

# User Guide for Axoclamp™ 900A

Computer-controlled  
Microelectrode Amplifier



**Molecular Devices**

Molecular Devices Corporation  
Sunnyvale, California 94089

Part Number 2500-0179 Rev. A



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**DECLARATION OF CONFORMITY**

(According to ISO/IEC GUIDE 22 and EN 45014)



**Molecular Devices**

Manufacturer's Name: **MOLECULAR DEVICES CORPORATION**

Manufacturer's Address: **3280 Whipple Road  
Union City, CA 94587 USA**

***DECLARES, THAT THE PRODUCT***

Product Name: **Voltage/Current Clamp**

Model Number: **Axoclamp 900A**

***-CONFORMS TO THE FOLLOWING EUROPEAN DIRECTIVES***

***Low Voltage Directive 73 / 23 / EEC  
EMC Directive 89 / 336 / EEC  
With Amendment 93 / 68 / EEC - CE Marking Directive***

Supplementary Information:


Safety: ***EN 61010-1:2001 (TUV Rheinland)***

EMC: ***EN 61326-1 w/A1 1998 (Immunity)  
EN 61326-1 w/A1 1998 Class A (Emissions)***

Year Mark First Applied: **2006**

I, the undersigned, hereby declare that the equipment specified above conforms to the above Directives and Standards.

Place: **Sunnyvale, California USA.**

Signature: 

Date: **Feb. 10, 2006**

Full Name: **Richard Armenta**

Position: **Quality Director**



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## Chapter 1: Introduction

Axoclamp 900A is the newest cutting-edge instrument from Molecular Devices, the world leader in high-quality, low-noise microelectrode amplifiers. Like the Axoclamp-2B that preceded it, the Axoclamp 900A offers several modes of operation that measure signals from single cells, tissue slices, and whole animal preparations. The advanced signal conditioning added to the Axoclamp 900A amplifier saves the expense of buying additional hardware and frees up valuable space in your electrophysiology setup.

By making the Axoclamp 900A computer-controlled, it enables us to add several powerful new features that make it easier to set up and run experiments and that keep experiments running longer. This exciting new instrument offers you the flexibility you need for today's experiments, as well as for experiments you decide to do in the future.

This versatile amplifier offers several modes of operation, making it suitable for a wide range of biological preparations and for maximum flexibility in experimental design:

- Current-clamp (I-Clamp) mode, for measuring voltage responses, is available in two channels with independent Bridge Balance and I=0 options.

- Two-electrode voltage clamp (TEVC) mode uses two microelectrodes, one for continuous recording of electrode voltage and the other for injection of current. TEVC mode can be used with large frog oocytes or smaller mammalian cells. The high output compliance of this mode makes it possible to voltage clamp currents that are larger than currents clamped with single-electrode techniques.
- Discontinuous current clamp (DCC) mode can be used for more accurate voltage measurements, and is especially useful when small changes in electrode resistance occur during an experiment.
- Discontinuous single-electrode voltage clamp (dSEVC) mode is used to voltage clamp small cells that cannot tolerate being impaled by two electrodes. Set up correctly, this mode eliminates the large series resistance inherent in many preparations.
- High-voltage current clamp (HVIC) mode is used primarily for extracellular is applications.

Amplifier performance is better than ever. The  $\pm 180$  V output compliance used for TEVC and HVIC modes makes it possible to pass larger currents and ensures faster clamp speeds. TEVC and dSEVC modes both have wide AC voltage-clamp gain ranges for excellent voltage control. When DC Restore is enabled, the DC voltage-clamp gain is greater than 1,000,000, ensuring optimal voltage control for constant-voltage measurements. The new dSEVC design is more stable and twice as fast as in the Axoclamp-2B, providing an excellent alternative to standard continuous single-electrode voltage clamping.

The Axoclamp 900A is essentially an analog input/output instrument similar to conventional amplifiers by Molecular Devices. Thus, BNC-type input and output connections are necessary to communicate with a digitizing interface, oscilloscope or other recording device. The Axoclamp 900A contains no front panel knobs and switches. Instead, the instrument is operated using a control panel program, the Axoclamp 900A Commander, which runs on a host computer and communicates with the amplifier via a USB cable.



Computer control of this next generation amplifier means that the knobs, dials and buttons are no longer needed and are replaced by an intuitive software control interface. Computer control provides several advantages over conventional amplifiers and makes the Axoclamp 900A amplifier easier to use and enables experiments to last longer. Automation of several standard tasks becomes possible, such as adjustment of Pipette Offset, Bridge Balance, and Pipette Capacitance Neutralization. Oscillations in the measured signal can be detected and suppressed automatically, in less than 1 ms, based on user-selected settings. Instant switching between modes can be automated (I-Clamp to dSEVC or TEVC and back again), with users defining the threshold, timing, and trigger (internal or external) for mode switching. In DCC and dSEVC modes, the monitor signal used for tuning can be displayed on the computer screen as well as on an oscilloscope. Slow current injection can be used in I-Clamp mode to prevent small, slow drifts in the membrane voltage that could end experiments prematurely.

The Axoclamp 900A Commander interface is completely independent of acquisition software. Thus, the Axoclamp 900A can be used with any data acquisition package. It is compatible with Digidata series digitizers (1200A or later) and pCLAMP 7 (or later) software. However, telegraphing is only supported in pCLAMP versions 10 and higher. Regarding third-party software, see our webpage “Developer Info” for a detailed Software Development Kit that describes how to read telegraph information.

We recognize that software control of an amplifier is an unusual step forward for some users. If computer mouse control is unsettling, consider the optional SoftPanel device to control the Axoclamp 900A. The SoftPanel is essentially a hardware extension of the Axoclamp 900A Commander software. Knobs and buttons replace mouse or keyboard control. For more information, visit our web site or call Molecular Devices Technical Support.

The Axoclamp 900A is a sophisticated instrument. Experienced and inexperienced researchers alike are advised to read this manual thoroughly and to familiarize themselves with the instrument. The following two chapters provide step-by-step instructions using the CLAMP-1U and MCO-2U model cells.

Molecular Devices is pleased to answer any questions regarding the theory and use of the Axoclamp 900A. Any comments and suggestions on the use and design of the Axoclamp 900A will be much appreciated. We welcome reprints of papers describing work performed with the Axoclamp 900A. Keeping abreast of your research helps us to design our instruments for maximum usefulness.

## Chapter 2: Installation and Basic Operation

### Installation

Carefully unpack all parts, and use the enclosed shipping list to verify that all parts have been received. Retain packing materials in case the instrument needs to be returned to the factory at a later date.

For the initial checkout, the Axoclamp 900A should be situated on a bench top away from other equipment. Do not install it in a rack until the checkout is complete.

### Check List

These installation and checkout procedures require the following:

1. Axoclamp 900A main unit with power cord.
2. HS-9A headstage with CLAMP-1U model cell.
3. 2 HL-U electrode holders.
4. USB (A/B-type) control cable.
5. Axoclamp 900A Commander host software (from CD or web site).

6. A PC running Windows 2000 or XP Pro operating system. The PC should have up to three spare USB ports: two for the amplifier and one if the optional SoftPanel is used.
7. External oscilloscope.

## **Installing Hardware**

1. Connect the appropriate end of the USB cable to the CONTROL USB connector on the Axoclamp 900A rear panel, and the other end to a free USB port on your PC. This is the main connection between the amplifier and the computer.
2. Connect a second USB cable between the SCOPE USB connector on the Axoclamp 900A rear panel and another free USB port on your PC. This connection delivers the monitor signal from the amplifier to the computer for discontinuous mode operation.
3. Connect the HS-9A headstage(s) to the CHANNEL 1 HEADSTAGE and CHANNEL 2 HEADSTAGE rear panel connectors. The amplifier CAN be left on when changing headstages. HOWEVER, DO NOT DISCONNECT HEADSTAGES WHILE THE SOFTWARE IS STARTING UP.
4. Connect the power cable, and turn on the Axoclamp 900A. The front panel POWER light should illuminate and the status light should eventually go out. The Windows operating system automatically recognizes the new USB hardware as a Human Interface Device (HID).
5. If you are using the optional SoftPanel device, connect it to a different computer USB port using the USB cable supplied with the SoftPanel.

## **Installing the Axoclamp 900A Commander**

1. Run the Axoclamp 900A Commander installer from the enclosed CD, or from the installation file downloaded from the Molecular Devices

web site. This installs all necessary files and generates a shortcut for *Axoclamp 900A* on your desktop.

2. Run *Axoclamp 900A Commander* by double-clicking on the *Axoclamp 900A* desktop icon. The first time the program is run, you are asked to update *Axoclamp 900A Commander*. If you have just installed the software from the CD, we suggest that you download the latest update. Molecular Devices is very responsive to customer feedback, thus it is likely that the web site contains a newer, updated version. We also recommend that you choose to be reminded every 30 days to check for a new download.
3. Next you see the *Select Device* dialog. In the *Axoclamp* tab select *Axoclamp Hardware*, then click the *Scan* button. The amplifier serial number is identified in the list when the instrument is successfully recognized.
4. If the program is unable to find a valid serial number, check that the *Axoclamp 900A* is switched on and that the USB cable is connected properly.
5. If you are using the optional *SoftPanel* device, click on the *SoftPanel* tab and click the *Scan* button. After this device is recognized, click the *OK* button.
6. The main *Axoclamp 900A Commander* window appears. If installed correctly, the *Axoclamp* serial number appears in the *900A Commander* window heading. If the optional *SoftPanel* is configured correctly, the *Configure SoftPanel* icon is enabled.

## Input/Output Connections

### Front Panel

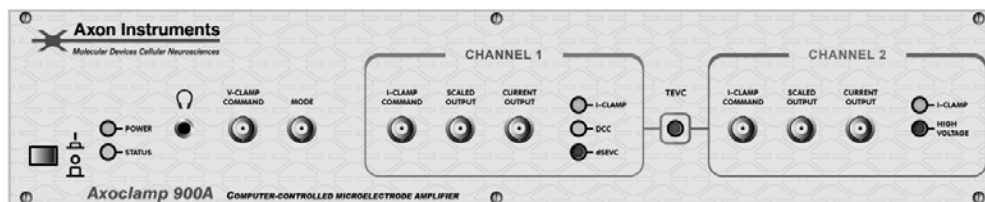


Figure 1. Axoclamp 900A Front Panel.

### Audio Output

The headphones socket drives headphones or a powered speaker to monitor the audio output of the Axoclamp 900A. The output is the same as that available at the rear panel headphones socket.

### Inputs

**V-CLAMP COMMAND:** Voltage commands to the Axoclamp 900A are accepted at this input. The External Command is enabled or disabled in the *Options / General* tab of Axoclamp 900A Commander.

**MODE:** This is enabled when the user has checked one of the *Switch to dSEVC/TEVC on ext input* checkboxes in the Headstage 1 or 2 section at the top of the Axoclamp 900A Commander. For example, a TTL Low (0–0.8 V) input at MODE could select one of the V-Clamp modes while a TTL High (2–5 V) input could select I-Clamp. These inputs can be a TTL Digital Signal controlled by pCLAMP. High and low behavior is user selectable in the *Options / Auto* tab of Axoclamp 900A Commander.

**I-CLAMP COMMAND:** Current commands to the Axoclamp 900A are accepted at this input. The External Command is enabled or disabled in the *General* tab under the *Options* toolbar button. The scaling depends on the headstage connected to each channel.

## Scaled Output

The output signal at this BNC is selected from the list in the *Scaled Output* section of the main window of the Axoclamp 900A Commander. The choices include:

- Membrane Current.
- Membrane Potential.
- Internal Command Potential (VC) or Internal Command Current (IC).
- External Command Potential (VC) or External Command Current (IC).
- Auxiliary Potential (if HS-9A headstage attached) or Auxiliary Current (if VG-9A headstage attached).

The same inputs and outputs are available for Channel 2.

## Current Output

The output signal at this BNC is the current output when in one of the V-Clamp modes. This output is not filtered and is scaled only by the headstage current injection resistor in the current-passing channel.

## Rear Panel

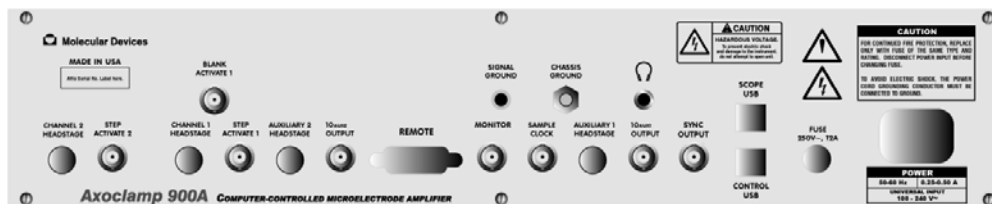


Figure 2. Axoclamp 900A Rear Panel.

CHANNEL 1 HEADSTAGE and CHANNEL 2 HEADSTAGE inputs: HS-9A headstages are plugged into these inputs. Note that CHANNEL 1 HEADSTAGE refers to CHANNEL 1 inputs/outputs on the front panel, and CHANNEL 2 HEADSTAGE refers to CHANNEL 2 inputs/outputs.

STEP ACTIVATE: Allows external command activation on either Channel 1 or Channel 2 independently.

BLANK ACTIVATE: Allows the Channel 1 voltage to be held at its previous value. Useful when response to external stimulus needs to be blanked.

AUXILIARY 1 HEADSTAGE and AUXILIARY 2 HEADSTAGE: The optional voltage-following (HS-9A) or bath clamp (VG-9A) headstage is plugged into these connectors. HS-2A and VG-2A headstages can also be plugged into these connectors with an appropriate optional adapter cable (1-2100-0934).

10<sub>AUX1</sub> OUTPUT and 10<sub>AUX2</sub> OUTPUT: These BNC outputs provide  $\times 10$  output signal for the respective AUXILIARY HEADSTAGE channels.

REMOTE: Most commonly used for connecting the remote BUZZ box. Can also be used for control of CLEAR ( $\pm$ ) and STEP ACTIVATE on either channel and for BLANK ACTIVATE on Channel 1.

MONITOR: The same signal as on the SCOPE USB. Used for tuning discontinuous modes: DCC and dSEVC.

SIGNAL GROUND: While the 1 mm gold ground socket on the headstage should be used for the bath ground, this 4 mm yellow socket can be connected to a central grounding bus and used to ground other sources of noise in your setup, such as the Faraday cage, perfusion system, etc.

CHASSIS GROUND: This provides an alternative chassis or power supply ground.

SAMPLE CLOCK: Used to trigger an external monitor scope if the SCOPE USB input to the computer is not used.



**AUDIO OUTPUT:** This can drive headphones or a powered speaker, like the front panel Audio Output.

**SYNC OUTPUT:** The signal available at this BNC connector is intended to be used as an external trigger for an oscilloscope when internal commands (Seal Test, Tuning, or Pulse) are activated, or to indicate the Mode state of the amplifier (commanded either externally by the MODE BNC or internally by the Auto Mode switch feature). The Sync Output sends out a 0 to (approximately) 5 V step aligned with the onset of the Seal Test, Tuning, or Pulse step. Or, when following automated Mode switching, 2–5 V (HIGH) corresponds to V-Clamp while 0–0.8 V (LOW) corresponds to I-Clamp. See the *Options / General* tab to select the function of the SYNC OUTPUT.

**CONTROL USB:** Connects to a USB port of the host computer to control the Axoclamp 900A.

**SCOPE USB:** Connects to a USB 2.0 port of the host computer to provide Scope window data. This is a self-contained single-channel 5 MHz acquisition system that allows the user to see critical switched membrane voltage signals during the switching period. It helps in the setup of neutralization and gain in discontinuous modes (DCC and dSEVC).

## Overview

The purpose of this section is to describe the basic controls of the Axoclamp 900A Commander. The following chapter *Setup and Tutorials* guides you step-by-step through the various recording configurations.

Axoclamp 900A Commander is the control platform for all Axoclamp 900A functions, from basic parameter settings to various “smart” automated features. Not being bound by the physical space restrictions imposed by amplifier panels, software control allows a greater range of control features to be offered. Software control also allows seamless integration with data acquisition programs, such as Molecular Devices’ pCLAMP 10.

Physical “knobs and buttons” control of the Axoclamp 900A is still possible with the SoftPanel, an optional hardware extension that provides an alternative interface to Axoclamp 900A Commander.

With Commander, the Axoclamp 900A can telegraph a broadened range of information to other applications, without the need for any cabling beyond the USB port connection to your computer.

For detailed information on each Axoclamp 900A Commander feature, please consult the *Commander Reference* in Chapter 5.

## **Axoclamp 900A Commander Interface Overview**

The Axoclamp 900A Commander front panel is designed to resemble hardware instrument controls. It is divided into five main sections:

- Toolbar
- Meters
- Mode buttons
- Mode tabs
- Scaled Output tabs

The Axoclamp 900A Commander panel can be shrunk by dragging any edge or corner. Scroll bars allow you to display the controls you are most interested in when you have done this. Use the *Resize Window* tool button to toggle between the full panel, Meters display, and the sizing you have set.

### **Toolbar**

The Toolbar contains all the program-specific functions in Axoclamp 900A Commander. Resting your mouse over a toolbar button when the Commander window is active displays a brief tool tip describing the

function of each of the buttons, as well as the keyboard shortcut for that function.

The *Options* tool button opens a dialog box where many basic Axoclamp 900A Commander parameters are set.

## Meters

The Meters at the top of the Axoclamp 900A Commander main window are grouped into two headstages (unless you have one headstage only, in which case only one headstage appears).

By default, each channel displays the potential in millivolts (mV) and the current in picoamps (pA) or nanoamps (nA).

### *Total Resistance, Rt*

Check the *Total Resistance* (Rt) checkbox to display resistance in M $\Omega$  on the top meter for either channel. This can be activated in either voltage-clamp or current-clamp modes, but not I=0 mode or with slow current injection. Note that to measure resistance the Axoclamp 900A Commander generates a command signal, thus the current reading fluctuates markedly when resistance measurement is enabled.

## Mode Buttons

The Mode buttons set the amplifier mode—current clamp at zero (I=0) or current clamp (IC) for each of the two headstages:

- Headstage 1 supports discontinuous current clamp mode (DCC) and discontinuous single-electrode voltage clamp mode (dSEVC).
- Headstage 2 supports high-voltage current clamp (HVIC) and two-electrode voltage clamp mode (TEVC) when used in conjunction with headstage 1.

Click on a mode button to switch to the selected mode. The change is confirmed by the indicator lights on the front panel of the Axoclamp 900.

DCC or dSEVC modes cannot be used at the same time as HVIC or TEVC and vice versa.

I=0 is a special mode of current clamp in which all command inputs are disconnected. In a real cell, membrane potential returns to its resting potential. This mode is generally used in transitions between voltage-clamp and current-clamp modes.

When you choose a mode in a particular channel, the relevant Mode tab is automatically brought forward. You can shift between tabs without changing mode.

Amplifier mode is telegraphed to compatible data acquisition programs. You can also synchronize a TTL output to the mode selection, using the Sync Output setting on the *Options / General* tab.

### ***External Mode Control***

The mode buttons can be operated from an external source. Check the *Switch to dSEVC on ext input* or *Switch to TEVC on ext input* checkbox to enable external switching. See further details at *External [or Remote] Mode Switching*.

### ***Automatic Mode Control***

The Axoclamp 900A can be set to switch automatically from current to voltage clamp if triggered by a change in the recorded membrane potential. Configure this on the *Options / Auto* tab. When configured, the ext input checkbox changes to *Switch to dSEVC at threshold* or *Switch to TEVC at threshold*.

## **Mode Tabs**

The Mode tabs are used for switching between the controls for different modes for each headstage.

You can set controls for a mode while the Axoclamp 900A is operating in another mode, as the Mode tabs select mode controls only, and do not

cause Axoclamp to enter the mode. That is the function of the Mode buttons.


**Note:** If you are on Audio, you hear the channel the uppermost Mode tab belongs to.

## Scaled Output Tabs

The Scaled Output tabs are used to select signals from the headstage, internal commands and external commands.

You can set the gain, output offset, lowpass and highpass cutoff frequency for each selected signal. If you change the signal, the last used settings for that signal are restored automatically. When changing modes using the Mode buttons, all scaled output settings that were originally setup in that mode are restored in a similar manner.

## Setting Parameters in the Axoclamp 900A Commander

User-configurable numerical fields on the Axoclamp 900A Commander printed in **blue text** are called gliders. Placing the mouse over a glider changes the cursor to . Click the glider and it is displayed in **red text**, indicating that it is ready to accept mouse input. There are several ways you can adjust the setting once a field is active.

### *Mouse Gliding*

Drag the mouse up or move the mouse wheel forward to increase the value; drag it down or move the wheel backwards to decrease the value.

Many gliders have a checkbox that enables or disables hardware output. These checkboxes also allow you to control the glider without shifting the mouse to the glider control. Click the checkbox, then immediately start changing the glider level with the mouse.

The sensitivity of each glider can be altered from the right-click popup menu for the field:

- “×1 Fine” changes values by the smallest increment for the glider.
- “×2 Medium” doubles the glider sensitivity.
- “×5 Coarse” increases glider sensitivity five times.

With these basic sensitivity levels set for each glider, you can additionally boost sensitivity, while you are using the gliders, with one of two keyboard accelerator keys:

- <Shift> to boost sensitivity.
- <Ctrl> to boost sensitivity even further.

The <Shift> and <Ctrl> keys alter sensitivity by different amounts for each glider. If <Shift> boosts sensitivity by 10 and <Ctrl> by 100, then at Medium setting they increase sensitivity 20 and 200 times respectively, while with a basic Coarse setting these are 50 and 500 times respectively.

### ***Keyboard Gliding***

- <Up Arrow> and <Right Arrow> both increase the value.
- <Down Arrow> and <Left Arrow> both decrease the value.

Keyboard gliding uses the same sensitivity options as mouse gliding, described above:

- Three basic popup menu settings: Fine, Medium and Coarse.
- Additional "real-time" sensitivity boost with <Shift> and <Ctrl> buttons.

**Note:** Some settings individual increments in the glider value are too small to affect the glider display.

Other keyboard controls include:

- <Page Up> is equivalent to <Shift> + <Up Arrow>.

- <Page Down> is equivalent to <Shift> + <Down Arrow>.
- <Home> snaps to the minimum value for that control.
- <End> snaps to the maximum value for that control.

### ***Keyboard Numeric Input***

Some gliders can accept numeric input from the keyboard (*e.g.*, Holding). These have associated edit boxes.

Double-click on the blue text to display the edit box. Type a number and hit <Enter> or click on another control to apply the change. If the control has the focus (*i.e.*, if it is displayed with red text) you can type a number without double-clicking first.

**Note:** Some fields shift between units depending on the value displayed, *e.g.*, the Tuning amplitude field displays some values in pA and others in nA. When typing in values you must always express the value in terms of the smaller unit.

You can cancel an editing operation and dismiss the edit box leaving the glider unchanged by hitting the <Esc> key.

### ***Glider Menus***

Some gliders cannot accept numeric input from the keyboard (*e.g.*, Seal Test frequency). These have associated popup menus.

Double-click or right-click to display the popup menu. (Double-click always displays the alternate interface regardless of the type of glider.) Click on the desired value to make a selection and dismiss the menu.

### ***Auto***

A number of routine instrument adjustments have been automated in the Axoclamp 900A Commander. For example, the Bridge Balance control has

an Auto button that calculates optimal levels for this property and applies it accordingly. The value can also be set manually.

## **Toolbar Buttons in the Axoclamp 900A Commander**

At the top of the Axoclamp 900A Commander main window is a row of toolbar buttons that provide access to a number of special features.

Positioning the mouse cursor over each button displays, after a short delay, a tool tip for the button. This tool tip identifies the alternative keyboard shortcut that activates the feature. For example, the Resize Window button is associated with the <F2> key. This feature toggles the size of the Commander window between full-size, meters-only, or the user-adjusted size. Drag the lower-right corner of the Commander window to change the size, then click the <F2> key to toggle between window sizes.

Most other buttons are self-explanatory, with the possible exception of the Save Configuration <F5>, Load Configuration <F4> and Quick Select buttons. These buttons allow the user to store and retrieve parameter settings for the Axoclamp 900A Commander. The Quick Select buttons can be assigned to a particular set of parameter settings to facilitate rapid loading, or, alternatively, to run an executable command line. This might be useful for experiments that require different configurations, or when several users share the same recording setup, or when an external command is desired (for example, starting a custom script to initiate a software-controlled perfusion device).

## **Communication with the Axoclamp 900A**

The Axoclamp 900A has a number of LEDs that communicate the status of the amplifier.

1. When you power ON the Axoclamp 900A, the green POWER LED comes on and the yellow STATUS LED flashes ON then OFF temporarily, indicating that the microprocessor is running.



2. When the Axoclamp 900A receives a "non meter" command it flashes ON then OFF.
3. Toggle the Headstage 1 and Headstage 2 Mode buttons in the Axoclamp Commander, switching repeatedly between the different voltage-clamp and current-clamp modes.

The tabs immediately below the mode switches in the software should change appropriately. Also, LED indicator lights on the front panel of the Axoclamp 900A should change with the different modes. The green I-CLAMP LEDs should light for IC and I=0, a yellow LED for DCC, a red LED for dSEVC, and a blue LED for HVIC. For TEVC mode, the green CHANNEL 1 I-Clamp LED and the blue TEVC LED should light. Check to see that the LEDs change with the modes selected in the software.

4. If the USB cable is unplugged the STATUS LED flashes briefly ON then OFF.

## Model Cells

We recommend that you practice using the Axoclamp 900A with model cells. The CLAMP-1U model cell shipped with your Axoclamp 900A is a useful tool for learning the operation of the Axoclamp 900A and subsequently for verifying the correct operation of the Axoclamp 900A and the recording pathway.

### The CLAMP-1U Model Cell

The cell and microelectrode components simulate a medium sized cell having an input resistance of 50 M $\Omega$ , a membrane time constant of ~25 ms and microelectrode resistances of 50 M $\Omega$ . A switch allows the CLAMP-1U model cell to be configured as:

- BATH mode—two 50 M $\Omega$  microelectrodes to ground.

- CELL mode—two microelectrodes connected to a  $50\text{ M}\Omega // 470\text{ pF}$  cell.

The case of the model cell is connected to ground. Shielding between the two microelectrode resistors is effected by the body of the switch.

When the switch is in the BATH position, both microelectrode resistors are connected to ground. This is a convenient position for practicing bridge balancing techniques and offset adjustment.

When the switch is in the CELL position, both microelectrode resistors are effectively intracellular. In Bridge or DCC mode you should see exponential voltage responses to steps of current. In dSEVC mode you should be able to clamp the cell at gains of up to  $0.8\text{ nA/mV}$  using an HS-9A  $\times 0.1$  headstage, at sampling rates up to  $8\text{ kHz}$ . In TEVC mode, use one of the following microelectrode headstage combinations: two HS-9A  $\times 0.1$  headstages, two HS-9A  $\times 1$  headstages, or an HS-9A  $\times 0.1$  headstage for CHANNEL 1 and an HS-9A  $\times 1$  headstage for CHANNEL 2.

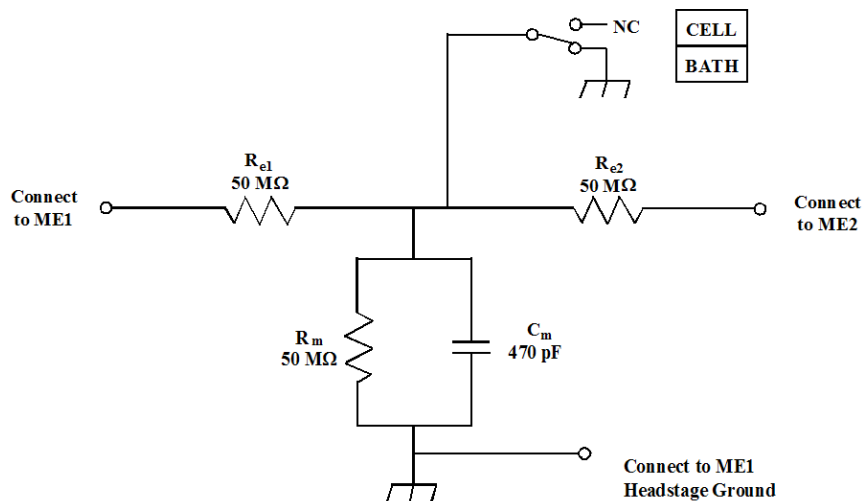


Figure 3. CLAMP-1U model cell.

## The MCO-2U Model Cell

If you intend to use your Axoclamp 900A for TEVC of oocytes, the MCO-2U model cell may be purchased separately. This model cell mimics the typical characteristics of the oocyte, the recording microelectrodes and the bath electrodes. The circuit diagram is given below.

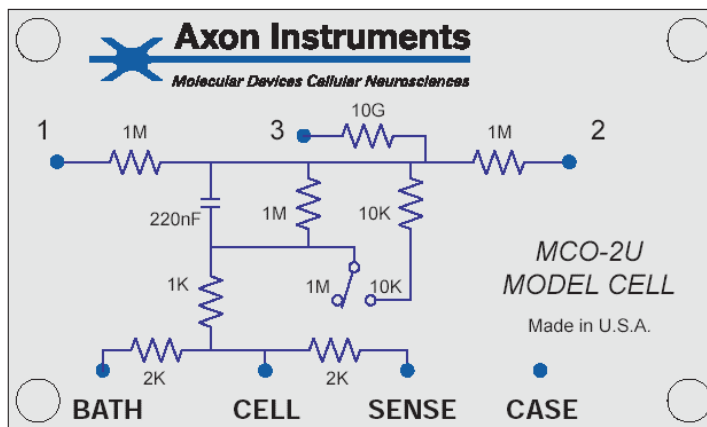


Figure 4. MCO-2U model cell.

## Installation of Model Cells

To install the model cell plug it into one or both of your headstages. Connect the gold-plated ground jack to the gold jack on the back of the CHANNEL 1 headstage using the cable provided.

## Power Supply

### Supply Voltage Selection

The Axoclamp 900A can be directly connected to all international supply voltages. The input range is from 85 to 240 V<sub>AC</sub>. No range switching is required.

## Changing the Fuse

The Axoclamp 900A uses a 2 A, 250 V slow acting 5 × 20 mm fuse. Before changing the fuse investigate the reason for its failure. To change the fuse:

1. Disconnect the power cord.
2. Use a screwdriver or a similar device to rotate the fuse holder counterclockwise.
3. Replace the fuse with another fuse of the same rating.
4. Reconnect the power cord.

## Glitches

The Axoclamp 900A has been designed to minimize the effects of power-supply transients (glitches). Although normally inconsequential, glitches could cause transients to appear on the voltage and current outputs that may corrupt high-sensitivity recordings.

The most effective way to gain immunity from mains glitches is to eliminate them at the source. Most glitches are due to the on/off switching of other equipment and lights on the same power-supply circuit.

Precautions to be taken include:

1. Avoid switching equipment and lights on or off while recordings are being made.
2. Water baths, heaters, coolers, etc. should operate from zero-crossing relays.
3. Radio Frequency Interference filters should be installed in glitch-producing equipment.

## Grounding and Hum

A perennial bane of electrophysiology is line-frequency pickup (noise), often referred to as hum. Hum can occur not only at the power line frequency but also at multiples of it.

The Axoclamp 900A has inherently low hum levels (less than 20  $\mu\text{V}$  peak-to-peak). To take advantage of these low levels great care must be taken when integrating the Axoclamp 900A into a complete recording system. The following procedures should be followed.

1. Only ground the preparation bath by directly connecting it to the yellow ground connector on the back of the CHANNEL 1 headstage (or to a virtual-ground headstage if used).
2. Place the Axoclamp 900A in a position in the rack where transformers in adjacent equipment are unlikely to radiate into its electronics. A thick sheet of steel placed between the Axoclamp 900A and the radiating equipment can effectively reduce the induced hum.
3. Initially make only one connection to the Axoclamp 900A: the oscilloscope from the Scaled Output. Ground the CHANNEL 1 headstage input through a 1 M $\Omega$  resistor to the yellow ground connector. After verifying that the hum levels are low, increase the complexity of the connections one lead at a time. Leads should not be draped near transformers inside other equipment. In desperate circumstances the continuity of the shield on an offending coaxial cable can be broken.
4. Try grounding auxiliary equipment from a ground distribution bus. This bus should be connected to the Axoclamp 900A via the yellow 0.16" (4 mm) socket on the rear panel. This socket is connected to the Axoclamp 900A's signal ground (*i.e.*, the outer conductors of all the BNC connectors) which is isolated from the chassis and power ground.

5. If more than one headstage is used, all the headstage cables should run from the Axoclamp 900A to the preparation in a bundle. The bundle can be formed either by gently twisting the cables together or by loosely tying them together.
6. Experiment. While hum can be explained in theory (*e.g.*, direct pickup, earth loops), in practice the ultimate theory is the end result. Following the rules above is the best start. The final hum level can often be kept to less than 100  $\mu\text{V}$  peak-to-peak referred to  $V_m$ . One technique that should not be used to reduce the hum is the delicate placement of cables so that a number of competing hum sources cancel out. Such a procedure is too prone to accidental alteration.

## Chapter 3: Setup and Tutorials

### Checklist

The setup and tutorial procedures require the following:

- Axoclamp 900A main unit with power cord.
- Two USB control cables.
- Axoclamp 900A Commander software.
- A PC running Windows 2000 or XP Professional.
- External two-channel oscilloscope.
- Three BNC cables.
- Four head stages:
  - HS-9A  $\times 0.1$ .
  - HS-9A  $\times 1$ .
  - HS-9A  $\times 10$ .

- o VG-9A  $\times 100$  (bath clamp head stage).
- Two model cells:
  - o CLAMP-1U.
  - o MCO-2U (oocyte).

Note that most of the tutorials use the HS-9A  $\times 0.1$  and HS-9A  $\times 1$  headstages and the CLAMP-1U model cell that come standard with the Axoclamp 900A. However, one of the TEVC tutorials requires a different set of headstages, HS-9A  $\times 10$  and VG-9A  $\times 100$ , and a different model cell, the MCO-2U. If you don't have the specific headstages or model cell called for in the tutorial, you can substitute your headstages in the tutorials. But be aware that some of the scaling will be different.

## Axoclamp 900A and Axoclamp 900A Commander Setup

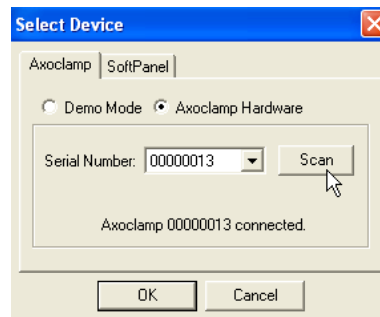
We recommend performing the initial setup and tutorials using the Axoclamp 900A, the associated computer and an oscilloscope in a standalone fashion on a bench top. The tutorials may also be performed at a later date with the equipment mounted in a typical laboratory setting.

1. Connect the Axoclamp 900A to power source with power cable.
2. Connect a USB cable between the computer and the CONTROL USB port at the back of the Axoclamp 900A.
3. Connect another USB cable between the computer and the SCOPE USB port at the back of the Axoclamp 900A.
4. Install the Axoclamp 900A Commander software on the computer to control the Axoclamp 900A. At the Hardware Installation message about the Universal Service Bus controllers, click Continue Anyway to allow installation to proceed to completion.

**There is no need to turn off the power to the Axoclamp 900A prior to connecting headstages. However, do not disconnect headstages while the software is starting up.**




5. Connect the HS-9A  $\times 0.1$  headstage connector to the CHANNEL 1 HEADSTAGE INPUT on the rear panel.
6. Connect the HS-9A  $\times 1$  headstage connector to the CHANNEL 2 HEADSTAGE INPUT on the rear panel.
7. Connect headstage 1 to the CLAMP-1U model cell at the ME 1 connector.
8. Ground the CLAMP-1U case to the signal ground socket on the rear of the headstage. The switch should be in the BATH position.
9. Place the headstage and model cell into a shielded enclosure (optional) ensuring that the shield is also connected to the signal ground.
10. Turn the Axoclamp 900A on. The Windows operating system should recognize that two HID devices are attached.
11. Run Axoclamp 900A Commander. The first time it is run the *Select Device* dialog is displayed automatically:



**Figure 5.** Select Device dialog box.

12. Press *Scan* and the serial number of the Axoclamp 900A is displayed. Press *OK* and the main Commander interface is displayed.

13. Press the *Reset to Program Defaults* button  and accept the warning by clicking on *Yes* to continue.

Information about the connected headstages is displayed at the top of the Commander interface, including the current injection resistance ( $R_0$ ) for each headstage. For the HS-9A  $\times 0.1$  headstage,  $R_0$  is 100 M $\Omega$ , and for the HS-9A  $\times 1$ ,  $R_0$  is 10 M $\Omega$ .

14. Connect a BNC cable between the SCALED OUTPUT for Channel 1 and an external oscilloscope.
15. Connect another BNC cable between the SCALED OUTPUT for Channel 2 and the oscilloscope.
16. Connect another BNC cable between the external trigger input of the oscilloscope and the SYNC OUTPUT BNC on the rear panel of the Axoclamp 900A.

## Tutorial 1: Current Clamp (IC)

Equipment required:

- CLAMP-1U model cell.
- HS-9A  $\times 0.1$ .
- HS-9A  $\times 1$ .

If you have just performed the Axoclamp 900A setup please skip to step 9.

**There is no need to turn off the power to the Axoclamp 900A prior to connecting headstages. However, do not disconnect headstages while the software is starting up.**

1. Connect the HS-9A  $\times 0.1$  headstage connector to the CHANNEL 1 HEADSTAGE INPUT on the rear panel.

2. Connect the HS-9A  $\times 1$  headstage connector to the CHANNEL 2 HEADSTAGE INPUT on the rear panel.
3. Connect headstage 1 to the CLAMP-1U model cell at the ME 1 connector.
4. Ground the CLAMP-1U case to the signal ground socket on the rear of the headstage. The switch should be in the BATH position.
5. Place the headstage and model cell into a shielded enclosure (optional) ensuring that the shield is also connected to the signal ground.
6. Turn the Axoclamp 900A power ON.
7. Run the Axoclamp 900A Commander software.
8. Press the *Reset to Program Defaults* button in the toolbar at the top of the Commander and accept the warning.

Information about the connected headstages is displayed at the top, including the current injection resistance for each headstage. For the HS-9A  $\times 0.1$  headstage,  $R_0$  is 100 M $\Omega$ , and for the HS-9A  $\times 1$ ,  $R_0$  is 10 M $\Omega$ .

9. In the I-Clamp 1 tab, with the CLAMP-1U switch in the BATH position, press the *Pipette Offset Zero* button.



Figure 6. Pipette Offset controls.

The corrected offset voltage for the headstage should be less than  $\pm 10$  mV.

10. The Channel 1 SCALED OUTPUT Signal selected should be “Headstage 1, Membrane Potential (10 mV/mV)”.

11. Check on the *Tuning* checkbox.  Tuning: 1.000 nA

The Oscilloscope should show a 500 mV<sub>p-p</sub> square wave at 10 Hz. This is the voltage expected from the 50 MΩ bath resistor in the model cell.

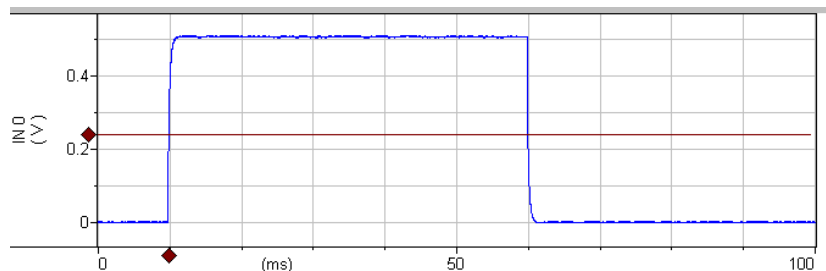


Figure 7. I-Clamp voltage measurement.

12. Press the *Auto* button in the Bridge Balance section.

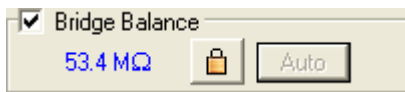
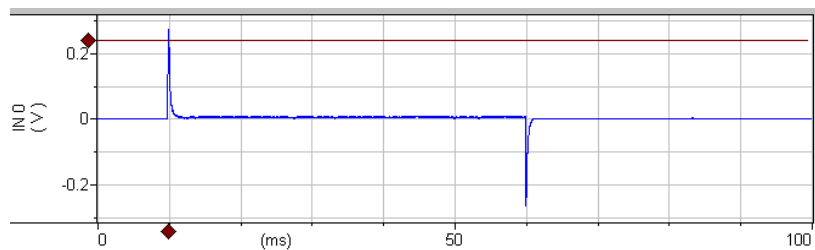


Figure 8. Bridge Balance controls.

The waveform should decrease to close to zero and the bridge resistance display should be 45 to 55 MΩ. If not, click on the *Lock* button and press the *Auto* button again. You should also be able to click on the Bridge resistance field and use the software glider to adjust the bridge balance resistance. Note that with all the software gliders, once you have clicked on the numeric field, you can use the mouse to 'glide' through the numbers or use the keyboard arrow keys to change the value. If you right click on the value field you can set the coarseness of the control. You can also enter the numbers directly into the field or select from allowed values, depending on the field.

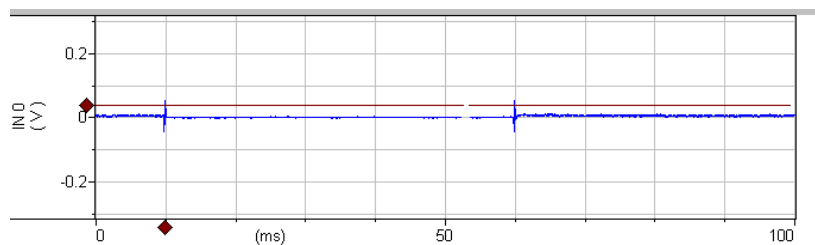
**Note:** *Auto Bridge Balance* works only in the bath.

13. Notice a 1 ms wide transient on each 10 Hz waveform edge. This transient is caused by uncompensated electrode capacitance.



**Figure 9.** Bridge Balance of voltage signal in bath.

14. Check the *Pipette Capacitance Neutralization* checkbox: Change the value in the numeric field until the capacitive transient decreases to near zero. The value should be between 1 and 3 pF.



**Figure 10.** Pipette capacitance neutralized in bath.

15. Move the CLAMP-1U switch to the CELL position. The waveform should be about  $400\text{ mV}_{p-p}$ , a triangle wave with exponential sides.

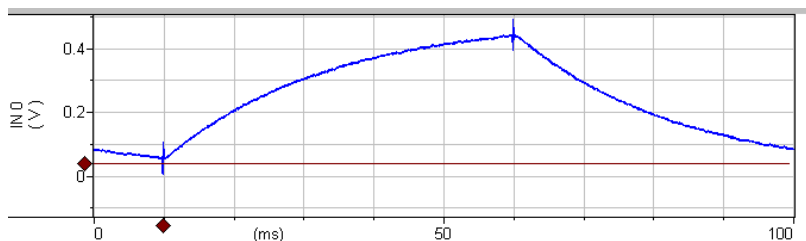


Figure 11. Compensated I-Clamp voltage signal.

**The following two steps should not be done if this tutorial is being performed solely in preparation for another tutorial.**

### Oscillation Detection

16. Click *Detect Oscillation* and select *Disable Neutralization*:

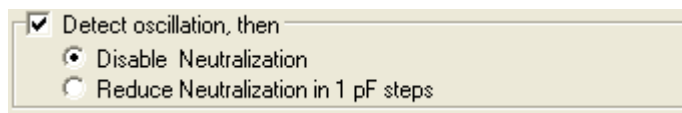


Figure 12. Oscillation detection controls for I-Clamp mode.

Click in the *Pipette Capacitance Neutralization* value field, type '5', and press <ENTER> to set the capacitance to 5 pF. This should initiate oscillation. A pop-up dialog should be displayed:



Figure 13. Oscillation detected message for I-Clamp mode.

The *Pipette Capacitance Neutralization* checkbox in the main menu should be disabled.

Set Pipette Capacitance Neutralization back to its previous value (~2 pF) and recheck the *Pipette Capacitance Neutralization* checkbox.

**Note:** Oscillation Detection is available in current-clamp modes of IC and DCC but not HVIC. It is also available in the two voltage-clamp modes, TEVC and dSEVC.

## Slow Current Injection

### 17. Slow current injection (track).

This can be used to maintain the baseline membrane potential in I-Clamp mode in order to offset electrode drift over the course of an experiment.

Select *Inject slow current to maintain potential at* and select 10 mV:

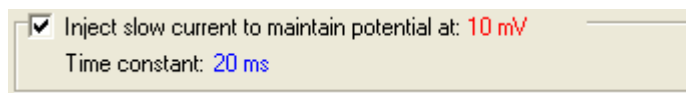
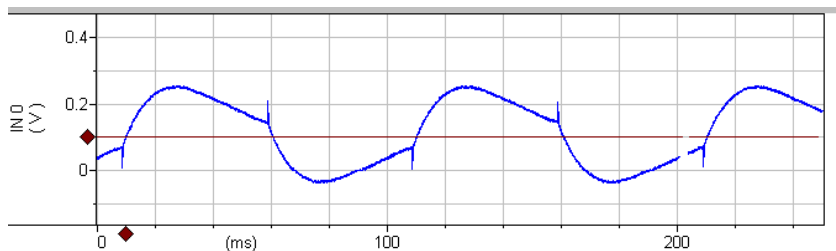


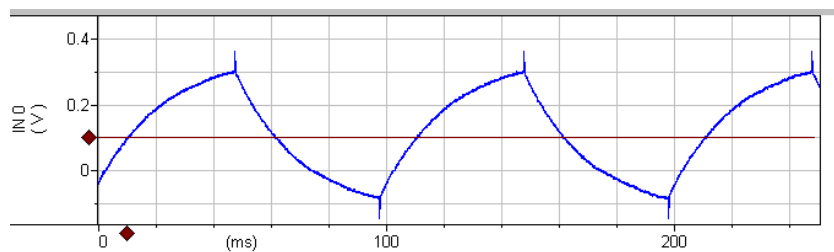
Figure 14. Inject slow current controls.



**Figure 15.** Slow current injection with short time constant.

**Note:** The average DC Scope level went to 100 mV (10 mV at the headstage) shown as the red line in the figure. Also notice the waveform shape is distorted compared with the previous waveform in current-clamp mode step 15 above. This is because the “Slow Current” correcting time constant is shorter than the test waveform frequency.

Change the slow current *Time constant* to 2 seconds. Deselect and reselect *Inject slow current*. Notice that the speed of establishing the average DC level slows down and the test waveform is no longer distorted.



**Figure 16.** Slow current injection with long time constant.

## Tutorial 2: Discontinuous Current Clamp (DCC)

Discontinuous current clamp mode can be used for accurate voltage measurement, and is especially useful when small changes in electrode resistance occur during an experiment. In DCC mode, CHANNEL 1 is





cyclically used to pass current. The voltage recorded at the tip of CHANNEL 1 is measured by a sample-and-hold circuit in between each current-passing period and after all transient voltages due to current passing have decayed. Thus the membrane potential can be recorded independently of the IR voltage drop across the microelectrode.

The advantage of DCC mode compared with I-Clamp mode with Bridge Balance is that it is tolerant of small changes in microelectrode resistance. The disadvantage is that DCC mode is noisier than I-Clamp mode. During DCC mode CHANNEL 2 can be used in I-Clamp mode for continuous current passing.

Equipment required:

- CLAMP-1U model cell.
- HS-9A  $\times 0.1$ .
- HS-9A  $\times 1$ .

Follow the steps of **Tutorial 1** before beginning these instructions.

1. In Channel 1 select DCC mode. 
2. Set DCC Rate to 10 kHz. 

**Note:** When DCC mode is selected the Scope window display, with the “monitor” waveform, should automatically appear on the computer screen.

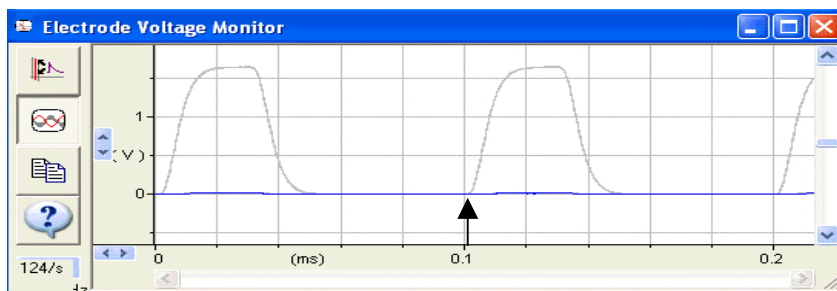


Figure 17. Monitor signal for DCC mode.

The monitor waveform rise and fall times should be less than 20  $\mu\text{s}$ . Most importantly, the voltage transients should decay completely by the time the samples are taken (arrow), even for the largest transients.

3. The waveform on the oscilloscope should be similar in shape to the IC waveform except it will have about double the noise:

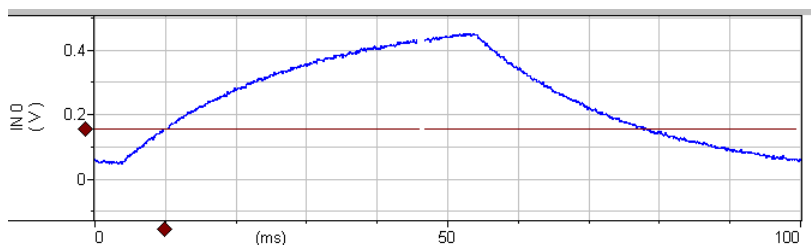
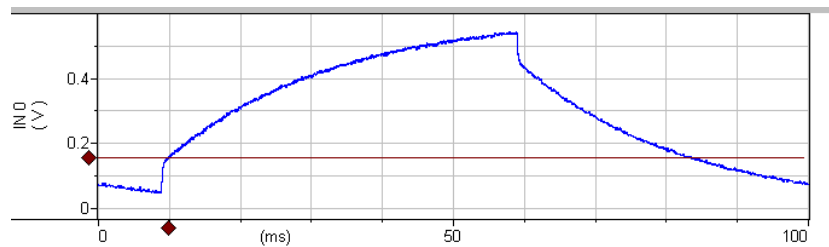


Figure 18. Membrane potential signal in DCC mode.

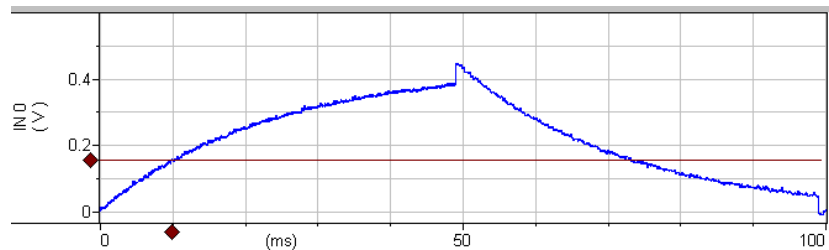
4. Adjust the *Pipette Capacitance Neutralization* value and *Rate* in the I-Clamp 1 tab. Observe how the two settings vary the waveform. The ideal waveform should have a rate as high as possible without the triangle wave splitting apart on the oscilloscope display. See the section on the *Theory of Recording Modes* in Chapter 4 for explanation on how to use the software Scope window to optimize these settings.

Neutralization set at 1.5 pF (under compensated):



**Figure 19.** Pipette Capacitance Neutralization under compensated in DCC mode.

Neutralization set at 3.0 pF (over compensated):



**Figure 20.** Pipette Capacitance Neutralization over compensated in DCC mode.

## Tutorial 3: Discontinuous Single-Electrode Voltage Clamp (dSEVC)

dSEVC mode is used to voltage clamp small cells that cannot be impaled by two electrodes. This mode can also eliminate the large series resistance inherent in many preparations.

Equipment required:

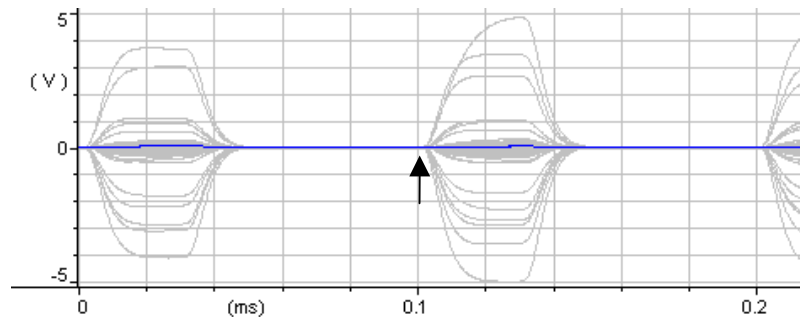
- CLAMP-1U model cell.
- HS-9A  $\times 0.1$ .

- HS-9A ×1.

Follow the steps of **Tutorial 1** (excluding the optional steps) before beginning these instructions.

1. Select DCC mode and setup as outlined in **Tutorial 2**.
2. Select dSEVC mode in Channel 1.

**Note:** When DCC and then dSEVC modes are selected the “monitor” waveform is displayed in the Scope window on the computer screen.

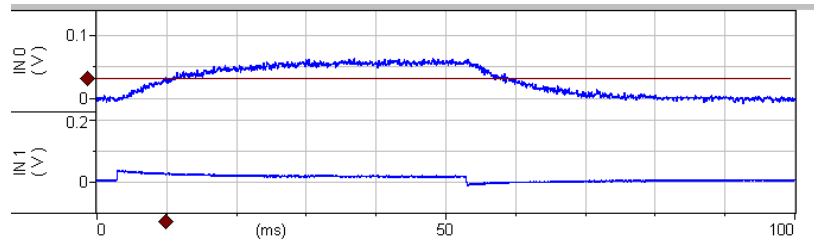


**Figure 21.** Monitor signal for dSEVC mode.

The important feature is that the voltage transients decay completely by the time the samples are taken (arrow) even for the largest transients. These traces show that switching frequency can be nearly doubled if required.

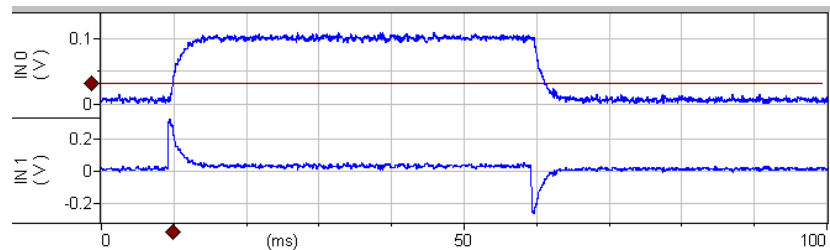
3. Click the *Seal Test* checkbox.
4. The signal for Channel 1 SCALED OUTPUT should be “Headstage 1, Membrane Potential (10 mV/mV)”.

5. The signal for Channel 2 SCALED OUTPUT should be “Headstage 1, Membrane Current (0.1 V/nA)”.



**Figure 22.** Voltage and current signals for dSEVC mode with low gain.

6. The membrane potential waveform on the oscilloscope should display about a 60 mV square wave (6 mV at the membrane) with about a 20 ms rise time. Note that we programmed a voltage of 10 mV but only got a 6 mV response, indicating a higher gain is required. Also, the current trace (IN1) shows a similar settling time. In the dSEVC control tab, glide the *Gain* from 0.03 to about 0.35 nA/mV. The oscilloscope waveform should display a 100 mV square wave that is now the correct amplitude (monitoring  $10 \times V_e$ ). The rise time should be about 2 ms.



**Figure 23.** Voltage and current signals for dSEVC mode with increased gain.

- Adjust *Pipette Capacitance Neutralization* and *Rate* to get a smooth exponential rise time, then increase *Gain* (nA/mV) to obtain the fastest rise time without overshoot. Optimum *Gain* should be greater than 1.5 nA/mV and the rise time should be less than 300  $\mu$ s with noise around 10 mV<sub>p-p</sub>. See the expanded time scale below:

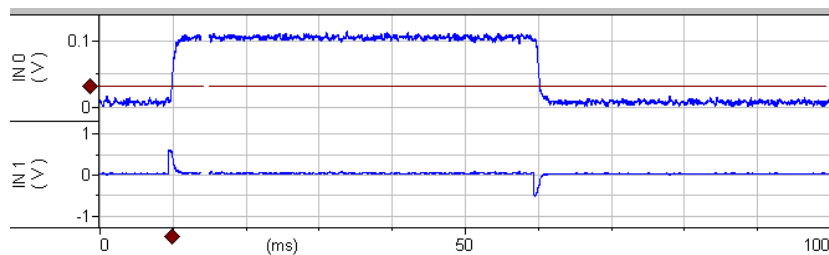


Figure 24. Voltage and current signals for dSEVC mode with further tuning.

- With practice you should be able adjust *Pipette Capacitance Neutralization* and *Rate* to increase *Gain* even higher so that the current trace settles in less than 0.5 ms (see expanded trace below).

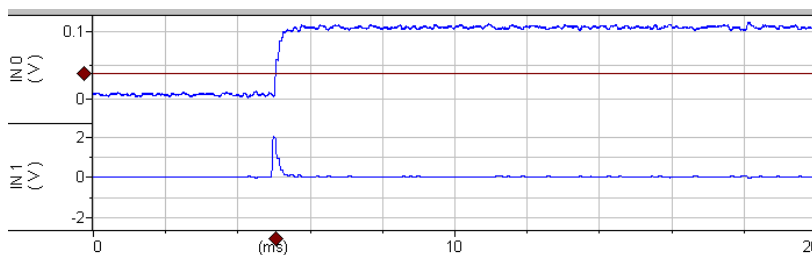
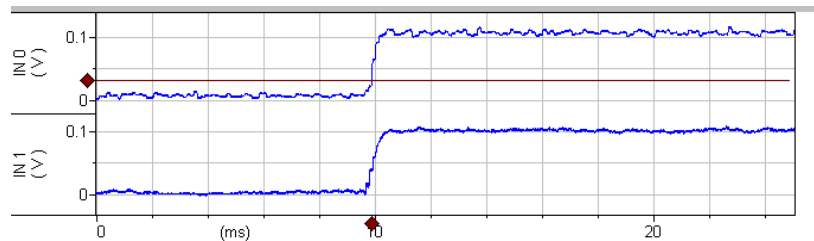


Figure 25. Voltage and current signals for dSEVC mode optimized.

9. Connect the headstage from CHANNEL 2 to the ME2 input of the CLAMP-1U model cell and select the “Headstage 2, Membrane Potential (10 mV/mV)” signal in the Channel 2 SCALED OUTPUT section. The Channel 2 headstage will now serve as a cell membrane voltage monitor. You should see that the Channel 2 SCALED OUTPUT signal is identical to the Channel 1 SCALED OUTPUT from Headstage 1 (100 mV<sub>p-p</sub>) as below. Channel 2 speed will be slightly slower due to capacitance loading. You can speed it up by optimizing Headstage 2 neutralization.



**Figure 26.** Monitoring the dSEVC voltage response with a second headstage. IN0 is the membrane voltage as measured by Headstage 1 (dSEVC mode). IN1 is the membrane voltage as measured by Headstage 2 with 2.5 pF Pipette Capacitance Neutralization.

## Tutorial 4: Two-Electrode Voltage Clamp (TEVC)

### I. TEVC Using the CLAMP-1U Model Cell


Two-Electrode Voltage Clamp is preferred to discontinuous Voltage Clamp (dSEVC) as it delivers a faster, lower noise and a more stable voltage clamp. However, it requires two electrodes to be inserted into the same cell, which is more difficult in smaller cells. Headstage 1 is always the “voltage monitoring” electrode and Headstage 2 is always the “current-passing” electrode.

Equipment required:

- CLAMP-1U model cell.

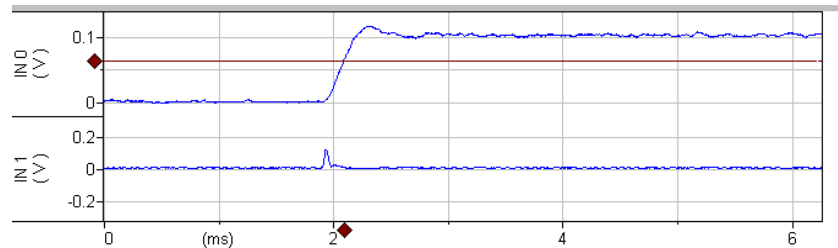
- HS-9A  $\times 0.1$  as the voltage monitoring headstage.
- HS-9A  $\times 1$  as the current passing headstage.

Instructions:

1. Connect headstages to the rear panel headstage inputs:
  - a. HS-9A  $\times 0.1$  to the CHANNEL 1 HEADSTAGE input.
  - b. HS-9A  $\times 1$  to the CHANNEL 2 HEADSTAGE input.
2. Connect:
  - a. Channel 1 SCALED OUTPUT to oscilloscope channel 1.
  - b. Channel 2 SCALED OUTPUT to oscilloscope channel 2.
3. Press the *Reset to Program Defaults* button  and accept the warning.
4. Connect:
  - a. Headstage 1 to the ME1 input of the CLAMP-1U model cell.
  - b. Headstage 2 to the ME2 input of the model cell.
  - c. The ground socket from one of the headstages to the ground socket on the model cell.
5. With the CLAMP-1U model cell in the BATH position, use the *Pipette Offset* in the I-Clamp 1 and I-Clamp 2 tabs to zero the voltage in each channel.
6. Select the I-Clamp 1 tab and neutralize pipette capacitance for I-Clamp 1 only:
  - a. Check the *Tuning* checkbox.



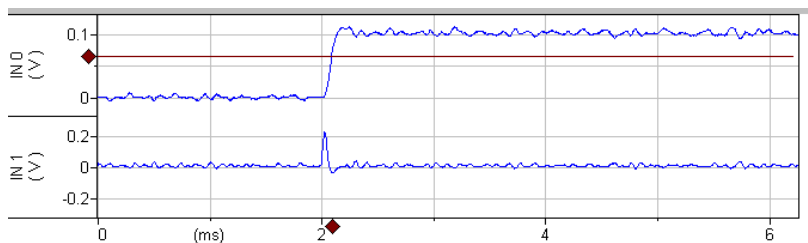
- b. Switch from the BATH to the CELL position on the CLAMP-1U model cell.
  - c. Check the *Pipette Capacitance Neutralization* checkbox.
  - d. Glide the neutralization value until the leading edge of channel 1 speeds up maximally without overshoot. The value should be between 1 and 3 pF.
7. Select TEVC mode on Channel 2.
  8. Enable *Seal Test*.  Seal Test: 10 mV 10 Hz
  9. For the SCALED OUTPUT signals select:
    - a. Channel 1 “Headstage 1, Membrane Potential (10 mV/mV)”.
    - b. Channel 2 “Headstage 2, 0.1× Membrane Current (1 V/μA)”.
  10. Adjust the *Gain* until channel 1 starts to overshoot. The Rise time should be about 200 μs.



**Figure 27.** Voltage and current signals for TEVC mode. IN0 is the membrane potential ( $\times 10$ ). IN1 is the membrane current (1 V/ $\mu$ A). The voltage-clamp Gain is 200. Pipette Capacitance Neutralization is 2.5 pF.

11. To optimize the voltage rise time, increase the *Pipette Capacitance Neutralization* up to an additional 1 pF until the overshoot disappears and then increase the gain. Do not increase capacitance neutralization or the gain too high, as oscillation will result. With practice you

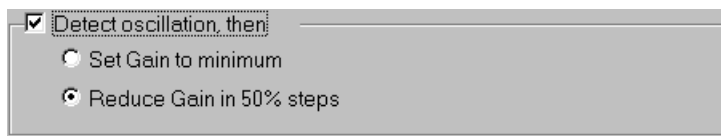
should be able to get a rise time of about 100  $\mu\text{s}$ , with a gain of about 350 to 400 and noise of less than 20  $\text{mV}_{\text{p-p}}$  (2 mV at the cell). Oscilloscope channel 2 monitors current and should display a waveform that settles in less than 200  $\mu\text{s}$ .



**Figure 28.** Optimizing TEVC mode. IN0 is the membrane potential ( $\times 10$ ). IN1 is the membrane current ( $1 \text{ V}/\mu\text{A}$ ). The voltage-clamp Gain is 388. Pipette Capacitance Neutralization is 3.5 pF.

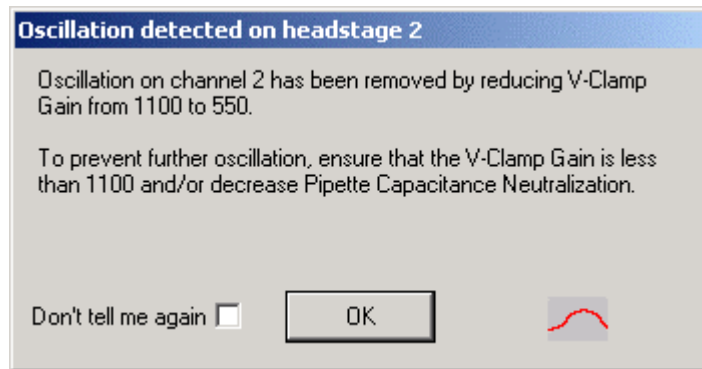
### Oscillation Detection

1. In the *Detect oscillation* section of the TEVC tab, you can choose to *Reduce gain in 50% steps* or *Set Gain to minimum*.



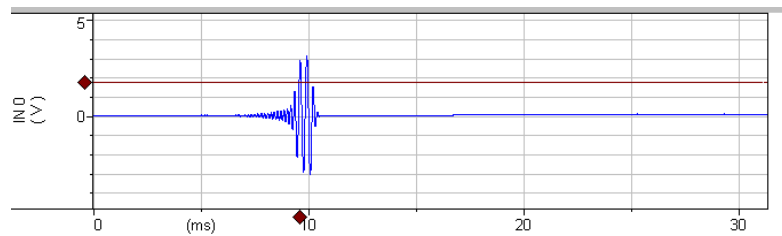
**Figure 29.** Oscillation detection settings for voltage-clamp modes (TEVC, dSEVC).

2. Enable *Detect oscillation*.
3. Increase the gain until the amplifier oscillates. The following message will appear when an oscillation is detected. This will typically happen at gains between 1000 and 2000 in TEVC mode.



**Figure 30.** Oscillation detected message for voltage-clamp modes (TEVC, dSEVC).

4. Below is an example of a typical oscillation, showing that it was detected and removed in about 2 ms.



**Figure 31.** Automated suppression of voltage signal oscillation in TEVC mode. IN0 is 10× the membrane voltage.

**Note:** Oscillation detection and correction is available in IC (both channels), DCC, dSEVC, and TEVC modes, but not in I=0 or HVIC modes.

## II. TEVC Using the MCO-2U Oocyte Model Cell and VG-9A Bath Clamp



Oocytes are large cells used to study ion channels heterologously. Membrane currents from these heterologously expressed channels can be quite high ( $> 50 \mu\text{A}$ ). When the currents are large, bath resistance, which is typically a few  $\text{k}\Omega$ , can cause significant errors in measurements. For example, a bath resistance of  $1 \text{k}\Omega$  will lead to cell voltage errors of  $1 \text{mV}$  per  $\mu\text{A}$ . Additionally, in ligand-gated channels, low membrane resistance can lead to loss of tight voltage clamp when high current is passed. This procedure explains how to overcome each of these problems.

Equipment required:

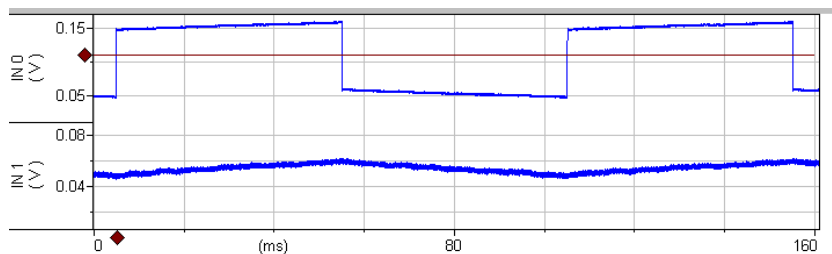
- MCO-2U model cell.
- HS-9A  $\times 1$  as voltage monitoring headstage.
- HS-9A  $\times 10$  as current-passing headstage.
- VG-9A  $\times 100$  bath clamp.

Instructions:

1. Connect headstages to headstage inputs:
  - a. HS-9A  $\times 1$  cable to the CHANNEL 1 HEADSTAGE.
  - b. HS-9A  $\times 10$  cable to the CHANNEL 2 HEADSTAGE.
  - c. VG-9A  $\times 100$  bath clamp cable to one of the auxiliary headstage inputs on the rear panel.
2. Connect:
  - a. HS-9A  $\times 1$  headstage to the E#1 input to the model cell.
  - b. HS-9A  $\times 10$  headstage to the E#2 input to the model cell.

- c. Connect the red (sense) and white (bath) sockets on the VG-9A headstage to the same colored terminals on the MCO-2U via short wires supplied with the MCO-2U model cell (< 2 inches with 2 mm pins on either end).
3. Make sure the MCO-2U switch is in the 1 M $\Omega$  position (typical membrane resistance).
4. Connect the SCALED OUTPUT BNCs for Channels 1 and 2 to oscilloscope channels 1 and 2 and connect the SYNC OUTPUT BNC on the rear panel to the oscilloscope external trigger input.
5. Press the *Reset to Program Defaults* button  and accept the warning.
6. Verify that the R<sub>0</sub> value for Headstage 1 is 10 M $\Omega$  and the R<sub>0</sub> value for Headstage 2 is 1 M $\Omega$ .
7. Zero the Pipette Offset for Channels 1 and 2 (outlined in the Current Clamp section).
8. Set the Headstage 1 *Tuning* level to 10 nA by clicking in the *Tuning* field, typing '10 <ENTER>' . Enable Tuning by checking the box.
9. For the SCALED OUTPUT signals select:
  - a. Channel 1 "Membrane Potential, 10 mV/mV".
  - b. Channel 2 "Membrane Potential, 10 mV/mV".
10. Oscilloscope channel 1 should show a positive 100 mV step followed by a 10 mV ramp and then a negative 100 mV step and 10 mV ramp.
11. The step should have a rise time of about 50  $\mu$ s. As this is already quite fast, no neutralization of pipette capacitance is normally required.

12. Since Headstage 2 is plugged into the model cell E#2 input, oscilloscope channel 2 should show a 10 mV<sub>p-p</sub> triangular wave.



**Figure 32.** IC mode with 10 nA Tuning pulse in channel 1. IN0 is 10× the membrane voltage in channel 1 and IN1 is 10× the membrane voltage in channel 2.

13. In the TEVC tab set the *Lag* to about 0.2 ms. Lag: 0.21 ms

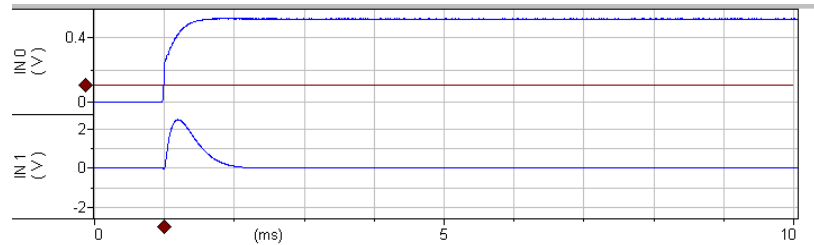
14. Select TEVC mode. I=0 IC HVIC TEVC

15. For the SCALED OUTPUT signals select:

- a. Channel 1 “Headstage 1, Membrane Potential, 10 mV/mV”.
- b. Channel 2 “Headstage 2, 0.1xMembrane Current (0.1 V/μA)”.

16. Turn on a 50 mV *Seal Test* pulse.  Seal Test 50 mV 10 Hz

17. Increase the Voltage Clamp Control (AC) *Gain* until the voltage trace in oscilloscope channel 1 begins to overshoot on the leading edge. This should occur between a gain of 3000 to 3500. Do not increase gain beyond this, as the system will oscillate. The scope should look like this (see below).



**Figure 33.** TEVC mode with the MCO-2U model cell. IN0 is 10× the membrane voltage. IN1 is the membrane current (0.1 V/μA). The voltage-clamp gain is 4500.

The current settling time (lower trace) should be around 1 ms. The fast rise time of the membrane voltage (top trace) is caused by the “1K” bath resistance in the model cell. As the current decreases to zero the cell voltage is clamped at the 50 mV command voltage.

18. Switch the MCO-2U switch to the 10K position. Notice the steady state current during the voltage pulse increases (channel 2 on the oscilloscope) by about 450 mV (4.5 μA) and the voltage clamp voltage drops to about 48 mV due to the extra load.

## DC Restore

DC Restore provides optimal voltage control for large currents in the absence of voltage steps. It is especially useful for recordings of ligand-gated channels or other measurements not requiring voltage steps. This feature works with both TEVC and dSEVC modes.

1. To demonstrate DC Restore, uncheck *Seal Test* and check  *Holding*.
2. Move the MCO-2U switch to 1 MΩ.
3. Set the  *Holding* level to 50 mV and verify that the voltage ‘V’ in the Headstage 1 section at the top of the Axoclamp 900A Commander reads 50 mV.
4. Move the MCO-2U switch to 10 kΩ.

5. The 'V' meter in the software will drop to about 47 mV.
6. Check *DC Restore*.
7. The 'V' meter will return to 50 mV. This demonstrates the DC gain is very high (typically > 1,000,000).
8. Reduce the Voltage Clamp Control (AC) Gain to 500 and note the 'V' meter stays at 50 mV indicating that the high DC gain is still clamping the voltage even at low AC gain.
9. Verify that the AC gain is low. Uncheck *DC Restore* and note that the 'V' meter reading drops to less than 40 mV, showing a > 10 mV loss of DC voltage clamp.

## Tutorial 5: Auto Mode Switching

In addition to manually controlled mode switching from Axoclamp 900A Commander, Axoclamp 900A has the ability to automatically switch between I-Clamp and V-Clamp modes and back again. Auto mode switching allows the user to set up parameters to switch automatically from I-Clamp mode to either dSEVC mode or TEVC mode and back to I-Clamp mode. The switching can be triggered by an external input or by a positive or negative  $V_m$  threshold crossing.

Prior to enabling Auto Mode Switching:

- I-Clamp mode must be configured.
- The voltage-clamp mode that will be switched into must also be configured (by following the procedures outlined in the appropriate tutorial).

The switch to V-Clamp mode can be initiated by:

- The external MODE BNC input going high (2–5 V) or low (0–0.8 V).



- The Headstage 1 Membrane Potential passing through a pre-determined level. The direction it passes through the level is also programmable (negative to positive or positive to negative).
- Going into V-Clamp can be instantaneous (< 2 ms) or can be delayed up to 500 ms.

Returning to I-Clamp mode can be programmed to be:

- Manual return.
- On external MODE BNC input going low or high, depending on how the logic is set for going into voltage-clamp mode.
- After a programmable time ranging between 2 ms and 500 seconds.

### Example of Auto Mode Switching

The following switches from I-Clamp to TEVC mode when membrane voltage in headstage 1 passes through +20 mV in a negative to positive direction. It stays in TEVC mode for 500 ms generating a 0 mV to 10 mV<sub>p-p</sub> 10 Hz Seal Test waveform and then returns to I-Clamp. The cycle then repeats.

Set up both I-Clamp and V-Clamp modes using the appropriate tutorials and model cell prior to going into AUTO mode. This will prevent oscillations when switching into a new mode.


Make sure to uncheck *Oscillation Detection* in the appropriate V-Clamp tab when setting up the V-Clamp mode.

To initiate an I-Clamp to V-Clamp transition, a waveform in headstage 1 that moves through +20 mV is required. To do this we will use a TUNE input from Headstage 2.

1. In Channels 1 & 2 select IC mode.

2. For the SCALED OUTPUT 1 signal select “Headstage 1, Membrane Potential (10 mV/mV)”.
3. For the SCALED OUTPUT 2 signal select “Headstage 2, Membrane Potential (10 mV/mV)”.
4. Connect SCALED OUTPUTS 1 & 2 to oscilloscope channels 1 & 2.
5. In the I-Clamp 2 tab set the TUNE input for Headstage 2 to 1 nA.
6. Oscilloscope Channel 1 should show a 50–400 mV triangular wave with rounded sides. This equates to a 5–40 mV waveform that will trigger the mode change when enabled.
7. Enable a 10 mV Seal Test waveform at 10 Hz when in TEVC mode. This pulse will be delivered once TEVC mode is entered.

## Setup of Auto Mode

1. Press the *Options* button. 
2. Select the *Auto* tab.

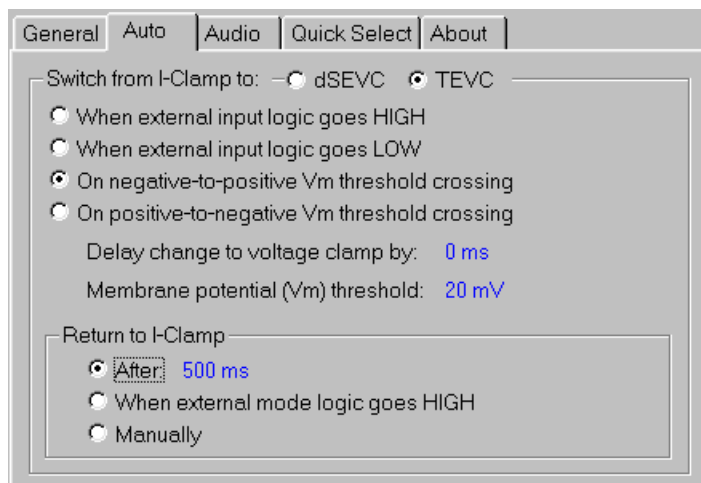
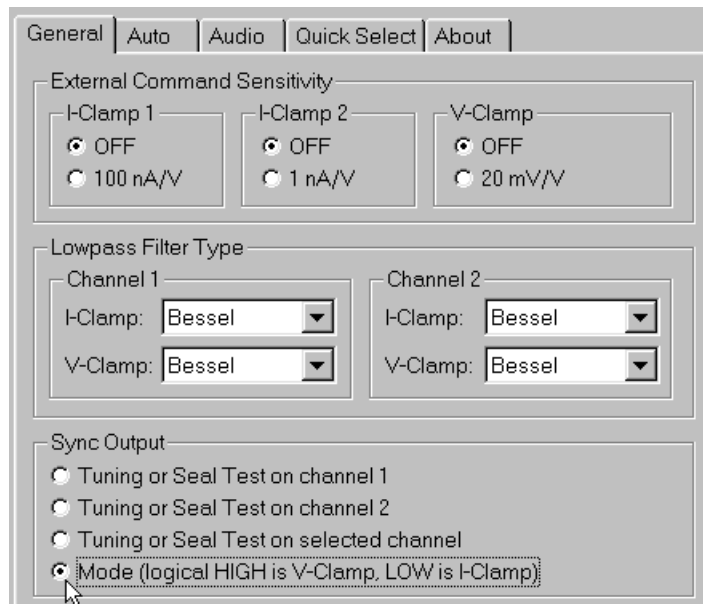


Figure 34. Settings dialog for Automatic Mode Switching.

3. Select *TEVC*.
4. Select *On negative to positive  $V_m$  threshold crossing*.
5. Set the Membrane potential ( $V_m$ ) threshold to +20 mV.
6. Set *Return to I-Clamp* after 500 ms.

In order to monitor mode changes on the scope we need to program the SYNC BNC to follow mode changes:

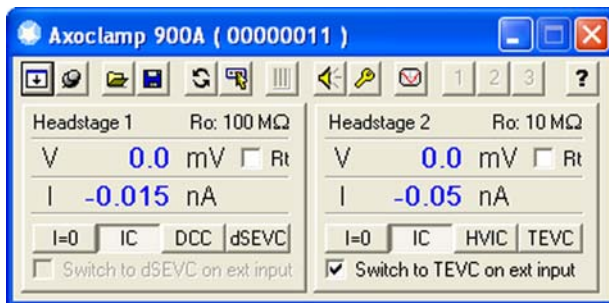
7. Click on the *General* tab.
8. In the *Sync Output* group click the *Mode* radio button. This makes rear panel SYNC BNC follow mode signals. LOW is I-Clamp and HIGH is V-Clamp.



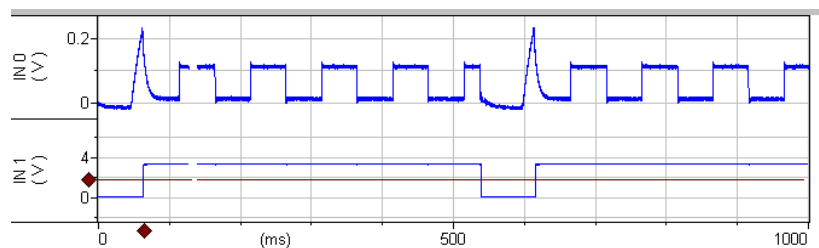
**Figure 35.** Setting up Sync Output.

9. Close the dialog.

10. Connect oscilloscope channel 1 to Headstage 1 SCALED OUTPUT.
11. Connect oscilloscope channel 2 to rear panel SYNC BNC.
12. In the Headstage 2 meter group, click *Switch to TEVC at threshold*.



The following waveforms should result:



**Figure 36.** Automated Mode Switching signals. IN0 is 10× the membrane potential in Channel 1. IN1 is the Mode signal. HIGH is V-Clamp mode and LOW is I-Clamp mode.

Explanation of the waveform:

- o The membrane potential starts at zero in I-Clamp mode.
- o Headstage 2 injects a 1 nA current into the membrane.
- o This current is picked up in Headstage 1 as seen at the 50 ms point with the exponentially rising membrane potential.

- o When the membrane potential reaches 20 mV the mode is changed to V-Clamp (lower trace).
- o Seal Test is started delivering 0 to 10 mV at 10 Hz voltage-clamp command.
- o 500 ms later, the programmed time to be in V-Clamp mode, the system returns to I-Clamp.
- o The cycle repeats.

## Tutorial 6: High-Voltage Current Clamp (HVIC)

High-voltage current clamp (HVIC) is only available on HEADSTAGE 2. HVIC is used primarily for extracellular iontophoresis applications, when large currents need to be injected into the preparation, or electrode resistance is very high. For example, if you want to pass 1  $\mu\text{A}$  current into the CLAMP-1U model cell it requires more than 100 V of dynamic range for the driving electrode.

The downside is that capacitance neutralization is not available, so speed is slower and noisier than normal current clamp.


**Note:** If you are using currents below 10 nA then you may see zero offsets. If this is so, you should use a small holding current to offset the error.

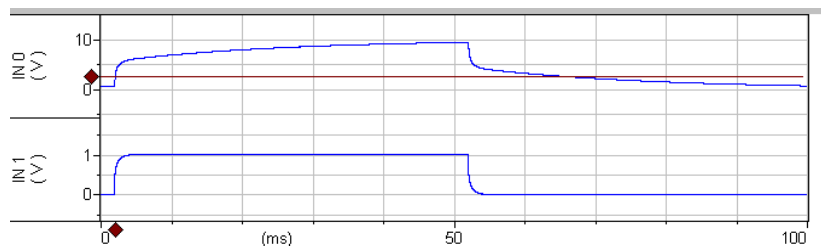
Equipment required:

- CLAMP-1U model cell.
- HS-9A  $\times$ 1.

Instructions:

1. Connect the HS-9A  $\times$ 1 headstage connector to HEADSTAGE 2.
2. Disconnect HEADSTAGE 1 (do not leave it connected as it will saturate with the test signals used in this section).

3. Connect the CLAMP-1U model cell to the HS-9A  $\times 1$  headstage, and the ground wire between the CLAMP-1U and the HS-9A  $\times 1$ .
4. Press the *Reset to Program Defaults* button  and accept the warning.
5. In the Headstage 2 tab select HVIC.
6. In I-CLAMP 2 tab, set 1000 nA of Tune current and enable the *Tuning* checkbox.
7. Connect scope channel 1 to SCALED OUTPUT 1.
8. The Channel 1 SCALED OUTPUT Signal selected should be “Headstage 2, 0.1 $\times$  Membrane Potential”.
9. Connect scope channel 2 to SCALED OUTPUT 2.
10. The Channel 2 SCALED OUTPUT Signal selected should be “Headstage 2, 0.1 $\times$  Membrane Current”.

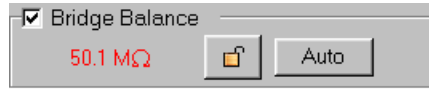


**Figure 37.** HVIC mode. IN0 is the headstage voltage  $\times 0.1$  ( $100 V_{p-p}$ ). IN1 is the membrane current ( $1 V/\mu A$ ). The rise time of the current signal is about 1 ms.

The waveform shapes are similar to normal I-Clamp. However, the speed is slower but compliance is much higher (up to  $\pm 180 V_{p-p}$ ).

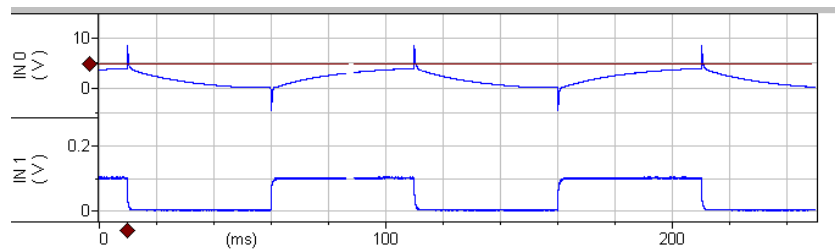
11. If the membrane voltage waveform is less than  $\pm 10 V_{p-p}$  then you can use *Bridge Balance* if the signal selection is “Membrane Potential ( $1 mV/mV$ )”.

12. Reduce the Tuning amplitude to 100 nA.
13. Select “Membrane Potential (1 mV/mV)”.



**Figure 38.** Bridge Balance settings.

14. Check *Bridge Balance* and balance bridge ~50 MΩ.



**Figure 39.** HVIC mode with Bridge Balance.

15. HVIC with Bridge Balance (50.1 MΩ).

The 1 ms spikes in the voltage trace are caused by slower response of HVIC.





## Chapter 4: Guide to Electrophysiological Recording

The purpose of this chapter is to provide practical advice on sharp microelectrode recording with the Axoclamp 900A. It includes both tutorial-style guidance and technical details for reference. This information has been distilled from textbooks on the subject (see References at the end of this manual) and from experienced researchers working in laboratories around the world. However, as is the case for all advice (and particularly that pertaining to research), the suggestions given here should be taken as provisional until they have been tested in your own circumstances.

### General Advice

#### Chamber Design

The tissue chambers used in many in vitro electrophysiological experiments usually have four main requirements:

- A perfusion system for keeping the tissue alive and applying drugs.
- A method for keeping the tissue mechanically stable.
- Optical properties suitable for observing the tissue and positioning electrodes.

- An electrically stable bath (reference) electrode.

## Perfusion

Normally the external solution used in vitro experiments is a pH-buffered salt solution that mimics the extra- or intracellular composition of the cells under study. Sometimes the solution is bubbled with CO<sub>2</sub> (to maintain the pH of bicarbonate-buffered solutions) and/or O<sub>2</sub> (to maintain the metabolic viability of the cells). Some cells (*e.g.*, those in retinal slices) have unusually high metabolic rates and require fast perfusion with high-O<sub>2</sub> solution to remain viable. Other cells (*e.g.*, neurons in dissociated cell culture) may not need any perfusion or bubbling at all. Because the health of the cells is the single most important factor in determining the success of your experiments, it is worth spending some time establishing the optimal conditions for cell survival.

## Mechanical Stability

Sharp microelectrode recordings are not robust in the presence of vibrations, and neither are they tolerant of large drifts in the tissue or electrode that tend to pull the electrode out of the cell. For this reason, it is important to use a good, drift-free micromanipulator for the electrode, and to secure the tissue or cells in the chamber so they cannot move very far. Tissue slices are commonly held in place in the chamber by a weighted “net” or “grid” of fine threads.

A grid is easily made as follows. Bend a piece of 0.2–0.4 mm diameter platinum wire into a ring small enough to fit in the bottom of your chamber, then flatten the wire in a vise. Using a pair of fine forceps, pull a single strand of nylon thread off a ~1 m length of unwaxed nylon dental floss. (It is very wispy but remarkably strong.) Wrap the thread tightly in a spiral around a strip of thin black card about 3 × 10 cm, securing each end with sticky tape. Bending the card slightly, slip the flattened platinum ring under the threads, and adjust its position and the spacing of the threads until the optimal grid pattern is obtained. Finally, add a tiny spot of cyanoacrylate glue to each thread crossing point and, after it is dry, cut the completed grid free.

## Optics

It is difficult to generalize about the optical requirements of the chamber, since the optical technology in use may range from a simple dissection scope to a multiphoton microscope. In general, however, it is probably best to build a chamber with a glass microscope coverslip forming the bottom, to ensure the best possible optical clarity.

## Bath Electrode

The simplest kind of bath electrode is a chlorided silver wire placed in the bath solution. However, if the chloride ion concentration of the bath is altered by perfusion during the experiment, this kind of electrode can introduce serious voltage offset errors. In this case it is essential to use a salt bridge for the bath electrode.

In any case, at the end of every experiment it is good practice to check for drift in electrode offsets. This is easily done by pressing the I=0 button on the Axoclamp 900A Commander. This displays on the meter the pipette voltage required for zero current through the electrode. If, for instance, the meter shows 2 mV, there has been a 2 mV drift since the electrodes were nulled at the beginning of the experiment, and your voltage values may be in error by at least this amount. Large offset errors may indicate that your electrode wires need rechloriding, or a fluid leak has developed in your chamber, causing an electrical short circuit to the microscope.

If you use both headstages on the Axoclamp 900A (*e.g.*, for making simultaneous recordings from pairs of cells) you may wonder whether one or both headstage ground sockets need to be connected to the bath electrode. We have found empirically that the noise in the recordings depends on which headstage is grounded and what mode it is in (V-Clamp or I-Clamp). It is helpful to have a wire connected from each headstage to the bath electrode, with the connection able to be switched off by a toggle switch without bumping the electrode. In this way the best grounding configuration can be established during the experiment.

## Interfacing a Computer

Because the Axoclamp 900A is a computer-controlled instrument, the installation of a computer in your electrophysiology rig is obligatory. The minimum computer configuration requires a USB port for communicating with the Axoclamp 900A. However, in order to make full use of the power and convenience of your computer, it is recommended that you also attach a digitizing interface, such as the Digidata 1440. An interface allows you to generate command signals and save the data in a very flexible manner, without the cost and complexity of a conventional system based on stimulators, digital oscilloscopes, laboratory tape recorders and chart recorders. Digitizing interfaces are typically connected to the computer via a card (*i.e.*, a USB card) that is provided with the interface.

Finally, it is necessary to install software to control the interface. Software is available from Molecular Devices (pCLAMP) or other vendors, or you can write your own. To be able to telegraph to the Axoclamp 900A you need to use pCLAMP 10. The beauty of the Axoclamp package is that you are not tied to any particular PC data acquisition software. Any PC-based software that is able to control the digitizing interface is acceptable, while the Axoclamp 900A Commander runs in the background controlling the Axoclamp 900A.

## Noise

### Measurement of Noise

Noise is reported in two different ways in this manual.

- Peak-to-peak ( $p-p$ ) noise. This measure finds favor because it is easily estimated from an oscilloscope and its meaning is intuitively obvious. A disadvantage is that it is very insensitive to structure in the noise (*e.g.*, different frequency components). For this reason, it is most commonly used for quantifying “white” noise (see *Filters* in this chapter).

- Root-mean-square (*rms*) noise. This is essentially the “standard deviation” of the noise and can be calculated using a computer or an electronic circuit designed for this purpose. For white noise, the rms noise is approximately one-sixth the peak-to-peak noise.

When reporting measured noise, the bandwidth (*i.e.*, filter cutoff frequency) must always be stated.

## Sources of Noise

### *Cell and Seal*

**V-Clamp:** The higher the resistance ( $R$ ) and the smaller the capacitance ( $C$ ) between the interior of the electrode and ground, the smaller the current noise. In whole-cell recordings (smaller  $R$ , larger  $C$ ) the noise of the cell usually dominates, meaning that subsequent noise sources (listed below) become less important.

**I-Clamp:** The voltage noise is dominated by the load resistance but is also affected by the stray capacitance. For a purely resistive load the noise is given approximately by  $12\sqrt{R} \mu V_{\text{rms}}$  (10 kHz bandwidth), where  $R$  is the parallel combination of the current injection resistor ( $R_0$ ) and the load resistance (*i.e.*, the electrode resistance plus input resistance of the cell). Thus, a low resistance electrode/cell combination is preferred. A large stray capacitance will reduce the noise by acting like an RC filter, but this will also reduce the measurement bandwidth. Increasing the Capacitance Neutralization setting will improve the bandwidth but increase the noise.

### *Electrode and Holder*

**V-Clamp:** Current noise increases markedly with electrode capacitance. This can be minimized by coating the electrode, and by other strategies. Increasing electrode resistance apparently decreases the current noise, but this is due to the RC filtering effect of the electrode resistance in parallel with the electrode capacitance. In fact, it is desirable to *decrease* the electrode resistance in order to maximize the bandwidth of the clamp, even if this apparently increases the noise of the recording.

**I-Clamp:** Voltage noise increases markedly with electrode capacitance and resistance. Thus, both should be minimized as much as possible (see *Sharp Microelectrode Recording* in this chapter).

### ***Headstage Circuit***

**V-Clamp:** Current noise decreases as the value of the current injection resistor ( $R_0$ ) is increased. Thus, for minimum noise the largest  $R_0$  should be chosen, subject of course to range limitations.

**I-Clamp:** Voltage noise decreases as the value of  $R_0$  is decreased, but  $R_0$  should be chosen so that it matches the load resistance (*i.e.*, sum of electrode and cell resistance) within a 1:10 ratio (a 1:5 ratio is optimal). Thus,  $R_0 = 50 \text{ M}\Omega$  will work optimally for loads between  $10 \text{ M}\Omega$  and  $250 \text{ M}\Omega$ . This range limitation is determined by the effectiveness of the Capacitance Neutralization circuit.

### ***Compensation Circuits***

**V-Clamp:** Increasing Voltage-Clamp Control Gain is required for optimal voltage control and bandwidth, but can decrease voltage-clamp stability and lead to oscillations that can kill cells. Changing the Lag is used to enable higher gains while preventing oscillation. These changes typically need to be made iteratively for best results.

**I-Clamp:** Increasing Pipette Capacitance Neutralization increases the voltage noise, because the compensation circuit employs positive feedback that injects noise back into the headstage.

### ***Power Supply***

Noise can arise from earth loops, power supply glitches and radiation from nearby instruments (see *Grounding and Hum*, and *Power Supply*).

### ***Computer Noise***

Digital computers can generate considerable electrical noise, both via the power ground and via radiative interference from the monitor. For optimal

noise performance of the Axoclamp 900A, careful attention should be paid to the placement of the computer. For example, the monitor should not be placed immediately above or below the Axoclamp 900A in the instrument rack.

## Bath Error Potentials

In most experiments, the bathing solution is grounded by a solid grounding electrode (such as an agar/KCl bridge) and all measurements are made relative to the system ground (on the assumption that the bath is also at ground). This assumption may not be true if  $\text{Cl}^-$  concentration or temperature of the bathing solution is significantly changed, if there is restricted access from the extracellular space to the grounding point, or if the membrane current is sufficiently large as to cause a significant voltage drop across the resistance of the grounding electrode. The latter circumstance would normally occur only when voltage clamping very large cells such as *Xenopus* oocytes, in which case the ionic current may be of the order of several microamperes or even several tens of microamperes.

Depending upon the method of grounding, the resistance of the bath-grounding electrode ( $R_b$ ) could be as much as 10 k $\Omega$ , although with care it is not difficult to achieve  $R_b$  values less than 1 k $\Omega$ .

In a simple TEVC setup, the voltage drop across  $R_b$  is indistinguishable from the membrane potential. That is, the potential recorded by the voltage-recording micropipette ( $V_1$ ) is the sum of the transmembrane potential ( $V_m$ ) and the bath potential ( $V_b$ ). Problems arise if the product of the clamp current ( $I_2$ ) and  $R_b$  is significant. For example, for  $I_2 = 5 \mu\text{A}$  and  $R_b = 2 \text{k}\Omega$ , the voltage error is 10 mV. In some experiments, a worst-case error of this magnitude might be tolerable, but if the error were to be much greater, the position of I-V curves and other responses would be seriously affected.

To record  $V_m$  faithfully, either  $V_b$  must be made equal to or nearly equal to zero, or the value of  $V_b$  must be independently measured and subtracted from the potential recorded by CHANNEL 1.

The following four procedures to minimize the effect of errors introduced by  $R_b$  are listed in the preferred order of implementation. Please see the summary at the end of this section.

### (1) Minimize $R_b$

Steps should always be taken to minimize  $R_b$ . There are three main contributors to  $R_b$ :

1. The cell access resistance from the membrane surface to the bath.
2. The resistance of the grounding pellet.
3. The resistance of the agar bridge (if used).

Typical values of the access resistance of a 1 mm diameter sphere in Ringer's solution (such as an oocyte) are on the order of 150–200  $\Omega$ . This is a given, and no amount of manipulation can alter this for a given set of experimental conditions; fortunately it is relatively small. On the other hand, the resistance of the grounding pellet and agar bridge are larger, but one can take precautions to minimize them. A 1 mm diameter Ag/AgCl pellet in Ringer's solution has a resistance of 300–600  $\Omega$ , depending on how much of the surface is in contact with the saline. The larger the surface area in contact with the saline, the smaller the resistance.

The resistance of an agar bridge depends on the length and diameter of the bridge, as well as what is inside (*i.e.*, Ringer's solution vs. 3 M KCl). For a 1 cm long bridge:

	<b>1 mm diameter</b>	<b>2 mm diameter</b>
<b>Ringer's</b>	10.2 k $\Omega$	2.6 k $\Omega$
<b>3 M KCl</b>	510 $\Omega$	130 $\Omega$

Therefore, to minimize  $R_b$ , it would be best to eliminate the agar bridge and ground the preparation directly with a Ag/AgCl pellet. The pellet should be as large as practical, and the area of it in contact with the solution should be maximized. However, if the bathing solution is changed during the



experiment, the DC offset of the Ag/AgCl pellet will change with the chloride activity. In these cases, it is essential to use an agar bridge to prevent the DC offset of the bath from changing. Another advantage of an agar bridge is that it prevents metal ions from the grounding electrode from entering the bathing solution.

In order to minimize  $R_b$  when using an agar bridge, it is best to fill the bridge with 3 M KCl instead of Ringer's solution. When the agar bridge is filled with 3 M KCl, the sum of all components of  $R_b$  is approximately 1–2 k $\Omega$ . If leakage of KCl from the agar bridge is a problem, it may be necessary to fill the agar bridge with Ringer. In this case,  $R_b$  is several kilohms.

One can actually measure  $R_b$  in Bridge mode by placing both micropipettes in the bath. Put the micropipette connected to CHANNEL 1 on the far side of the micropipette connected to CHANNEL 2, away from the bath electrode. A 100 Hz, 10 V<sub>p-p</sub> square wave command delivered to the EXT. CHANNEL 2 COMMAND input produces a 1  $\mu$ A<sub>p-p</sub> current. The response measured with the micropipette connected to CHANNEL 1 is 1 mV/k $\Omega$ . If the product of the measured bath resistance and the magnitude of the expected current is such that significant voltage errors may result, then it is necessary to clamp the bath potential to zero using a virtual ground circuit, as described below.

## (2) Clamp $V_b$ Using a Bath Clamp or Virtual Ground

Another means to eliminate the effect of the voltage drop across  $R_b$ , or minimize  $V_b$ , is to actively control the bath potential, measured near the outside surface of the cell. This is achieved using a two-electrode virtual-ground circuit:

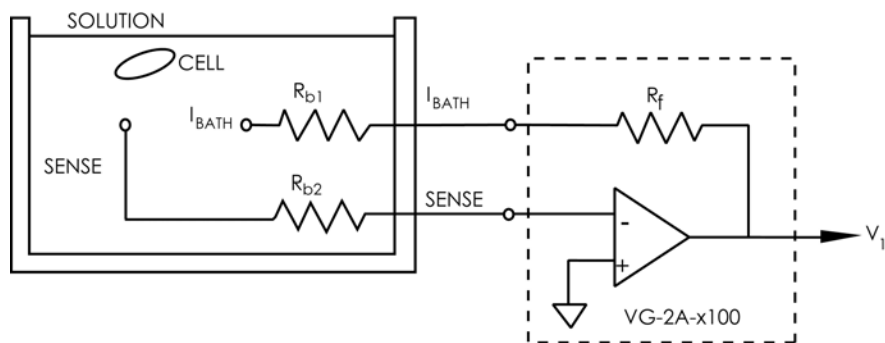


Figure 40. Bath clamp.

One electrode (SENSE) is a voltage-sensing electrode. It is placed in the bath near the cell surface. It is connected to the virtual-ground circuit by an agar bridge, or other means, of resistance  $R_{b2}$ . Since there is no current flowing through this electrode, there is no voltage drop across  $R_{b2}$ . The other electrode ( $I_{BATH}$ ), with resistance  $R_{b1}$ , is also placed in the bath. This electrode carries the ionic current. The feedback action of the operational amplifier ensures that the potential at the SENSE electrode is equal to the potential at the positive input; *i.e.*, 0 mV, irrespective of the voltage drop across  $R_{b1}$ .

This configuration is particularly recommended for voltage clamping oocytes. The VG-9A  $\times 100$  bath clamp is required for the fastest responses and guaranteed stability. Connect this headstage to the AUXILIARY 1 HEADSTAGE input.

### (3) Independently Measure $V_b$ and Subtract it from the CHANNEL 1 Potential

When an HS-9A headstage is connected to the rear-panel AUXILIARY 1 HEADSTAGE connector, measurements by both CHANNEL 1 and CHANNEL 2 are automatically made relative to the potential recorded by this headstage. Therefore  $V_m$  is unaffected by any changes in  $V_b$  produced by line-frequency pickup or by changes in the temperature or ionic composition of the bath solution. If  $V_b$  is not measured the system automatically reverts to using the system ground as the reference potential.

Any of the HS-9A series headstages can be used to record  $V_b$ . Headstage gains of  $\times 10$ ,  $\times 1$  and  $\times 0.1$  are suitable. The  $\times 10$  headstage can also be used, although at the risk of slightly greater high frequency noise.

A broken 3 M KCl-filled microelectrode or a blunt patch pipette filled with the normal external solution works well as an extracellular bath microelectrode. During recording it is positioned close to the cell, and is shielded from the current-passing electrode. The bath microelectrode cannot be used for current passing.

### *Grounding*

Irrespective of whether or not the bath potential is measured, the preparation bath should be grounded by directly connecting it to the yellow ground connector on the back of the CHANNEL 1 headstage (or to a virtual-ground headstage if used).

### *Bandwidth of $V_b$ Measurement*

The  $V_b$  bandwidth is internally filtered to a  $-3$  dB cutoff of 300 Hz before it is fed back and subtracted from the measurements made by CHANNEL 1 and CHANNEL 2. The voltage recorded by the bath microelectrode is available at the SCALED OUTPUT signal BNC on the front panel. You can select these auxiliary signals instead of the main headstage signals in the Commander software.

To be effective in compensating for  $R_s$  errors the frequency response of a bath voltage electrode should match the voltage recording electrode frequency response. At the same time it is most important for the bath voltage signal to be appropriately attenuated at high frequencies, since this signal is not inverted and, thus, presents a positive feedback pathway that can potentially destabilize the clamp. For optimum voltage-clamp performance, the bandwidth of the bath potential is limited to 300 Hz before it is subtracted from the potentials recorded by CHANNEL 1 and CHANNEL 2 (see Finkel & Gage, 1985). Therefore  $V_b$  is effective for series resistance compensation only up to frequencies of 300 Hz. This may not be sufficient for some purposes. In this case actively clamping the bath potential is a superior alternative method for  $R_p$  compensation.

## Summary

In summary, we recommend that in experiments where the voltage-clamp current is substantial you should take steps to minimize the value of  $R_b$  by appropriately selecting the bath electrodes. If this is insufficient, actively clamp the bath potential ( $V_b$ ) using a bath-clamp or virtual-ground headstage. Finally, if for some reason this approach is inappropriate, consider using an HS-2 or HS-9 series headstage to measure and subtract  $V_b$  from the recording pathway.

## Headstages

### HS-9A Series

HS-9A and VG-9A series headstages are the standard headstages used for the Axoclamp 900A and include HS-9A  $\times 0.1$ , HS-9A  $\times 1$ , HS-9A  $\times 10$ , VG-9A  $\times 10$ , and VG-9A  $\times 100$ . Two headstages come standard with each Axoclamp 900A amplifier: HS-9A  $\times 0.1$  and HS-9A  $\times 1$ . Other headstages may be substituted on request at the time of purchase.

All headstages record voltage at unity gain. Several headstage current gains (H) are available. Axoclamp 900A Commander Scaled Outputs indicate the scaling of signals from each headstage, including the output gain. All H values are powers of 10. Small H values are used with high-resistance cells and electrodes. Large H values are used to pass large currents.

H =  $\times 10$ ,  $\times 1$ ,  $\times 0.1$  are for recording and clamping.

### VG-9A Series

The virtual ground headstages are optional and are not required for normal operation. Virtual-ground headstages measure the total bath current and are recommended for two-electrode voltage clamp. They are also useful in clamping the bath when there may be changes in the bath potential caused by changes in temperature or ion concentration in the bath (the VG-9A  $\times 100$

is recommended if this is the only intended use). Virtual Ground output attenuation (VG) specifies the sensitivity. The smaller VG is more sensitive and it is used for low currents.

## Current Clamp Circuit

In I-Clamp mode a separate headstage circuit is used, called a voltage follower. The essential features of a voltage follower are shown in Figure 41. A1 is an (effectively) infinite input resistance, unity-gain op amp, the output of which is the pipette voltage,  $V_p$ . A2 is a summing amplifier used for injecting current into the cell. The voltage across the headstage resistor  $R_0$  is equal to  $V_{cmd}$  regardless of  $V_p$ . Thus the current through  $R_0$  is given exactly by  $I = V_{cmd} / R_0$ . If stray capacitances are ignored, all of this current is injected into the cell.

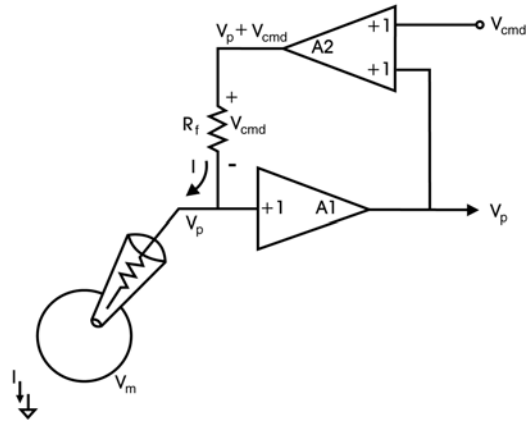


Figure 41. Voltage follower headstage.

**Note:**  $V_{cmd}$  is limited to 12 V in the Axoclamp 900A, which in turn limits the maximum amount of current that can be injected through the headstage resistor into the electrode. For example, with  $R_0 = 100 \text{ M}\Omega$ , the maximum current that can be injected is  $12 \text{ V}/100 \text{ M}\Omega = 120 \text{ nA}$ . These current limits are listed in Table 1, page 74.

## Mounting the Headstage

For maximum mechanical rigidity, the HS-9A headstage should be mounted directly to the head of the micromanipulator using the dovetailed mounting plate.

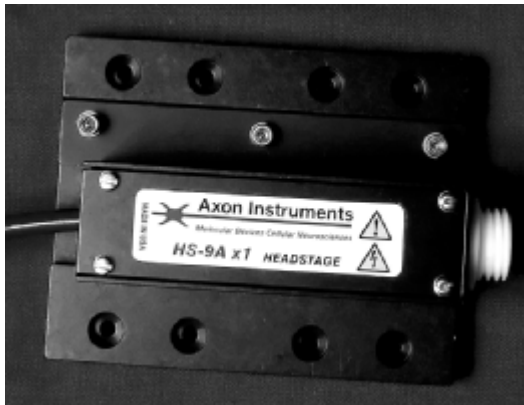


Figure 42. HS-9A headstage attached to a mounting plate.

## Bath Connection

The bath (or ground) electrode should be connected to the gold-plated 1 mm plug on the rear of the headstage. The bath should not contact any other ground (*e.g.*, Signal Ground).

## Static Precautions

The headstage can normally be safely handled. However, if you are in a laboratory where static is high (*i.e.*, you hear and feel crackles when you touch things) you should touch a grounded metal object immediately before touching the headstage.

## Acoustic Pick-up

Rare cases have been reported in which the headstage was susceptible to low amplitude acoustic pick-up. For example, the audible hum of a nearby

isolation transformer can acoustically couple to the input of the headstage. This was traced to the silver wire of the electrode and was solved by trimming off a fraction of the wire, thus changing its resonant frequency.

## Compatibility of the HS-2 and HS-2A with the Axoclamp 900A

HS-2 and HS-2A headstages can be used with the Axoclamp 900A, but they need to be connected using an adapter cable. Once this is done the Axoclamp 900A Commander recognizes the headstage as a custom type and prompts for current injection resistance and capacitance values. This message appears on startup of the Commander.

Once a custom headstage is connected correctly the Commander can be shut down and restarted without prompting for values again. To change a custom headstage from one type to another the user can unplug and re-plug the cable from the back of the unit.

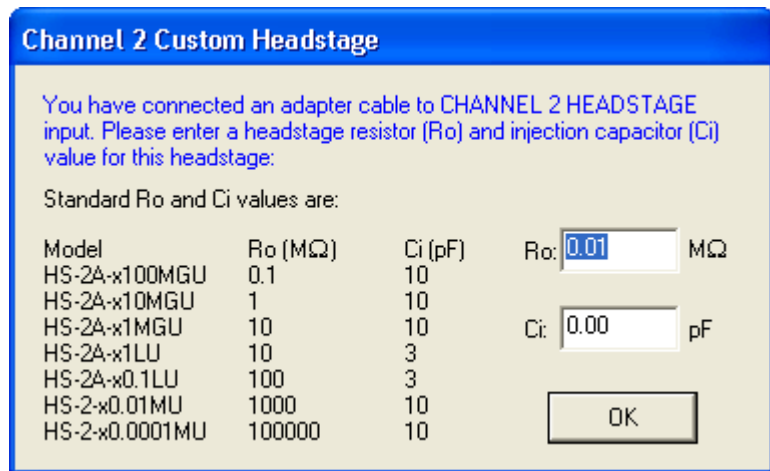


Figure 43. Custom headstage dialog.

## General Characteristics

The HS-9A headstage buffers the high impedance of the microelectrode, making the potential recorded by the microelectrode available to the rest of

the circuitry. It also provides the means for injecting current into the microelectrode and for neutralizing the input capacitance.

Current in each microelectrode is continuously measured during both voltage clamp and current clamp. This measurement does not include currents from sources other than the microelectrode (*e.g.*, hum, iontophoresis, the other microelectrode) and indicates zero if the microelectrode blocks.

## The Meaning of H

A precision resistor ( $R_0$ ) inside the headstage sets the headstage current gain (H). The particular value of H used affects the Bridge range, the sensitivity to current commands, the sensitivity of the current monitors and the gain in SEVC mode. Since the effects always appear in multiples of ten, they are easy to calculate. For your convenience, Table 1 summarizes these effects. Note that voltage commands during voltage clamp and recorded voltages are not affected by the headstage current gain value.

<b>H</b>	<b>×10</b>	<b>×1</b>	<b>×0.1</b>
$R_0$	1 MΩ	10 MΩ	100 MΩ
Max. Bridge Balance	8 MΩ	80 MΩ	800 MΩ
Max. Step Command	±10,000 nA	±1000 nA	±100 nA
Max. DC Current Command	±1000 nA	±100 nA	±10 nA
Ext. Command	100 nA/V	10 nA/V	1 nA/V
Max Total Current <sup>1</sup>	12 μA	1.2 μA	0.12 μA
I Output	1 mV/nA	10 mV/nA	100 mV/nA
Max. Gain in dSEVC	3000 nA/mV	300 nA/mV	30 nA/mV
Max. Gain in TEVC	50000	50000	50000

**Table 1.** How H affects control and measurement range.

<sup>1</sup> Assumes that electrode resistance is negligible.



## Guidelines for Selecting a Headstage

The H value required depends on the typical input resistances,  $R_{in}$ , of your cells. The recommended values given in Table 2 are for micropipettes. When patch pipettes are used,  $H = 0.1$  should be selected as a matter of course.

Some overlap in these recommendations is allowable. The guiding principles are these:

$H = \times 10$	for 200 k $\Omega$	$< R_{in}$	$< 5$
$H = \times 1$	for 2 M $\Omega$	$< R_{in}$	$< 50$
$H = \times 0.1$	for 20 M $\Omega$	$< R_{in}$	$< 500$

**Table 2.** Recommended H values for various cell input resistances.

1. For maximum sampling rates in dSEVC and DCC modes use the largest feasible H value. (This is because the current-passing response is best with low values of  $R_0$ .)
2. A limitation on using large H values is that as  $R_0$  becomes smaller the input leakage current of the headstage becomes more prone to increase with time and temperature (see the discussion of *Input Leakage Current* later in this section).
3. A further limitation on using large H values is that if  $R_0$  is less than the microelectrode resistance,  $R_e$ , the high-frequency noise is worse.
4. The H value sets the current-passing sensitivity in Bridge and DCC modes and the Gain in SEVC modes. Hence it should be chosen for sensitivities suitable for your cell. These sensitivities are listed in Table 2 above.
5. If  $R_e \gg R_{in}$  a smaller H value should be favored.

### *General Rule for Choosing a Headstage*

The general rule for intracellular microelectrode recording is that the resistor in the headstage ( $R_0$ ) should be between 1/3 and 3 times the value of the electrode resistance,  $R_e$ . If the  $R_0$  value is less than 1/3  $R_e$ , the wideband noise will increase and the headstage leakage current might cause DC errors. If the  $R_0$  value is greater than 3 times  $R_e$ , the voltage drop across  $R_0$  during current passing might be excessive and the bandwidth might be compromised.

If the value of  $R_0$  is higher or lower than the recommended range, there is a gradual deterioration in performance. There is no catastrophic diminishment of performance, and in many instances, the user will be blessed with adequate performance even if  $R_0$  is as small as 1/10 or as large as  $\times 10 R_e$ .

<b>USE</b>	<b>RECOMMENDATION</b>
NORMAL INTRACELLULAR (BRIDGE) RECORDING	Follow the general rule.
EXTRACELLULAR RECORDING	Since there is no current passing, there is no upper limit on $R_{in}$ . For example, the $\times 1$ and $\times 0.1$ headstages are equally suitable for a 1 M $\Omega$ extracellular electrode.
dSEVC and DCC	$\times 1$ and $\times 0.1$ give the best dynamic performance.
TEVC	Usually use the largest reasonable H value for the current-passing microelectrode (CHANNEL 2) and the lowest reasonable H value for the voltage-recording microelectrode (CHANNEL 1). For example, use $\times 10$ or $\times 1$ for CHANNEL 2 and $\times 1$ or $\times 0.1$ for CHANNEL 1.
IONTOPHORESIS	To electrically eject charged molecules out the microelectrode tip into the preparation, it is best to use low resistance microelectrodes and high H values. $\times 1$ is the best general-purpose headstage for iontophoresis of drugs or dyes.

**Table 3.** Recommended headstages for different applications.

## Headstage Connection

The threaded white socket is the microelectrode input. The connection between the microelectrode and this socket should be kept as short as possible.

### *Ground Output Connector (Gold)*

The gold ground socket of the CHANNEL 1 headstage is the signal ground and is used for grounding the preparation. Using this connection as the preparation ground minimizes hum.

## Interchangeability

Any unity-gain headstage in the HS-9A series can be used for CHANNEL 1 or CHANNEL 2. The headstages should not be exchanged while the Axoclamp 900A is switched on.

## Cleaning

To clean salt spills from the input connectors wipe with a damp cloth. Avoid spilling liquids on the headstage.

## Input Leakage Current

All DC-connected systems suffer from the problem of drift. With changes in temperature and the passage of time the DC transfer functions of all semiconductor devices can drift by many millivolts away from their initial values. The major worry in a microelectrode system is that the cumulative effects of drift in various parts of the circuit may lead to the development of a DC offset across the resistor ( $R_0$ ) used to set the H value. As a result, an undesirable DC leakage current is injected into the microelectrode.

Careful consideration of this problem has been applied throughout the design of the Axoclamp 900A and the overall DC offset has been made as insensitive as possible to the drift in the integrated circuits. As well, special low-drift integrated circuits have been used in all critical positions. The

magnitude of the DC leakage current increases with H. This normally introduces no greater error in the DC offset voltage developed across the microelectrode or the cell membrane because larger H's are usually used with lower-resistance cells and microelectrodes.

## **Input Resistance**

The input resistance of the headstages is predominantly related to  $R_0$ . A circuit inside the Axoclamp 900A called a constant current source (CCS) controls the voltage across  $R_0$ . Ideally, the voltage across  $R_0$  is independent of the microelectrode voltage. The accuracy of the CCS in controlling the voltage across  $R_0$  is preset at the factory. Extremely stable components are used in the CCS so that the accuracy will not deteriorate with time. In general the CCS is effective to one part in  $10^4$  so that the input resistance is  $R_0 \times 10^4$ .

Other possible factors which would decrease the input resistance are minimized. For example, the field effect transistor (FET) input of the headstage is referenced to the input voltage rather than to ground. This technique is known as bootstrapping. Thus the effective resistance of the input is much greater than the already high resistance of the FET. Leakage current and resistive loading through the insulation of the input socket are minimized by using Teflon insulation and by driving the case with the DC input voltage.

## **Holders**

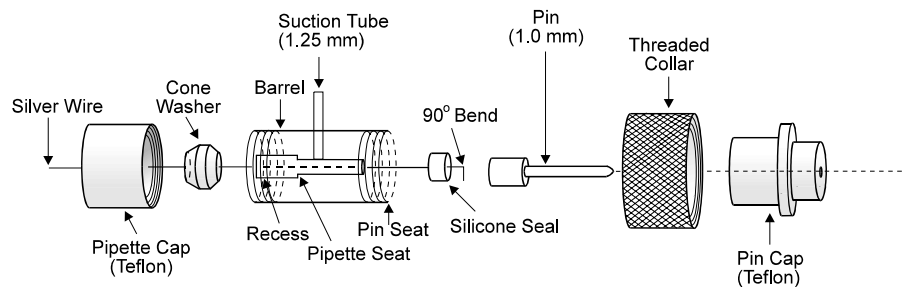
The HL-U series holder provides a universal fit for a very wide range of electrode diameters and will fit any of the U-type headstages of Molecular Devices amplifiers, including the HS-9A and VG-9A headstages.

### **Holder Design**

The barrel of the holder is made of polycarbonate for lowest noise. There are two different barrel lengths (16 mm and 28 mm). The shorter length contributes less to instrument noise and is therefore suited to single-channel patch clamp recordings. Although the longer barrel will contribute

more to the noise, the greater length may provide the needed clearance between the headstage case and other components in the experimental setup. To further minimize the noise contributed by the holder in single-channel recording, the holder uses a small (1 mm) pin for the electrical connection and a large amount of insulating Teflon.

Mechanical stability of the electrode is assured in several ways (see Figure 44). As the pipette cap is closed, the cone washer is compressed on the electrode from the force applied to the front and back of the cone washer. The cap also forces the blunt end of the electrode against the rear wall of the holder bore. (The electrode should always be inserted as far as it will go in the holder.) The holder mates with the threaded Teflon connector on U-type Molecular Devices headstages and is secured in place with a threaded collar.



**Figure 44.** Exploded view of the HL-U holder.

The bore size of the HL-U accepts pipettes with an outer diameter (OD) of 1.0–1.7 mm. Pipettes are secured by a cone washer with an inner diameter (ID) that accommodates the pipette OD. Color-coding aids identification of the four sizes of cone washers: 1.0 mm (orange), 1.3 mm (clear), 1.5 mm (orange) and 1.7 mm (clear). When the pipette OD falls between two sizes of cone washers, the larger size cone washer should be used. For instance, if the pipette OD is 1.6 mm, then use a cone washer with an ID of 1.7 mm.

An Ag/AgCl pellet offers no greater stability than properly chlorided silver wire. Moreover, the diameter of the pellet (1 mm) restricts its use to pipettes with a large ID (> 1.1 mm). Therefore, the HL-U is supplied with 0.25 mm silver wire, which must be chlorided before use (see below).

Spare components included with each holder are: one 50 mm length of silver wire, 40 cone washers (10 of each size), and one 70 mm length of silicone tubing. Cut into 2 mm lengths, the silicone tubing will yield approximately 30 replacement silicone seals. Additional cone washers, silicone tubing, pins and silver wire can be purchased from Molecular Devices, as well as optional Ag/AgCl pellet assemblies.

## Optional Ag/AgCl Pellets

The HL-U holder will accommodate a 1 mm diameter Ag/AgCl pellet that should provide many months of DC-stable recordings. The inner diameter (ID) of the pipette must be > 1 mm. A wax-sealed Teflon tube surrounds the silver wire. This ensures that the electrode solution only contacts the Ag/AgCl pellet. Three pellet assemblies are sold as HLA-003.

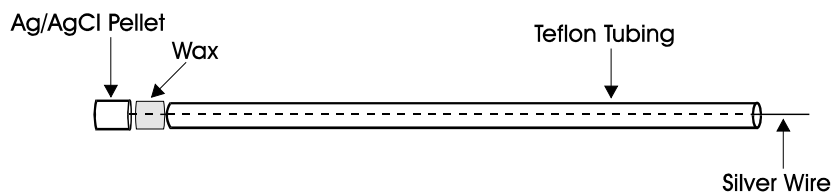


Figure 45. Ag/AgCl pellet assembly.

## Holder Use

### *Insertion of Electrode*

Make sure the electrode cap is loosened so that pressure on the cone washer is relieved, but do not remove the cap. Push the back end of the electrode through the cap and cone washer until it presses against the end of the bore. Gently tighten the cap so that the electrode is gripped firmly.

To minimize cutting of the cone washer by the sharp back end of the electrode, you can slightly smooth the edges by rotating the ends of the electrode glass in a Bunsen burner flame prior to pulling.

### *Filling Electrodes*

Only the taper and a few millimeters of the shaft of the pipette should be filled with solution. The chlorided tip of the wire should be inserted into this solution. Avoid wetting the holder since this will increase the noise.

### *Silver Chloriding*

It is up to you to chloride the end of this wire as required. Chloriding procedures are contained in many electrophysiology texts. For easy-to-use recipes see *Microelectrode Methods for Intracellular Recording and Iontophoresis* by Purves (page 51) and *The Axon Guide* (page 83). Typically the chlorided wire will need to be replaced or rechlorided every few weeks. A simple yet effective chloriding procedure is to clean the silver wire down to the bare metal using fine sand paper and immerse the cleaned wire in bleach for about 20 minutes, until the wire is uniformly blackened. This provides a sufficient coat of AgCl to work reliably for several weeks. Drifting or otherwise unstable offsets during experiments is suggestive of the need for rechloriding. The chlorided region should be long enough so that the electrode solution does not come in contact with the bare silver wire.

Heat smoothing the back end of the electrode extends the life of the chloride coating by minimizing the amount of scratch damage. Another way to protect the AgCl coating is to slip a perforated Teflon tube over the chlorided region.

## **Holder Maintenance**

### *Cleaning*

For lowest noise, keep the holder clean. Frequently rinse the holder with distilled water. If more thorough cleaning is required, briefly wash in ethanol or mild soapy water. Never use methanol or strong solvents.

### *Replacing the Silver Wire*

To replace the silver wire, insert the nonchlorided end through the hole of the silicone seal and bend the last 1 mm of wire over to an angle of 90°. Press the wire into the back of the barrel making sure that the silicone seal is flush with the back of the barrel. Slip the threaded collar over the back of the barrel.

## **Sharp Microelectrode Recording**

The HS-9A headstage of the Axoclamp 900A contains a voltage-follower circuit that is activated when I=0 or IC (I-Clamp) mode is selected. Although the I-Clamp circuit is designed to be used with high-resistance sharp microelectrodes, it can also be used with lower-resistance patch electrodes, which in some cases offer advantages. In this chapter it is assumed for the most part that sharp microelectrodes are being used for the I-Clamp recording.

### **Microelectrode Properties**

Users of sharp microelectrodes spend far more time than patch clampers worrying about the properties of their electrodes. This is because the higher resistance of sharp microelectrodes may introduce a number of undesirable properties. For best results, the microelectrode voltage must settle rapidly after a current pulse, and the microelectrode must be able to pass current without large changes in resistance.

The important factors that need to be considered are discussed below.

#### *Electrode Glass*

Borosilicate glass is often used; however, through trial and error one type of glass supplied by a specific glass manufacturer may have been shown to yield the best results. It is suggested that the literature be consulted prior to selecting glass for recording.



***Tip Resistance***

Tip resistance ( $R_e$ ) should be as low as possible and consistent with good impalements of the cell. Low values of  $R_e$  allow for greater stability and faster settling time of the microelectrode.

**Stability**

$R_e$  of most microelectrodes changes with time and with current passing.  $R_e$  is affected not only by the magnitude of the current but also by its polarity. In general, microelectrodes of lower resistance are more stable during current passing than those of higher resistance.

**Settling time**

The decay time constant of the microelectrode voltage after a current pulse depends strongly on  $R_e$ . Thus, lower  $R_e$  values produce faster settling times. As well, high  $R_e$  values are sometimes associated with a slow final decay even after the electrode capacitance has been eliminated.

***Microelectrode Capacitance***

The settling time of a microelectrode depends not only on  $R_e$  but also on the transmural capacitance ( $C_t$ ) from the inside of the microelectrode to the external solution. For fastest settling,  $C_t$  must be as small as possible.  $C_t$  is usually 1–2 pF per mm of immersion. In order to reduce the effect of  $C_t$ , two approaches may be taken. One is to electronically compensate  $C_t$  using the Pipette Capacitance Neutralization control in the Axoclamp 900A Commander. This is discussed below, in the section on “Impaling Cells”. The other approach is to minimize the problem by careful experimental design, as follows.

In an isolated preparation, lowering the surface of the solution as far as possible can reduce  $C_t$ . For a long slender microelectrode, 200  $\mu\text{m}$  or less is regarded as a low solution level; 500  $\mu\text{m}$  is tolerable. Deep is regarded as 1 mm or more. For a microelectrode that tapers steeply (*i.e.*, a stubby microelectrode) deeper solutions can be used with less loss of performance. When working with very low solution levels there is a risk of evaporation

exposing the cells to the air unless a continuous flow of solution is provided across or through the preparation. If evaporation is a problem, try floating a layer of mineral oil on the surface of the solution. If used, this layer of oil has the additional advantage of automatically coating the microelectrode as it is lowered into the solution.

Precautions must be taken to prevent surface tension effects from drawing a thin layer of solution up the outer wall of the microelectrode. If this film of saline is allowed to develop,  $C_t$  will increase substantially. Because the film of saline has axial resistance the contribution to  $C_t$  will be very nonlinear, and the voltage decay after a current pulse will either be biphasic or slow, even when capacitance neutralization is used. To prevent the saline film from developing, the microelectrode should be coated with a hydrophobic material. This can be done just before use by dipping the filled microelectrode into a fluid such as silicone oil or mineral oil. Another method is to coat the microelectrode with Sylgard #184 or Q-dope (model airplane glue). The selected material should be painted onto the electrode to within 100  $\mu\text{m}$  of the tip.

### ***Tip Potentials***

During the passage of current, a slowly changing voltage may be generated at the tip of a microelectrode. Changes in this tip potential are indistinguishable from changes in the membrane potential and can therefore be a serious source of error.

### **Identifying Tip Potentials**

- While the microelectrode is outside the cell, press the *Pipette Offset* button to zero the offset. In IC mode, pass a constant current into the bath for about 10 seconds; this can be done by setting a Holding current in the Axoclamp 900A Commander and checking the *Holding* checkbox. The current magnitude should be the same as the maximum sustained current likely to be passed during the experiment. When the current is switched off the recorded potential should return to zero within a few milliseconds at most. Some

microelectrodes either return very slowly to zero potential, or not at all. These micropipettes should be discarded.

- While the experiment is in progress, occasionally check the resistance of the microelectrode. Changes in tip potential are usually accompanied by changes in microelectrode resistance.

### **Preventing Tip Potentials**

Not much can be done to prevent tip potentials from changing but the following may be helpful.

- Sometimes the slow changes in tip potentials are worse when a AgCl pellet is used instead of a Ag/AgCl wire. Some holders are acceptable while other, ostensibly identical, holders are not. Therefore holders should be tested and selected.
- The variability of the tip potentials may be related to pressure developed when the microelectrode is pressed into an unvented holder. The suction port on the HL-U series holders provided with the Axoclamp 900A should therefore be left open.
- Using filling solutions with low pH, or adding small concentrations of polyvalent cations like  $\text{Th}^{4+}$ , may reduce the size of the tip potential and therefore the magnitude of any changes (Purves, 1981).

### **Filling Solutions**

The best filling solution to use depends on the preparation under investigation and the experience of the investigator. Although KCl gives one of the lowest tip resistances for a given tip diameter, a KCl-filled electrode is not necessarily the fastest to settle after a current pulse; K-citrate is sometimes faster.

It is important to be aware that during current passing, large amounts of ions from inside the microelectrode can be iontophoresed into the cell. For example, if current is passed by the flow of ion species A from the microelectrode into the cell, then after 50 seconds of current at 1 nA (or 1 s

of current at 50 nA) the change in concentration of A inside a cell 100  $\mu\text{m}$  in diameter is 1 mM. If A is an impermeant ion, the cell may swell due to the inflow of water to balance the osmotic pressure. The injection of a permeant ion, such as chloride, can significantly alter the equilibrium potential for that ion.

## Impaling Cells

Start with the Axoclamp 900A in IC mode (I-Clamp):

- Fill a microelectrode with internal solution and secure it firmly in the pipette holder.
- Be sure to support the headstage with your other hand so that the micromanipulator does not have to absorb your force.
- Advance the electrode until its tip enters the bath.
- Press the *Pipette Offset* button to null the offset.
- Check the stability of the bath electrode and microelectrode. Drifts in Scaled Output: Membrane Potential indicates that the electrode wires probably need to be rechlorided. Also check for a changing tip potential by passing a steady current, as described above.
- Check the *Tuning* checkbox and observe the Scaled Output: Membrane Potential on a scope.
- Move the electrode tip close to where cells are likely to be encountered, and then increase *Pipette Capacitance Neutralization* in the Axoclamp 900A Commander to give the fastest step response. It is advisable to adjust the capacitance neutralization with the microelectrode as close as possible to the final position, since moving the electrode can change  $C_t$  and invalidate the setting. It may be wise to slightly under-compensate, because changes in the solution level could lead to oscillations that may destroy the cell.

- Press the *Bridge Balance* button. The value (M $\Omega$ ) found for optimal balance gives the resistance of the electrode; see Chapter 5, *Bridge Balance*, for further details.
- Sometimes the cell is impaled as soon as the microelectrode is pressed against the cell surface. More often the microelectrode is advanced until there is a slight deflection in the tip potential. At this point the cell can be impaled by pressing the Buzz button or the *Clear +/Clear -* buttons. If these fail, vibrating the microelectrode tip by lightly tapping on the micromanipulator sometimes works. When the electrode penetrates the cell there is a sudden change in the Membrane Potential trace, reflecting the intracellular potential. The voltage response to the Tuning steps will be slower and much larger, reflecting the membrane time constant and input resistance.
- After impaling the cell, it is often helpful to back off the microelectrode slightly and allow the penetration to stabilize for a few minutes. For some cells it may help to apply a small DC current to the electrode (enough to produce several mV hyperpolarization) during the penetration. Selecting the *Holding* checkbox and slowly increasing the Holding value can apply this DC current.
- Once the penetration has stabilized, you should recheck the Bridge Balance and Pipette Capacitance Neutralization. Further details on this are given in Chapter 5. It is sometimes useful to inject a small, brief current pulse at the start of each sweep of data collection in order to continually check the Bridge Balance setting during the course of an experiment.

## Theory of Recording Modes

### Current-Clamp Mode

In I-Clamp mode the microelectrode voltages are monitored continuously, and continuous currents can be injected down CHANNEL 1 or CHANNEL 2.

Associated with the current flow ( $I$ ) in a microelectrode is a voltage drop across the microelectrode that depends on the product of the current and the microelectrode resistance ( $R_e$ ). This unwanted IR voltage drop adds to the recorded potential. The Bridge Balance control can be used to balance out this voltage drop so that only membrane potential is recorded. The term “Bridge” refers to the original Wheatstone Bridge circuit used to balance the IR voltage drop and is retained by convention.

The particular setting required to balance the Bridge is a measure of the microelectrode resistance, and the microelectrode resistance can be read from the Axoclamp 900A Commander.

## Discontinuous Current Clamp (DCC) Mode

In Discontinuous Current Clamp (DCC) mode, CHANNEL 1 is cyclically used to pass current. The voltage recorded at the tip of CHANNEL 1 is memorized by a sample-and-hold circuit in between each current-passing period after all transient voltages due to current passing have decayed. Thus the membrane potential can be recorded independently of the IR voltage drop across the microelectrode.

The advantage of DCC mode compared with I-Clamp mode is that it is tolerant of small changes in microelectrode resistance. The disadvantage is that DCC mode is noisier than I-Clamp mode. During DCC mode CHANNEL 2 is still in I-Clamp mode and can be used for continuous current passing.

The principles of operation are outlined in the block diagram and timing diagram of Figure 46.

The voltage recorded by the microelectrode ( $V_1$ ) is buffered by a unity-gain head stage (A1). Assume that  $V_1$  is exactly equal to the instantaneous membrane potential ( $V_m$ ). Switch S2 briefly closes thereby enabling the voltage on the holding capacitor ( $C_H$ ) to charge up to the value of  $V_m$ . S2 opens again after the “sample” period and  $V_m$  is held by  $C_H$ . A buffer amplifier (A2) interfaces  $C_H$  to the recording apparatus. This switch,

capacitor and buffer amplifier arrangement constitute an analog memory known as a sample-and-hold amplifier.

Immediately after the sample period, the current injection period begins when switch S1 changes over from the zero volts position to the current-command voltage ( $V_{cmd}$ ) position. This connects  $V_{cmd}$  to a differential amplifier (A4) arranged so that its output is  $V_1 + V_{cmd}$ . The voltage appearing across  $R_0$  is exactly equal to  $V_{cmd}$  thereby forcing the current ( $I_0$ ) into the microelectrode to be equal to  $V_{cmd} / R_0$ . Amplifiers A4 and A1 and resistor  $R_0$  constitute a controlled-current source (CCS) which injects a current into the microelectrode directly proportional to the voltage at the input of the CCS irrespective of the resistance of the microelectrode or the voltage at its tip.

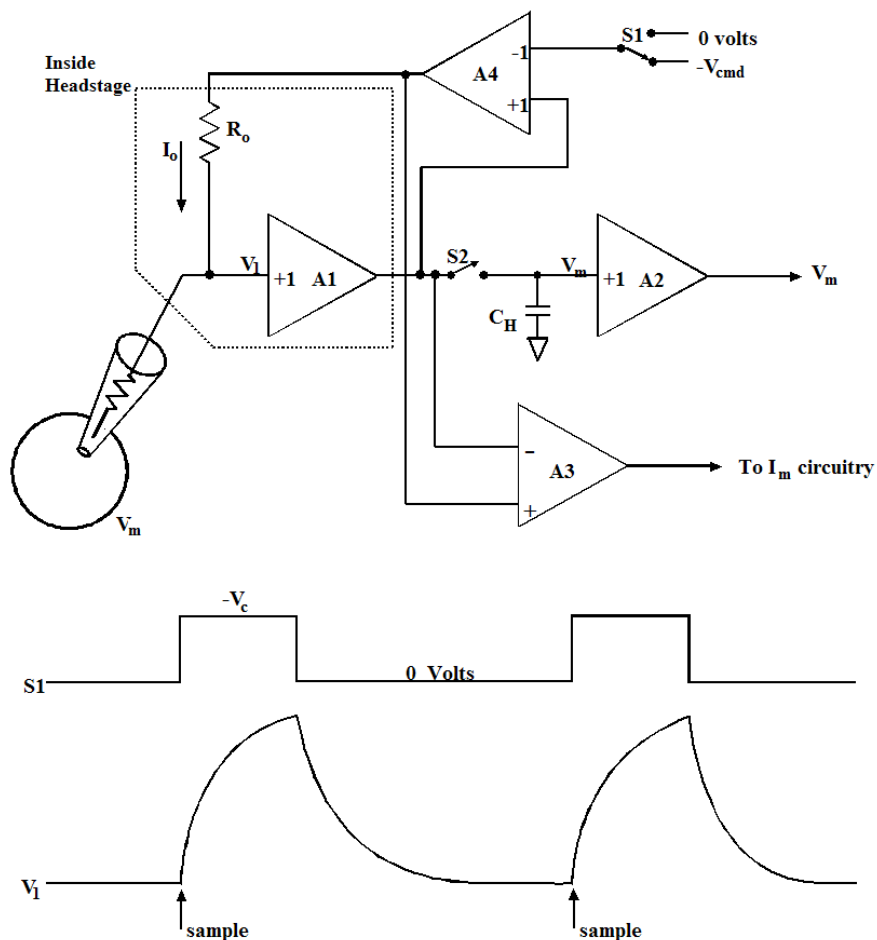


Figure 46. DCC Mode block diagram and timing diagram.

During the current-injection period a square pulse of current proportional to  $V_{cmd}$  is injected into the microelectrode. Because of this current  $V_1$  rises. The rate of rise of  $V_1$  is limited by the parasitic effects of capacitance through the wall of the glass microelectrode to the solution, and capacitance at the input of the buffer amplifier. The final value of  $V_1$  reached consists mostly of the IR voltage drop across the microelectrode resistance. Only a tiny fraction of  $V_1$  consists of the membrane potential recorded at the tip.



After 30% of one cycle has elapsed, the voltage-recording period begins when S1 changes back to the zero volts position. Passive decay occurs because the input of the CCS is zero volts and thus its output current is zero. Sufficient time must be allowed during the voltage-recording period for the voltage to decay to within a millivolt or less of  $V_m$ . At the end of the passive decay period S2 is again briefly closed and a new sample of  $V_m$  is taken to begin a new cycle.

The actual voltage used for recording purposes is the sampled voltage. The Scaled Output Headstage 1 Membrane Potential signal is the sampled membrane potential.

The instantaneous current into the microelectrode is monitored by a differential amplifier (A3). The output of A3 is taken to an averager (not shown) that samples, smoothes and scales the current pulses and this average value is available on the Scaled Output Headstage 1 Membrane Current signal.

During DCC mode the input to the CCS and the output of the CHANNEL 1 current monitor are automatically scaled so that they represent the true membrane current even though the instantaneous current flows for only 30% of the time.

The cycling (sampling) rate must be chosen so that there are ten or more cycles per membrane time constant. This enables the membrane capacitance to smooth the membrane voltage response to the current pulses.

## **Discontinuous Single-Electrode Voltage Clamp (dSEVC) Mode**

Although two-electrode voltage clamping is faster, the Axoclamp 900A allows very fast discontinuous single-electrode voltage clamping. In a model cell (10 M $\Omega$ /1 nF) using a 10 M $\Omega$  resistor to model the microelectrode, the 10–90% rise time is only 100  $\mu$ s. In a real setup the response speed is limited by the microelectrode characteristics, but membrane potential rise times (without overshoot) of less than 1 ms have been regularly achieved in a variety of cell types. The discontinuous single-

electrode voltage clamp mode can be used with either micropipettes or patch pipettes.

In discontinuous single-electrode voltage clamp mode the tasks of voltage recording and current passing are allocated to the same microelectrode. Time-sharing techniques are used to prevent interactions between the two tasks. The principles of operation have been published (Brenneke & Lindemann, 1974; Wilson & Goldner, 1975; Finkel & Redman, 1985) and are outlined in the block diagram and timing diagram of Figure 47, and in the following discussion.

A single microelectrode (CHANNEL 1) penetrates the cell and the voltage recorded ( $V_1$ ) is buffered by a unity-gain headstage (A1). To begin the discussion, assume that at this moment  $V_1$  is exactly equal to the instantaneous membrane potential ( $V_m$ ). A sample-and-hold circuit (SH1) samples  $V_m$  and holds it for the rest of the cycle.

The sampled membrane potential is compared with a command voltage ( $V_{cmd}$ ) in a differential amplifier (A2). The output of this amplifier becomes the input of a controlled-current source (CCS) if the switch S1 is in the current-passing position. The gain of the CCS is  $G_T$ . The CCS injects a current into the microelectrode that is directly proportional to the voltage at the input of the CCS irrespective of the resistance of the microelectrode.

The period of current injection is illustrated at the start of the timing waveform. S1 is shown in the current-passing position during which a square pulse of current is injected into the microelectrode. Because of this current,  $V_1$  rises.

The rate of rise is limited by the parasitic effects of the capacitance through the wall of the glass microelectrode to the solution, and the capacitance at the input of the buffer amplifier. The final value of  $V_1$  mostly consists of the IR voltage drop across the microelectrode due to the passage of current  $I_0$  through the microelectrode resistance  $R_e$ . Only a tiny fraction of  $V_1$  consists of the membrane potential recorded at the tip.

S1 then switches to the voltage-recording position. When the input of the CCS is zero volts, its output current is zero and  $V_1$  passively decays. During

the voltage-recording period  $V_1$  decays asymptotically towards  $V_m$ . Sufficient time must be allowed for  $V_1$  to reach within a millivolt or less of  $V_m$ . This requires a period of up to nine electrode time constants ( $\tau_e$ ). At the end of the voltage-recording period a new sample of  $V_m$  is taken and a new cycle begins.

The actual voltage used for recording purposes is the sampled voltage. As illustrated in the bottom timing waveform the sampled value of  $V_m$  moves in small increments about the average value. The difference between  $V_{m(\text{average})}$  and  $V_{\text{cmd}}$  is the steady-state error ( $\epsilon$ ) of the clamp that arises because the gain ( $G_T$ ) of the CCS is finite. The error becomes progressively smaller as  $G_T$  is increased.

The duty cycle used in dSEVC mode is current passing for 30% of each cycle, and voltage recording for 70% of each cycle.

The cycling rate (sample rate) must be chosen so that there are ten or more cycles per membrane time constant. This enables the membrane capacitance to smooth the membrane voltage response to the current pulses.

When optimally adjusted, the circuit enables the first steady-state measurement of voltage to be taken 1 to 2 cycle periods after the onset of a membrane conductance change or a change in the command voltage.

The Lag control (not shown in the Figure) alters the frequency response of the differential amplifier (A2). It can be used to compensate for the complicated frequency characteristics of a real cell (see the description of *Lag* in Chapter 5).

The Gain control alters  $G_T$ . Its operating range is  $(100 \times H)$  nA/mV.

While CHANNEL 1 is used dSEVC mode it is still possible to independently use CHANNEL 2. For example, CHANNEL 2 could be used for recording from and stimulating other cells that make connections to the cell being voltage-clamped.

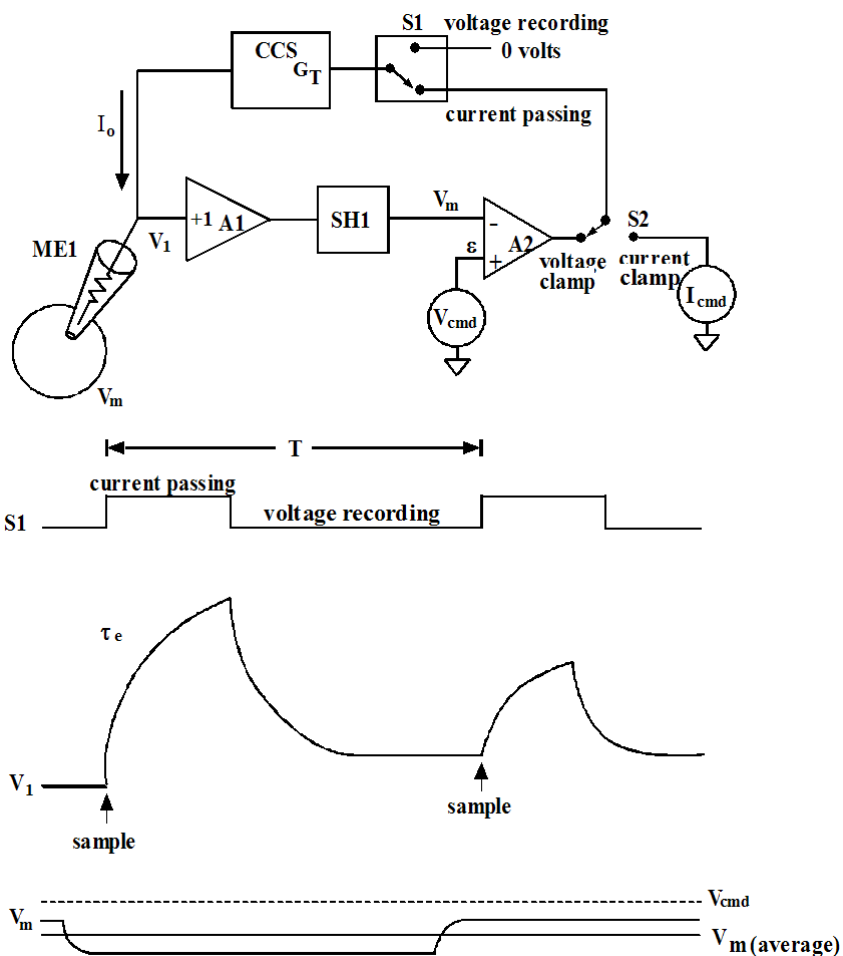


Figure 47. dSEVC block diagram and timing waveforms.

### Minimum Sampling Rate And Maximum Gain

If the sampling rate is too slow the dSEVC becomes unstable. This is because the long current-passing period charges the membrane potential past the desired potential before the clamp has an opportunity to take a new sample of potential and adjust the current accordingly. The larger the cell membrane capacitance ( $C_m$ ) the slower the sampling rate ( $f_s$ ) that can be

used for a given average gain ( $G$ ). The stability criterion is (see Brenneke & Lindemann, 1974; Finkel & Redman, 1985):

$$0 < \frac{G}{C_m f_s} < 2$$

For critical damping we require:

$$\frac{G}{C_m f_s} = 1$$

Thus for a given  $G$ , if  $C_m$  is small  $f_s$  must be large.

As an example, if  $G = 1$  nA/mV and  $C_m = 100$  pF, then  $f_s$  must be 10 kHz for critical damping. If  $f_s$  is less than 10 kHz in this example, the step response will overshoot and at 5 kHz the clamp will oscillate destructively.

If the sampling rate in this example cannot be as great as 10 kHz because the microelectrode response is too slow, then a lower value of  $G$  will have to be used to maintain stability.

### ***Clamp Error***

With finite gains in the voltage-clamp circuit  $V_m$  does not quite follow  $V_c$ . The error is  $\varepsilon = V_{cmd} - V_m$ .

Similarly, if  $V_{cmd}$  is constant and the cell membrane conductance changes, then there is an error in the measurement of the current underlying the conductance change. This error is similar in percentage to the voltage error.

Usually the gain of the voltage-clamp circuit can be increased so that  $\varepsilon$  is 10% or less. The percentage error depends on the frequency of the command signal or of the conductance change. It is smallest for slow signals and DC, and largest for the fastest signals. Thus very fast transients (such as the rising phase of synaptic currents) will be clamped less well than slower transients (such as the decay phase of synaptic currents).

## ***Gain***

The clamp gain during dSEVC mode is given in nA/mV. This refers to how many nanoamperes the output current will change by for each millivolt of difference between  $V_m$  (the membrane potential) and  $V_{cmd}$  (the command potential). The value indicated on the front panel is the average value ( $G$ ). The average value depends upon the instantaneous gain during the current-passing period ( $G_T$ ) and upon the duty-cycle.

## ***Series Resistance, $R_s$***

In dSEVC mode the effect of series resistance,  $R_s$ , is to slow the microelectrode response. Thus, the current does not completely decay to baseline before the voltage measurement. Since the potential recorded by the membrane potential is the sum of the voltage across  $R_s$  and the membrane resistance,  $R_m$ , there is an error in the membrane potential recorded. The added consequence is that  $R_s$  limits the maximum rate at which the membrane can be charged. To eliminate  $R_s$  altogether, watch the MONITOR output and make sure the transient decays completely before the next sample is taken.

## ***Advantages of a Patch Pipette***

It turns out that a patch pipette is ideal for dSEVC. That is, when  $R_e$  (microelectrode resistance) is very small, its time constant is fast. In addition, the magnitude of the voltage transient across the microelectrode for a given current is proportional to  $R_e$  and therefore small when  $R_e$  is small. This double advantage of low  $R_e$  values means that the dSEVC can be cycled very rapidly without introducing a sampling error.

## **Noise in DCC and dSEVC Modes**

The noise inherent in discontinuous single-electrode clamps (discontinuous current clamp or discontinuous single-electrode voltage clamp) is four or more times greater than the noise in continuous electrode clamps (bridge current clamp or two-electrode voltage clamp) when the discontinuous

electrode clamps are adjusted for the same dynamic response and accuracy as the continuous electrode clamps.

There are two major reasons for this inherent deterioration in noise performance.

The first is due to capacitance neutralization. A fundamental property of all capacitance neutralization circuits is that they introduce noise in excess of what is contributed by the thermal noise of the recording microelectrode and the input noise of the buffer amplifier. The excess noise becomes progressively larger as the microelectrode time constant is reduced. In discontinuous systems the microelectrode time constant must be deliberately reduced more than in continuous systems so that after a current pulse the microelectrode voltage decays to  $V_m$  within the time allotted for passive recording. The excess noise due to optimizing the capacitance neutralization can vary from a factor of about two in a system where primary efforts have been taken to keep the input capacitance low, to much larger factors in systems where large amounts of capacitance-to-earth and capacitance-to-shield are tolerated.

The second major reason for the deterioration in noise performance of discontinuous single-electrode clamps has to do with the sampling process. Sampling processes alias the noise in the input signal spectrum into a larger-magnitude spectrum confined to a bandwidth equal to half of the sampling rate ( $f_s$ ). The normal procedure used in digitizing systems to avoid aliasing is to reduce the bandwidth of the input signal to  $f_s/2$  or below. This is not possible in discontinuous single-electrode clamping because reducing the bandwidth of the microelectrode increases the time constant and therefore prevents adequate settling. The amount of aliased noise depends in part on the duty cycle used in the discontinuous clamp. The 30% duty cycle used in the Axoclamp 900A has been chosen to give a good compromise between aliased noise and dynamic performance (Finkel & Redman, 1984b). With this duty cycle the increase in noise due to aliasing is a factor of about two.

The two contributions to noise discussed above lead to a factor of four or more deterioration in noise. To keep the deterioration as small as this, the experimenter should try to do the following:

1. Keep the real value of  $C_{in}$  as small as possible so that only minimal capacitance neutralization must be used. (Avoid using coaxial cable to connect the microelectrode to the headstage.)
2. Increase the cycle rate so that the amount of aliased noise is minimized.

Finally, the amount of noise recorded can be reduced to some extent by using as much output filtering as possible. However, the output filtering should never be increased to the extent that dynamic information such as rise time is lost. Usually, output filtering at  $f_s/10$  is a good compromise. The best way of reducing noise in the records is by averaging repetitive responses. This well-known procedure reduces the noise by the square root of the number of averages without affecting the time course of the signal.

Although the noise performance of discontinuous single-electrode voltage clamps is poorer than that of the two-electrode voltage clamp, the single-electrode technique is extremely rewarding because it allows voltage clamping to be performed in preparations where two-electrode voltage clamping is not feasible such as when the cell is buried beneath many layers of other cells. In addition, the signal-to-noise ratio in many preparations during discontinuous single-electrode voltage clamp is, despite the above considerations, adequate for data to be analyzed without averaging.

## TEVC Mode

In two-electrode voltage clamp (TEVC) mode the Axoclamp 900A acts as a conventional voltage clamp with an output compliance of  $\pm 180$  V. CHANNEL 1 is the voltage-recording microelectrode and CHANNEL 2 is the current-passing microelectrode.



The output of the clamp is a voltage source that is connected to CHANNEL 2. The voltage-clamp Gain control determines the steady-state accuracy and the response speed. The Gain control is marked in units of V/V. This refers to how many volts the output changes for each volt of difference between  $V_m$  (the membrane potential) and  $V_{cmd}$  (the command potential). For example, when the gain is at a value of 10,000 V/V, a 100  $\mu$ V difference between  $V_m$  and  $V_{cmd}$  causes the output to shift by 1 V. If the resistance of CHANNEL 2 is 10 M $\Omega$  there is a current shift of 100 nA.

Several other controls affect the voltage-clamp response. The Lag introduces phase lag in the voltage-clamp amplifier. The Holding control shifts the clamped membrane potential. The Pipette Capacitance Neutralization setting of CHANNEL 1 affects the voltage-clamp response. The Seal Test setting delivers a voltage pulse.

The best settings of the voltage-clamp parameters are found by setting up the best possible response to a step change in  $V_c$ . Usually, the ability of the voltage clamp to follow a step change in command is identical to the ability of the voltage clamp to follow a step change in membrane conductance (Finkel & Gage, 1985).

### ***General Considerations***

It is worthwhile to examine the pros and cons of the TEVC technique. While the TEVC technique is more powerful than techniques using single microelectrodes, it is considerably more complicated and difficult to implement. However, the extra effort will be well rewarded. If you are unfamiliar with the theory behind TEVC systems then Taylor (1991) gives a good general overview with a number of useful references. Finkel and Gage (1985) provides a more thorough discussion of the theoretical aspects.

The main advantages of the TEVC technique are:

1. High current-passing capacity: for larger cells single-electrode clamps do not work adequately, if at all. An Axoclamp 900A in TEVC mode can supply current in the mA range. A common application for two-electrode voltage clamps is recording currents in amphibian oocytes.
2. Excellent time resolution: the rise time for a voltage step with a TEVC can be a factor of 3 or more faster than a single-electrode clamp implemented using similar microelectrodes. A high frequency response might be required to record very rapid tail currents or gating currents associated with voltage-gated channels, for example.
3. Low noise: current noise levels with a TEVC are generally lower than single-electrode voltage clamps implemented using microelectrodes of comparable resistance, even given the higher frequency response of the TEVC.

The major drawbacks to TEVC systems are that two microelectrodes must be applied to one cell and that there is added complexity.

### *Configuration of the Voltage Clamp System*

Before setting up the voltage clamp you have to decide how to record the membrane potential ( $V_m$ ) and how to measure the membrane current ( $I_m$ ). The decisions you make are determined by what you want to achieve and the particular characteristics of your preparation and bath. The brief discussions of the various options that follow will help you assess your particular requirements.

#### **Voltage Recording and Series Resistance**

The reference point for the voltage recorded by CHANNEL 1 is the system ground and under ideal conditions a bath ground electrode holds the extracellular solution at a constant voltage relative to the system ground. After CHANNEL 1 is offset to zero volts in the bath and then applied to the

cell, it provides the voltage-clamp circuit with an accurate measure of  $V_m$  provided that there is no net current flow.

Under normal conditions however, there is current flowing across the membrane and this current not only produces a voltage drop across the membrane but also across any resistance that is in series with the membrane and the system ground. These extraneous resistances can be lumped together as “series resistance” ( $R_s$ ). The voltage drop across  $R_s$  is called the series resistance error or bath error potential. The voltage drop across  $R_s$  causes the bath potential to deviate from the zero current potential by an amount directly proportional to the current flowing. Thus, for example, if you have a series resistance of 2 k $\Omega$  and a 5  $\mu$ A peak current occurs during a voltage step, then the peak membrane voltage error is 10 mV.

For a detailed discussion of this type of series resistance and the methods used to minimize it, see *Bath Error Potentials* on page 65.

### **Selection of the Membrane Voltage Recording Headstage (CHANNEL 1)**

Usually you will use a low-noise type headstage for the voltage-recording microelectrode, as this allows for some capacitance compensation to be applied if necessary while taking advantage of the very low-noise voltage recording characteristics of these headstages.

The headstages are designed to be compact and robust so that they can be mounted on the micromanipulator as close as possible to the recording point. This strategy greatly reduces the input capacitance. For this reason, the electrode holders are designed to plug directly into the headstage input.

It is recommended that you use the simplest configuration and add more complex features as the need arises. In this case all that is required are two headstages, one for voltage (CHANNEL 1) and the other for current passing (CHANNEL 2). The bath ground electrode is plugged into the ground jack on the CHANNEL 1 headstage.

The reader is referred to the section on Headstages in this chapter for specific recommendations regarding the selection of headstages.

### Selection of the Current Recording Method

There are two techniques available for recording current. For almost all applications the built-in current monitor gives excellent performance, however some users may wish to bypass the current sensing resistor and, therefore, have to measure current using the virtual ground technique. The two techniques are discussed in turn below.

If you are interested in obtaining accurate subtraction of the linear capacitive currents at the make and break of a voltage step, then you must ensure that the current measuring device has enough compliance to fully resolve these large transients. Table 4 summarizes the current passing capability of the HS-9A headstages.

Headstage	$R_0$ (M $\Omega$ )	Short-Circuit Current	Conversion
HS-9A $\times 0.1$	100	$\pm 1.8 \mu\text{A}$	100 mV/nA
HS-9A $\times 1$	10	$\pm 18 \mu\text{A}$	10 mV/nA
HS-9A $\times 10$	1	$\pm 180 \mu\text{A}$	1 mV/nA

Table 4. Current passing capacities in TEVC mode.

### Series Current Measurement (Internal)

To monitor the clamp current it is recommended that you use the internal current monitor in the current-passing headstage. Advantages are simplicity and the fact that only the clamp current is recorded, and not other currents injected into the chamber from iontophoretic electrodes, for example.

Disadvantages are that the current measuring circuit has slightly more high frequency noise and also loads the output of the voltage clamp. In some circumstances this extra load can affect performance. For example, the  $\times 1$  headstage has a 10 M $\Omega$  resistance in series with the output, and the clamp

current is measured from the voltage drop across this resistor. If your current-passing microelectrode resistance is 5 MΩ and the output voltage 180 V, then ~120 V drops across the current sensing resistor and only ~60 V across the microelectrode. The effective gain of the clamp is reduced by one third and thus the frequency response is compromised (see Tuning the Voltage-Clamp—Fidelity and Stability section). Generally this is not a significant problem since the high output compliance of the TEVC simply allows a higher clamp gain to be used. If you are using series current measurement and have headstages with different H values, use the headstage with the higher H value (which has the lower value for the current sensing resistor) for the current-passing microelectrode.

### *Tuning the Voltage-Clamp—Fidelity and Stability*

The object of tuning the voltage clamp is to maximize the feedback gain. There are two reasons, first the bandwidth of the clamp is strongly dependent on the gain and second, the fidelity, or accuracy, with which the voltage clamp tracks the command potential is dependent on the gain.

#### **Fidelity**

The steady-state membrane potential ( $V_m$ ) after a step change in the command voltage ( $V_{cmd}$ ) is:

$$V_m = V_{cmd} \frac{\mu K}{\mu K + 1}$$

where  $\mu$  is the gain of the clamp amplifier and K is the attenuation of the clamp amplifier caused by the cell membrane resistance ( $R_m$ ) and the resistance ( $R_{e2}$ ) of the output microelectrode (CHANNEL 2).

$$K = \frac{R_m}{R_m + R_{e2}}$$

As the product  $\mu K$  becomes very large, the difference between  $V_m$  and  $V_{cmd}$  becomes very small. Ideally, the error is very low, just a fraction of one percent.  $\mu$  is set by the Gain glider in the V-Clamp Control section of the TEVC tab of the Axoclamp 900A Commander. In TEVC mode the gain can

range from 20 to 50,000. If K were unity, the error would vary from 3 percent down to 0.01 percent. However, K is always less than unity, so the error is worse. If the output micropipette resistance is 90 MΩ and the membrane resistance is 10 MΩ, K is 0.1 and the error is ten times worse than if K were unity. Further, during activation of membrane currents  $R_m$  can drop dramatically, and K becomes equal to  $R_m/R_{e2}$ . Thus, as a rule of thumb it is desirable to use an output micropipette whose resistance is as low as possible, ideally smaller than the resting membrane resistance.

### Step Response and Bandwidth

After a step command, the membrane potential relaxes exponentially towards its new value. For  $\mu K \gg 1$ , the time constant for the relaxation is:

$$\tau = \frac{R_{e2} C_m}{\mu}$$

Increasing the clamp gain decreases the time constant for the step response. For example, if  $R_{e2} = 10 \text{ M}\Omega$ ,  $C_m = 1000 \text{ pF}$  and  $\mu = 100$ , the time constant is 100  $\mu\text{s}$ . Stated differently, increasing the clamp gain also increases the bandwidth with which  $V_m$  can follow changes in  $V_{cmd}$ . The -3 dB frequency of the bandwidth is:

$$f_{3dB} = \frac{\mu}{2\pi R_{e2} C_m}$$

### Stability

An ideal voltage clamp is unconditionally stable. The membrane capacitance provides a 90° phase shift, which is required for stability in all negative feedback circuits. Unfortunately, in the real world other factors combine to make the circuit unstable at high clamp gains.

The coupling capacitance ( $C_c$ ) between the microelectrodes is extremely destabilizing. Values as small as 0.01 pF can lead to oscillation if  $\mu$  has a magnitude of several hundred or more.

Another destabilizing factor is the non-ideal nature of the membrane. In theory the membrane is simply modeled as a parallel resistor and capacitor. In practice, a distributed model applies. The capacitance elements are themselves non-ideal; they should be modeled by an ideal capacitor with a series resistance component. For real membranes, the phase shift at high frequencies is less than  $90^\circ$ . In the Axoclamp 900A, a phase-shift control is included to allow the user to empirically add some phase lag to the circuit to build the total high-frequency phase shift up to  $90^\circ$ .

The input capacitance of the voltage-recording microelectrode (CHANNEL 1) adds another frequency-dependent variable into the system, which also tends to decrease the stability. The effect of this input capacitance is usually minimized by carefully adjusting the capacitance neutralization control to maximize the bandwidth of CHANNEL 1.

### *Inter-Electrode Coupling Capacitance and Shielding*

When the voltage-recording microelectrode and current-passing microelectrode are applied to a cell there is considerable coupling capacitance ( $C_x$ ) between them due to their proximity. This capacitive coupling introduces a low impedance feedback pathway at high frequencies that completely bypasses the cell membrane. The presence of this capacitance is the single most significant destabilizing influence on the voltage clamp, and for this reason it is essential that it be reduced as far as is practical. In point of fact, coupling capacitance as low as 0.01 pF can destabilize the response at high gain settings.

There are three ways to reduce  $C_x$ :

1. Introduce the two microelectrodes into the preparation at a wide angle, preferably greater than  $90^\circ$ . Keep the tips of the microelectrodes as far apart as possible. Generally it is better to have the tip of the current microelectrode nearer the center of the cell, so that field potentials do not affect the local membrane potential unevenly (see *Eliminating the Voltage Error Due to Coupling Between Intracellular Microelectrodes* on page 106).

2. Place a grounded metal shield between the two microelectrodes. If the shield cannot be placed in the middle, then place it nearer the current-passing microelectrode where added stray capacitance is not so critical. A shield near CHANNEL 1 increases the input capacitance and thus increase noises levels. The shield should block all line-of-sight pathways between the two microelectrodes and their holders and should extend as close as possible to the tip of the current-passing microelectrode without coming into direct electrical contact with the bath solution. In extreme cases you can coat CHANNEL 2 very close to the tip with conductive silver paint that can then be insulated by a coat of Sylgard. One effect of the shield is to vastly increase the output capacitance of the current-passing microelectrode that may affect the high-frequency measurement of the current unless the capacitance neutralization of CHANNEL 2 is properly set. Under these circumstances the capacitance neutralization of CHANNEL 2 may have a significant effect on the rise time of the clamp step response since the output capacitance and the microelectrode resistance will effectively form a lowpass filter for the output voltage.
3. Measures that reduce the stray capacitance of the microelectrodes will also reduce  $C_x$ . This would include coating the microelectrodes with Sylgard.

**Note:** The use of a driven shield on CHANNEL 1 is not recommended as this will increase the noise; it is far better to place a grounded shield on the current-passing microelectrode.

### ***Eliminating the Voltage Error Due to Coupling Between Intracellular Microelectrodes***

Another potential source of voltage error in TEVC mode is introduced by the field potential that can develop around the tip of the current-passing microelectrode when current densities are high. The potential differences across this field can be quite high.



The equation for the access resistance,  $R_a$ , in a sphere is:  $R_a = \rho/4\pi r$ . The resistivity,  $\rho$ , is typically 100  $\Omega$  cm for physiological saline and the radius,  $r$ , of the sphere is in cm.

If the potential picked up by the voltage microelectrode is to be less than 5 mV and the current is 10  $\mu$ A,  $R_a$  must be less than 500  $\Omega$ . This requires a separation of 160  $\mu$ m between the microelectrode tips, a significant distance even on the scale of amphibian oocyte dimensions. For a 100 nA current the separation need only be 1.6  $\mu$ m. Clearly, as current becomes very large the voltage error due to the coupling of intracellular microelectrodes worsens. This may present a special problem when the concentration of heterologously expressed excitable proteins in amphibian oocytes is very large. If the current generated is larger than 10  $\mu$ A, an alternative technique such as measuring the current using the macropatch technique might be considered.

It is good practice to keep the tips of the two intracellular microelectrodes as far apart as possible inside the cell. Generally, it is better to put the tip of the current-passing microelectrode, CHANNEL 2, near the middle of the cell so that the field potentials near its tip do not unevenly affect the local membrane potential.

## Filters

The theory behind the design and choice of appropriate filters is very extensive, as you will see from any book on signal processing. Here we provide just a few basic principles to assist you in choosing the filter type and cutoff frequency that are most suited to your experiments.

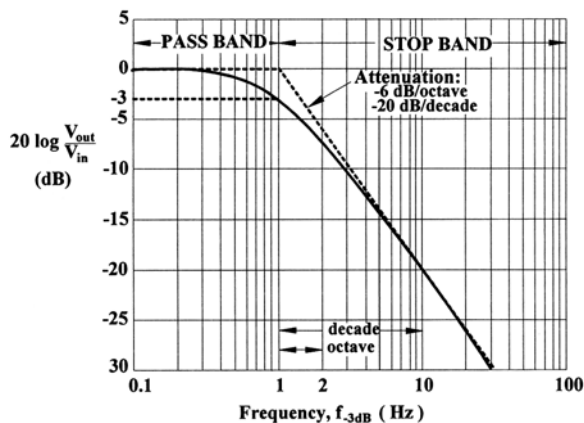
### -3 dB Frequency

The -3 dB, or cutoff, frequency ( $f_c$ ) of a filter is the frequency at which the output signal *voltage* (or *current*) is reduced to  $1/\sqrt{2}$  (i.e., 0.7071) of the input. Equivalently,  $f_c$  is the frequency at which the output signal *power* is reduced to half of the input. These terms arise from the definition of decibel (dB):

$$\text{Voltage: } \text{dB} = 20 \log(V_{\text{out}}/V_{\text{in}})$$

$$\text{Power: } \text{dB} = 10 \log(P_{\text{out}}/P_{\text{in}})$$

For a lowpass filter, the frequency region below  $f_c$  is called the pass band, while that above  $f_c$  is called the stop band. In the stop band, the signal attenuates (or 'rolls off') with a characteristic steepness. The steepness of the roll-off at higher frequencies is determined both by the type of filter (see below) and the number of poles of the filter: the larger the number of poles, the faster the roll-off. The lowpass on the Scaled Output of the Axoclamp 900A are 4-pole filters. Filters with more poles can be constructed, but they are more complex to implement and yield diminishing returns.



**Figure 48.** Filter characteristics, illustrated for a single-pole, lowpass filter. The spectrum has been normalized so that the signal magnitude in the pass band is 0 dB. The -3 dB frequency has been normalized to unity.

## Types of Filters

There are many types of filters, distinguished by their effects on both the amplitude and phase of the signal. The two most common filters used in electrophysiology are the Bessel filter and the Butterworth filter, both of which are implemented in the Axoclamp 900A.

***Bessel Filter***

This is the analog filter used for most signals for which minimum distortion in the time domain is required. The Bessel filter does not provide as sharp a roll-off as the Butterworth filter, but it is well behaved at sharp transitions in the signal, such as might occur at capacitance transients or single-channel current steps.

***Butterworth Filter***

This is the filter of choice when analyzing signals in the frequency domain, *e.g.*, when making power spectra for noise analysis. The Butterworth filter has a sharp, smooth roll-off in the frequency domain, but introduces an overshoot and “ringing” appearance to step signals in the time domain.

***Choosing the Cutoff Frequency***

In practice, there are two important considerations when selecting the filter cutoff frequency.

**Aliasing**

If the digitizing interface samples at 2 kHz, for example, any noise in the sampled signal that has a frequency greater than 1 kHz will appear in the digitized trace as extra noise in the range 0 to 1 kHz. In other words, higher-frequency noise (> 1 kHz) will appear under the alias of lower-frequency noise (< 1 kHz). This error is called aliasing. A fundamental principle of signal analysis, called the Nyquist Principle, therefore states that, in order to avoid aliasing, the digitizing frequency ( $f_d$ ) should be at least twice the filter cutoff frequency ( $f_c$ ):

$$f_d \geq 2f_c$$

The minimum permissible digitizing frequency (exactly twice  $f_c$ ) is called the Nyquist frequency. In practice, it is better to sample at two or more times the Nyquist frequency. Thus,  $f_d = 5f_c$  is commonly used. This means that, if the Axoclamp 900A filter is set at 5 kHz, your interface should be capable of digitizing at 25 kHz.

## Risetime

The risetime is typically given as the time taken for a signal to increase from 10% to 90% of its peak value. The more heavily a step response is filtered, the greater the 10–90% risetime. For the 4-pole Bessel filter in the Axoclamp 900A, the filtered 10–90% risetime ( $T_r$ , in ms) of a step input depends on  $f_c$  (in kHz) approximately as:

$$T_r \approx 0.35/f_c$$

(This can be measured by applying Seal Test to the model BATH in V-Clamp mode and looking at “Scaled Output: Membrane Current” while changing the filter setting.) Suppose you are interested in measuring action potentials, for which you expect the 10–90% risetime to be about 0.4 ms.

You would then choose the filter cutoff frequency to be high enough that the filter risetime is about ten times faster than 0.4 ms so the action potentials are minimally distorted by the filter. According to the above equation, then, the appropriate filter setting would be 10 kHz. In practice, you may need to make other compromises. For example, if the signal is very noisy you may wish to filter more heavily and accept that the action potential risetime is artifactually slowed.

## *Highpass Filter*

The Scaled Output signal can be highpass filtered by setting the AC value in the Output Gains and Filters section of the main Axoclamp 900A Commander panel. This is typically done in order to remove a DC component of the signal. When the filter cutoff is set to DC this highpass filter is bypassed.

## Blanking

A common problem when using stimulating electrodes is that some of the stimulus is directly coupled into the recording microelectrode. This can saturate the coupling capacitors of subsequent AC recording circuits. The saturation effects may take tens or hundreds of milliseconds to subside.

The best way to minimize or even eliminate this artifact is at the source, by using small stimuli, isolated stimulators, placing a grounded shield between the stimulating electrodes and the microelectrodes, etc. Often, though, it is not possible to reduce the artifact to manageable levels.

The Axoclamp 900A can circumvent the effects of the stimulus artifact by Blanking. At the moment the logic level of the BLANK ACTIVATE 1 input goes high, the value of  $V_m$  is sampled and saved. For the duration of the HIGH signal, this saved value is used instead of the actual potential.

In voltage-clamp modes the voltage-clamp current during the Blanking period will be held at the level that existed at the start of the period. A small deviation from the command potential may develop during the Blanking period as a result of comparing the command to the sampled value of  $V_m$  instead of the instantaneous value of  $V_m$ . This deviation will only be seen when the Blanking period ends. Usually this deviation is preferable to the situation that can occur if Blanking is not used. If Blanking is not used the artifact picked up by CHANNEL 1 is treated by the voltage-clamp circuit as an attempt by the cell to change its potential. Therefore, the voltage-clamp circuit causes a current to be passed into the cell to clamp this presumed membrane potential change. If the stimulus artifact is large, the consequent current artifact can be large enough to damage the cell.

The width of the Blanking period should be no longer than the minimum width required to cover the period of the stimulus artifact. It is important not to Blank for longer than necessary since during Blanking no updating of  $V_m$  is allowed. Even when Blanking is used, attempts should still be made to minimize the artifact at the source.

## Current Measurement

The current injected down each microelectrode is independently measured. The measurement is true. Thus, if the microelectrode blocks, the measured current falls to zero even though a current command may exist.

Two current outputs apply to CHANNEL 1 in discontinuous modes (DCC and dSEVC), the instantaneous current and the sampled current. The

instantaneous current switches from zero to some finite value at the sample rate. This is because for 30% of each period CHANNEL 1 is used for passing current while for the remaining 70% of each period no current is passed and the IR voltage drop due to the previous current is allowed to passively decay. On the other hand, the sampled current is the true membrane current. It is recovered from the instantaneous current by a circuit that samples the current pulses, retains the samples during the passive-decay period, then scales the samples to yield the average current for the whole period. The sampled current output, selected from either the Scaled Output (Membrane Current signal) or from the Current Output, is smoothed by the output filter.

The whole current into the bath can be separately measured using a virtual-ground headstage (see below).

## Virtual-Ground Current Measurement

A Virtual-Ground headstage can be used to ground the preparation bath. Connect the headstage to the AUXILIARY 1 or 2 HEADSTAGE input. All of the current flowing into the Virtual-Ground input is measured and a voltage proportional to the current is provided at the  $10_{\text{AUX1}}$  or  $10_{\text{AUX2}}$  OUTPUT. The output gain is 1 mV/nA when VG is  $\times 10$ , and 0.1 mV/nA when VG is  $\times 100$ .

	$R_0$ (M $\Omega$ )	Full scale range	Conversion
VG-9A $\times 10$	1	$\pm 10$ $\mu\text{A}$	1 mV/nA
VG-9A $\times 100$	0.1	$\pm 100$ $\mu\text{A}$	0.1 mV/nA

**Table 5.** Current recording ranges for virtual ground headstages.

A Virtual-Ground headstage is not required for normal use of the Axoclamp 900A because built-in current-measurement circuits are provided for each microelectrode. However, in TEVC mode the current output of the Virtual-Ground headstage has slightly less high-frequency noise than the output of the built-in current-measurement circuit.

The Virtual-Ground circuit measures all currents into the preparation bath. Thus if an iontophoretic microelectrode is to be used, it must have its own separate current return microelectrode (adding yet another electrode to the bath), otherwise the iontophoretic current will appear in the clamp current. A more insidious problem arises because the bath effectively becomes a very sensitive antenna and will pick up very low levels of power line radiation that will be evident as line frequency noise in the current signal. Saline-filled tubing acts as an excellent antenna. To prevent them carrying hum, long saline-filled tubes should have the saline pathway broken by an air-filled drip near the preparation.

Another potential source of error using this current-measurement technique is the fact that there will be direct capacitive coupling between the current-passing microelectrode and the ground electrode. This current bypasses the cell membrane but appears on the current signal. Generally this will not pose a significant problem, provided that the current-passing microelectrode is carefully shielded.

This output is also available on CHANNEL 1 and CHANNEL 2 scaled outputs.





## Chapter 5: Commander Reference

This chapter provides details of the theory and operation of each Axoclamp 900A Commander feature. It is organized by the name of the software feature, in alphabetical order.

Please consult the Index if you are having trouble locating a particular item.

Before using this chapter, it may be helpful to first read the Appendix describing Polarity Conventions. This summarizes the conventions used for the polarities of currents and voltages in all amplifiers manufactured by Molecular Devices.

### Always On Top

Always On Top <F3> puts Axoclamp 900A Commander on top of other applications, even when it is not the active window.

### Bridge Balance

Bridge Balance subtracts a value from the Membrane Potential signal to compensate for the voltage drop across the electrode when current is being injected. The adjustable field shows the estimated electrode resistance that Bridge Balance compensates for.

Bridge Balance is available only in I-Clamp mode, when Membrane Potential is selected as the Scaled Output signal.

For the Bridge Balance algorithm to work correctly, always use Pipette Capacitance Neutralization first.

How to use Bridge Balance:

- **Manual:** Enter an estimate of the electrode resistance in the adjustable field, and then check the checkbox to activate the circuit. If typing in the value, express it in M $\Omega$ . Once Bridge Balance has been applied you can, if necessary, adjust the value of the estimated resistance to tune the response.
- **Auto:** Click the *Auto* button to automatically apply compensation. Bridge Balance generates a test pulse to calculate electrode resistance and applies compensation accordingly. It automatically sets the adjustable field to the resistance it is compensating for, and checks the checkbox.

Auto can be used whether or not Bridge Balance has already been activated.

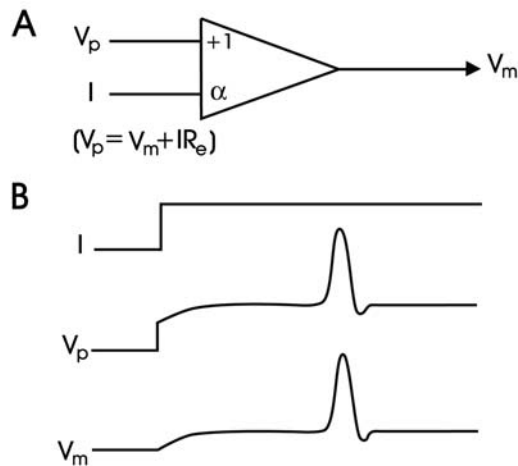
You can fine-tune the resistance value manually after Auto has been applied.

## Bridge Balance Overview

In some experiments it may be desired to inject a current (I) into a cell in current-clamp mode, *e.g.*, to depolarize the cell and evoke action potentials. The flow of I through the microelectrode produces a voltage drop across the electrode that depends on the product of I and the microelectrode resistance ( $R_e$ ). This unwanted  $IR_e$  voltage drop adds to the recorded potential. The Bridge Balance control can be used to balance out this voltage drop so that only the membrane potential is recorded. The term “Bridge” refers to the original Wheatstone Bridge circuit used to balance the IR voltage drop and is retained by tradition, even though operational amplifiers have replaced the original circuitry.

The technique is illustrated schematically in Figure 5.5A. A differential amplifier is used to subtract a scaled fraction of the current  $I$  from the voltage recorded at the back of the microelectrode,  $V_p$ . The scaling factor is the microelectrode resistance ( $R_e$ ). The result of this subtraction is thus the true membrane potential,  $V_m$ .

Figure 49 shows how bridge balance is done in practice. When the current is stepped to a new value (top), there is a rapid voltage step on  $V_p$  due to the ohmic voltage drop across the microelectrode (middle). Following this instantaneous step, there is a slower rise in  $V_p$  largely due to the membrane time constant of the cell. Correct adjustment of the bridge amplifier removes the instantaneous step, leaving the corrected  $V_m$  trace (bottom). Although this adjustment is done with a step current injection, the correction remains valid for any arbitrary waveform of injected current, provided the microelectrode maintains a constant resistance.



**Figure 49.** Schematic bridge balance circuit and adjustment procedure.

## Bridge Balance in the Bath

Some investigators like to set Bridge Balance in the bath, before attempting to impale cells. This is to make it easier to see when a cell has been penetrated.

Check the Tuning checkbox and set the parameters to  $-1$  nA and 50 Hz. Observe the Membrane Potential on Scaled Output. Press the *Auto Bridge Balance* button; the fast voltage steps seen at the start and finish of the current step should be eliminated. You may need to manually adjust the Bridge Balance M $\Omega$  value for optimum balance. The M $\Omega$  value is the resistance of the electrode.

## Bridge Balance in the Cell

The Bridge Balance should be frequently checked when inside a cell, because the electrode resistance can drift. While setting Bridge Balance, Pipette Capacitance Neutralization should also be set (see *Capacitance Neutralization*). Both settings can be monitored continuously through the experiment by injecting a small current step near the beginning of each data sweep.

It is recommended that Pipette Capacitance Neutralization be set at the same time as Bridge Balance, because both the electrode capacitance and the electrode resistance cause errors if left uncompensated. Also, it is easier to correctly balance the bridge when electrode capacitance is minimized, because the “break” between the rapidly decaying voltage across the microelectrode and the slowly decaying voltage across the cell’s membrane resistance is more distinct.

The balancing procedure is the same as in the bath, except that the trace appears more rounded because of the time constant of the cell membrane. Because the Tuning pulse width is typically brief compared with the membrane time constant, the voltage response looks like a series of ramping straight lines. To make it easier to see the fast voltage step in  $V_p$  on an oscilloscope, it is recommended that the scope input be AC coupled to remove the resting membrane potential from the signal. The scope gain can

then be turned up without the annoying offset. The  $M\Omega$  value found by Bridge Balance is the resistance of the electrode, which may be slightly higher than the value in the bath because of partial blockage of the tip during penetration.

The residual transient at the start and finish of the current step is due to the finite response speed of the microelectrode, which is determined in part by the capacitance of the electrode. The transient can be minimized by correctly setting the Pipette Capacitance Neutralization control (see *Capacitance Neutralization*). Adjust Pipette Capacitance Neutralization for the most rapid decay without causing an overshoot.

## **Buzz**

The *Buzz* button and adjustable field for pulse duration are located at the bottom of the I-Clamp tab.

In I-Clamp mode only, Buzz drives a brief, large current oscillation through the micropipette. The oscillation is achieved by increasing the Pipette Capacitance Neutralization by a small amount over the currently set level. Therefore, it is essential that you set Pipette Capacitance Neutralization to the correct level prior to using Buzz. If the Pipette Capacitance Neutralization level is too low, Buzz may fail to activate. Buzz duration can be set to discrete values between 100  $\mu$ s and 500 ms. View the duration options by right-clicking the field.

Depending on the microelectrode and the preparation, this method can aid in clearing blocked electrode tips. When used while the tip of the microelectrode is pressing against the membrane, Buzz may also cause the micropipette to penetrate the cell. The exact mechanism is unknown, but it may involve attraction between the charge at the tip of the electrode and bound charges on the inside of the membrane.

For some small cells a long duration Buzz can be deadly. An appropriate duration can be found for most cells that is sufficiently long to allow penetration of the membrane but short enough that the cell is not damaged after penetration.

## Clear

The Clear buttons, Clear+ and Clear-, are located in the I-Clamp tabs.

In I-Clamp mode only, the Clear+ and Clear- commands drive large positive and negative current steps, respectively, down the micropipette. Clear+ delivers a depolarizing current (one that causes a positive shift in the membrane voltage), while Clear- delivers a hyperpolarizing current (one that causes a negative shift in the membrane voltage).

Clear is used for two purposes:

- Clearing blocked microelectrodes. If the microelectrode resistance in the bath seems much higher than it should be, the electrode can often be cleared by rapidly toggling the Clear switch from plus to minus. Because of the large current passed this should only be done extracellularly.
- Penetrating cells. Sometimes microelectrode tips press against the cell membrane but fail to penetrate. A quick press on the Clear buttons will often force the electrode to penetrate. Whether to use a hyperpolarizing or depolarizing current depends on the preparation and must be determined by trial and error. Like Buzz, the mechanism for impalement is unknown.

Clear generates a Sync Output. The meters in Axoclamp Commander are disabled for approximately 1 second after a Clear command to avoid overloading the meter inputs.

## Configure SoftPanel

The Axoclamp SoftPanel is an optional hardware extension that gives you control of the Axoclamp 900A from a dials and buttons interface, rather than with computer keyboard and mouse. Attached to your computer's serial or USB port, it has 8 knobs and 8 buttons configurable to control your choice of Axoclamp 900A Commander functions (or you can use default settings).

Having attached a SoftPanel to a port of your computer, you must open the Select Axoclamp Device dialog box in order for the Axoclamp 900A Commander to recognize the device. If you have no SoftPanel, or Axoclamp 900A Commander does not recognize the device, the SoftPanel tool button is disabled.

## Default SoftPanel Settings

See below for the default SoftPanel settings for the Axoclamp 900A.

These settings can be customized to the individual user.

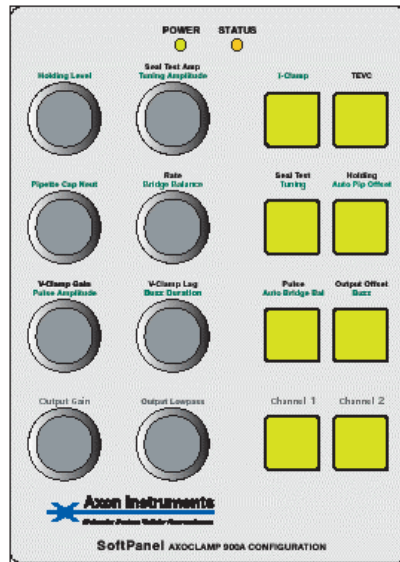


Figure 50. SoftPanel Overlay.

## How to Configure SoftPanel

- Open the *Configure SoftPanel* dialog box with the tool button or <F8>.
- On each of the I-Clamp and V-Clamp tabs, select from the list boxes the parameter you want to associate with the particular knob or button.

- Besides parameter controls, the buttons on the SoftPanel have a range of auxiliary functions they can be associated with. Some of these mimic the functionality of keyboard keystrokes used in association with glider controls, to increase the sensitivity of other knobs and buttons on the SoftPanel.

**Note:** The SoftPanel buttons can also be used to call Quick Select Buttons.

The options and their functions are:

Option	Function
<Shift>	Increases the sensitivity of any knob that is adjusted while the button is pressed.
<Ctrl>	Increases knob sensitivity to a greater extent than <Shift>.
<Page Up>	Increases the parameter that is currently selected in Commander in increments equivalent to a knob-click with <Shift>.
<Page Down>	Decreases the parameter that is currently selected in Commander in increments equivalent to a knob-click with <Shift>.
<Home>	Takes the parameter that is currently selected in Commander to its least value.
<End>	Takes the parameter that is currently selected in Commander to its greatest value.
Minimize Window	Minimizes the Commander interface.
Restore Window	Displays the Commander interface after it has been minimized.

**Table 6.** SoftPanel default settings.

## Defaults

Click the *Defaults* button to set the SoftPanel controls configuration to factory defaults. These settings are printed on the magnetic overlay that comes with the SoftPanel. Current clamp commands are printed in green; voltage clamp commands are printed in black.



The *Reset To Program Defaults* command sets the SoftPanel to its default values, as well as all other program settings.

## Detect Oscillation

The art of pipette capacitance neutralization is to increase the neutralization as far as possible without provoking oscillations that may be harmful to your cell. But no matter how carefully you compensate capacitance to begin your experiment, it is still possible to experience oscillations later in an experiment because electrode properties (*e.g.*, resistance, junction potential) may change over time.

## Current Clamp

The Axoclamp 900A provides you with options to protect your cell from harmful oscillations during a current-clamp experiment by automatically disabling or reducing Pipette Capacitance Neutralization.

Use the Detect Oscillation functionality as follows:

- Check the *Detect Oscillation* checkbox, and select the *Disable Neutralization* option.
- Now, increase the Pipette Capacitance Neutralization until you reach a value that evokes full-scale oscillations. The automatic protection circuit will work quickly to disable the Pipette Capacitance Neutralization, and several things will happen:
  - To the right of the “Disable...” field, a small icon appears briefly to display repeated images of a sine wave that is reduced to a flat line.
  - The Pipette Capacitance Neutralization feature is disabled (box is unchecked).
  - You hear an audible tone.

- o A warning message appears to indicate the detection of oscillations and the disabling of Pipette Capacitance Neutralization.

To prevent the warning message from appearing again check “Don’t tell me again” in the warning dialog. Alternatively, go to the *Options / General* tab, and uncheck the “Show warning when oscillation suppressed” option.

Instead of disabling Neutralization entirely, you can choose to reduce it iteratively by 1 pF steps until oscillations are no longer detected.

## Voltage Clamp

In voltage-clamp mode oscillations are suppressed by changing the Gain setting.

## Current Injection Resistor, $R_0$

The current injection resistor in the headstage is automatically detected and displayed in the  $R_0$  field at the top of the Headstage tabs. All relevant parameters in the Commander interface are adjusted based on this value for the resistor.

## Holding

The Holding command is located at the top of both V-Clamp and I-Clamp tabs. It has an on/off checkbox and adjustable field for voltage (V-Clamp) or current (I-Clamp) settings.

The Holding command is available in both voltage-clamp and current-clamp modes.

In voltage-clamp mode, Holding allows the user to apply a membrane DC holding potential of  $\pm 200$  mV.

In current-clamp mode, Holding allows the user to apply a DC holding current of:

- $\pm 1000$  nA with the 1 M $\Omega$  headstage current injection resistor selected.
- $\pm 100$  nA for the 10 M $\Omega$  resistor.
- $\pm 10$  nA for the 100 M $\Omega$  resistor.

Current clamp Holding is disabled if you run Slow Current Injection.

## How to Use Holding

- Set the voltage (V-Clamp) or current (I-Clamp) to the desired value. If, in I-Clamp mode, you enter values expressed in units of nA.
- Check the Holding checkbox to apply the holding current or potential. Settings can be adjusted after the command has been activated.

Uncheck the checkbox to stop the holding current or potential.

## Keyboard Shortcuts

### Hot Keys

Where a command name on the Axoclamp 900A Commander interface has a letter underlined, pressing the key for that letter will activate (or deactivate) that command.

### Shortcut Keys

The table below has shortcut key combinations for:

- Toolbar.
- Meters and Mode Selection.
- I-Clamp Tab.
- dSEVC and TEVC Tabs.

- Adjustable Numerical Fields.
- General.

<b>Command</b>	<b>Shortcut</b>
Toolbar	
Help	F1
Resize Window	F2
Always On Top	F3
Load Configuration	F4
Save Configuration	F5
Reset To Program Defaults	F6
Select Device	F7
Configure SoftPanel	F8
Configure Audio Monitor	F9
Options	F10
Monitor Scope	F11
Channel 1 Scaled Output Scope	Shift+F11
Quick Select Button #1	Ctrl+1
Quick Select Button #2	Ctrl+2
Quick Select Button #3	Ctrl+3
<b>Meters and Mode Selection</b>	
Headstage 1 Total Resistance (Rt)	Ctrl+R
Headstage 2 Total Resistance (Rt)	Shift+R
Headstage 1 I=0 Mode	Ctrl+Z
Headstage 1 I-Clamp Mode	Ctrl+I
Headstage 1 DCC Mode	Ctrl+D
Headstage 1 dSEVC Mode	Ctrl+S
Headstage 1 Ext/Auto Mode Switch	Ctrl+A
Headstage 2 I=0 Mode	Shift+Z
Headstage 2 I-Clamp Mode	Shift+I
Headstage 2 HVIC Mode	Shift+H

Headstage 2 TEVC Mode	Shift+T
Headstage 2 Ext/Auto Mode Switch	Shift+A
<b>I-Clamp Tab</b>	
Zero Pipette Offset Lock	Ctrl+Alt+L
Zero Pipette Offset	Ctrl+Alt+P
Auto Bridge Balance Lock	Ctrl+Shift+L
Auto Bridge Balance	Ctrl+Shift+B
Clear+	Ctrl+J
Clear-	Ctrl+K
Pulse	Ctrl+Shift+P
Buzz	Ctrl+Alt+B
Zero Scaled Zero	Ctrl+Shift+O (letter)
<b>V-Clamp Tab</b>	
Zero Scaled Output	Ctrl+Shift+O (letter)
Pulse	Ctrl+Shift+P

<b>Keystroke</b>	<b>Function</b>
<b>Adjustable Numerical Fields</b>	
<Up Arrow>	Increases value. For dual control field, increases value in field to the left.
<Down Arrow>	Decreases value. For dual control field, decreases value in field to the left.
<Right Arrow>	Increases value. For dual control field, increases value in field to the right.
<Left Arrow>	Decreases value. For dual control field, decreases value in field to the right.

<Shift+Arrow>	As for Arrow commands above, but with greater increment.
<Ctrl+Arrow>	As for Arrow commands above, but with greatest increment.
<Page Up>	Increases value with greater increment: equivalent to <Shift+Up Arrow>.
<Page Down>	Decreases value with greater increment: equivalent to <Shift+Down Arrow>.
<Home>	Go to minimum value for field.
<End>	Go to maximum value for field.
<b>General</b>	
<Tab>	Go to next active field.
<Shift+Tab>	Go to previous active field.
<Ctrl+Tab>	Go to next tab.
<Shift+Ctrl+Tab>	Go to previous tab.
<Arrow>	Move between options (in non-numerical fields). Adjust values in numerical fields (see above: <i>Adjustable Numerical Fields</i> ).
<Space Bar>	Toggle on/off (checkbox only).
<Enter>	Accept changes: equivalent to pressing OK button.
<Esc>	Cancel.

Table 7. Shortcut keys.

## Load Configuration

You use Load Configuration <F4> to restore Axoclamp 900A Commander to a particular configuration of control settings by opening the Axoclamp Configuration (\*.acc) file in which the configuration was saved.

The Load Configuration tool button opens a standard Windows dialog box from which you can open such files.

Configuration files can also be opened with the Quick Select tool buttons or by using the desktop shortcut command line switch /C[ConfigFileName].

See *Save Configuration*.

## Mode Switching

The Mode Switch checkboxes are located beneath the Mode buttons for each headstage in the top section of the Commander interface:

- When external mode switching is configured, the checkbox label reads *Switch to dSEVC / TEVC on ext input*.
- When automatic mode switching is configured, the checkbox label reads *Switch to dSEVC / TEVC at threshold*.

Checking the Mode Switch when it is configured for external switching allows you to switch between current- and voltage-clamp modes with a remote device (*e.g.*, a Clampex digital output) for one channel at a time.

Alternatively, the Mode Switch enables automatic changes from current to voltage clamp (triggered by the membrane potential crossing a specified threshold) when this is configured.

Configure Mode Switch behavior on the *Options / Auto* tab.

Enabling either switching option disables the mode buttons.

## Connecting an External Command Signal

Connect an external digital command source to the MODE input BNC on the front panel of the Axoclamp 900A and check the *Switch to dSEVC / TEVC on ext input* box (but see note 1 below).

Axoclamp 900A now reads:

- 0 V MODE input as command for current clamp.

- 4–5 V as command for voltage clamp.

If the Mode Switch checkbox is not labeled *Switch to dSEVC / TEVC on ext input*, change it by selecting *When external mode logic goes HIGH* or *When external mode logic goes LOW* in the *Switch from I-Clamp to dSEVC / TEVC* section on the *Options / Auto* tab.

A step to 2.5 V should be adequate to switch the command, but a setting greater than 4 V is safer. It is important that there is sharp step between the two command signals, as a slowly increasing voltage will not be sensed. Rise time should be less than 20  $\mu$ s. Input impedance is 12.2 k $\Omega$ .

## Automatic Mode Switching

Automatic switching from current to voltage clamp, on crossing a stipulated membrane potential threshold, is configured on the *Options / Auto* tab.

When automatic mode switching is configured the Mode Switch label changes to *Switch to dSEVC / TEVC at threshold*, but the automatic switching is not enabled until the checkbox is checked.

## Additional Note

Before checking the *Switch to dSEVC / TEVC on ext input*. checkbox, ensure that a signal consistent with your actual Commander mode setting is being received. For example:

- If you are in V-Clamp mode then input at the MODE BNC must be +5 V before the *Switch to dSEVC / TEVC on ext input* checkbox is checked, so that the next input voltage transition to 0 V forces Commander to change to I-Clamp mode.
- If you are in I-Clamp mode then input at the MODE BNC must be 0 V before the *Switch to dSEVC / TEVC on ext input* checkbox is checked, so that the next input voltage transition to greater than 4 V forces Commander to change to V-Clamp mode.



## Monitor Scope

Monitor Scope <F11> launches a scope window to monitor the effects of changing the rate glider in DCC or dSEVC mode. In these modes the Axoclamp 900A repetitively cycles between current passing (through the electrode) and voltage measuring, at a frequency set by the rate glider.

This button also connects the scope to the Channel 1 scaled output signal when the <Shift> key is held down. The corresponding shortcut is <Shift+F11>.

## Options

The Options <F10> dialog box has five tabs on which many Commander parameters are set.

The Options tabs are:

- *Options / General.*
- *Options / Auto.*
- *Options / Audio.*
- *Options / Quick Select.*
- *Options / About.*

## Options / General

Use the General tab under the Options <F10> tool button to control the external command sensitivity of I-CLAMP and V-CLAMP COMMAND inputs, the scaled output lowpass filter type, the output to the SYNC OUTPUT BNC, view auxiliary headstage information and control other miscellaneous functions.

## External Command Sensitivity

Although the Axoclamp 900A Commander provides some simple built-in command stimuli (*e.g.*, via the *Pulse* button), it is expected that most experiments require more complex stimulus protocols. These must be supplied by an external pulse generator or a computer program like pCLAMP. External stimulus commands are supplied to the Axoclamp 900A via the COMMAND BNC on the front panel (one BNC for each Channel). Note that this is a DC-coupled input, so be sure that the external pulse generator is correctly calibrated so that zero volts really correspond to zero.

In V-Clamp mode, the purpose of External Command Sensitivity is to scale down the command signal in order to reduce the effect of noise in the external pulse generator. There are two settings: 20 mV/V and OFF. 20 mV/V means that a 1 V step applied to the COMMAND BNC appears to the cell as a 20 mV step; *i.e.*, external commands are divided down by 50-fold. This setting should be used when you want to minimize noise as much as possible.

In I-Clamp mode, the purpose of External Command Sensitivity is to scale a voltage COMMAND signal into current. For example, 100 nA/V means that a 1 V step applied to the COMMAND BNC appears to the cell as a 100 nA step injection of current. The Sensitivity setting changes as the value of the headstage current injection resistor is changed, since the amount of current that can be injected by the headstage depends on this resistor.

External Command Sensitivity settings are telegraphed to compatible data acquisition programs.

### *Additivity of Commands*

All command stimuli applied by the Axoclamp 900A are additive. That is, the external command is algebraically added to Holding, Pulse and Seal Test or Tuning commands before the sum is applied to the cell.

## Lowpass Filter Type

Choose the type of lowpass filter you want to apply to the Scaled Output, for each channel in each amplifier mode.

The options are Bessel or Butterworth. Bessel filters are preferred to Butterworth filters for time-domain analysis. Butterworth filters provide lower noise than Bessel filters, but they add considerable overshoot and are best used for frequency-domain analysis (*i.e.*, noise analysis).

The selected filter type is displayed in the Scaled Output tabs of the Axoclamp 900A Commander, at the bottom. Set the filter cutoff frequency in the adjustable field to the right of the filter type.

See also *Scaled Output—Lowpass Filter*.

## Sync Output

The SYNC OUTPUT BNC on the rear panel of the Axoclamp 900A outputs a TTL signal (HIGH, 2–5 V; LOW, 0–0.8 V) synchronized with the feature selected in this section. This can be used to send triggers to an oscilloscope, data acquisition software, or other equipment.

You can choose to follow internal commands or amplifier mode on either channel exclusively, or on the channel that is currently selected. A channel is selected when one of its Mode tabs is uppermost on the main Commander interface.

### *Tuning or Seal Test*

For the Seal Test and Tuning the TTL output follows the seal test or tuning pulse, going HIGH at the greater voltage or current phase of the pulse cycle, and LOW at the lesser phase.

A HIGH output is also generated for the duration of Pulse and Clear commands. There is no TTL output synchronized with Buzz.

## ***Mode***

A HIGH output is generated for voltage clamp, and a LOW output for current clamp and  $I=0$ . By including this signal as an extra channel in the recorded data file sections of data in each mode can be identified and analyzed separately. This is useful during auto mode switching when mode changes can be very fast.

## **Auxiliary Headstage Information**

If you are using additional headstages, *e.g.*, bath headstages, connected to the AUXILIARY HEADSTAGE connections on the rear panel of the Axoclamp 900A, this section reports the headstage types and the gain they apply, prior to gain applied with the gain settings in the Axoclamp 900A Commander interface.

Signals from both auxiliary headstages are available for selection on the Scaled Output.

## **Miscellaneous**

Other miscellaneous options at the bottom of this dialog are:

- *Show monitor scope in DCC or dSEVC mode.* Enable this checkbox to automatically start the monitor scope when mode is changed to DCC or dSEVC.
- *Show warning when oscillation detected.* Enable this checkbox to show an error message dialog after an oscillation has been detected and removed.
- *Show advanced signals in scaled output tabs.* Enable this checkbox to show additional signals from all headstages in the scaled output tabs.

## Options / Auto

The *Auto* tab under the *Options* tool button has configuration options for amplifier mode switching and action options for when oscillation is detected.

### Switch to from I-Clamp to dSEVC / TEVC

Configure amplifier mode switching, for each headstage, for when you enable the *Mode Switching* checkbox in the top section of the Commander interface.

All four major options in this section offer means to trigger a change to voltage clamp from current clamp. Options for how to trigger a return to current clamp after a change to voltage clamp are at the bottom of the section.

Changes are not triggered from I=0 mode.

You can synchronize a TTL output signal to amplifier mode selection, with Sync Output on the *Options / General* tab.

#### ***When External Input Logic Goes HIGH***

Select to change to dSEVC or TEVC when TLL goes HIGH. When this option is selected, the *Mode Switching* checkbox is labeled *Switch to dSEVC / TEVC on ext input*.

#### ***When External Mode Logic Goes LOW***

Select to change to dSEVC or TEVC when TLL goes LOW. You must configure the delay field below the option buttons for this selection. When this option is selected, the *Mode Switching* checkbox is labeled *Switch to dSEVC / TEVC on ext input*.

### ***On Negative-To-Positive $V_m$ Threshold Crossing***

Select to automatically change to dSEVC or TEVC when membrane potential goes over a stipulated threshold value. When this option is selected, the *Mode Switching* checkbox is labeled *Switch to dSEVC / TEVC at threshold*.

### ***On Positive-To-Negative $V_m$ Threshold Crossing***

Select to automatically change to dSEVC or TEVC when membrane potential falls below a stipulated threshold value. When this option is selected, the *Mode Switching* checkbox is labeled *Switch to dSEVC / TEVC at threshold*.

You must configure the delay field below for any of these selections and configure the membrane potential threshold field if you have selected a threshold option.

### ***Delay Change to Voltage Clamp By***

From the time a change to voltage clamp is triggered by any of the four options above, you can delay the actual change by up to 500 ms in increments of 2 ms.

### ***Membrane Potential ( $V_m$ ) Threshold***

For either of the two membrane potential threshold-crossing options above, enter the potential ( $\pm 200$  mV) at which you want the change to voltage clamp triggered.

## **Return to Current Clamp**

Select a means to return to current clamp following a change to voltage clamp made with one of the options above.

### *After*

Enter a duration up to 500 s to stay in voltage clamp before returning to current clamp. This is timed from when the amplifier went into voltage clamp, not from when the change to voltage clamp was triggered. The glider increments are 10 ms.

### *When External Mode Logic Goes LOW*

The Axoclamp stays in voltage clamp until a change to LOW mode logic (0–0.8 V TTL signal) on the MODE BNC.

This option can be selected irrespective of whether an external HIGH logic signal was used to switch to voltage clamp in the first place, *e.g.*, you might have switched to voltage clamp using an automatic threshold-crossing option. However, you must ensure that when the Axoclamp first switches to voltage clamp, the external TTL signal is HIGH, so that a change to LOW triggers a return to current clamp at the correct time.

### *Manually*

The Axoclamp remains in voltage clamp until you change the mode using the Commander mode buttons.

## **Options / Audio**

The Audio tool button <F9> opens the *Audio* tab in the Options dialog, where you can configure audio monitoring of output signals.

Audio output can be monitored by making connections to the Axoclamp 900A in one of three different ways:

1. Connect the rear panel AUDIO OUTPUT to the Line IN connector of your computer sound card. This allows the Axoclamp 900A to use the computer's speaker.
2. Connect headphones or remote powered speakers to the front panel PHONES output or the rear panel AUDIO OUTPUT. This allows

dedicated use of the headphones or external speakers by the Axoclamp 900A.

3. Connect the Line OUT of your computer sound card to the rear panel AUDIO INPUT of the Axoclamp 900A, and the rear panel AUDIO OUTPUT to external powered speakers. This is the same as option 2, except that now the Axoclamp 900A audio output is mixed with the computer's audio output to external speakers.

**Warning:** Never connect the computer's microphone jack to Audio connectors on the Axoclamp 900A. This could lead to large voltages being sent to the Axoclamp 900A, with the possibility of causing damage to its circuitry.

## Mute

Check this option to mute the audio monitor.

## Volume

Use the slide to set the volume of the audio monitor: right to increase volume, left to decrease.

## Audio Mode

The audio monitor operates in two different modes, which can be used for different types of recording.

### *Direct Signal Monitoring*

Direct signal monitoring uses a power amplifier that applies the signal directly to the speaker, thereby allowing signals in the audio bandwidth to be heard. Each spike is heard as an audible click, and the rate and volume of clicking is a good indicator of the muscle or nerve activity. This type of monitoring is frequently used in EMG monitoring or in central nervous system recording.



## ***Voltage Controlled Oscillator (VCO)***

Voltage controlled oscillator (VCO) uses a tone generator whose frequency depends on the amplitude of the input signal. The oscillator frequency increases with increasingly positive signals. It is often used for intracellular recordings, *e.g.*, to monitor a DC signal such as membrane potential. The default setting for the VCO is 2200 Hz at 0 V ranging to 300 Hz at -100 mV.

In the previous generation of Molecular Devices amplifiers, VCO was called tone mode.

## **Audio Signal**

Select from the list boxes the output signals you want to listen to with the audio monitor. You can listen to a different output for each channel in each mode.

Whether you hear Channel 1 or Channel 2 at any time depends on which tab is uppermost on the front panel. If a Channel 1 tab is on top, the Channel 1 output is audible, while Channel 2 output is audible when either of the Channel 2 tabs is uppermost.

## **Options / Quick Select**

The *Quick Select* tab under the *Options* tool button is used to configure the Quick Select Buttons on the toolbar. Use these buttons to open saved configurations files (\*.acc) of the Axoclamp 900A Commander, or to start an executable file (\*.exe).

To set a Quick Select button use one of the following methods:

- On the *Quick Select* tab, type the file-path of a saved configuration (\*.acc) or executable (\*.exe) file into the field associated with the Quick Select button that you want to configure. Closing the dialog box links the button to that file.

- Click the *Browse* button for your chosen Quick Select button, and navigate to a saved configuration (\*.acc) or executable (\*.exe) file in the Open File dialog. Closing the dialog box links the button to that file.

See *Load Configuration; Save Configuration*.

## Options / About

Access the *About* tab from the *Options* tool button in the main toolbar.

This tab contains:

- Version information for Axoclamp 900A Commander—important for you to know when contacting Molecular Devices Technical Support.
- Serial number of the Axoclamp 900A that Commander is associated with, and version information of Controller and DSP Firmware. This information is automatically displayed.
- A link to the Axoclamp 900A Commander Update page at Molecular Devices web site. Users are advised to check this page at regular intervals to ensure they have the latest bug fixes, Help updates and any new features that might be added to Commander between major version releases.

To help remind you of updates, check the option in this group and set the number of days (between 7 and 365) for reminder messages to be posted. A dialog box with a link to the Update page is displayed on startup of Commander whenever the number of days chosen has passed since you were last prompted with the reminder.

## Output Offset

The Output Offset command is located in the Scaled Output tabs in the Axoclamp Commander, at the bottom. It consists of an on/off checkbox, a

*Zero* button and a glider control for manual adjustment following use of the *Zero* button.

Output Offset is used to zero the reading of the Scaled Output by removing the DC voltage. It is useful for recording small signals that are riding on a large constant offset current or voltage. With the *Zero* button, it samples output current or voltage for around 70 ms immediately after pressing, and then subtracts the value found from subsequent Scaled Output signals. In most cases it is recommended that Output Offset is not used, as useful information may be lost. When used, the *Zero* command is generally adequate.

Unlike Leak Subtraction or Bridge Balance, Output Offset does not account for currents or voltages that change as a result of time-varying command pulses; it simply provides a constant offset adjustment.

The Zero Output Offset only affects the signal on the Scaled Output. In other words, the cell is not affected by the Output Offset command. No other input or outputs are affected.

The offset is reported in mV only. When the Scaled Output is set to read voltages—in either voltage-clamp or current-clamp modes—it gives a correct reading. When the Scaled Output is set to read current (in either mode) the reported offset value must be divided by the Scaled Output scale factor to give the offset in terms of current, *e.g.*, if the Scaled Output scale factor is 1 V/nA and the Output Offset field reports 2000 mV, the current offset is 2 nA.

The amount of offset required remains invariant under most changes in the scaled output gain. When output gain is less than 10, there is  $\pm 3000$  mV offset available. If the gain is 10 or greater, there is a limit of  $\pm 300$  mV.

## How to Use Output Offset

### *Auto*

Click the *Zero* button to automatically calculate and apply an offset value. The Output Offset checkbox is checked automatically to show the newly

calculated offset is being applied. Offset value is displayed in the adjustable field.

You can fine-tune the offset value manually after pressing *Zero*.

### ***Manual***

Check the checkbox and then adjust the value to optimize the Scaled Output signal (but note that *Zero* is generally used for this command).

### **Additional Notes**

Output Offset can fail if the DC voltage is greater than  $\pm 3$  V.

## **Pipette Capacitance Neutralization**

### **Input Capacitance**

The capacitance ( $C_{in}$ ) at the input of the headstage amplifier is due to the capacitance of the amplifier input itself ( $C_{in1}$ ) plus the capacitance to ground of the microelectrode and any connecting lead ( $C_{in2}$ ).  $C_{in}$  combined with the microelectrode resistance ( $R_e$ ) acts as a lowpass filter for signals recorded at the tip of the microelectrode. For optimal performance at high frequencies this RC time constant must be made as small as possible.

Two techniques may be used to increase the recording bandwidth.

- Use microelectrodes with the lowest possible resistance compatible with stable recording, and take steps to minimize the contribution to  $C_{in}$  by the capacitance of the microelectrode. In practice, this means using patch electrodes where possible, or using sharp microelectrodes with minimal capacitance (see Chapter 4, *Sharp Microelectrode Recording*).
- Electronically neutralize  $C_{in}$ .

The second approach has been implemented in the Axoclamp 900A in two ways.

### *Primary Method for Neutralizing $C_{in}$*

In some cases the steps discussed above may not be sufficient to decrease the RC time constant of the voltage-recording microelectrode, particularly in situations where high resistance microelectrodes must be used. For this reason an effective, though less desirable, technique is provided that can electrically reduce the *effective* magnitude of  $C_{in2}$ . The technique is known as “capacitance compensation”, “negative capacitance” or “capacitance neutralization”. A compensation amplifier at the output of the unity gain buffer drives a current injection capacitor connected to the input. At the ideal setting of the compensation-amplifier gain, the current injected by the injection capacitor is exactly equal to the current that passes through  $C_{in2}$  to ground.

### *Secondary Method for Neutralizing $C_{in}$*

A special technique is used in the HS-9A headstage to keep the contribution to  $C_{in}$  from the input amplifier as small as possible. The technique is known as “bootstrapping”. Unity gain feedback is used to reduce the component of stray capacitance that exists between the amplifier input and its power supplies and case. Sophisticated circuitry is used to superimpose the unity-gain output of the buffer amplifier back onto its own power supplies, fixing the voltage drop across  $C_{in1}$  to a constant value, thereby preventing current flow through  $C_{in1}$ . The effective value of  $C_{in1}$  is thus reduced to well below its real value. This eliminates the high-frequency current loss through the power supply capacitance, thereby increasing the bandwidth. Since the power supply capacitance is present whether or not the power supply is bootstrapped, there is no noise penalty due to implementing this technique.

## **Adjusting Capacitance Neutralization**

Check the Tuning checkbox and choose amplitude (nA) and frequency (Hz) parameters that result in a sawtooth pattern of about 10 mV amplitude on “Scaled Output: Membrane Potential”. Carefully increase the Pipette Capacitance Neutralization value until overshoot just starts to appear in the step response. This is easiest to see if you have already adjusted Bridge

Balance (see *Bridge Balance* in this chapter). If you go too far the overshoot may become a damped oscillation, which may escalate into a continuous oscillation, killing the cell.

Sometimes the overshoot is difficult to see. In this case, you may prefer to look at the “Scaled Output: Membrane Potential” trace at high gain on an oscilloscope, advancing the Pipette Capacitance Neutralization value until the trace becomes noisy and oscillations seem imminent. It is usually prudent to reduce the Pipette Capacitance Neutralization setting slightly from the optimal, in case the capacitance changes during the experiment.

## Limitations of Capacitance Neutralization

Use of capacitance neutralization is less desirable than physically minimizing  $C_{in2}$ , since the neutralizing circuit adds noise to the voltage signal.

It is important to recognize that the capacitance neutralization circuit is not more than 90% effective even for ideal microelectrodes. This is because of the finite frequency responses of the headstage amplifiers and the capacitance neutralization circuit, and also because  $C_{in2}$  does not behave ideally as a linear lumped capacitor. Consequently, the amount of  $C_{in2}$  that the circuit must neutralize should be kept as small as possible (see Chapter 4, *Sharp Microelectrode Recording*).

## Oscillation Detection and Removal

The Axoclamp 900A supports automatic oscillation detection and removal when using Pipette Capacitance Neutralization; see *Detect Oscillation*.

## Pipette Offset

The Pipette Offset control is located in the upper right corner of both V-Clamp and I-Clamp tabs. It consists of a *Zero* button with a padlock button to “lock” it, and a glider control for manual adjustment following use of the *Zero* button.

Pipette Offset is used at the beginning of each experiment, when the pipette is first introduced to the bath, to compensate for potentials caused by differences in the concentration of bath and pipette solutions.

## How to Use Pipette Offset

- Introduce the pipette to the bath and click the *Zero* button. A test pulse is generated, potential calculated, and offset automatically applied.
- The associated padlock button “locks” and the *Pipette Offset* button is disabled.
- Manual adjustments can be made to the setting following use of the *Zero* button. Adjust the glider control until the reading in the current meter is zero. The allowed range is  $\pm 250$  mV. The glider control allows fine adjustments with increments of 20  $\mu$ V for current clamp.
- To reset the Pipette Offset, click on the “locked” padlock button to “unlock” it, and click the *Zero* button again. Note the cautionary point below, however.

## Additional Notes

- Auto Pipette Offset can fail if the DC voltage is greater than  $\pm 250$  mV.
- To avoid introducing errors, you should not change the pipette offset after penetrating a cell with a sharp electrode, or after switching from voltage-clamp to current-clamp mode.

## Pulse

The Pulse command is located near the bottom of both V-Clamp and I-Clamp tabs. It includes an activation button, and adjustable fields for pulse amplitude and duration.

Pulse provides a way of generating simple command currents (I-Clamp) and command voltages (V-Clamp). The amplitude and duration of these can be set in the associated fields.

More complex command stimuli can be generated by an external pulse generator or a computer program like pCLAMP.

## How to Use Pulse

- Set the desired voltage or current Pulse amplitude in the left-hand field. If typing in the value in current clamp, use pA.
- Set the desired Pulse duration in the right-hand field. Note that only certain values are available; right-click on the field to see these.
- Click the *Pulse* button to activate the pulse.

## Additional Notes

Pulse generates a Sync Output.

## Quick Select

The three Quick Select buttons can be customized to:

- Open saved Configuration files (\*.acc) of Axoclamp 900A Commander.
- Run an executable file on your computer or network.

Configure the buttons in the *Options / Quick Select* dialog box, which you can access with the toolbar *Options* button <F10>.

**Note:** You can configure your SoftPanel to activate Quick Select Buttons.

## Configuration Files

Use the first option to have Axoclamp 900A Commander configurations at your fingertips. Once the buttons are set, clicking on a button opens its associated configuration (of all Axoclamp 900A Commander settings) directly into the program.



Configuration files can also be opened with the *Load Configuration* tool button <F4>.

## Executable Files

Use the second option to start any executable file on your computer that you want to run concurrently with your experiment.

## Reset to Program Defaults

Clicking the *Reset to Program Defaults* <F6> button resets all Axoclamp 900A Commander options to their default values. Regardless of which mode tab is on top when this button is clicked, all tabs are reset, including the SoftPanel.

## Resize Window

*Resize Window* <F2> resizes the Axoclamp 900A Commander interface. It toggles the interface between three states:

- Full display: the whole interface is shown.
- Meter panel: only the toolbar, meters and mode buttons are shown.
- Manually resized: only enabled if you have resized the interface by dragging the edges or corners, the program remembers the new sizing and interface positioning within the window.

## Save Configuration

Use *Save Configuration* <F5> to save all the control settings current at any time (e.g., the amplifier mode setting, settings for Holding and Pipette Offset, etc.). These configurations are saved as Axoclamp Configuration files (\*.acc). This allows the user to restore these settings at a later date, simply by opening the appropriate file.

The *Save Configuration* tool button opens a standard Windows dialog box in which you can save the current settings configuration.

When naming configuration files it is advisable to not use spaces in the name. For example, if you want to open a configuration file using a desktop shortcut command line switch, files with spaces in their names are not recognized.

Once a configuration has been saved, you can reinstate it by opening the appropriate file, either with *Load Configuration*, *Quick Select*, or by using a desktop shortcut command line switch.

See *Load Configuration*.

## Scaled Output

The Scaled Output field is located in the lower part of each of the I-Clamp tabs and in the dSEVC and TEVC tabs.

The Scaled Output field determines the output signal at the SCALED OUTPUT BNC. The scale factor displayed beside the signal name is calculated from the selected output gain and headstage current injection resistor.

See below for diagram to explain potential-reading signals.

## Voltage Clamp

The signals available in voltage clamp are: TEVC, HIVC, dSEVC, DCC.

### *Membrane Current*

*The current measured at the top of the pipette.*

### *Membrane Potential*

$$V_m + V_{rs}$$

Membrane Potential includes the effects of pulse commands (such as the Seal Test), the Holding level, and external commands. Pipette offset is removed from the signal, but the voltage drop across the pipette is not.

### ***External Command Potential***

The command signal input at the COMMAND input BNC.

### ***Auxiliary 1 Potential***

Potential from the rear-panel AUXILIARY HEADSTAGE # 1 connection (see note below).

### ***Auxiliary 2 Current***

Current from the rear-panel AUXILIARY HEADSTAGE # 2 connection (see note below).

## **Current Clamp**

The signals available in current clamp are:

### ***Membrane Potential***

Membrane Potential includes the effects of pulse commands (such as the Tuning), the Holding level, and external commands. Pipette offset is removed and Bridge Balance is applied.

### ***Membrane Current***

The current measured at the headstage, including all corrections such as Pipette Capacitance Neutralization and Bridge Balance.

### ***Command Current***

The current sent to the headstage before any corrections have been applied.

### ***External Command Current***

Reads the command signal input at the COMMAND input BNC.

### ***Auxiliary 1 Potential***

Potential from the rear-panel AUXILIARY HEADSTAGE # 1 connection (see note below).

### ***Auxiliary 2 Current***

Current from the rear-panel AUXILIARY HEADSTAGE # 2 connection (see note below).

## **Auxiliary Headstages**

HS-2 and VG-2 headstages can be used as auxiliary headstages at the AUXILIARY 1 HEADSTAGE and AUXILIARY 2 HEADSTAGE inputs, typically as headstages for extracellular recordings. The scale factor, determined by the headstage, is displayed beside the signal name when connected on the *Options / General* tab.

## **Telegraphs**

Scaled output signal type, units and scaling factors are telegraphed to compatible data acquisition programs.

## **Conditioning the Scaled Output**

The following fields below the Scaled Output field set parameters for conditioning the output signal:

- Gain.
- Lowpass Bessel or Butterworth Filter.
- Highpass Filter.

The scale factor of the signal when reading current is also affected by the selected current injection resistor.

## Highpass Filter

This option is for measurement of very small signals, *e.g.*, extracellular voltages in current clamp. It offers highpass filtering from DC to 100 Hz to minimize the effects of drift and can be applied to the headstage Scaled Output signal along with the lowpass filter. The Highpass field is located in the Scaled Output section beneath the V-Clamp and I-Clamp tabs. There are 8 cutoff frequency options offered (right-click the field to see these).

### *How To Set Highpass Filtering*

- Use glider controls or select from the right-click popup menu to set the cutoff frequency desired. Filtering begins immediately.
- Set the field to DC to bypass this filter.

## Gain

Scaled Output Gain is in the Scaled Output section in the lower part of both V-Clamp and I-Clamp tabs. It has one adjustable field determining the gain value.

Scaled Output Gain determines the amplification of the raw output signal to the SCALED scaled output BNC, *e.g.*, a gain setting of 2 doubles the strength of the output signal, of 20 increases it 20×, and so on.

Gain can be set at certain values only; right-click on the field to see these.

As Scaled Output Gain is altered, the scale factor displayed beside the signal type in the *Scaled Output* field is updated accordingly.

### *How To Set Gain*

- Use glider controls to adjust the field or right-click and select the gain you want. Changes are implemented immediately.

### ***Additional Notes***

Output signal gain is also affected by the headstage current injection resistor selected.

Scaled Output Gain is telegraphed to compatible data acquisition programs.

### **Lowpass Filter**

The type of lowpass filter enabled, Bessel or Butterworth, is displayed in the Scaled Output section in the lower half of both V-Clamp and I-Clamp tabs.

Filter cutoff frequencies are displayed in the adjustable field to the right of the filter type.

The Axoclamp 900A Commander offers a choice of two different lowpass filters to the Scaled Output: Bessel or Butterworth. Select these on the *Options / General* tab.

Different cutoff frequency settings are available for the two filter types. Right-click on the adjustable field to see the options.

Bessel is the default lowpass filter, as it is preferred for time-domain analysis.

### ***How To Set the Lowpass Filter***

- Check you have the correct filter type. To change it go to the *Options / General* tab.
- Adjust the field value using glider control, or select from the right-click popup menu, to set the desired filter cutoff frequency. Changes apply immediately.

### *Additional Notes*

Lowpass filter information is telegraphed to compatible data acquisition programs.

See Chapter 4 for a background discussion of filtering methods.

## **Seal Test**

The Seal Test controls are located in the top section of the V-Clamp tab.

In V-Clamp mode only, Seal Test generates test pulses to be used during seal formation, or any other time you need a regular input pulse in order to monitor some parameter in your experimental setup.

You can choose potentials from 0 to  $\pm 1000$  mV.

Discrete frequency settings between 2 and 5000 Hz are available. View the options by right-clicking on the frequency field.

### **How To Use Seal Test**

- Set the Seal Test amplitude and frequency fields to the desired values.
- Start the Seal Test pulse by checking the checkbox. Pulse parameters can be changed after Seal Test has commenced.
- Uncheck the checkbox to stop Seal Test.

### **Additional Notes**

- Seal Test is the voltage clamp analog of Tuning in current-clamp mode.
- Seal Test generates a Sync Output.

## Select Device

The *Select Device* <F7> tool button opens a dialog box that allows the user to:

- Configure Axoclamp 900A Commander to run either in Demo mode or connected to an Axoclamp 900A amplifier.
- Configure the serial or USB port to which a SoftPanel is connected.

## Axoclamp Tab

### *Demo Mode*

Click this button to operate in Demo mode. This allows you to explore Axoclamp 900A Commander without being connected to an Axoclamp 900A instrument.

### *Axoclamp Hardware*

Click this button to connect the Axoclamp 900A Commander software to your Axoclamp 900A amplifier. If you want to run more than one Axoclamp 900A amplifier at a time, simply open a new instance of the Axoclamp Commander for each amplifier and select which amplifier you want to control within this dialog.

### *Scan*

Click this button to detect all Axoclamp 900As connected to the computer. All Axoclamp 900A amplifiers that are connected are listed, even if these are already under control from another instance of Axoclamp 900A Commander.

### *Serial Number*

The serial numbers of the Axoclamp 900A amplifiers that Axoclamp 900A Commander recognizes as physically connected to your computer (after pressing the Scan button) are displayed in this list box. If there is more than



one amplifier, select the one that you want this instance of Axoclamp 900A Commander to control.

If an amplifier in the list is already controlled by another instance of Axoclamp 900A Commander, you are not permitted to connect to it. In this case an error message reports that the Axoclamp has been connected by another instance of Axoclamp 900A Commander.

Connections can also be automatically established on startup using the desktop shortcut command line switch /S[SerialNumber]. This option is useful if you want to connect to more than one Axoclamp 900A amplifier without manually selecting the correct device each time.

## SoftPanel Tab

Use this tab to associate a SoftPanel with Axoclamp 900A Commander.

### *Scan*

Click the *Scan* button to search all serial and USB ports for SoftPanels. Results are displayed in the Port field.

Once you have attached a SoftPanel, Axoclamp 900A Commander automatically attaches it each time you start up, as long as it is plugged in to your computer.

### *Port*

Identifying numbers for SoftPanels connected to the computer are listed in this list box (after pressing the Scan button). If there is more than one SoftPanel attached, select the one you want this instance of Axoclamp 900A Commander to use, to control the amplifier that is connected to the instance.

If a SoftPanel in the list is already connected to another instance of Axoclamp 900A Commander, the dialog reports that it is unavailable.

Different identifiers are used for serial and USB SoftPanels:

- RS232 (serial) SoftPanels are identified by the serial (COM) port to which they are attached.
- USB SoftPanels are identified by a “USB” prefix, and the SoftPanel’s serial number.

## Slow Current Injection

The Slow Current Injection section is in the upper half of the I-Clamp tab.

In current-clamp mode you can track a preset membrane potential by automatically injecting a slow current into the headstage. You must enter both the membrane potential you want to track, and a time to set the speed at which current is injected to follow that potential.

Slow current injection cannot be enabled at the same time as current clamp Holding.

The speed at which current is injected into the cell depends on the current injection resistor and load as well as the setting in the *Time constant* field. The time constant values shown in the glider are correct for the HS-9A  $\times 1$  headstage and the CLAMP-1U model cell.

Current injection duration is proportional to the resistance of the injection resistor, *i.e.*, the higher the resistance of the injection resistor the longer it takes to reach the desired voltage. On the other hand, the current injection duration is inversely proportional to the load, *i.e.*, the higher the resistance of the load the faster it reaches the desired voltage. For example, voltage-tracking with this feature is slow when the electrode is in the bath, and fast when in the cell.

In summary:

- Injection resistor: High resistance—slow response.
- Load: High resistance—fast response.

## How To Use Slow Current Injection

- Set the potential at which to maintain the cell membrane.
- Set the time for current injection (see discussion above).
- Turn slow current injection on with the checkbox. If the checkbox is disabled, you must turn off Holding.

## Telegraphs

Axoclamp 900A Commander informs other applications (including Molecular Devices' pCLAMP in versions 10 and higher) of a range of its parameter settings. These telegraphs are handled by the program, using the Windows messaging system. The benefit of this is that no additional telegraph cabling is required: the computer connection to the Axoclamp 900A transmits all telegraphs.

Axoclamp 900A Commander telegraphs the following state information:

- Amplifier mode.
- Scaled Output signal type.
- Scaled Output Gain.
- Scaled Output Lowpass Filter cutoff.
- Scaled Output scale factor when the Gain is 1.
- Scaled Output scale factor units.
- External Command Sensitivity.
- Axoclamp 900A Commander application version.
- Firmware version—low memory.

- Firmware version—high memory.
- Amplifier Serial Number.

Third-party software developers can contact Molecular Devices Technical Support for details of Axoclamp 900A Commander telegraphs.

## Tuning

The Tuning control is located in the top section of the I-Clamp tab.

In current-clamp mode only, the Tuning command generates an AC current that is fed to the electrode. Current amplitude range depends on the headstage current injection resistor selected:

Injection Resistor	Current Amplitude
1 M $\Omega$	$\pm 12 \mu\text{A}$
10 M $\Omega$	$\pm 1.2 \mu\text{A}$
100 M $\Omega$	$\pm 120 \text{nA}$

**Table 8.** Headstage current amplitude range.

Frequency settings for Tuning range in discrete values between 2 and 5000 Hz. Right-click the field to view the options.

## How To Use Tuning

- Set current amplitude and frequency to the values desired, and then turn the signal on by checking the checkbox. If typing in the amplitude, enter the value in pA. Frequency values can be selected from the right-click popup menu.
- Signal parameters can be adjusted once the command has been activated.
- Uncheck the checkbox to turn off the Tuning current.

## Additional Notes

- Tuning generates a Sync Output.
- Tuning is the current clamp equivalent of Seal Test in voltage clamp.

## V-Clamp Control

### Gain

See *Detect Oscillation*.

### Lag

A voltage clamp is a negative-feedback circuit and as such it requires a  $90^\circ$  phase shift within the circuit. Ideally this phase shift is supplied by the capacitance of the membrane. In practice, membranes introduce significantly less than  $90^\circ$  phase shift (see discussion by Finkel & Gage, 1985).

The frequency response of the voltage-clamp circuit can be modified by the Lag control. The voltage-clamp circuit can thereby be adjusted to compensate for the non-ideal phase response of real membranes. The controls are in the Axoclamp 900A Commander, in the Voltage Clamp Control section of the TEVC and dSEVC tabs.

Phase lag cuts the high-frequency gain of the voltage-clamp circuit. This can be used to reduce the noise but at the same time it slows the response and introduces ringing. In the extreme lag position the phase-control circuit introduces pure lag.

In some instances no phase lag is required (*e.g.*, with an RC cell model the best TEVC or dSEVC is achieved when no phase lag is used).

### *How to Use Lag*

Lag can be used during voltage clamp to compensate for the frequency characteristics of membranes that are not well modeled by a parallel resistance and capacitance. Both the membrane voltage and current step responses should be improved by using the Lag. If only the membrane voltage step response is improved it is likely that there is a resistance ( $R_s$ ) in series with the membrane.

In some cases using some lag reduces the current noise during voltage clamp. See the discussions on each type of voltage-clamp mode for more details.

### **DC Restore**

One potential source of a small but variable input leakage current is due to DC current flow through the dielectric of the capacitor ( $C_n$ ) used for pipette capacitance neutralization. For example, the pipette potential might be 200 mV (although the experimenter does not see this potential because of pipette offset compensation). To compensate several microfarads of input capacitance, the gain of the pipette capacitance neutralization might be 2. Thus 400 mV would be fed back to  $C_n$ , resulting in 200 mV across this capacitor. If the dielectric resistance of  $C_n$  were  $10e^{11}$  ohms, there would be 2 pA through this capacitor.

To eliminate this source of leakage current, the DC Restore circuit removes the DC voltage from across  $C_n$ . There may be a transient shift in the electrode voltage while the pipette capacitance neutralization is being adjusted.

## Chapter 6: Troubleshooting

It has been our experience at Molecular Devices that the majority of troubles reported to us have been caused by faulty equipment connected to our instruments.

If you have a problem, please physically disconnect all instruments connected to the Axoclamp 900A except for the oscilloscope. Ideally, remove the Axoclamp 900A from the rack. Work through the Setup and Tutorials. This can often uncover a problem that is in your setup. If the problem persists, please call us for assistance.

Another common problem is caused when dirt or corrosion build up in the headstage connector socket, which can cause unstable current and voltage offsets. It is important to keep the holders and the headstage inputs clean.

## Voltage Clamp Problems

Some problems and possible causes are listed below.

### dSEVC

**Symptom:** The V-Clamp Gain cannot be increased to the level appropriate to clamp the cell even after adjusting the Pipette Capacitance Neutralization.

**Possible cause:** The microelectrode response may be too slow.

**Suggestion:** Try a lower resistance microelectrode such as a patch pipette.

### TEVC

**Symptom:** The voltage clamp becomes unstable even at low gains. Oscillation is seen on the current trace during voltage steps.

**Possible cause:** The inter-electrode coupling capacitance may be too high.

**Suggestion:** Check that the shield between the microelectrodes is correctly placed and adequately grounded.

**Symptom:** The voltage clamp is slow to respond. There may also be a DC error.

**Possible causes:** The voltage-clamp gain is too low.

**Suggestions:** Use phase lag if necessary to enable the gain to be increased. If the problem is present even at maximum clamp gains then the current-passing microelectrode is probably blocked. This is most likely to occur when using high resistance micropipettes. Withdraw the current-passing microelectrode, replace it, and try again. Microelectrodes filled with 4 M K-acetate or K-citrate tend to pass current better than KCl filled electrodes.



**Symptom:** The time-course of the voltage step is slower than the current transient. The peaks of the current transients are clipped (have a flat top).

**Possible cause:** The current-passing side of the voltage clamp is saturating. If this is the case then variation in the gain around its maximum level will have little effect on the voltage rise time. Most likely the output amplifier is saturating, but if you are using a virtual ground headstage to measure current then the saturation may be occurring there.

**Suggestions:** If the rise time is adequate and non-linear capacitive currents are acceptable, then there is no problem. If the saturation is not acceptable, then reducing the resistance of the current-passing microelectrode may help considerably. If you halve this resistance you halve the output voltage required to drive the same current. If the problem lies in the virtual-ground unit, use another one with higher range.

## All Voltage-Clamp Modes

**Symptom:** Unable to offset the voltage microelectrode voltage to zero.

**Possible causes:** There may be a break in the connection between the headstage input and ground, causing the input to float. The capacitance neutralization circuit may be oscillating.

**Suggestions:** Check the electrical continuity and DC stability of the voltage recording and bath ground electrode holders. Check for bubbles in the microelectrodes. Observe the voltage signal at a wide bandwidth to check that the capacitance neutralization circuit is not in oscillation.

**Symptom:** Persistent overshoot during a voltage step. High voltage and current noise. Cannot be “tuned” out.

**Possible cause:** If you are using an external voltage command, examine the command signal on an oscilloscope. The command signal itself may overshoot during a voltage step. Some D/A converters can also have high levels of digital switching noise.

**Suggestions:** Either get another D/A converter or lowpass filter the voltage command signal. Select the filter cut-off frequency such that the rise time of  $V_{cmd}$  is faster than the rise time of the CHANNEL 1 microelectrode voltage. The  $V_{cmd}$  signal should not be the limiting factor determining the clamp step response rise time. Never apply filtering to  $V_{cmd}$  as a way of compensating for performance in the voltage clamp setup—this is a false benefit.

**Symptom:** Extraneous noise in the  $V_m$  signal.  $V_m$  may drift several mV/min.

**Possible cause:** The Ag/AgCl pellet or Ag wire in an electrode holder may be defective.

**Suggestions:** Check the DC stability of the various voltage recording and bath ground electrode holders and replace where necessary.

**Symptom:** Time-course of the voltage step is faster than the current transient.

**Possible causes:** 1) A large series resistance or 2) the cell is not isopotential. The latter is common when you voltage clamp a neuronal soma with an axon and dendrites attached. The slower components in the current transient are due to charging of the distributed capacitance of these processes. In the absence of significant series resistance the voltage recorded is an accurate representation of the soma potential, but not that in the processes. Series resistance exaggerates the difference in time course.

**Suggestions:** 1) Reduce the series resistance (see *Series Resistance* and, if using TEVC, *Bath Error Potentials* in Chapter 4) or 2) if your cell is not isopotential there is not much you can do short of cutting off or ligating the processes.

## Space Clamp

There is one limitation to the performance of the voltage clamp that cannot be electrically compensated. This is the deviation of the cell from a sphere centered on the tip of the voltage-recording microelectrode. The voltage clamp is maintained at the tip of the voltage-recording microelectrode. If all

portions of the cell membrane are separated from this tip by equal access resistance, then the membrane will be uniformly voltage clamped. However, many cells have processes such as axons, dendrites and filopodia attached to the cell body (where the microelectrodes are usually located). The membranes of these processes are separated from the cell body by an axial access resistance whose value depends on the distance to each portion of the membrane and the cross section in that region of the cell. Thus there is a voltage drop across the access resistance that becomes substantial for distal components of the membrane. Even though the somatic membrane potential may be well controlled, the axonal or dendritic membrane potential may be very poorly controlled. In these cases, the time course of synaptic currents, regenerative currents and measurements of reversal potentials may be grossly distorted.

As a general rule, the voltage clamp is considered to be acceptable if the length of the attached axon or dendrites is no more than 0.1 length constants. (Even this short length will cause significant distortion of fast currents. See Figure 7 in Rall and Segev, 1985). Calculation of the length constant for a cell is complicated since it depends on the geometry of the particular cell under investigation. Some of the common ways to avoid the problems of poor space clamping are as follows:

- Restrict investigations to spherical cells. Many cultured cells are convenient.
- Ligate attached axons. For example, the axon of large molluscan neurons can be tied off with nylon thread.
- Use short segments. For example, short segments (100  $\mu\text{m}$ ) of arteriolar syncytia can be separated from the arteriole by careful cutting with a razor blade.
- Restrict the range of the clamp to a short segment of the cell. This is the essence of the “sucrose gap” technique sometimes used on axons.
- Restrict the measurement to currents that are generated close to the microelectrodes. For example, the end plate currents in muscle fibers

can be well clamped, even though the bulk membrane current is very poorly clamped.

## Noise

To realize optimal noise performance, the user must pay close attention to noise sources. All potentially contributing noise sources must be minimized. Specifically, the headstage, the pipette glass, the holder and, in the case of patch pipettes, the membrane seal contribute significant noise even under circumstances where extraneous noise pickup from the environment is negligible. It is absolutely crucial that the entire preparation be properly shielded, and that hum from power supply, mains and other sources be negligible.

## Chapter 7: Specifications

### Functional Specification

#### Instrument Usage

Unless otherwise specified:

- $T_A = 20^\circ \text{C}$ , 1 hr warm-up time.

#### Main Unit

- Line voltage: universal 100–240 VAC.
- Line frequency: 50–60 Hz.
- Power: 30 W (maximum).
- Fuse: 5 mm × 20 mm, 250 V, 2A slow.
- Case (H × W × L): 4.3" × 19" × 14.3" (109 mm × 483 mm × 363 mm).
- Weight: 9.5 lbs (4.3 kg).
- Rack use: standard 19" rack-mount (2U) with handles.

- Tabletop use: bayonet feet.

## Headstage

- (H × W × L): 0.75" × 0.70" × 2.25" (19 mm × 18 mm × 57 mm).

## Axoclamp 900A Headstages

- HS-9A ×0.1    unity gain voltage-follower     $R_0 = 100 \text{ M}\Omega$ .
- HS-9A ×1     unity gain voltage-follower     $R_0 = 10 \text{ M}\Omega$ .
- HS-9A ×10    unity gain voltage-follower     $R_0 = 1 \text{ M}\Omega$ .
- VG-9A ×10    bath clamp                     $R_0 = 1 \text{ M}\Omega$ .
- VG-9A ×100   bath clamp                     $R_0 = 0.1 \text{ M}\Omega$ .

$R_0$  is the current passing resistor in the headstage.

## Output Compliance

- CHANNEL 1:  $\pm 12 \text{ V}$  in all modes.
- CHANNEL 2:  $\pm 12 \text{ V}$  in I-Clamp.

$\pm 180 \text{ V}$  in TEVC and HVIC.

## Pipette Capacitance Neutralization

- CHANNEL 1:  $-10$  to  $35.5 \text{ pF}$  in all modes.
- CHANNEL 2:  $-10$  to  $35.5 \text{ pF}$  in I-Clamp.

No neutralization on CHANNEL 2 in TEVC and HVIC.

## Oscillation Detection and Correction

Oscillation is detected on membrane voltage of both CHANNEL 1 and CHANNEL 2.

AC signals  $> 140 \text{ mV}_{\text{p-p}}$  in I-Clamp or  $80 \text{ mV}_{\text{p-p}}$  in voltage clamp at frequencies  $> 4 \text{ kHz}$  for longer than 2 ms are defined as an oscillation.

### *I-Clamp and DCC*

Oscillation correction reduces neutralization by 1 pF. Optionally, it can reduce neutralization to zero.

### *SEVC and TEVC*

Oscillation correction reduces voltage clamp gain by 2:1. Optionally, it can reduce gain to minimum.

## Sample Rate

600 Hz to 50 kHz (Discontinuous mode).

## DCC Mark/Space

30% command 70% settling (Discontinuous mode).

## Phase Lag

54  $\mu\text{s}$  to 50 ms (128 steps).

## dSEVC Gain

1 to 2500 /  $R_0$  A/V (e.g., 1 to 2500 nA/mV for HS-9A  $\times 10$ ).

## TEVC Gain

20 to 50,000.

## **DC Restore**

When selected in TEVC or dSEVC modes, DC restore increases DC Voltage-Clamp Gain to ~1,000,000. This means the holding level is restored to > 99% of the programmed value in < 100 ms.

## **Buzz**

Capacitance neutralization increased by 4 pF for a programmable time (0.1 ms to 500 ms). This is also available remotely via a tethered buzz box (> 2.5 V = buzz).

## **Clear (+/-)**

Maximum positive or negative current—manual up to 0.5 s.

## **Step Activate**

Available on each channel, STEP ACTIVATE 1 and STEP ACTIVATE 2 inputs work independently, setting external timing for going between the holding level and a programmable pulse with external timing up to 50 kHz. External timing is set with a TTL high pulse input to the STEP ACTIVATE BNC for the duration of the step. The amplitude of the step is set by the Tuning amplitude in current clamp and by the Seal Test amplitude in voltage clamp.

## **Blank Activate**

Available on rear panel BLANK ACTIVATE 1 BNC or REMOTE connector. Used for blanking response to external stimuli on Channel 1 only.



## Scope

The scope is a self-contained single channel 5 MHz acquisition system with its own USB plug and a PC software scope. It allows the user to see critical switched membrane voltage signals during the switching period, which helps in the setup of neutralization and gain in discontinuous modes (DCC and dSEVC).

The monitor signals are also available on the rear panel (MONITOR and SAMPLE RATE BNCs) if the user prefers an analog scope.

- Interface: Dedicated USB 2.0 connector.
- Acquisition rate: 2 kHz to 5 MHz.
- Resolution: 14 bits.
- Monitor signal: Raw membrane potential (before sample and hold).
- PC display: PC digital scope that is launched whenever DCC or dSEVC is selected. It can be inhibited and manually launched from Axoclamp 900A Commander.

## Options

### *Buzz Box*

Allows remote buzzing of cells in either channel via long life Hall effect switches with endurance of more than 1,000,000 operations. Plugs into REMOTE connector.

### *Adapter for HS-2 and VG-2 Headstages*

Allows Axoclamp 2 headstages (HS-2 and VG-2) to be used in the Axoclamp 900A (part number 1-2100-0934). Presence of this connector is automatically detected by Axoclamp 900A Commander and user is asked to input calibration parameters.

## Internally Generated Signals

### *I-Clamp*

- Pipette offset:  $\pm 200$  mV with auto zero.
- Bridge Resistance:  $8 R_0$  to  $R_0/100$ .
- Track level:  $\pm 1000$  mV (slow voltage clamp).
- Track speed: Programmable 20 ms to 2 s.
- Test level: Up to  $\pm 10/R_0$  (e.g., 10  $\mu$ A for HS-9A  $\times 10$ ).
- Test Frequency: 2 Hz to 5 kHz; includes exact 50 Hz and 60 Hz.
- Holding level: Up to  $\pm 1/R_0$  (e.g., 1  $\mu$ A for HS-9A  $\times 10$ ).

### *dSEVC*

- Test level: Up to  $\pm 4/R_0$  (e.g., 4  $\mu$ A for HS-9A  $\times 10$ ).
- Test Frequency: 2 Hz to 5 kHz.
- Holding level: Up to  $\pm 1/R_0$  (e.g., 1  $\mu$ A for HS-9A  $\times 10$ ).

### *TEVC*

- Test level: Up to  $\pm 1000$  mV.
- Test Frequency: 2 Hz to 5 kHz.
- Holding level: Up to  $\pm 200$  mV.

***HVIC***

- Test level: Up to  $\pm 100/R_0$  (e.g., 100  $\mu\text{A}$  for HS-9A  $\times 10$ ).
- Test Frequency: 2 Hz to 5 kHz.
- Holding level: Up to  $\pm 10/R_0$  (e.g., 10  $\mu\text{A}$  for HS-9A  $\times 10$ ).

**External Command Inputs*****I-Clamp (Channel 1 I-CLAMP Command BNC)***

Command gain:  $V/10 R_0$  (e.g., 100 nA/V for HS-9A  $\times 10$ ).

***dSEVC (Channel 1 I-CLAMP Command BNC)***

Command gain:  $V/10 R_0$  (e.g., 100 nA/V for HS-9A  $\times 10$ ).

***TEVC (V-CLAMP Command BNC)***

Command gain: 20 mV/V.

***HVIC (Channel 2 I-CLAMP Command BNC)***

Command gain:  $V/R_0$  (e.g., 1000 nA/V for HS-9A  $\times 10$ ).

**External Current Outputs (2 off)**

Current gain:  $R_0 V/\mu\text{A}$  (e.g., 10 V/ $\mu\text{A}$  for HS-9A  $\times 10$ ).

**Auxiliary Inputs & Outputs (2 off)**

There are two auxiliary connectors that can accept either VG-9 bath clamps or HS-9 headstages. The rear panel 10AUX BNC connectors have a fixed gain of 10:1.

### ***HS-9 Headstages***

When HS-9 headstages are plugged into AUX1 or AUX2 sockets they act as voltage followers only (cannot pass any current).

- Output Calibration: 10 V/V.
- Input loading: < 5 pF (un-neutralized).
- Bias current: < 2 pA.

### ***Bath Clamp Headstages***

Bath Clamp Headstage plugged into AUX1 or AUX2 sockets.

- Clamp Current: 100  $\mu$ A max for VG-9A  $\times 100$ .
- Output Calibration: 1  $\mu$ A/V for VG-9A  $\times 100$ . Since there is a fixed gain of 10:1, the BNC output saturates at 1/10<sup>th</sup> of the maximum current but clamp continues to operate up to the maximum specified.

## **Scaled Outputs**

Two identical scaled outputs are provided with front panel output BNCs.

- Gain: 1 to 2,000 in 1:2:5 sequence.
- Highpass Filter: DC-300 Hz.
- Lowpass Filter: Bessel, 2 Hz to 30 kHz; Butterworth, 3 Hz to 45 kHz.
- Output (DC) Offset: Auto zero or manual setting up to  $\pm 3$  V on gains < 10 and  $\pm 300$  mV on gains  $\geq 10$ .

## **Metering (4 off)**

- Resolution: 16 bits.

- Refresh Rate: 600 ms.
- Monitor Signals: All membrane current, voltage and resistances.

### Audio Monitor

- Outputs: One on rear panel and one on front panel suitable for driving headphones or powered speakers.
- Monitor Signal: Voltage or current from either channel.
- Mode: Direct or Voltage Controlled Oscillator (VCO).
- VCO:  $-100\text{ mV} \sim 500\text{ Hz}$ ,  $0\text{ mV} \sim 2400\text{ Hz}$ ,  $100\text{ mV} \sim 4000\text{ Hz}$ .
- Level: Programmable volume up to  $1\text{ V}_{\text{p-p}}$ .
- Output Impedance:  $500\ \Omega$  (short circuit protected).
- Noise:  $< 1\text{ mV}_{\text{rms}}$ .

## Performance Specification

### I-Clamp (Channels 1 & 2)

Internal holding level max:  $\pm 10\text{ nA}$ ,  $\pm 100\text{ nA}$ ,  $\pm 1000\text{ nA}$   
(depends on headstage).

External command sensitivity: 1, 10, or 100 nA/V (depends on headstage).

### DCC (Channel 1)

Internal holding level max:  $\pm 10\text{ nA}$ ,  $\pm 100\text{ nA}$ ,  $\pm 1000\text{ nA}$   
(depends on headstage).

External command sensitivity: 1, 10, or 100 nA/V (depends on headstage).

## HVIC (Channel 2)

Internal holding level max:  $\pm 0.13$ – $13.0 \mu\text{A}$  (depends on headstage).

External command sensitivity: 10, 100, or 1000 nA/V (depends on headstage).

## dSEVC (Channel 1)

Internal holding level:  $\pm 200 \text{ mV}$ .

AC voltage-clamp gain: 0.003–30 nA/mV, 0.03–300 nA/mV, 0.3–3000 nA/mV (depends on headstage).

External command sensitivity: 20 mV/V.

Voltage rise time: 250  $\mu\text{s}$ .

Current settling time<sup>2</sup>: 500  $\mu\text{s}$  to 10% of peak value.

Voltage noise<sup>2</sup>: 180  $\mu\text{V}_{\text{rms}}$ .

Current noise<sup>2</sup>: 0.30  $\text{nA}_{\text{rms}}$ .

## TEVC (Channels 1 & 2 together)

Internal holding level:  $\pm 200 \text{ mV}$ .

AC voltage-clamp gain: 20 to 50,000.

Voltage-clamp lag: 5.4  $\mu\text{s}$ –52 ms.

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<sup>2</sup> Model cell with two 50 M $\Omega$  resistors to simulate the electrode resistances and a 50 M $\Omega$  resistor and 470 pF capacitor in parallel to simulate the cell membrane. 10 mV step. Lowpass filter, 10 kHz. Voltage-clamp Gain, 2.2 nA/mV; Lag, minimum. Sample rate, 20 kHz. Pipette Capacitance Neutralization, 1.5 pF. Set for flattest membrane step response. HS-9A  $\times 0.1$  headstage.

External command sensitivity:	20 mV/V.
Voltage rise time:	60 $\mu$ s.
Current settling time:	80 $\mu$ s to 10% of peak value.
Voltage noise:	23 $\mu$ V <sub>rms</sub> .
Current noise:	70 nA <sub>rms</sub> .

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<sup>3</sup> Model cell with two 1 M $\Omega$  resistors to simulate the electrode resistances and a 1 M $\Omega$  resistor and 220 nF capacitor in parallel to simulate the oocyte membrane. Lowpass filter, 10 kHz. Voltage-clamp gain, 9300; Lag, 0.019 ms. Adjusted for fastest rise time. HS-9A  $\times$ 1 headstage for voltage electrode and HS-9A  $\times$ 10 for current electrode.





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## Appendix ° : Important Safety Information

### DISCLAIMER

THIS EQUIPMENT IS NOT INTENDED TO BE USED AND SHOULD NOT BE USED IN HUMAN EXPERIMENTATION OR APPLIED TO HUMANS IN ANY WAY.

### WARNING

IF THIS EQUIPMENT IS USED IN A MANNER NOT SPECIFIED BY THE MANUFACTURER, THE PROTECTION PROVIDED BY THE EQUIPMENT MAY BE IMPAIRED.

### Power-Supply Voltage Selection and Fuse Changing.

#### Supply Voltage

The Axoclamp 900A can be directly connected to all international supply voltages. The input range is from 100 to 240 V~. No range switching is required. Alternatively, the instrument can be powered by a DC voltage of 120 to 310 V.

#### Changing the Fuse

The Axoclamp 900A uses a 250 V~, T2A, 5 × 20 mm fuse.

In the event of fuse failure, disconnect the power cord.

Before changing the fuse investigate the reason for its failure.

To change the fuse:

1. Disconnect the power cord.
2. Use a screwdriver or a similar device to rotate the fuse holder counterclockwise.
3. Replace the fuse with another fuse of the same rating.
4. Reconnect the power cord.

## Important Safety Information

### **Basic Equipment Setup and Safety**

1. Connections: Use the included IEC power cord to connect the instrument to a GROUNDED power receptacle.
2. Mounting: Table or rack.
3. Assembly: The headstage connects to the instrument through the rear panel, 25 pin D-sub connector marked "Headstage". Power should always be turned OFF when connecting headstages to the main unit.
4. Use: Do not operate this equipment with covers or panels removed.
5. Cleaning: Wipe the headstage connector with a damp cloth to clean salt spills. Avoid spilling liquids on the headstage.

The Teflon input connector should be kept very clean. Effective cleaning can be done by swabbing carefully with denatured alcohol or deionized water. If possible, avoid the use of Freon since it is thought to be detrimental to the environment.

### **Safe Environmental Conditions**

1. Indoor use.
2. Mains supply fluctuations: not to exceed  $\pm 10\%$  of the nominal voltage.
3. Temperature: between 5 °C and 40 °C.
4. Altitude: up to 2000 m.
5. This instrument is designed to be used under laboratory conditions. Operate in a clean, dry environment only. Do not operate in a wet or damp environment.

### **Static Precautions**

If you are in a laboratory where static is high (*i.e.*, you hear and feel crackles when you touch things), you should touch a grounded metal object immediately before touching the headstage.

### **Shipping the Axoclamp 900A**

The Axoclamp 900A is a solidly built instrument designed to survive shipping around the world. However, in order to avoid damage during shipping, the Axoclamp 900A must be properly packaged.

In general, the best way to package the Axoclamp 900A is in the original factory carton. If this is no longer available, we recommend that you carefully wrap the Axoclamp 900A in at least three inches (75 mm) of foam or "bubble-pack" sheeting. The wrapped Axoclamp 900A should then be placed in a sturdy cardboard carton. Mark the outside of the box with the word FRAGILE and an arrow showing which way is up.

We do not recommend using loose foam pellets to protect the Axoclamp 900A. If the carton is dropped by the shipper, there is a good chance that the Axoclamp 900A will shift within the loose pellet packaging and be damaged.

If you need to ship your Axoclamp 900A to another location, or back to the factory, and you do not have a means to adequately package it, Molecular Devices can ship the proper packaging material to you for a small fee. This may seem like an expense you would like to avoid, but it is inexpensive compared to the cost of repairing an instrument that has sustained shipping damage.

It is your responsibility to package the instrument properly before shipping. If it is not, and it is damaged, the shipper will not honor your claim for compensation.

# Renseignements Importants

## LIMITE DE RESPONSABILITE

CE MATERIEL N'A PAS ETE CONCU POUR DES EXPERIENCES SUR LES ETRES HUMAINS; ET NE DOIT DONC PAS ETRE UTILISE A CETTE FIN.

### ATTENTION

L'EMPLOI DE CE MATERIEL D'UNE MANIERE DIFFERENTE A CELLE SPECIFIEE PAR LE FABRICANT AFFECTERA LE NIVEAU DE PROTECTION FOURNIT PAR L'APPAREIL.

### Sélection du voltage et changement du fusible.

#### Voltage d'alimentation

Le Axoclamp 900A peut être directement branché sur toutes alimentations comprises entre 100 et 240 V~. Aucun changement n'est nécessaire afin de sélectionner le voltage de l'appareil. En outre, l'appareil peut être aussi alimenté en courant continu (DC) de 120 à 310 V.

#### Changement du fusible

Le Axoclamp 900A emploie un fusible de 250 V~, T2A, 5 × 20 mm.

En cas de rupture du fusible, débrancher la prise de courant.

Avant de changer le fusible, chercher la raison de la panne.

Pour changer le fusible:

1. Débrancher la prise de courant.
2. A l'aide d'un tournevis ou autre outil de ce genre, faire tourner le support du fusible dans le sens opposé des aiguilles d'une montre.
3. Remplacer le fusible par un fusible de même valeur.
4. Rebrancher la prise de courant.

#### Installation du matériel et sécurité

1. Branchement: Employer le fil électrique IEC fourni pour brancher l'appareil à une prise de courant comprenant UNE TERRE.
2. Pose: Table ou rack.
3. Montage: La tête de l'amplificateur ("headstage") est connectée à l'appareil sur le panneau arrière, par l'intermédiaire d'une prise D-sub à 25 fiches portant l'indication "Headstage".
4. Emploi: Ne pas utiliser ce matériel sans son couvercle et ne pas le couvrir lors de son utilisation.
5. Nettoyage: Essuyer la prise du "headstage" avec un linge humide pour nettoyer les traces de sel. Eviter de renverser des liquides sur le "headstage".
6. La prise d'entrée en Téflon doit être maintenue très propre. Un nettoyage efficace consiste à vaporiser de l'alcool ou à essuyer soigneusement avec de l'eau désionisée Si possible, éviter l'emploi de Fréon, ce produit étant considéré comme nuisible pour l'environnement.

#### Conditions à respecter pour un emploi sans danger

1. Emploi à l'intérieur.
2. Fluctuations du réseaux d'alimentation: ne doivent pas dépasser ±10% de la tension nominale.

3. Température: entre 5 °C et 40 °C.
4. Altitude: jusqu'à 2000 m.
5. Cet appareil a été étudié pour l'emploi en laboratoire et il doit être situé dans un environnement sec et propre. Ne pas l'utiliser dans un environnement mouillé ou humide.

### **Précautions statiques**

Le "headstage" peut être maniée sans danger. Cependant, dans un laboratoire avec un niveau élevé d'électricité statique (c'est-à-dire lorsque vous sentez et voyez des décharges électriques), touchez un objet métallique pour une mise à la terre avant de toucher le "headstage".

*Ne pas* d'ébrancher le Axoclamp 900A lors de la manipulation de l'entrée du "headstage", ceci risque de déranger son équilibre thermique.

### **Expédition du Axoclamp 900A**

Le Axoclamp 900A est un appareil de construction robuste, étudié en vue d'expéditions dans le monde entier. Cependant, l'appareil doit être correctement emballé pour éviter tout dommage pendant son transport.

En général, la meilleure façon d'emballer le Axoclamp 900A est de le mettre dans son carton d'origine. Si celui-ci n'est plus disponible, il est recommandé d'envelopper soigneusement le Axoclamp 900A dans au moins trois inches (75 mm) de mousse ou de feuilles d'emballage à bulles. Le Axoclamp 900A ainsi protégé devra alors être placé dans un carton solide. Indiquer la mention FRAGILE sur l'extérieur de la boîte ainsi qu'une flèche vers le haut montrant la position verticale.

Il n'est pas recommandé d'employer des boulettes de mousse pour protéger le Axoclamp 900A. En cas de chute de la boîte durant son transport, le Axoclamp 900A pourrait se déplacer à l'intérieur et être endommagé.

Si vous devez expédier le Axoclamp 900A à un autre endroit, ou le renvoyer au fabricant, et si les matériaux d'emballage nécessaires corrects ne sont pas disponibles, ces derniers peuvent être obtenus chez Molecular Devices pour un prix minime. Bien que ceci puisse sembler être une dépense que vous pourriez éviter, elle est cependant insignifiante en comparaison à celle que coûterait la réparation d'un appareil endommagé pendant le transport.

La responsabilité vous incombe de bien emballer l'appareil avant son expédition. Si ceci n'est pas fait, le transporteur ne pourra pas satisfaire vos réclamation de compensation en cas d'avaries.



# Wichtige Informationen

## UNZULÄSSIGE VERWENDUNG

DIESER APPARAT IST NICHT VORGESEHEN, BEI MENSCHLICHEN VERSUCHEN VERWENDET ZU WERDEN UND AUCH NICHT AN MENSCHEN IN IRGENDWEISE ANWENDBAR.

### WARNUNG

WENN DIESER APPARAT IN EINER ART UND WEISE ANGEWENDET WIRD, DIE NICHT VOM HERSTELLER SPEZIFISCH ERWÄHNT WIRD, KANN DIE SCHUTZVORRICHTUNG DES APPARATES BEEINTRÄCHTIGT WERDEN.

### Spannungswahl für die Stromversorgung und Auswechseln der Sicherung.

#### Netzspannung

Der Axoclamp 900A kann direkt an alle internationalen Netzspannungen angeschlossen werden. Die Eingangsspannung reicht von 100 bis 240 V~. Ein Umschalten des Spannungsbereichs ist nicht erforderlich. Das Instrument kann auch mit Gleichstromspannungen von 120 bis 310 V betrieben werden.

#### Auswechseln der Sicherung

Der Axoclamp 900A verwendet eine 250V~, T2A, 5 × 20 mm Sicherung.

Im Falle des Ausfalls der Sicherung das Netzkabel ausschalten.

Vor dem Auswechseln der Sicherung den Grund für ihren Ausfall untersuchen.

Schritte zum Auswechseln der Sicherung:

1. Das Netzkabel ausschalten.
2. Die Fassung der Sicherung mit einem Schraubenzieher oder einem ähnlichen Werkzeug entgegen dem Uhrzeiger drehen.
3. Die Sicherung mit einer anderen Sicherung mit gleicher Nennleistung ersetzen.
4. Das Netzkabel wieder anschließen.

#### Grundlegende Hinweise zu Installation und Sicherheit der Ausrüstung

1. Netz- und Erdungsanschlüsse: Das Instrument mit dem beigefügten IEC Netzkabel an einen Erdungsschalter anschließen.
2. *Anbringung: Tisch oder Rahmengerüst.*
3. Montage: Der Vorverstärker ("headstage") wird über einen mit der Aufschrift "Headstage gekennzeichneten 25 Pin D-Unterstecker an der Rückwand des Instrumentes verbunden.
4. Gebrauch: Dieser Apparat darf nicht mit abgenommenen Abdeckungen oder Platten in Betrieb gesetzt werden.
5. Reinigung: Zur Reinigung von verschüttetem Salz den Vorverstärkeranschluß mit einem feuchten Tuch abwischen. Das Verschütten von Flüssigkeiten auf den "headstage" ist zu vermeiden.

Der Teflon-Eingangsstecker sollte in sehr sauberem Zustand gehalten werden. Durch Besprühen mit Alkohol oder vorsichtigem Abtupfen mit entionisiertem Wasser ist eine wirksame Reinigung möglich. Die Benutzung von Freon ist nach Möglichkeit zu vermeiden, da diese Substanz als umweltschädigend angesehen wird.

### **Umweltsichere Betriebsbedingungen**

1. Verwendung in Innenräumen.
2. Netzschwankungen: darf nicht  $\pm 10\%$  der Nennspannung überschreiten.
3. *Temperatur: zwischen 5 °C und 40 °C.*
4. Höhe: bis zu 2000 m.
5. Dieses Instrument ist für den Gebrauch unter Laborbedingungen vorgesehen. Nur in sauberer, trockener Umgebung in Betrieb setzen. Nicht in nasser oder feuchter Umgebung in Betrieb setzen.

### **Schutzmaßnahmen gegen statische Aufladung**

Der "headstage" kann normalerweise sicher gehandhabt werden. Falls Sie sich jedoch in einem Labor mit höher statischer Aufladung befinden (*D.h.* Sie hören und fühlen beim Berühren von Objekten ein Knacken), sollten Sie unmittelbar vor dem Berühren der "headstage" ein geerdetes Objekt aus Metall anfassen.

Bei Handhabung des Vorverstärkereingangs sollten Sie die Stromzufuhr zum Axoclamp 900A *nicht* abschalten, um das Temperaturgleichgewicht nicht zu stören.

### **Versand des Axoclamp 900A**

Bei dem Axoclamp 900A handelt es sich um ein solide gebautes Instrument, das beim weltweiten Versand keinen Schaden nehmen sollte. Um jedoch Versandschäden zu verhindern, muß der Axoclamp 900A ordnungsgemäß verpackt werden.

Im allgemeinen läßt sich der Axoclamp 900A am besten im Originalkarton des Werks verpacken. Ist dieser nicht mehr vorhanden, empfehlen wir, den Axoclamp 900A vorsichtig in mindestens 75 mm starkem Schaumstoff oder Bubblepackungen einzuwickeln. Der so eingewickelte Axoclamp 900A sollte dann in einen festen Pappkarton gesetzt werden. Die Außenseite des Kartons ist mit dem Worten ZERBRECHLICH (FRAGILE) und einem Pfeil, der auf die Oberseite des Kartons weist, zu kennzeichnen.

Sollte der Karton vom Spediteur fallengelassen werden, besteht eine gute Möglichkeit, daß der Axoclamp 900A innerhalb der losen Schaumstoffkugelverpackung bewegt wird und dadurch beschädigt werden kann.

Wenn Sie den Axoclamp 900A an einen anderen Ort oder zurück ans Werk senden müssen und Ihnen kein angemessenes Verpackungsmaterial zur Verfügung stehen, kann Molecular Devices Ihnen das geeignete Verpackungsmaterial gegen eine kleine Gebühr zustellen. Sie mögen dies zwar als unnötige Zusatzkosten betrachten, doch ist dieser Aufwand im Vergleich zu den Reparaturkosten für ein während des Transports beschädigtes Instrument gering. Sie sind selbst für das richtige Verpacken des Instruments vor dem Versand verantwortlich. Bei einer nicht ordnungsgemäßen Verpackung, die eine Beschädigung zur Folge hat, wird der Spediteur ihren Schadensersatzanspruch nicht anerkennen.

# Importante Información sobre la Seguridad

## LÍMITE DE RESPONSABILIDADES

ESTE EQUIPO NO ESTÁ DISEÑADO PARA USO EN HUMANOS Y NO DEBE USARSE PARA EXPERIMENTACIÓN O APLICACIÓN EN SERES HUMANOS BAJO NINGUNA CIRCUNSTANCIA.

## ADVERTENCIA

SI ESTE EQUIPO SE USA DE MANERA NO ESPECIFICADA POR EL FABRICANTE SE PODRÍA PERDER LA PROTECCIÓN PROVISTA POR EL EQUIPO.

### Selección del suministro de corriente y cambio de fusibles.

#### Voltaje de entrada

El Axoclamp 900A puede conectarse directamente a todos los suministros de energía. El límite de voltaje va entre 100 y 240 V~. No es necesario efectuar cambios en el selector. Además, el instrumento puede ser alimentado a partir de una fuente de corriente continua con voltajes entre 120 y 310 V.

#### Cambio de fusible

El Axoclamp 900A utiliza un fusible de 250 V~, T2A, 5 × 20 mm.

En el caso de que un fusible falle, desconecte el cordón eléctrico.

Antes de cambiar el fusible investigue la causa de la falla.

Para cambiar el fusible:

1. Desconecte el cordón eléctrico.
2. Use un destornillador o un dispositivo similar para girar el portafusibles en sentido contrario al de las manecillas del reloj.
3. Reemplace el fusible existente con otro de la misma capacidad.
4. Conecte nuevamente el cordón eléctrico.

#### Instalación básica y seguridad del equipo

1. Suministro de corriente y conexión a tierra: Use el cordón eléctrico IEC incluido para conectar el instrumento a una toma de corriente CON CONEXIÓN A TIERRA.
2. Montaje: Sobre una mesa o en un estante.
3. Ensamblaje: El cabezal ("headstage") se conecta al instrumento en el tablero posterior con el conector de 25 clavijas D-sub, marcado "Headstage".
4. Uso: No utilice este equipo sin las cubiertas o paneles.
5. Limpieza: Limpie el conector del "headstage" con un paño húmedo a fin de quitar los derrames de sales. Evite derramar líquidos sobre el "headstage".

El conector de entrada fabricado de Teflon debe mantenerse muy limpio. Puede hacerse una limpieza efectiva rociando con alcohol o con un algodón humedecido con agua desionizada. En la medida de lo posible evite el uso del gas freón, puesto que es dañino para el medio ambiente.

**Condiciones de seguridad ambiental**

1. Para uso interior.
2. Fluctuaciones eléctricas en la fuente de suministro: no deben exceder  $\pm 10\%$  del voltaje nominal.
3. Temperatura: entre 5 °C y 40 °C.
4. Altitud: hasta 2.000 m.
5. Este instrumento está diseñado para ser usado en condiciones de laboratorio. Debe operarse únicamente en un ambiente limpio y seco. No lo use en un ambiente húmedo ni mojado.

**Precauciones contra la estática**

El “headstage” puede manejarse con seguridad, bajo condiciones normales. Sin embargo, si usted se encuentra en un laboratorio donde la estática es alta (por ejemplo, si escucha y percibe chispas cuando toca los objetos), usted debería tocar inmediatamente un objeto metálico que esté en contacto con tierra, antes de tocar el “headstage”.

No apague el interruptor principal del Axoclamp 900A cuando manipule la entrada del “headstage” ya que esto afectará el equilibrio térmico.

**Envío del Axoclamp 900A**

El Axoclamp 900A es un instrumento de construcción sólida, diseñado para soportar el transporte a cualquier parte del mundo. Sin embargo, para evitar los daños que pudieran ocurrir durante su envío, el Axoclamp 900A debe empacarse adecuadamente.

En general, la mejor manera de empacar el Axoclamp 900A es en la caja original de fábrica. Si ésta ya no se encuentra disponible, le recomendamos que envuelva cuidadosamente el Axoclamp 900A en una funda o sábana de espuma o de “empaques de burbujas” con un espesor mínimo de 3 pulgadas (75 mm). El Axoclamp 900A, envuelto así, deberá colocarse en una caja de cartón resistente. Marque el exterior de la caja con la palabra FRÁGIL y una flecha que indique la posición hacia arriba.

No recomendamos el uso de bolitas de espuma sueltas para proteger el Axoclamp 900A. Si la caja se cae accidentalmente durante el transporte, es muy probable que el Axoclamp 900A se desplace dentro del contenedor con las bolitas de espuma sueltas y se dañe.

Si necesita enviar su Axoclamp 900A a otra localidad, o de regreso a la fábrica, y no posee el empaque adecuado, Molecular Devices puede enviarle el material necesario por un cargo mínimo. Esto podría parecerle un gasto superfluo que preferiría evitar, pero es económico comparado con lo que costaría la reparación de un instrumento que ha sufrido daños durante el envío.

Es su responsabilidad empacar el instrumento adecuadamente antes de enviarlo. Si no lo hace así y resulta dañado, el transportista no será responsable ni aceptará su reclamo de indemnización.

## Appendix “ : Polarity Conventions

### Biological Polarity Conventions

#### Inward Current

Current (carried by positive ions) that flows across the cell membrane, from the outside surface to the inside surface.

#### Outward Current

Current that flows from the inside to the outside surface of the cell.

#### Membrane Potential

The potential inside the cell minus the potential outside the cell:

$$V_m = V_{in} - V_{out}$$

#### Depolarization

A positive shift in  $V_m$  (e.g., from  $-60$  mV to  $+80$  mV) caused by a flow of inward current.

## Hyperpolarization

A negative shift in  $V_m$ .

## Axoclamp Polarity Conventions

The conventions described here apply to all amplifiers manufactured by Molecular Devices.

To prevent confusion, Molecular Devices always uses current and voltage conventions based on the instrument's perspective. That is, the current is defined with respect to the direction of flow into or out of the headstage. Molecular Devices amplifiers do not have switches that reverse the current or the voltage command polarities. This prevents forgetting to move the switch to the correct position. The data are recorded unambiguously and the correct polarity can be determined during subsequent data analysis.

### Positive Current

Current that flows *out* of the headstage into the electrode and out of the electrode tip into the cell.

### Positive/Negative Potential

A positive/negative voltage at the headstage input with respect to the bath ground.

With these definitions it is easy to work out the correct polarity for every recording configuration. For example, in the whole-cell or outside-out patch configuration the electrode tip is on the intracellular face of the cell. Thus, a *negative* potential,  $V_p$ , at the headstage input (=electrode interior) is a *negative* potential inside the cell. The cell's membrane potential under voltage clamp is therefore  $V_m = V_{in} - V_{out} = V_p - 0 = V_{cmd}$ . *Positive* current flowing out of the electrode must then flow from the inside to the outside surface of the cell, which means that it is *outward* current.

## Appendix 2 : Glossary

$C_{in}$	Total input capacitance of the headstage due mainly to the microelectrode and any connecting cable.
$C_m$	Membrane capacitance of cell.
DCC	Discontinuous current clamp.
dSEVC	Discontinuous single-electrode voltage clamp.
$f_s$	Sampling rate; rate for switching from current passing to voltage recording in DCC and dSEVC modes.
G	The average gain during dSEVC.
$G_T$	The instantaneous gain of the controlled current source during dSEVC.
H	Headstage current gain.
$I_1, I_2$	Current flow in microelectrode 1, Current flow in microelectrode 2.
$I_m$	Membrane current flow.

Lag	High-frequency cut.
$R_b$	Bath electrode resistance.
$R_e$	Intracellular electrode resistance.
$R_f$	Feedback resistor, terminology used interchangeably with $R_0$ .
$R_0$	Current injection resistance.
$R_s$	Resistance in series with membrane.
RMP	Resting membrane potential.
$R_m, R_{in}$	Input resistance of cell membrane.
SEVC	Single-electrode voltage clamp.
$T_A$	Ambient temperature.
TEVC	Two-electrode voltage clamp.
VC	Voltage Clamp.
VG	Virtual-ground output attenuation.
$V_m$	Membrane potential recorded by microelectrode 1.
$\mu$	Gain of voltage clamp.



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