<tr>
<td>17</td>
<td>IN-1</td>
<td>18</td>
<td>NC</td>
</tr>
<tr>
<td>19</td>
<td>GND</td>
<td>20</td>
<td>GND</td>
</tr>
<tr>
<td>21</td>
<td>GND</td>
<td>22</td>
<td>GND</td>
</tr>
<tr>
<td>23</td>
<td>NC</td>
<td>24</td>
<td>STROBE</td>
</tr>
<tr>
<td>25</td>
<td>OUT-0</td>
<td>26</td>
<td>OUT-8</td>
</tr>
<tr>
<td>27</td>
<td>OUT-1</td>
<td>28</td>
<td>OUT-9</td>
</tr>
<tr>
<td>29</td>
<td>OUT-2</td>
<td>30</td>
<td>OUT-10</td>
</tr>
<tr>
<td>31</td>
<td>OUT-3</td>
<td>32</td>
<td>OUT-11</td>
</tr>
<tr>
<td>33</td>
<td>OUT-4</td>
<td>34</td>
<td>OUT-12</td>
</tr>
<tr>
<td>35</td>
<td>OUT-5</td>
<td>36</td>
<td>OUT-13</td>
</tr>
<tr>
<td>37</td>
<td>OUT-6</td>
<td>38</td>
<td>IN-8</td>
</tr>
<tr>
<td>39</td>
<td>OUT-7</td>
<td>40</td>
<td>NC</td>
</tr>
</tbody>
</table>

Table 12.1: PIN assignment of the Digital I/O Connector (EPC 10 USB). IN = Digital Input Line; OUT = Digital Output Line; GND = Ground Line; NC = Not Connected; STROBE = Internal line for communicating with HEKA devices

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

![Figure 12.1: Digital I/O connector (40-Pin, male) of the EPC 10 USB](image)
12.2 Digital In Connector

The *Digital In* connector at the rear panel can be used to read TTL trigger signals from external devices.

<table>
<thead>
<tr>
<th>PIN #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal</td>
<td>IN-3</td>
<td>IN-2</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>IN-1</td>
</tr>
</tbody>
</table>

Table 12.2: PIN assignment of the Digital IN Connector (EPC 10 USB), upper row. IN = Digital Input Line; GND = Ground Line

![Digital In Connector](http://www.heka.com)

12.3 Digital Out Connector

The *Digital Out* connector at the rear panel of the EPC 10 USB USB amplifiers can be used to trigger external devices, which require TTL inputs. The signals of pin 13, 12 and 2 (*OUT-0*, *OUT-1* and *OUT-2*, respectively) can also be accessed from the BNC trigger outputs at the front panel of the amplifier.

<table>
<thead>
<tr>
<th>PIN #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal</td>
<td>OUT-3</td>
<td>OUT-2</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>GND</td>
<td>OUT-1</td>
</tr>
</tbody>
</table>

Table 12.4: PIN assignment of the Digital IN Connector (EPC 10 USB), upper row. OUT = Digital Output Line; GND = Ground Line

![Digital Out Connector](http://www.heka.com)
12.4 Breakout Cable

On request there is an optional breakout cable (order number: 895214) available. Via this cable the first 8 digital outputs (Dig-Out 0 - Dig-Out 7) or digital inputs (Dig-In 0 - Dig-In 7) are accessible via BNC connectors.

![Figure 12.4: DSub-25, female, to 8x BNC breakout cable](image)

Please contact sales@heka.com to get further information on this cable.

12.5 Connecting Cables

12.5.1 DG 4/5 / Lambda 10-2 / Lambda 10-3 to EPC 10 USB

On request there is a connecting cable available to connect either the DG 4/5 wavelength switcher or the Lambda 10-2 / Lambda 10-3 optical filter changer (order number: 895102).

![Figure 12.5: DSub-25 (male) to DSub-25 (female) connecting cable](image)
### Table 12.6: Pin assignments of Digital Out Connector of EPC 10 USB and Parallel Port connector of DG 4/5.

<table>
<thead>
<tr>
<th>PIN #</th>
<th>Signal</th>
<th>PIN #</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>OUT-0</td>
<td>2</td>
<td>Command Bit 0 (Filter Position)</td>
</tr>
<tr>
<td>12</td>
<td>OUT-1</td>
<td>3</td>
<td>Command Bit 1 (Filter Position)</td>
</tr>
<tr>
<td>2</td>
<td>OUT-2</td>
<td>4</td>
<td>Command Bit 2 (Filter Position)</td>
</tr>
<tr>
<td>1</td>
<td>OUT-3</td>
<td>5</td>
<td>Command Bit 3 (Filter Position)</td>
</tr>
<tr>
<td>14</td>
<td>OUT-4</td>
<td>6</td>
<td>NC</td>
</tr>
<tr>
<td>15</td>
<td>OUT-5</td>
<td>7</td>
<td>NC</td>
</tr>
<tr>
<td>16</td>
<td>OUT-6</td>
<td>8</td>
<td>NC</td>
</tr>
<tr>
<td>17</td>
<td>OUT-7</td>
<td>14</td>
<td>NC</td>
</tr>
<tr>
<td>18</td>
<td>OUT-8</td>
<td>9</td>
<td>NC</td>
</tr>
<tr>
<td>19</td>
<td>OUT-9</td>
<td>16</td>
<td>NC</td>
</tr>
<tr>
<td>4,11</td>
<td>GND</td>
<td>24</td>
<td>GND</td>
</tr>
</tbody>
</table>

### Table 12.7: Pin assignments of Digital Out Connector of EPC 10 USB and Parallel Port connector of Lambda 10-2.

<table>
<thead>
<tr>
<th>PIN #</th>
<th>Signal</th>
<th>PIN #</th>
<th>Signal</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>OUT-0</td>
<td>2</td>
<td>Command Bit 0 (Filter Position)</td>
</tr>
<tr>
<td>12</td>
<td>OUT-1</td>
<td>3</td>
<td>Command Bit 1 (Filter Position)</td>
</tr>
<tr>
<td>2</td>
<td>OUT-2</td>
<td>4</td>
<td>Command Bit 2 (Filter Position)</td>
</tr>
<tr>
<td>1</td>
<td>OUT-3</td>
<td>5</td>
<td>Command Bit 3 (Filter Position)</td>
</tr>
<tr>
<td>14</td>
<td>OUT-4</td>
<td>6</td>
<td>Command Bit 4 (Speed)</td>
</tr>
<tr>
<td>15</td>
<td>OUT-5</td>
<td>7</td>
<td>Command Bit 5 (Speed)</td>
</tr>
<tr>
<td>16</td>
<td>OUT-6</td>
<td>8</td>
<td>Command Bit 6 (Speed)</td>
</tr>
<tr>
<td>17</td>
<td>OUT-7</td>
<td>14</td>
<td>Command Bit 7 (Shutter)</td>
</tr>
<tr>
<td>18</td>
<td>OUT-8</td>
<td>9</td>
<td>Command Bit 8 (Wheel Select Bit)</td>
</tr>
<tr>
<td>19</td>
<td>OUT-9</td>
<td>16</td>
<td>NC</td>
</tr>
<tr>
<td>4,11</td>
<td>GND</td>
<td>24</td>
<td>GND</td>
</tr>
</tbody>
</table>
### Table 12.8: Pin assignments of Digital Out Connector of EPC 10 USB and Parallel Port connector of Lambda 10-3. OUT = Digital Output Line; GND = Ground Line

<table>
<thead>
<tr>
<th>EPC 10 USB</th>
<th>Lambda 10-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIN #</td>
<td>Signal</td>
</tr>
<tr>
<td>13</td>
<td>OUT-0</td>
</tr>
<tr>
<td>12</td>
<td>OUT-1</td>
</tr>
<tr>
<td>2</td>
<td>OUT-2</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>14</td>
<td>OUT-4</td>
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<td>15</td>
<td>OUT-5</td>
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<td>16</td>
<td>OUT-6</td>
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<tr>
<td>17</td>
<td>OUT-7</td>
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<tr>
<td>18</td>
<td>OUT-8</td>
</tr>
<tr>
<td>19</td>
<td>OUT-9</td>
</tr>
<tr>
<td>4,11</td>
<td>GND</td>
</tr>
</tbody>
</table>

12.5.2 ValveLink 8 to EPC 10 USB

On request there is a connecting cable available to connect the ValveLink 8 controller (order number: 895113).

![Connecting Cable](http://www.heka.com)

Figure 12.6: DSub-25 (male) to DSub-9 (male) connecting cable
### Appendix I: Digital Inputs/Outputs

#### Table 12.9: Pin assignments of Digital Out Connector of EPC 10 USB and connector of ValveLink 8.

<table>
<thead>
<tr>
<th>EPC 10 USB</th>
<th>ValveLink 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIN #</td>
<td>Signal</td>
</tr>
<tr>
<td>13</td>
<td>OUT-0</td>
</tr>
<tr>
<td>12</td>
<td>OUT-1</td>
</tr>
<tr>
<td>2</td>
<td>OUT-2</td>
</tr>
<tr>
<td>1</td>
<td>OUT-3</td>
</tr>
<tr>
<td>14</td>
<td>OUT-4</td>
</tr>
<tr>
<td>15</td>
<td>OUT-5</td>
</tr>
<tr>
<td>16</td>
<td>OUT-6</td>
</tr>
<tr>
<td>17</td>
<td>OUT-7</td>
</tr>
<tr>
<td>18</td>
<td>OUT-8</td>
</tr>
<tr>
<td>19</td>
<td>OUT-9</td>
</tr>
<tr>
<td>4,11</td>
<td>GND</td>
</tr>
</tbody>
</table>

OUT = Digital Output Line; GND = Ground Line; NC = Not Connected

Please contact sales@heka.com to get further information on these cables.
13. Appendix II: Probe Adapters

The pre-amplifiers of the EPC 10 patch clamp amplifiers are available with two different mounting plates for mechanical connection of the pre-amplifier to e.g. various micromanipulator system:

13.1 Standard Mounting Plate

The probe is mounted on a 38 mm x 90 mm x 4 mm plate which has 4 wholes with 3 mm diameter. Since the plate is wider than the probe, there is room for custom mounting wholes on both sides of the probe.

![Figure 13.1: Adapterplate 1](image1)

13.2 Dovetail Mounting Plate

The dovetail adapter (order number: 895020) can be used to connect e.g. to Sutter MP 285 or HEKA MIM 4 micromanipulator. The dimensions of the dovetail match the probe housing.

![Figure 13.2: Adapterplate 2](image2)
14. Appendix III: S-Probe

The unique feature of the S-Probe is the massively reduced size and weight compared to our standard headstages. This increases the area of applications e.g. wherever the experimental space is limited or where the weight of the headstage itself matters. The cable is very flexible and therefore prevents cable stress.

The electrical specifications of the S-Probe are identical to our standard Red Star Headstage. In addition it has the feature of an optional bath sense connection which gives it the opportunity to operate in the 3 Electrode Mode.

The headstage has a thread connector to provide a safer electrical connection of the headstage and the pipette holder (see 15.2 on page 71).

**Important note:** The pipette holder may be tied with fingers only. Do not use tools to tighten it!

The headstage can be used either with an EPC 10 (revision 'O' or higher) or an EPC 10 USB (revision 'N' or higher) or the EPC 800 USB amplifier missing with 3 Electrode mode though.

For connecting the S-Probe to the model circuit (MC 10) of the EPC 10 USB an appropriate adapter is included.
15. Appendix IV: Pipette Holder

Due to the fact that the EPC 10 USB can be used either with the Red Star Headstage or the S-Probe different types and sizes of pipette holders are available.

*Important note:* More information about the different pipette holders and their accessories can be found on the HEKA website.

### 15.1 BNC-Type

The BNC-type connector does only fit to the Red Star Headstage.

![Figure 15.1: New BNC-type Pipette Holder for the Red Star Headstage](image)

In respect to different outer diameters of the glass electrodes the following pipette holders are available:

- Pipette Holder BNC-Type 1.0 mm (Order number: 895227)
- Pipette Holder BNC-Type 1.3 mm (Order number: 895228)
- Pipette Holder BNC-Type 1.5 mm (Order number: 895229)
- Pipette Holder BNC-Type 1.7 mm (Order number: 895230)
- Pipette Holder BNC-Type 2.0 mm (Order number: 895231)

Please contact sales@heka.com to get further information on this product.

### 15.2 SMA-Type

The SMA-type connector does only fit to the S-Probe (see chapter 14 on page 69). In respect to different outer diameters of the glass electrodes the following pipette holders are available:
- Pipette Holder SMA-Type 1.0 mm (Order number: 895148)
- Pipette Holder SMA-Type 1.3 mm (Order number: 895149)
- Pipette Holder SMA-Type 1.5 mm (Order number: 895150)
- Pipette Holder SMA-Type 1.7 mm (Order number: 895151)
- Pipette Holder SMA-Type 2.0 mm (Order number: 895152)

Please contact sales@heka.com to get further information on this product.
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