

# Warner Instruments Whole Cell/Patch Clamp Amplifier Model PC-505B



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The **PC-505B** Whole Cell/Patch Clamp Amplifier is a low noise, resistive-feedback patch clamp designed for whole-cell, single channel, and bilayer applications. The unique circuitry and dedicated design of this amplifier allows Warner Instruments to present a superior quality instrument at a cost significantly below that of many of our competitors.

Principal features of the **PC-505B** include:

- ✓ Low noise levels of 0.038 pA RMS at 1 kHz
- ✓ Built-in RMS noise monitor
- ✓ Voltage and current clamp modes with independently selectable  $V_{\text{hold}}$  and  $I_{\text{hold}}$
- ✓ Bandwidth to 25 kHz
- ✓ Internal 4-pole low-pass Bessel filter with rear panel filter telegraph
- ✓ Built-in test generator
- ✓ Automatic junction potential compensation
- ✓ Two-range fast capacitance compensation
- ✓ Series resistance and C-slow capacitance compensation
- ✓ % Correction circuitry
- ✓ Adjustable duration zap circuit for whole cell membrane penetration
- ✓ Output gain selection with rear-panel gain telegraph
- ✓ Front/rear panel controls and connectors are color coded and organized for quick reference and convenient operation

**THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED  
FOR USE ON HUMAN SUBJECTS**

## NOMENCLATURE

### Text conventions

This manual refers to amplifier controls at three functional levels; control blocks, specific controls within a block, and settings of specific controls. To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

- Warner Instrument product numbers are presented using **bold type**.
- References to instrument panel control blocks are specified using UNDERLINED SMALL CAPS.
- References to specific controls within a block are specified using SMALL CAPS.
- References to individual control settings are specified using *italic type*.
- Special comments and warnings are presented in **highlighted text**.

Any other formatting should be apparent from context.

### Device panel abbreviations

Many controls on the **PC-505B** have abbreviations associated with them. Several of these abbreviations are listed here for quick reference. In addition, these and other terms have been collected and are included in a Glossary at the back of this manual.

Term	Meaning	Sections
CAP COMP	capacitance compensation	<u>FAST CAP COMP</u>
$I_m$	output current	<u>OUTPUT, METER, REAR PANEL</u>
$V_m$	membrane voltage	<u>OUTPUT, METER, REAR PANEL</u>
$V_c$	COMMAND IN voltage	<u>OUTPUT, METER, REAR PANEL</u>
$\Sigma V_c$	sum of all command and compensation voltages	<u>METER</u>
$V_c + h_{IN}$	$V_c$ plus HOLDING voltage	<u>METER</u>
P STAT	potentiostat mode	<u>OUTPUT, REAR PANEL</u>

### Signal polarity conventions

#### **Membrane current ( $I_m$ )**

$I_m$  is indicated as positive when cations flow outward from the pipet tip, through the cell membrane and into the bath, and/or when anions flow from the bath into the pipet.

With outside-out patch and whole cell preparations, this corresponds to the conventional physiological definition of outward transmembrane current. With inside-out or cell-attached patches, the physiological transmembrane current equals minus the indicated  $I_m$ .

***Membrane potential ( $V_m$ )***

Membrane potential is defined as pipet potential minus bath potential. With outside-out patch and whole cell preparations, this corresponds to the conventional physiological definition of transmembrane potential. With inside-out or cell-attached patches, the physiological transmembrane potential equals minus the indicated  $V_m$ .

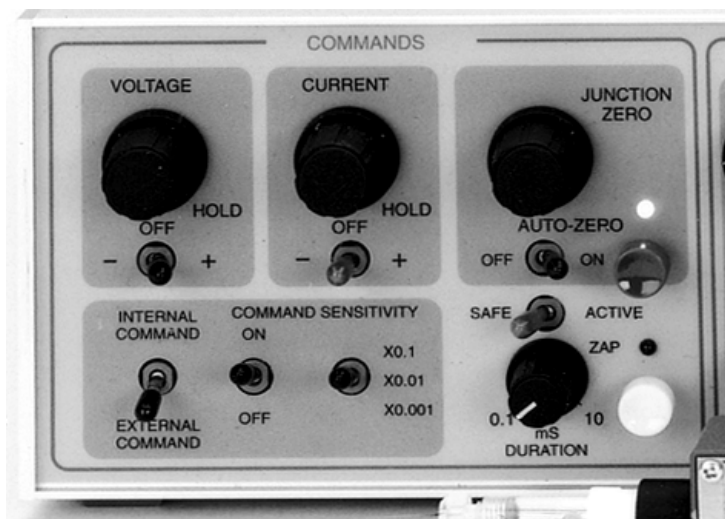
## CONTROL DESCRIPTION

The instrument front panel is divided into several control blocks. Controls within these blocks are dedicated towards a common functional purpose. Each control block is outlined in blue and is clearly labeled as COMMANDS, FAST CAP COMP, WHOLE CELL, and OUTPUT. A final section contains the LCD METER, as well as the METER SELECT and POWER switches. This section will be referred to as the METER block.

### Front panel

#### COMMANDS block

The COMMANDS block contains the VOLTAGE and CURRENT HOLDING controls, the JUNCTION ZERO controls, and a COMMAND SELECT toggle switch selecting internal or external command inputs. This block also contains several controls for adjusting the sensitivity of input commands, as well as controls for the ZAP function.



#### Voltage and current commands

The VOLTAGE and CURRENT HOLD controls provide independent modification of holding potential and holding current settings within the ranges  $\pm 200$  mV and  $\pm 1.0$  nA, respectively. A MODE toggle switch in the OUTPUT command block is used to select between voltage clamp ( $V_c$ ) or current clamp ( $I_c$ ) modes. Placing the instrument in current or voltage clamp mode activates either the VOLTAGE or CURRENT HOLD controls, respectively. This structure allows switching between voltage and current clamping configurations without the need to readjust settings. VOLTAGE and CURRENT HOLD controls are not attenuated by COMMAND SENSITIVITY settings.

#### Internal command, external command, and command sensitivity

The COMMAND SELECT toggle switch selects between an internally generated (*internal command*) or an externally generated (*external command*) command that is applied to the COMMAND IN BNC located on the instrument rear panel.

**NOTE:** When set to *external command*, the COMMAND SENSITIVITY controls attenuate the signal applied to the COMMAND IN BNC located on the instrument rear panel. When set to *internal command*, the COMMAND SENSITIVITY controls select the scaling of the internally generated TEST PULSE.

The COMMAND SENSITIVITY controls are comprised of an *on/off* toggle and a sensitivity selector. The SENSITIVITY SELECTOR attenuates either the internally generated TEST PULSE or



any externally applied command voltages connected to the COMMAND IN input BNC by factors of  $\times 0.1$ ,  $\times 0.01$ , and  $\times 0.001$ .

Internal or external commands are activated by moving the COMMAND SENSITIVITY toggle into the *on* position. When switched *off*, these modifiers are disconnected from the COMMAND pathway.

### Test pulse

When *internal command* is selected and COMMAND SENSITIVITY is switched *on*, a 1V p-p square wave test pulse (line frequency; 50/60 Hz) is generated by the **PC-505B**. The test pulse is attenuated by the SENSITIVITY SELECTOR toggle and is available for adjustment of capacitance compensation, measurement of pipet resistance, or for monitoring the formation of a gigohm seal at the electrode tip.

SENSITIVITY SELECTOR setting	Amplitude of test pulse square wave (p-p)
$\times 0.1$	100 mV
$\times 0.01$	10 mV
$\times 0.001$	1 mV

When *external command* is selected, the internal TEST PULSE circuitry is disabled and commands appearing at the COMMAND IN BNC are attenuated and available to be applied to the headstage.

### Junction Zero and Auto Zero

These controls set the pipette current to zero after the pipette is placed in the test solution and prior to seal formation. This circuitry is used to compensate for electrode potentials, liquid junction potentials and other offset voltages, and establishes a zero baseline reference potential. The 10-turn JUNCTION ZERO control manually adjusts  $I_m$  between  $\pm 120$  mV with immediate response.

With the AUTO ZERO toggle switch in the *on* position (auto zero LED *on*) depressing the associated pushbutton zeros the current automatically. The settling time is rapid when used with  $M\Omega$  pipette resistances, but can take several seconds if used with  $G\Omega$  resistances. For complete compensation, hold the pushbutton in until  $I_m$  on the METER reads *zero*.

**NOTE:** With AUTO ZERO turned *on*, the manual junction control remains active. In this mode, depressing the auto zero pushbutton will zero any manual setting, holding potential, and time-averaged test pulse or command voltage present. Switching AUTO ZERO *off* restores the uncompensated current and voltages. To re-zero, repeat using either manual or auto zeroing.

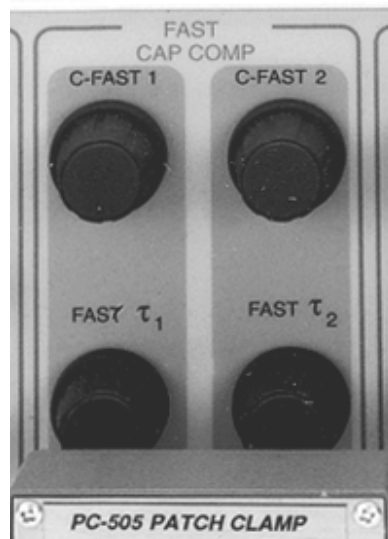
## Zap

The ZAP function generates an internal +1.5 V pulse which is applied to the headstage. The pulse duration may be adjusted from 0.1 to 10 ms as indicated. A *safe/active* toggle switch arms the circuitry and the pulse is initiated when the ZAP button is *depressed*.

## FAST CAP COMP (fast capacitance compensation) command block

The two pairs of controls, C-FAST 1/FAST  $\tau_1$  and C-FAST 2/FAST  $\tau_2$ , are used to adjust the amplitude and time constant (*tau*) of the circuitry that compensates for capacitive currents due to the pipet and other stray capacitances. This is achieved by applying an appropriate capacitive countercurrent to the headstage input. These controls are disabled in current clamp mode.

When properly adjusted, FAST CAP COMP controls have two important functions: 1) to closely align the voltage clamp waveform at the cell membrane to the command signal waveform, and 2) to minimize large current transients associated with rapid changes in applied membrane potential. These effects, if uncompensated, can drive the headstage amplifier into saturation, which can result in the loss of several ms of data while the headstage recovers.



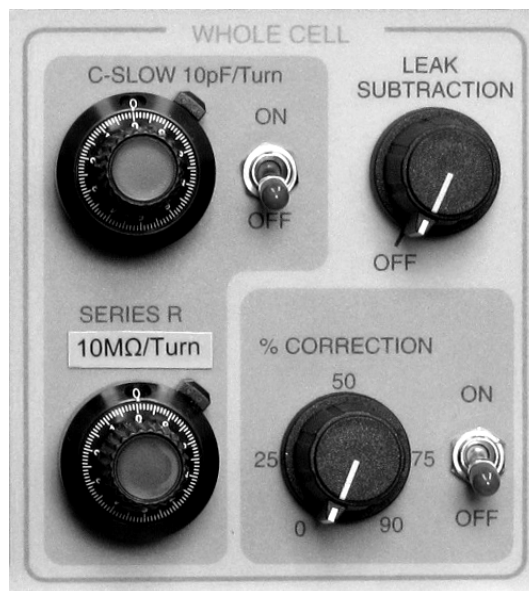
## WHOLE CELL COMMAND BLOCK

The WHOLE CELL command block contains controls for C-SLOW, SERIES R, % CORRECTION, and LEAK SUBTRACTION. These controls are used to compensate for the effects of membrane capacitance and access resistance when the amplifier is used in whole cell mode. With the exception of LEAK SUBTRACTION, these controls are only available when the PROBE RESISTER (in the OUTPUT command block) is in *low mode*.

### C-Slow

This control is used to compensate for the whole-cell membrane capacitance. The compensated capacitance can be read directly from the lockable 10-turn control. The associated toggle switch disables C-SLOW allowing comparison with the uncompensated signal.

This control is not available in patch mode since the FAST CAP COMP circuitry is used to address capacitances in this mode.



## Series R

Series resistance compensation is used to compensate for the voltage drop across the electrode and access resistance in the experimental setup. This control is used to adjust the time constant of the C-SLOW control facilitating measurement of membrane resistance which can be read from the dial.

This control is not available in single channel recording mode since series resistances are negligible when compared with cell membrane and single channel resistances.

## % Correction

% Correction is used to apply voltage compensation to the amplifier to correct for the voltage drops due to the series resistance. This control is adjusted until the output signal just begins to oscillate at the leading edge (ringing) of the TEST PULSE during experimental setup. The control is activated by a toggle switch.

## Leak subtraction

Leak subtraction is used to compensate for leakage currents to the bath through the pipet/membrane seal resistance ( $R_S$ ) or through the membrane patch. For 'leaky' seals ( $R_S \sim 1 \text{ G}\Omega$ ) it is important to correct for the error caused by the shunt resistance of the leak. This error can usually be ignored with 'tight' seals ( $R_S \sim 10$  to  $100 \text{ G}\Omega$ ). Fully counterclockwise rotation of this control switches it off.

**NOTE:** Do not use LEAK SUBTRACTION if using % CORRECTION in whole cell mode. The interaction between these two controls can introduce a systematic error into your data!

## OUTPUT command block

The OUTPUT command block contains controls for selecting different operating modes and headstage resistors. The instrument gain and filter controls can also be found here.

### Probe resistor select

The headstage resistor is selected with the PROBE RESISTOR select switch. The *high* position selects the  $50 \text{ G}\Omega$  resistor for both patch and bilayer headstages (models **LC-201**, **HC-202**, and **HB-205**, respectively). The *low* position is used for whole cell recording and selects the  $500 \text{ M}\Omega$  resistor in the **LC-201** headstage and the  $50 \text{ M}\Omega$  resistor in the **HC-202** headstage. It is not available in the **HB-205** headstage.

The low resistor is automatically selected when in current clamp mode,



### Clamp mode switch

In *voltage clamp* mode (MODE switch in upper position), all command controls are active with the exception of the CURRENT HOLD command (in COMMANDS block). The range of voltage clamp potentials at the headstage input is  $\pm 200$  mV.

*Zero current* mode (MODE switch in center position) is a transition mode between voltage clamp and current clamp. It disengages all commands and functions from the headstage with the exception of the ZAP controls.

The primary uses for zero current mode are: 1) to protect the preparation when switching between voltage and current clamp modes, and 2) to preset the voltage hold or current hold settings prior to switching to voltage or current clamp modes, respectively.

In *current clamp* mode (MODE switch in lower position), all commands are again active. The current range at the preparation is limited by the  $\pm 10$  V input maximum and the headstage resistor, as follows:

- $\pm 20$  nA with the 500 M $\Omega$  resistor
- $\pm 200$  nA with the 50 M $\Omega$  resistor

**NOTE:** Current clamp mode does not operate with the PROBE SELECT toggle set to *high*. The low headstage resistance is automatically selected when in current clamp mode.

### Gain

The GAIN control selects the amplifier gain in units of mV/pA. The gain scale on this control is modified by the PROBE RESISTOR select toggle (*x0.1* or *x1.0* for the *low* setting, and *x10* for the *high* setting).

LED's indicate the selected gain scale modifier. The clipping LED lights when the instrument  $I_m$  output exceeds the amplifier limits.

### 4-Pole Bessel filter

The  $I_m$  output signal can be filtered using the internal low-pass 4-pole Bessel filter selectable from 50 Hz to 20 kHz in 9 steps. The full amplifier bandwidth of 25 kHz is available by selecting *bypass* with the ACTIVE/BYPASS toggle switch. The filtered  $I_m$  signal is present at two BNC outputs labeled  $I_m$  located at both the front and rear panels.

### Voltammetry

The **PC-505B** can be used as a potentiostat for voltammetric measurements. A rear panel POTENTIOSTAT SWITCH increases maximum output of the VOLTAGE HOLD control (in the COMMANDS block) to  $\pm 1$  V and increases the maximum COMMAND IN signal to  $\pm 2$  V. The P STAT LED lights to indicate *on* status for this switch.

## METER section

The six METER switch settings interact with other controls as noted. Due to the limiting bandwidth of the meter display, any high frequency signals presented will be reported as its DC time average value.

**Junction Zero :** This selection reports the voltage supplied by the JUNCTION ZERO control used to compensate offset voltages present in the setup. Full scale reading is  $\pm 120$  mV.

**$V_c + h$  IN:** This selection reports the sum of the COMMAND IN voltage ( $V_c$ ; after attenuation by command sensitivity) and the internal holding voltage ( $h$ ). Full scale reading is  $\pm 200$  mV.

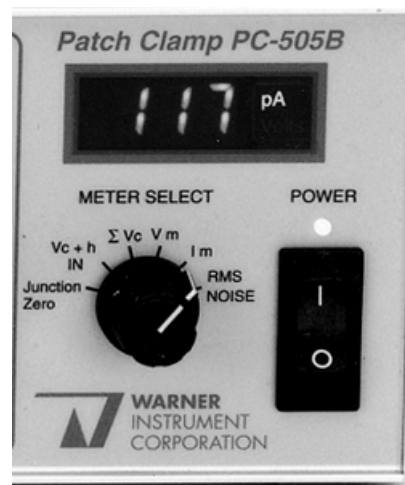
**NOTE:** To read  $h$  alone (in order to set the holding voltage or holding current) turn COMMAND SENSITIVITY *off* or set the external signal to zero.

**$\Sigma V_c$ :** This selection reports the sum of all command voltages. Mathematically,  $\Sigma V_c = (V_c + h \text{ IN}) + (\text{junction zero}) + (\text{auto zero}) + (\text{Series R})$ . It does not include *leak subtraction*. Full scale range is  $\pm 200$  mV.

**$V_m$ :** This selection reports the transmembrane potential when in current clamp mode. Full scale range is  $\pm 200$  mV.

**$I_m$ :** This selection reports the transmembrane current when in voltage clamp mode. Full scale range is  $\pm 1999$  pA.

**RMS noise:** This selection reports the root mean square (RMS) value of the noise filtered to a bandwidth of 1 kHz. This reading is valid only when the PROBE RESISTOR select is set to *high* since the amplifier gain changes for other settings. The expected reading for the 50 G $\Omega$  resistor (with open input and properly shielded from 60 Hz interference) is approximately 0.040 pA. Full scale range is 1.999 pA RMS.



## Front and rear panel BNC's and connectors

The **PC-505B** has input and/or output BNC's on both front and rear panels. These include  $I_m$  and  $V_m$  outputs,  $V_c \times 10$  and  $V_m \times 10$  outputs, GAIN and FILTER TELEGRAPHS,  $I_m/V_m$  mode telegraph, SYNC OUTPUT, and COMMAND INPUT.

Front and rear panel layouts are described below. With the exception of the rear panel COMMAND IN BNC, all connector sleeves are connected to circuit ground and are insulated from the chassis.

### Front panel BNC's

$V_c \times 10$  – Output BNC reporting the sum of all voltages applied to the headstage input.

$V_m \times 10$  – Output BNC reporting the transmembrane potential (active only in current clamp mode).

$I_m$  – Output BNC reporting membrane current in both voltage and current clamp modes.

### Rear panel BNC's

$I_m/V_m$  OUT– Output BNC reporting membrane current when instrument is in voltage clamp mode, and  $V_m \times 10$  when instrument is in current clamp mode.

$I_m/V_m$  TELEGRAPH – Provides TTL logic to indicate voltage clamp or current clamp mode.  
0=I clamp, 1= V clamp

COMMAND IN – Differential input BNC for voltage command from external sources (e.g. signal generator or computer). Since the center pin and sleeve are the (+) and (-) inputs, respectively, the sleeve is not grounded. The applied voltage is attenuated by the COMMAND SENSITIVITY switch and is available in both voltage and current clamp modes.

The COMMAND IN input BNC is disengaged when TEST PULSE is active (*internal command*), when the COMMAND SENSITIVITY toggle switch is *off*, or when the amplifier is in zero current mode (MODE selector switch set to  $I_0$ ).

GAIN TELEGRAPH – Output BNC reporting  $I_m$  gain as a combination of the GAIN switch selection and the gain multiplier ( $\times 0.1$ ,  $\times 1.0$  or  $\times 10$ ) dependent on the headstage in use. Telegraphs range from 0.5 to 7.0 V in 0.5 V steps as shown below.

Gain telegraph settings

$I_m$ gain (mV/pA)	telegraph out (V)
0.05	0.5
0.1	1.0
0.2	1.5
0.5	2.0
1.0	2.5
2.0	3.0
5.0	3.5
10	4.0
20	4.5
50	5.0
100	5.5
200	6.0
500	6.5
1000	7.0

FILTER TELEGRAPH – Output BNC reporting the filter setting in use. Telegraphs range from 0.2 to 2.0 V in 0.2 V steps as shown below.

Filter telegraph settings

Frequency (Hz)	telegraph out (V)
50	0.2
100	0.4
200	0.6
500	0.8
1k	1.0
2k	1.2
5k	1.4
10k	1.6
20k	1.8
Bypass	2.0

SYNC OUT – Sync out provides a TTL pulsed output for synchronizing an oscilloscopes or other equipment with the internally generated 50/60 Hz TEST PULSE or SPEED TEST signal.

### Rear panel connectors and controls

In addition to input and output BNC's, the instrument rear panel also contains the headstage cable connector, calibration trimmers, and selector/function switches for PSTAT and SPEED TEST modes.

Headstage cable connector – The headstage connects to the instrument via an 8-pin DIN connector.

PSTAT switch – The **PC-505B** is converted to operate as a potentiostat for voltammetric measurements by a clockwise rotation of this switch. When switched *on*, the front panel PSTAT indicator LED will light. In this mode, the maximum electrode voltage hold potential is increased to  $\pm 1$  V and the COMMAND IN attenuator is modified to allow command inputs up to  $\pm 2$  V.

SPEED TEST is used to re-adjust the rear panel boost trimmers, normally only required when replacing a headstage. These adjustments are factory set for the headstage supplied with the instrument.

CMR, BOOST, and GAIN trimpots are factory set. User adjustment may be required if a headstage is replaced. Adjustments instructions are provided with the new headstage.



Grounds – Circuit and chassis ground connectors (black and green, respectively) are binding posts supplied with a shorting link. For most recording situations, the shorting link can remain connected with no detrimental effect in amplifier performance. However, there are occasions where line noise can be reduced if the two grounds are isolated from each other. We recommend that you test both connected and unconnected configurations to determine which is best for your setup.

### Headstage

The headstage, or probe, is a low leakage current, solid state, current-to-voltage converter which is switch selectable between two feedback resistors.

The headstage input terminal is a 1 mm jack connector that accepts a 1 mm pin on the electrode holder. Pin jacks on the side of the headstage provide for grounded and/or driven shield applications.

The headstage housing is a metal enclosure which serves as a shield which is driven by the command signal. The attached metal rod is used for mounting to a micromanipulator and is insulated from the headstage housing. A 2.5 m cable with 8-pin DIN connector attaches the headstage to the mating connector on the instrument rear panel.

**NOTE:** Due to the requirement of maintaining the lowest possible instrument noise, headstage input protection cannot be employed. As such, *strict handling precautions are necessary to avoid damage to the headstage by static discharge.* (See page 18 for details.)

### Headstage types, applications and features

Headstage Types	Application	Features
LC-201	Patch / Whole Cell	50 G $\Omega$ /500M $\Omega$ headstage. Single channel currents up to $\pm 200$ pA. Whole cell currents up to $\pm 20$ nA.
HC-202	Patch / Whole Cell	50 G $\Omega$ /50M $\Omega$ headstage. Single channel currents up to $\pm 200$ pA. Whole cell currents up to $\pm 200$ nA.
HB-205	Bilayer studies	50 G $\Omega$ headstage for bilayer. Currents up to $\pm 200$ pA. Bilayer capacitance up to 250 pF.



### ***Electrode holders***

Electrode holders connect the glass micropipet electrode to the headstage. A fine chlorided silver wire, the actual electrode, makes electrical contact between the headstage input and the electrolyte solution in the micropipet.

Holders are machined from polycarbonate to minimize electrical noise and are custom bored to accommodate various diameter pipet electrode glasses. The micropipet is secured with a rubber gasket and a polycarbonate screw-cap matching the OD of the pipet glass. A 1 mm pin makes electrical contact with the silver wire and plugs the holder onto the headstage. A 1/16" OD access port is provided for applying suction to seal the pipet tip to the cell membrane.

**NOTE:** The standard holder used with the **PC-505B** is type **QSW-AxxP** (straight body) where "xx" specifies the glass OD in mm.

### ***Model Cell***

A model cell is included with the instrument. This model cell has three connectors, one to simulate the bath (10M $\Omega$  to ground), one each to simulate a membrane patch, and one to simulate a whole cell application. An additional model cell is included with the **HB-205** bilayer headstage to simulate a planar lipid bilayer membrane.

The model cell is used in the following familiarization instructions and is a valuable tool in troubleshooting problems with actual setups.

## SETUP AND INITIAL TEST

### Line voltage

Power line voltage requirements for the **PC-505B** are specified on the serial number nameplate attached to the chassis rear. They are wired for either 100-130 VAC or 220-240 VAC at either 50 or 60 Hz. Check to be sure the **PC-505B** is wired for the line voltage and frequency to be used.

### Instrument grounding

The power cord is fitted with a three-prong grounding type plug and should be plugged into a properly wired three-wire grounded receptacle. This internally grounds the **PC-505B** chassis to the power receptacle ground and insures safe operation of the equipment.

### Headstage precautions

The **PC-505B** headstage is a high-impedance, static-sensitive device as noted on the protective envelope in which it is shipped. The device can be seriously damaged by static discharge or inadvertent grounding. Therefore, to insure proper operation and long life, we recommend you follow these precautions:

1. Always discharge static electricity from your body before handling the headstage.

Your body has a capacitance of around 100-200 pF to ground and can acquire enough static charge (by handling Styrofoam, touching the face of a video monitor, walking across a dry carpet, wearing polyester clothing, etc) to alter your electric potential by as much as 10 kV relative to ground. A discharge of this magnitude through the headstage can render it useless. We recommend that you electrically discharge yourself either by firmly contacting a securely-grounded part of the setup with a lightly moistening a finger, or by wearing a grounded wrist strap available from any electronics store.

2. Do not ground or apply a low-impedance signal the headstage input connector pin.
3. Do not ground the headstage case when the power is on. This includes allowing the headstage case to inadvertently contact any grounded components, This is necessary since the case is both isolated from ground and is driven at the command potential.
4. Two 1 mm pin jacks are provided on the side of the headstage for grounded and/or driven shield applications.

The circuit ground pin jack is insulated from the case and is identified by a black collar. It is intended to be used for the bath ground and/or shielding around the electrode and holder. *It is not intended as a general equipment ground.*

The uninsulated pin jack makes direct contact with the headstage case and is driven at the command potential. It can be used to drive any additional guard shielding such as a foil covering or conductive paint applied to the pipette electrode. *Insure that the guard shielding never touches ground.*

## Headstage preparation

Using the proper handling precautions described above, connect the headstage cable connector into the rear panel probe receptacle and connect the  $I_m$  output BNC to an oscilloscope. The headstage probe normally requires a grounded enclosure (Faraday cage) to shield it from 50/60 Hz line interference.

The shield should be grounded to the (black) circuit ground jack on the rear of the **PC-505B** chassis. Place the headstage into the shield enclosure and run the grounding cable to the amplifier in the same bundle as the headstage cable.

**NOTE:** As a general rule, cables and wires running to the same location should be bundled to minimize stray capacitances.

## Test Procedures

To perform the following tests of the instrument you will need these tools and components.

- The **PC-505B** amplifier
- The headstage mounted into a shielded enclosure
- An oscilloscope with BNC cables
- An adjustable DC voltage source
- The model cell shipped with the instrument

### Initial connections

Place the amplifier on a counter and make the following connections between the instrument and oscilloscope.

1. Connect the  $I_m$  output BNC on the instrument front panel to the oscilloscope.
2. Connect the DC voltage source to the COMMAND IN BNC on the instrument rear panel.
3. Connect the headstage to the amplifier and place the headstage into the shielded enclosure (Faraday cage). Do not make attachments to the headstage inputs at this time. Insure that the Faraday cage is grounded as described above.
4. Plug the amplifier, voltage source, and oscilloscope in.
5. Power up the voltage source and oscilloscope.

### Initial configuration

Set all controls on the **PC-505B** to the values specified in the following table. With the exception of the power switch, this initial configuration will be used to return the amplifier to a known condition to begin each sub-section during the instrument checkout.

Begin each checkout section by returning the **PC-505B** to this known configuration.

Headstage inputs: *Open*

Front panel controls:

Control	Control block	Initial setting
POWER	<u>METER</u>	<i>off</i>
VOLTAGE HOLD	<u>COMMANDS</u>	<i>fully CCW, toggle switch off</i>
CURRENT HOLD	<u>COMMANDS</u>	<i>fully CCW, toggle switch off</i>
COMMAND SELECT	<u>COMMANDS</u>	<i>external command</i>
COMMAND SENSITIVITY	<u>COMMANDS</u>	<i>off, x0.1</i>
JUNCTION ZERO control	<u>COMMANDS</u>	<i>zero mV</i>
AUTO ZERO toggle	<u>COMMANDS</u>	<i>off</i>
ZAP toggle	<u>COMMANDS</u>	<i>safe</i>
ZAP control	<u>COMMANDS</u>	<i>0.1 ms</i>
C-FAST 1	<u>FAST CAP COMP</u>	<i>fully CCW</i>
C-FAST 2	<u>FAST CAP COMP</u>	<i>fully CCW</i>
FAST $\tau_1$	<u>FAST CAP COMP</u>	<i>fully CCW</i>
FAST $\tau_2$	<u>FAST CAP COMP</u>	<i>fully CCW</i>
C-SLOW	<u>WHOLE CELL</u>	<i>fully CCW</i>
C-SLOW toggle	<u>WHOLE CELL</u>	<i>off</i>
SERIES R	<u>WHOLE CELL</u>	<i>fully CCW</i>
LEAK SUBTRACTION	<u>WHOLE CELL</u>	<i>fully CCW, click off</i>
% CORRECTION	<u>WHOLE CELL</u>	<i>fully CCW</i>
% CORRECTION toggle	<u>WHOLE CELL</u>	<i>off</i>
PROBE RESISTOR select	<u>OUTPUT</u>	<i>high</i>
MODE	<u>OUTPUT</u>	$V_c$
GAIN	<u>OUTPUT</u>	<i>10 mV/pA</i>
FILTER	<u>OUTPUT</u>	<i>10 kHz</i>
FILTER toggle	<u>OUTPUT</u>	<i>active</i>
METER select	<u>METER</u>	$I_m$

Oscilloscope setup:

Control	Setting
Time base	2 ms/div
Voltage base	1 V/div
Trigger	line triggered
	Connect $I_m$ output on instrument front panel to the oscilloscope

**RMS noise checkout**

1. Set the METER SELECT switch to the *RMS noise* position.
2. Check for a noise reading on the METER at or below  $0.038 \text{ pA}$ . If the value reported is high, then relocate or adjust the headstage and shielding to minimize noise inputs.

 **$I_m$  output checkout**

1. Set scope voltage base to  $0.5 \text{ V/div}$ .
2. Set GAIN to  $100 \text{ mV/pA}$
3. Set FILTER to  $1 \text{ kHz}$ .
4. Check that the METER reads  $\pm 0 \text{ pA}$ .
5. Switch PROBE RESISTER toggle from *high* to *low*. Verify that the METER reading stays within  $\pm 0 \text{ pA}$
6. Switch PROBE RESISTER toggle from *low* to *high*.
7. Check that oscilloscope reads  $0.0 \pm 0.5 \text{ pA}$  (i.e. within 1 div of zero on oscilloscope for this setting.)
8. Set PROBE RESISTER toggle from *high* to *low*.
9. Set FILTER to  $50\text{Hz}$ .
10. Set oscilloscope voltage base to  $50 \text{ mV/div}$ .
11. Again, check that oscilloscope reads  $0.0 \pm 0.5 \text{ pA}$  (i.e. within 1 div of zero on oscilloscope for this setting.)
12. Set PROBE RESISTER toggle from *low* to *high*.
13. Set FILTER to  $1 \text{ kHz}$ .
14. Set oscilloscope voltage base to  $0.5 \text{ V/div}$ .
15. Move oscilloscope cable from  $I_m$  OUTPUT on instrument *front* panel to  $I_m/V_m$  OUTPUT on instrument *rear* panel.
16. Redo steps 7–11.
17. Return amplifier to initial settings.

**Junction zero checkout**

1. Set the METER SELECTOR switch to the *Junction Zero*
2. Adjust the JUNCTION ZERO control (COMMANDS block) until the meter reading is  $10 \text{ mV}$ .
3. Verify that  $V_m$  and  $\Sigma V_c$  on the METER SELECTOR switch give the same meter reading as *Junction Zero* ( $10 \text{ mV}$ ).
4. Set METER SELECT to  $V_c+h \text{ IN}$  and check that the METER reads *zero*.
5. Place METER SELECT switch in *Junction Zero* position.
6. Adjust the JUNCTION ZERO control to its maximum setting (fully CW).
7. Check that the METER reads  $\geq 120 \text{ mV}$  for *Junction Zero*,  $V_m$ , and  $\Sigma V_c$  METER SELECT settings.

8. Adjust the JUNCTION ZERO control to its minimum setting (fully CCW).
9. Check that METER reads  $\leq 120\text{ mV}$  for *Junction Zero*,  $V_m$ , and  $\Sigma V_c$  METER SELECT settings.
10. Set METER SELECT to *Junction Zero*.
11. Adjust JUNCTION ZERO until METER reads *zero*.
12. Return amplifier to initial settings.

### **Internal DC voltage command checkout**

1. Connect oscilloscope to  $V_c \times 10$  BNC on front panel.
2. Set scope to  $50\text{ mV/div}$ .
3. Set the METER SELECT switch to the *Junction Zero* setting and adjust meter reading to  $10\text{ mV}$  with the JUNCTION ZERO control.
4. Verify that scope reads 10 times the panel METER reading (2 div at these settings).
5. Using JUNCTION ZERO control adjust METER to  $0\text{ mV}$ .
6. Set the METER SELECT switch to the  $\Sigma V_c$  position. Verify that METER reads *zero*.
7. Set VOLTAGE COMMAND toggle to *positive (+)*.
8. Adjust VOLTAGE HOLD control *fully CW*.
9. Verify the METER reads a minimum of  $\pm 200\text{ mV}$ .
10. Set oscilloscope to  $1\text{ V/div}$ . Verify that scope reads 10x the meter reading. (e.g.  $2\text{ V}$  for a meter reading of  $200\text{ mV}$ ).
11. Switch VOLTAGE HOLD toggle to *minus (-)*. Redo steps 9 and 10.
12. Switch VOLTAGE HOLD toggle to *positive (+)*.
13. Using VOLTAGE HOLD control, adjust until meter reads  $150\text{ mV}$ .
14. Switch METER SELECT to *Junction Zero*.
15. Adjust JUNCTION ZERO until METER reads  $+100\text{ mV}$ .
16. Set METER SELECT to  $V_c+h\text{ IN}$ . Verify that METER reads holding potential of  $150\text{ mV}$ .
17. Set METER SELECT to  $\Sigma V_c$ . Verify that *meter* reads sum of holding potential and junction offset ( $250\text{ mV}$ ).
18. Set METER SELECT control to  $V_c+h\text{ IN}$ . Verify that the adjustment of JUNCTION ZERO control does not affect the meter reading.
19. Set the VOLTAGE HOLD toggle to *off*.
20. Set the METER SELECTOR switch to *Junction Zero* and adjust the METER to *zero*.
21. Return amplifier to initial settings.

### **External DC Voltage command checkout**

1. Apply a  $+1\text{ VDC}$  signal to COMMAND IN BNC on instrument rear panel.
2. Connect oscilloscope to  $V_c \times 10$  output BNC on instrument front panel.
3. Set the METER SELECT switch to  $\Sigma V_c$ .

4. Set COMMAND SELECT (COMMANDS block) to *external command*.
5. Set COMMAND SENSITIVITY to  $\times 0.1$ .
6. Turn COMMAND SENSITIVITY toggle to *on*.
7. Set oscilloscope to 0.5 V/div.
8. Verify that METER reads  $100 \pm 1$  mV and that oscilloscope reads 1 V (2 div at these settings).
9. Set COMMAND SENSITIVITY toggle to  $\times 0.01$ .
10. Set oscilloscope to 50 mV/div.
11. Verify that METER reads 10 mV and that oscilloscope reads 100 mV (2 div at these settings).
12. Set COMMAND SENSITIVITY toggle to  $\times 0.001$ .
13. Set oscilloscope to 5 mV/div.
14. Verify that METER reads 1 mV and that oscilloscope reads 10 mV (2 div at these settings).
15. Return amplifier to initial settings.

### ***Internal AC voltage command checkout***

1. Set COMMAND SELECT (COMMANDS block) to *internal command*.
2. Set COMMAND SENSITIVITY to  $\times 0.1$ .
3. Set COMMAND SENSITIVITY toggle to *on*.
4. Connect  $V_c \times 10$  output BNC to oscilloscope.
5. Set oscilloscope voltage base to 0.5 V/div and time base to 5 ms/div.
6. Verify that oscilloscope displays appropriate square wave (1 V p-p at line local frequency of 50 or 60 Hz).
7. Return amplifier and oscilloscope to initial settings.

### ***Auto Zero control checkout***

1. Disconnect voltage source from COMMAND IN BNC.
2. Connect model cell to headstage in *patch mode*.
3. Set METER SELECT to  $I_m$ .
4. Set PROBE RESISTER to *high*.
5. Set COMMAND SENSITIVITY toggle to *off*.
6. Set COMMAND SELECT (COMMANDS block) to *external command*.
7. Use JUNCTION ZERO control to set  $I_m$  output on METER to  $+10$  pA.
8. Switch AUTO ZERO *on* and press AUTO ZERO BUTTON until meter reads *zero*.
9. Switch AUTO ZERO *off*.
10. Re-zero  $I_m$  output using *manual* JUNCTION ZERO control.

11. Return amplifier to initial settings.

### ***ΣVc commands checkout***

1. Connect model cell to headstage in *patch mode*.
2. Connect  $I_m$  output BNC to oscilloscope.
3. Set oscilloscope to 1 V/div.
4. Set METER to  $I_m$ .
5. Verify PROBE RESISTER is set to *high*.
6. Set COMMAND SELECT to *external command*.
7. Set COMMAND SENSITIVITY toggle to *off*.
8. Set VOLTAGE HOLD to *positive (+)*.
9. Use VOLTAGE HOLD to set  $I_m$  output to +10 pA on METER (positive hold adjustment should give positive current output).
10. Set GAIN to 10 mV/pA.
11. Verify that oscilloscope reads +1 V (1 div at these settings)
12. Set VOLTAGE HOLD to *negative (-)*.
13. Verify that METER reads -10 pA.
14. Verify that oscilloscope reads -1 V (1 div at these settings)
15. Return amplifier to initial settings

### ***Cap Comp checkout***

1. Connect model cell to headstage in *patch mode*.
2. Set PROBE RESISTOR to *high*
3. Set VOLTAGE HOLD control to *off*.
4. Set scope to 5 V/div.
5. Set COMMAND SELECT to *internal command*.
6. Set COMMAND SENSITIVITY toggle to *on*.
7. Scope should now display large amplitude positive and negative pulses. Clipping LED will be lit.
8. Using FAST CAP COMP controls, minimize amplitude and duration of pulses. (Leading edge transients will remain due to characteristics of model cell.)
9. Set PROBE RESISTER to *low*.
10. Connect model cell to headstage in *whole cell mode*.
11. Turn C-SLOW toggle switch *on*.
12. Adjust C-SLOW and SERIES R controls until leading edge of square wave transitions smoothly from one peak to the other. Full transition should take approximately 1 ms. Remaining leading edge transients can be minimized by small adjustments to the FAST CAP COMP controls.



13. Turn % CORRECTION toggle switch *on*. Verify that control decreases transition time without increasing amplitude of spike.
14. Turn % CORRECTION *off*.
15. Turn LEAK SUBTRACTION *on*.
16. Verify that control adjusts amplitude of square wave.
17. Remove model cell from headstage.
18. Return amplifier to initial settings.

### **Current clamp checkout**

1. Attach model cell to headstage in *bath mode*.
2. Set MODE toggle to  $I_o$ .
3. Set METER SELECT to  $V_m$ .
4. Using JUNCTION ZERO control adjust METER to  $0\text{ mV}$ .
5. Set METER SELECT to  $V_c+h\text{ IN}$
6. Set CURRENT HOLD toggle to *positive (+)*
7. Adjust CURRENT HOLD control until METER reads  $50\text{ mV}$ .
8. Set METER SELECT to  $V_m$ .
9. Verify that METER reads  $0\text{ mV}$ . (e.g. CURRENT HOLD command is disabled in  $I_o$  mode.)
10. Set MODE toggle to  $I_c$ .
11. Set METER SELECT to  $I_m$ .
12. Adjust CURRENT HOLD control until METER reads  $500\text{ pA}$ .
13. Set METER SELECT to  $V_m$ .
14. Verify that meter reads  $5 \pm 1\text{ mV}$  (corresponding to the holding potential required to pass a  $500\text{ pA}$  current through a  $10\text{ M}\Omega$  resistor).
15. Return amplifier to initial settings.

**This completes the functional checkout of the instrument.** Please contact our technical support staff if you have any questions or experience any problems.

## OPERATION

### Initial settings

This section assumes that the following items are in place:

- data acquisition system or oscilloscope
- air table / Faraday cage
- micromanipulator
- microscope
- recording dish or chamber
- prepared patch pipettes (capillaries)
- electrode holder with flexible tubing attached to the side port
- Ag/AgCl reference electrode with or without KCl-agar salt bridge
- solutions for bath and pipettes
- cells

With the power off, set the amplifier controls to the known configuration shown below.

Control	Control block	Setting
POWER	<u>METER</u>	<i>off</i>
VOLTAGE HOLD	<u>COMMANDS</u>	<i>fully CCW, toggle switch off</i>
CURRENT HOLD	<u>COMMANDS</u>	<i>fully CCW, toggle switch off</i>
COMMAND SELECT	<u>COMMANDS</u>	<i>external command</i>
COMMAND SENSITIVITY	<u>COMMANDS</u>	<i>off, x0.001</i>
JUNCTION ZERO control	<u>COMMANDS</u>	<i>midpoint</i>
AUTO ZERO toggle	<u>COMMANDS</u>	<i>off</i>
ZAP toggle	<u>COMMANDS</u>	<i>safe</i>
ZAP control	<u>COMMANDS</u>	<i>0.1 ms</i>
C-FAST 1	<u>FAST CAP COMP</u>	<i>fully CCW</i>
C-FAST 2	<u>FAST CAP COMP</u>	<i>fully CCW</i>
FAST $\tau_1$	<u>FAST CAP COMP</u>	<i>fully CCW</i>
FAST $\tau_2$	<u>FAST CAP COMP</u>	<i>fully CCW</i>
C-SLOW	<u>WHOLE CELL</u>	<i>fully CCW</i>
C-SLOW toggle	<u>WHOLE CELL</u>	<i>off</i>
SERIES R	<u>WHOLE CELL</u>	<i>fully CCW</i>
LEAK SUBTRACTION	<u>WHOLE CELL</u>	<i>fully CCW, click off</i>
% CORRECTION	<u>WHOLE CELL</u>	<i>fully CCW</i>
% CORRECTION toggle	<u>WHOLE CELL</u>	<i>off</i>
PROBE RESISTOR select	<u>OUTPUT</u>	<i>high</i>
MODE	<u>OUTPUT</u>	<i><math>V_c</math></i>
GAIN	<u>OUTPUT</u>	<i>10 mV/pA</i>
FILTER	<u>OUTPUT</u>	<i>10 kHz</i>
FILTER toggle	<u>OUTPUT</u>	<i>active</i>
METER select	<u>METER</u>	<i><math>I_m</math></i>

Mount the headstage onto the micromanipulator and turn the power on.

## Preliminary

### **Mount the electrode**

Position the Ag-AgCl reference electrode or KCl-agar salt bridge into the recording chamber and connect to the ground pin on the headstage case.

**NOTE:** If using a Ag/AgCl wire or pellet reference electrode, make sure that your Ag wire/pellet is fully chlorided. Any bare  $\text{Ag}^{2+}$  that contacts your solutions will introduce an undesired junction potential. This is also true for the Ag/AgCl wire in the recording electrode in your pipette holder.

Insert a properly pulled and filled micropipette into the electrode holder and gently but firmly insert the prepared electrode holder into the headstage.

**NOTE:** Stray capacitance can be reduced by Sylgard coating your pulled pipettes. (But not the tip!) For a detailed discussion on this and other noise sources, see Chapter 1 in Patch-Clamp Applications and Protocols, by Levis and Rae (1995) Humana Press.

### **Submerge the electrode tip**

The following operations need to be performed quickly since any delay will foul the tip and inhibit seal formation. Also, it is advisable to locate the cell you want to patch before lowering the tip into solution.

Just before lowering the electrode pipette tip into the bath, carefully aspirate the surface of the bath with a small tube connected to a vacuum line, or quickly wipe the surface with a small clean piece of lens tissue. This will remove any debris on the surface.

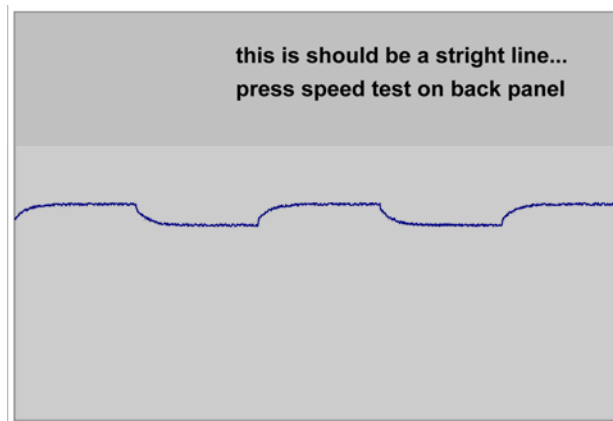
Next, apply continuous, gentle, positive air pressure (about 1-2 kPa or 10-20 cm of water) to the side port of the electrode holder to prevent any remaining debris from fouling the pipette tip during insertion and lower the tip into the bath.

Keep the pipette tip in the bath and maintain the air pressure in the electrode holder as you proceed with the steps below.

### **Zero the pipette offset**

On entering the bath, the junction potential between the pipette and reference electrodes will induce an appreciable current through the pipette. This is indicated by the panel meter  $I_m$  reading in pA, and on your data acquisition system or oscilloscope from the  $I_m$  output terminal.

**Note:** If the data trace is not a straight line but instead looks like the one to the right, then push (once) the SPEED TEST button on the rear of the instrument.



To establish a *zero baseline reference potential*, activate the junction zero circuit by placing the AUTO-ZERO toggle switch into the *on* position. This turns the auto-zero LED *on*. Next, *depress and hold* the AUTO-ZERO PUSHBUTTON to activate the automatic correction circuit. The settling time is rapid when used with M $\Omega$  pipette resistances, but can take several seconds if used with G $\Omega$  resistances. For complete compensation, keep the pushbutton depressed until  $I_m$  on the METER reads *zero*. If needed, small corrections to the offset can be made by adjusting the JUNCTION ZERO control.

Releasing the AUTO-ZERO PUSHBUTTON locks the offset potential to the value set during this operation. The value will remain in effect until the toggle is switched *off* or the pushbutton is *re-depressed*. Switching the AUTO-ZERO toggle to *off* restores the uncompensated current and voltage.

**NOTE:** Applied holding potentials remain active when the AUTO-ZERO toggle is turned *on*. Depressing the AUTO-ZERO PUSHBUTTON, however, zeros all manual settings (including holding potential, time-averaged test pulse, or command voltage) during the zeroing function. Manual settings are re-applied when the PUSHBUTTON is released.

After zeroing the current, the magnitude of the junction potential can be read from the meter ( $V_c+h$  setting) or on the oscilloscope from the  $V_c \times 10$  output. The zero baseline setting should remain stable so long as the AUTO-ZERO TOGGLE switch is set to *on*. If the value drifts, then make sure the reference electrode is properly submerged. If the zero setting continues to drift, then check your setup for errors (e.g., chloriding of the electrodes).

**IMPORTANT!** Once set, do not make further adjustments to the zero setting until you take a new pipette. If you change the zero setting after forming a gigaseal, then you'll not know the true transmembrane potential!

### **Measure the pipette resistance (RP)**

Measuring the resistance of the pipette is a routine method for determining the pipette's condition before attempting to record data. Useful pipettes for patch recording can have resistances within the range of 1-10 M $\Omega$  and those for whole cell recording can range from 1-5 M $\Omega$ . The specific resistance you'll see is highly dependent on the solutions used and the size of the tip. Generally speaking, smaller tips have higher resistance, more easily make gigaseals, and are desired for patch work. Larger tips are better for whole cell work since it is easier to rupture the membrane and the access resistance will be lower.

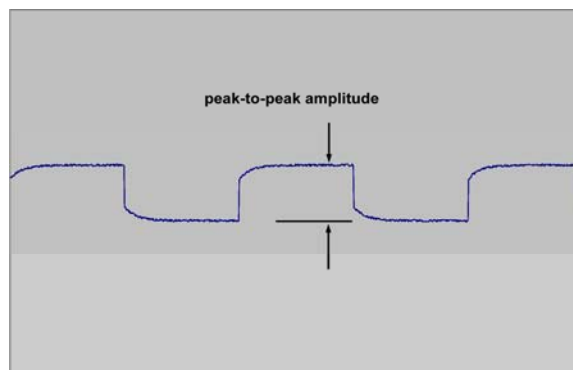
Pipettes with much higher resistance are likely to be blocked with debris from poorly filtered pipette or bath solutions, or to have constricted tips from over-polishing. Those with much lower resistance are probably broken. In either case, take a new pipette and start over.

The pipette resistance is measured with the pipette in the bath and a TEST PULSE applied. A test pulse function can be driven from software (e.g., pClamp, PatchMaster) or from the amplifier. In the amplifier, the test pulse is activated by setting the COMMAND SELECT toggle to *internal command*, the SENSITIVITY SELECTOR toggle to  $\times 0.001$ , and the

COMMAND SENSITIVITY toggle to *on*. These settings activate and apply a line frequency, 1 mV peak-to-peak square wave to the pipette. Adjust your acquisition system or oscilloscope and amplifier so as to clearly display 1-2 complete cycles of the output current signal. An example trace is shown to the right.

**Note:** If needed, the amplitude of the TEST PULSE can be set to a different value (1, 10, or 100 mV) as needed to see a clear signal. This is achieved by adjusting the SENSITIVITY SELECTOR (for example,  $\times 0.01 = 10$  mV).

Read the amplitude of the current (peak-to-peak on the square wave in pA or nA) on your oscilloscope or acquisition system. From the voltage amplitude of the test pulse and the output current you can calculate RP in  $M\Omega$  from Ohm's law ( $V=IR$ ).



**Notes:**

(1) If reading from an oscilloscope, be sure to take into account the GAIN MULTIPLIER just above GAIN SELECTOR switch.

(2)  $1 \text{ mV/pA} = 1 \text{ G}\Omega$

## Voltage clamp

### *Attach to the cell and form gigaseal*

Leave the TEST PULSE active and advance the pipette tip to contact the cell. Once the pipette begins to dimple the cell membrane, the amplitude of the test current should begin to decrease. When this happens, release the applied positive air pressure in the electrode holder and watch the test pulse for seal formation.

If a seal does not immediately form, then apply a pulse of gentle suction (2-3 kPa or less) to the electrode holder and watch the test current closely for seal formation (usually 10-30 s). Repeat as necessary and once a seal forms, quickly release suction to avoid going into whole-cell.

You'll know a seal has formed when the test current quickly decreases, then goes flat. The signal appears flat since the current is now too small to see at the scale your recording system is set to. You can increase the signal amplitude by making the following adjustments in order: (1) increase the COMMAND SENSITIVITY, (2) increase the amplifier GAIN, and (3) increase the y-axis scaling on your recording system.

**Notes:**

1. If a seal doesn't form within about 30 seconds, try alternately releasing and re-applying suction for about 10-30 s each.
2. If a seal appears but forms very slowly, continue alternating the suction, or advance the pipette very slightly against the cell.

3. If a seal doesn't form within about 5-10 min, it probably will not. Take a new pipette and start again.
4. Never attempt to clean and re-use a used pipette. It will never make a seal.

### Set electrode capacitance compensation

Once a seal has formed, apply electrode capacitance compensation.

Leave the TEST PULSE active and set the COMMAND SENSITIVITY to  $\times 0.01$  (10 mV). Adjust your recording system so you can visualize the trace as shown in the top figure to the right.

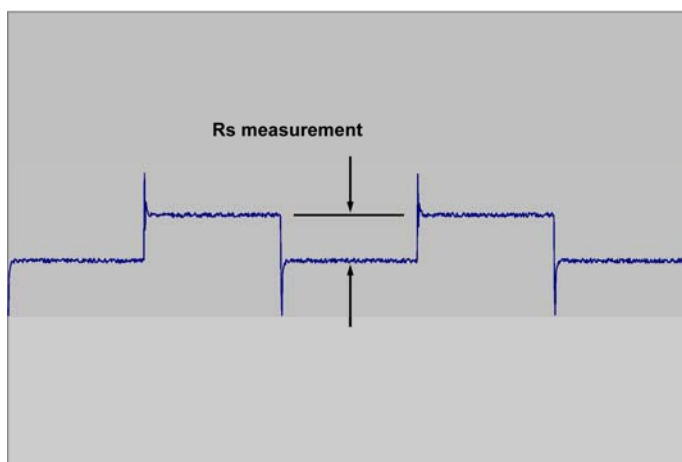
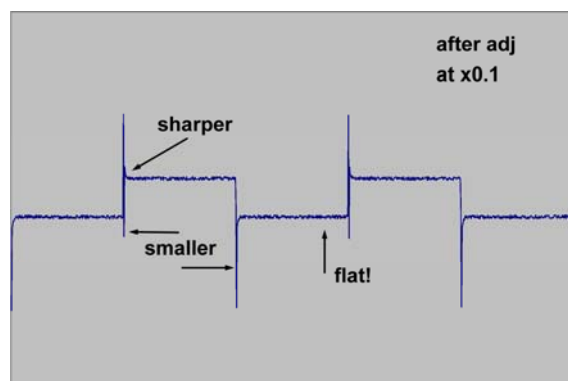
Use the FAST CAP COMP controls (C-FAST and FAST  $\tau$ ) to minimize the overshoot and balance the shape of the excursions on the waveform. Because the two banks of CAP COMP controls (1, 2) interact with each other, some trial and error in making a good adjustment is required.

Compensation is optimal when the square wave has as sharp a rise and fall as possible, a flat top and bottom, and minimum overshoot. (See the bottom figure on previous page.) Rise time improves with higher frequency BESSEL FILTER settings, however, higher frequency settings introduce a tradeoff between fast response and increased noise.

Once an optimal trace has been found at the  $\times 0.01$  setting, increase the COMMAND SENSITIVITY to  $\times 0.1$  (100 mV) and repeat. An appropriately adjusted trace is shown to the right.

### Measure seal resistance (RS)

You can measure seal resistance with the electrode still attached to the cell or after you've pulled a membrane patch (cell attached patch



or excise patch configurations, respectively).

Keep the TEST PULSE *on* and measure the peak-to-peak 'leak' current on the oscilloscope. An example is shown on the previous page. Since the test pulse has a known voltage amplitude (100 mV in this case), you can calculate RS by use of Ohm's law ( $R=V/I$ ). A good gigaseal resistance is typically in the range 1-10 G $\Omega$ .

### ***Patch or whole cell recording?***

At this point you can perform either patch clamp or whole cell recording. The following sections address additional setup steps to be performed prior to making measurements for each technique.

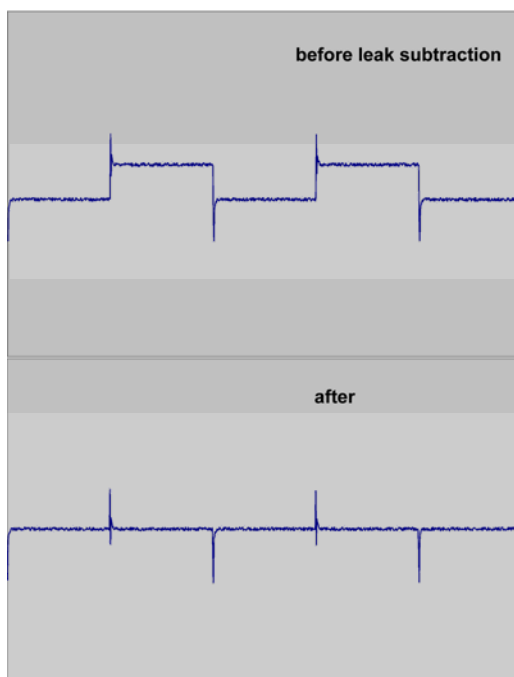
Both patch and whole cell recording can be performed with either the **LC-201B** low current or **HC-202B** high current headstages. In either case, whole cell recordings are performed with the PROBE RESISTOR SELECT toggle switch set to the *low* position (500 M $\Omega$  or 50 M $\Omega$ , respectively for each headstage).

### ***Patch recording - Leak subtraction***

If RS is only a few G $\Omega$ , it may be necessary to compensate for membrane voltage errors lost to the leak pathway.

With TEST PULSE still active, turn the LEAK SUBTRACTION control clockwise until the  $I_m$  trace on your acquisition system or oscilloscope becomes flat except for residual capacitance transients. (Alternatively, with the TEST PULSE *off*, run the holding potential up and down over a range that doesn't evoke channel currents and adjust the LEAK SUBTRACTION control so that the current trace on the oscilloscope remains fixed.) An example is shown to the right.

If RS is very high, leak subtraction may not be needed. If so, turn the LEAK SUBTRACTION control to 0 (*off*).



### ***Patch recording – Excised patch***

Watch with the microscope as you excise the patch. To pull a patch, quickly move the pipette away from the cell with the manipulator, then up and away using the fine vertical controls. During patch excision, the cell should stay attached to the bottom of the dish. If the excision is successful, the critical vibration-sensitive phase of the operation is complete.

If the cell remains attached to the pipette, try jiggling the micromanipulator controls, or tap gently on the micromanipulator, or bring the cell and pipette up briefly into the air

for no more than 1 second. If the patch still does not separate from the cell, you should consider proceeding with a cell-attached patch recording instead.

### **Patch recording - Cell-attached patch**

If the cell moves freely with the pipette, raise the pipette and cell up from the bottom of the dish, but keep everything submerged. If the cell is not free to move, then you can work with the cell adhered to the dish but take great care to avoid vibration while recording.

The **PC-505B** controls can be set to values needed to perform your work. However, some controls should not be moved or measurement artifacts or patch rupture can occur. These are shown in the table below.

Control	Control block	Setting
JUNCTION ZERO control	<u>COMMANDS</u>	Do not adjust!
AUTO ZERO toggle	<u>COMMANDS</u>	Leave on!
ZAP toggle	<u>COMMANDS</u>	<i>Safe</i>

Voltage clamp waveforms are usually applied to the COMMAND IN BNC as externally generated test protocols from your acquisition software. COMMAND IN inputs can be summed with internally generated inputs. To pre-set the internal holding voltage ( $V_h$ ) to a fixed value before beginning to record, or to reset it to another fixed value at any time during an experiment, first set the meter to  $V_c + h$  IN, then set the COMMANDS VOLTAGE POLARITY toggle switch to - or +. Finally, set the COMMANDS VOLTAGE HOLDING control to give the desired voltage reading on the meter.

The COMMANDS HOLDING VOLTAGE control can also be used as a quick check for voltage-gated channel activity. Turn COMMAND SENSITIVITY *off* to disengage the external inputs and vary  $V_h$  in either direction.

### **Whole-cell recording – Initial conditions**

Once the pipette is attached to cell with a gigaseal, you can rupture the membrane patch under the seal to gain pipette access to the cell interior. Before proceeding place the amplifier into whole-cell mode by switching the PROBE SELECT toggle to *low*. Also, switch the COMMAND SENSITIVITY toggle to  $\times 0.01$ .

### **Whole-cell recording - Rupture the membrane patch**

Leave the TEST PULSE active. You can use the ZAP function or a suction pulse to rupture the membrane patch. If using ZAP, start with a DURATION of  $0.1$  ms. Press the ZAP button to activate the function and increase the duration by  $0.1$  ms on successive tries as necessary.



Rupture is signaled by a sudden large increase in the TEST PULSE current when in *voltage clamp* mode, or as a sudden decrease in TEST PULSE voltage at the  $V_m \times 10$  terminal when in *current clamp* mode. Once the membrane is ruptured, immediately apply a resting holding potential appropriate to the cell under study.

An example of a ruptured membrane signal is shown to the right.

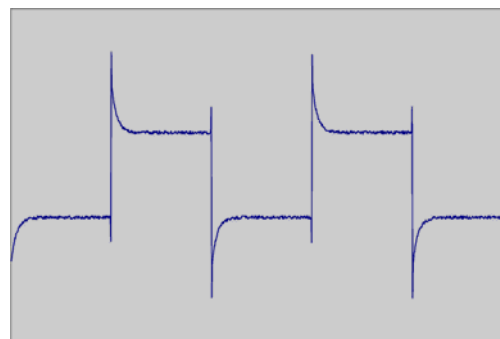
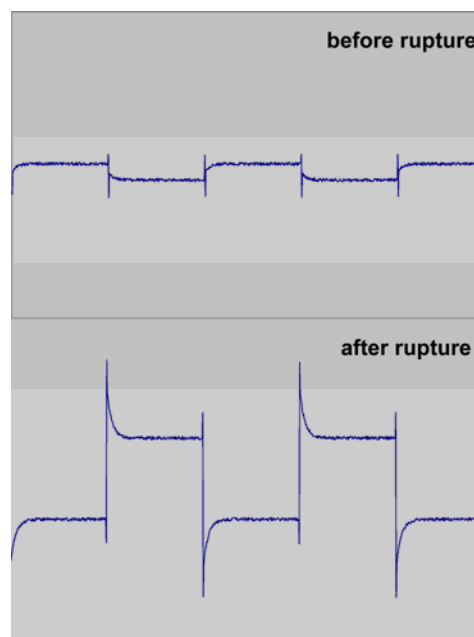
### Whole-cell recording - Series R compensation

In single channel recording, the pipette electrode resistance (RP) is negligible compared to the patch and seal resistances. However, in whole cell recording this electrode resistance becomes a significant factor and will slow the response of the amplifier.

Series R compensation is adjusted by using the SERIES R and C-SLOW controls. The SERIES R control compensates for the resistance of the pipette opening at the cell and the C-SLOW control compensates for the cell membrane capacitance.

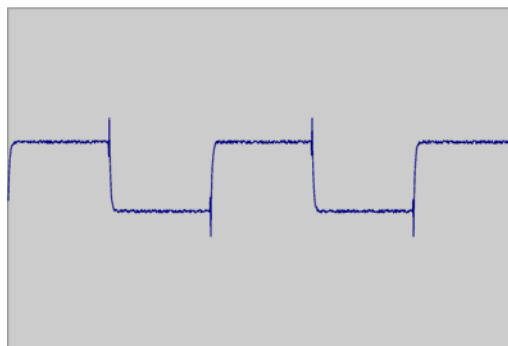
A good adjustment for Series R is important but can be a subtle operation. A basic approach is outlined below.

- Verify that the TEST PULSE is active and set to  $\times 0.01$ . The trace should appear like the example to the right.
- If you've previously measured the pipette resistance (RP), then initially set the SERIES R dial to this value. **NOTE:** The SERIES R control is calibrated to 10 M $\Omega$ /turn, so if your RP=4.5 M $\Omega$ , then set the dial to 0.45.
- If you do not know the pipette resistance, then set the SERIES R control to 5.0.
- Next, turn on C-SLOW and adjust up until a slight overshoot appears on the leading edge of the trace. An example is shown to the right.



- Return to SERIES R and adjust the overshoot away. Go back and forth between the C-SLOW and SERIES R controls until you've completely removed the leading spike and made the leading corner as sharp as possible. An example is shown to the right.

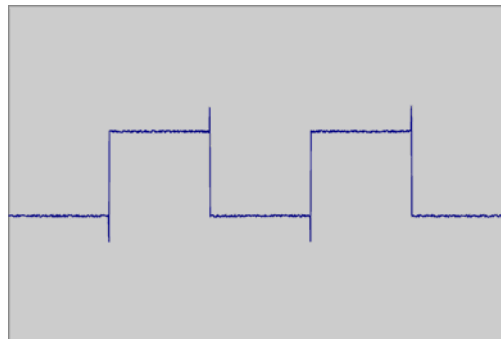
The value of Series R can now be read directly from the SERIES R dial.



### ***Whole-cell recording - % correction***

Once the series resistance has been measured and corrected, you can further optimize the amplifier by applying a signal boost in the form of % correction. Generally speaking, the maximum correction that can reasonably be applied is 80%.

Activate the % CORRECTION control and raise its value to 5% or so. Doing so will distort the previously Series R settings, so you will need to lower C-SLOW to compensate. Gradually go back and forth, increasing % CORRECTION and decreasing C-SLOW until you have maximized the sharpness of the leading corner on the trace without ringing. An example is shown to the right.



## Current clamp

Current clamping is used mainly with whole-cell recording. In current clamp mode, the VOLTAGE HOLD, JUNCTION ZERO, AUTO ZERO, and CAP COMP controls are *inactive*. CURRENT HOLD becomes *active*, as well as the  $V_m \times 10$  output.

In current clamp mode, an error voltage drop  $I_m R_P$  is generated by current through the electrode. Series R comp ( $10 \text{ m}\Omega/\text{turn}$ ) cancels the error voltage by feeding back a portion of the output current.

### Switching into current clamp

- 1) Begin by switching the MODE SELECTOR toggle (in the OUTPUT section) to  $I_o$  (zero current).
- 2) Turn off all applied commands and test signals to prevent inadvertently applying unwanted signals that could damage the cell preparation when you switch to current clamp or voltage clamp.

**Notes:** Although command signals are disengaged in zero current mode, they become active immediately on switching out of zero current mode.

Do not press zap. It remains active in zero current mode and can break the patch.

Do not change the cap comp or leak subtraction setting from those made while in voltage clamp mode. They are still valid when switching back to voltage clamp mode. Changes made now can't be monitored, can result in over-compensation, feedback oscillation, and can immediately destroy the patch, or at least may cause errors in series resistance compensation.

- 3) Pre-set the holding current. Unfortunately, this is not as straightforward as one would hope. Briefly:
  - Select  $V_c + H \text{ IN}$  on the meter. The meter will display units of mV. Disregard the units.
  - Set the polarity of the HOLDING CURRENT toggle switch (eg, + or -).
  - Adjust the CURRENT HOLD knob until the meter reads the desired current.

**IMPORTANT NOTE ! The actual current at the pipette is 10x that of the meter display. For example, when the meter reads 20 mV, then the actual current is 200 pA.**

- Then switch the HOLDING CURRENT toggle switch *off* until you are ready to use it.
- 4) Finally, switch the MODE SELECTOR toggle from  $I_o$  (zero current) to  $I_c$  (current clamp).

The amplifier is now ready to apply a current clamp when desired.

Activate the clamp mode by turning the HOLDING CURRENT toggle switch to *on* (eg, + or -).

### Recording in current clamp

Commands can now be applied with the pre-set holding current, an externally generated test protocol applied to the COMMAND IN BNC, or manually with the HOLDING CURRENT control knob. Commands from all of the sources are summed by the amplifier and applied. Command currents can be monitored on the meter or at the  $I_M$  OUTPUT BNC.

### External command currents

Maximum current capability produced by an externally applied command signal depend on the COMMAND SENSITIVITY selected. A 10 V signal applied to the COMMAND IN BNC will produce pipette currents as shown in the table below. (These values assume the headstage is set with the PROBE RESISTOR toggle set to *low*.)

COMMAND SENSITIVITY setting	Attenuated command voltage with 10 V input	Maximum current capability
x0.1	1 V	1 nA
x0.01	100 mV	100 pA
X 0.001	10 mV	10 pA

A positive command voltage produces a positive (cation-outward) current from the pipette.

### Bilayer recording

Bilayer recording is performed using the **HB-205B** headstage. The **HB-205B** is a switchable (50 G $\Omega$ /500 M $\Omega$ ) headstage modified to allow for compensating the relatively large capacitance of the bilayer membrane. Up to approximately 250 pF can be compensated. As such, the noise level for this headstage is about 10% greater than for the standard 50 G $\Omega$  headstage due to the increased capacitance compensation.

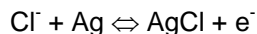
Bilayers are usually formed across an aperture in a cup contained within a Faraday cage. Because of the high impedance of the bilayer, the assembly *must* be shielded from interference to obtain low-noise recordings. Warner has a complete Bilayer Workstation available, and if needed, the amplifier used with that system can be replaced with the **PC-505B**.

Regardless if you're using the **PC-505B** with Warner's Bilayer Workstation or with a custom rig, Plug the **HB-205B** headstage into the PROBE RECEPTACLE on the rear of the instrument and turn the PROBE SELECT knob to *high*. The amplifier automatically recognizes the headstage and illuminates the front panel BILAYER light. You can proceed with voltage or current clamp measurements as desired.

## THEORETICAL CONSIDERATIONS

### Chloriding silver wire

Silver-silver chloride electrodes act as signal transducers by converting ionic currents in solution to an electric current within a wire. This is achieved by utilizing a reversible oxidation/reduction reaction between the electrode and  $\text{Cl}^-$  ions in solution. The chemical reaction is:



The potential developed by one electrode is proportional to the standard electrochemical potential for Ag/AgCl plus the  $\text{Cl}^-$  concentration at the solution/electrode interface. Since this potential is dependent on  $\text{Cl}^-$ , a voltage bias will be introduced by changing the solution  $\text{Cl}^-$  concentration. Therefore, we recommend that Ag/AgCl electrodes be connected to the bath through agar salt bridges to maintain a constant  $\text{Cl}^-$  concentration near the electrode. In addition, the isolation provided by the agar bridge will prevent  $\text{Ag}^+$  ions from contaminating the baths.

First clean the wire by wiping with a clean tissue wet with alcohol or a standard laboratory detergent, then rinse well with distilled water. Wiping in this way can help to straighten the wire. If using alcohol or similar solvent, avoid getting it onto the polycarbonate holder body as this can weaken the structure.

### *Chloriding by electrolysis*

Dip the Ag wire to the required depth in a solution of 0.1M NaCl or KCl, optionally made slightly acidic with HCl, and arrange to pass positive current from the Ag into the solution. For the indifferent electrode in the solution, a cleaned carbon rod from a discarded 6 V lantern battery works well. Another, thicker, Ag wire will also work, but most other metals are likely to contaminate the AgCl coat. Pass current at a density of about  $1 \text{ mA/cm}^2$  for about 1 minute or until adequately plated. For a 2 cm length of 0.01" wire, this is about  $150 \mu\text{A}$ . When well plated, the surface should be uniformly light grey.

Reversing the current polarity occasionally while plating, ending with the Ag positive, tends to make a more stable electrode. If available, a low-frequency signal generator at about 0.1 Hz, with a slight positive bias is also convenient. The electrolyte solution can be saved and reused indefinitely.

### *Chloriding chemically*

Immerse the clean Ag wire in Clorox solution until the wire is uniformly light gray, about 1-10 min. Rinse well with tap water, then with distilled.

### Electrode holders

The standard holder supplied with **PC-505B** is the **QSW-AxxP** (straight body style). It uses a 0.010" diameter, 99.99% pure silver wire to couple the signal from the micropipet solution to

the input pin of the headstage amplifier. Before use, the wire must be plated with silver chloride (AgCl) to within 2-3 mm of the end cap which secures the micropipet.

A 2 mm OD port on the side of the holder is used for applying pressure or suction through standard 1/16" ID flexible plastic tubing. Each holder is made to accommodate a single specified diameter of pipet electrode glass, designated by "xx" in the part number, where xx=10x the OD in mm. For example, **QSW-A15P** specifies 1.5 mm OD pipets.

### ***Care and use of holders***

Both ends of the pipet tubing should be lightly fire polished before pulling micropipets, in order to avoid scraping AgCl from the wire surface and to prolong the life of the rubber gasket that holds the pipet in place.

Fill pipets with only enough electrolyte to cover several mm of the AgCl coating when inserted into the holder. This minimizes stray capacitance and the noise level while recording. Take care to avoid getting pipet filling solutions onto exposed bare silver above the AgCl coating in the pipet, within the body of the holder, into the suction port, or especially onto the pin jack which could damage the headstage. Should this happen, disassemble the holder, rinse thoroughly with distilled water, dry thoroughly, and reassemble.

### ***Cleaning and storage***

After use, rinse any deposits from the holder body, the Ag wire, pin jack, and suction port and allow to dry. To protect the silver wire from getting bent in storage, the holder can be capped with a short piece of 3/8" ID plastic tubing, or a 50 x 10 mm ID *plastic* vial with a small nylon set screw.

### ***Replacing holder parts***

The rubber seal at the pipet end of the holder can be replaced if it becomes damaged with repeated pipet insertions. A spare is supplied with the holder and additional gaskets can be ordered as required. The gasket is easily removed with a small pair of blunt forceps.

The silver wire is also replaceable. Replacement is necessary when the wire becomes hopelessly kinked or badly jammed, as by carelessly inserting into pipets.

To replace the wire, unscrew the pin connector and remove the rubber seal. Pull the wire from the small hole in the center of the seal and replace it with a new piece about 6 cm long. Insert the wire through the seal from the pipet side so that 5 mm extends beyond the pin connector side. Bend this end by 90° at the seal and fold the end of the wire back across the seal to insure good contact with the pin connector. Reassemble the wire and seal into the holder taking care that the seal sits squarely in the hole and that the wire doesn't jam in the holder on its way through.

## **Reference electrodes**

A reference electrode in the recording chamber maintains the bath at circuit ground potential, the reference potential for all measurements. It is also the return path for currents

from the pipet electrode. A variety of Ag-AgCl reference electrodes are available from Warner Instruments.

A simple reference electrode can be made from a silver wire. Use wire somewhat thicker than the one in the pipet electrode holder. The end applied to the bath should be chlorided as described on page 26 such that the Ag-AgCl coating can be submerged with no bare Ag exposed to solution. The free end is connected to circuit ground.

Any exposed Ag surface that could potentially contact the bathing solution should be insulated with a waterproof coating of epoxy cement, insulating varnish, or similar durable waterproof coating. This precaution provides a stable baseline as long as the bath solution is not changed.

When bath solutions are to be changed during an experiment, a KCl salt bridge can be used to minimize changes in the junction potential that accompany these solution changes. A small glass or plastic U tube filled with saturated or 3 M KCl in warmed 2% agar gel is usually employed for this purpose. The AgCl reference electrode goes into one end of the tube and the other end is applied to the bath.

## APPENDIX

## Specifications

HEADSTAGES	
<b>LC-201B Headstage</b> (50 G $\Omega$ /500 M $\Omega$ )	single channel currents to 200 pA whole cell currents to 20 nA.
<b>HC-202B Headstage</b> (50 G $\Omega$ /50 M $\Omega$ )	single channel currents to 200 pA whole cell currents to 200 nA
<b>HB-205B Bilayer Headstage</b> (50 G $\Omega$ modified)	for artificial bilayer capacitances up to 250 pF currents to 200 pA
<b>Noise (referred to input)</b> Measured with an 8-pole Bessel filter, input open, 50 G $\Omega$ resistor:	DC to 1 k Hz    0.038 pA RMS DC to 5 k Hz    0.170 pA RMS
<b>Bandwidth</b>	25 kHz
VOLTAGE CLAMP COMMANDS	
<b>Command In</b>	$\pm 10$ V maximum, AC or DC applied to input
<b>Voltage Hold</b>	$\pm 200$ mV with 10-turn control
<b>Junction Zero</b>	$\pm 100$ mV with 10-turn control
<b>Internal Test Pulse</b>	1 Volt 50/60 Hz (line frequency) square wave attenuated by COMMAND SENSITIVITY
<b>Command Sensitivity</b>	attenuates at x0.1, x0.01, and x0.001
<b>Zap</b>	1.0 Volt Pulse adjustable duration from 0.1 to 10 ms
CURRENT CLAMP COMMANDS	
<b>Command In</b>	$\pm 1000$ pA maximum with Command Sensitivity @ x0.1 $\pm 100$ pA maximum with Command Sensitivity @ x0.01 $\pm 10$ pA maximum with Command Sensitivity @ x0.001
<b>Current Hold</b>	$\pm 1$ nA with 10-turn control
<b>Internal Test Pulse</b>	1 nA 50/60 Hz square wave
<b>Command Sensitivity</b>	attenuates at x0.1, x0.01, and x0.001
CAPACITANCE COMPENSATION	
<b>C-FAST 1</b> (Voltage Mode)	0.1 to 1.75 $\mu$ s 0 to 5 pF
<b>C-FAST 2</b> (Voltage Mode)	0.33 to 8.5 $\mu$ s 0 to 15 pF
<b>C-SLOW</b>	0 to 100 pF with 10-turn control
<b>Series R</b>	0 to 100 M $\Omega$ with 10-turn control
<b>% Correction</b>	0 to 90% of series R
<b>Leak Subtraction</b>	50 G $\Omega$ Headstage Resistor: $\infty$ to 50 G $\Omega$ 500 M $\Omega$ Headstage Resistor: $\infty$ to 500 M $\Omega$



	50 M $\Omega$ Headstage Resistor: $\infty$ to 50 M $\Omega$
<b>FRONT PANEL OUTPUTS</b>	
<b>I<sub>m</sub> (membrane current)</b>	gains of 0.05 to 10 mV/pA with 50 M $\Omega$ headstage resistor gains of 0.5 to 100 mV/pA with 500 M $\Omega$ headstage resistor gains of 5 to 1000 mV/pA with 50 G $\Omega$ headstage resistor
<b>V<sub>c</sub> x10</b>	summation of all commands amplified by 10
<b>V<sub>m</sub> x10</b>	membrane voltage amplified by 10
<b>I<sub>m</sub> Output Low-Pass Filter</b>	(4-Pole Bessel -3 dB points) 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 kHz Bypass allows full bandwidth
<b>Panel Meter</b>	3-1/2 digit LCD (full scale reading)
<b>Junction Zero</b>	$\pm 199.9$ mV
<b>V<sub>c</sub> + h IN</b>	sum of all commands and V hold $\pm 199.9$ mV
<b><math>\Sigma V_c</math></b>	Sum of all commands $\pm 199.9$ mV
<b>V<sub>m</sub></b>	membrane voltage in current clamp mode $\pm 199.9$ mV
<b>I<sub>m</sub></b>	membrane current $\pm 1999$ pA
<b>RMS noise</b>	1.999 pA
<b>REAR PANEL OUTPUTS</b>	
<b>Gain Telegraphs *</b>	0.5 to 7.0 V in 0.5 V steps
<b>Filter Telegraphs *</b>	0.2 to 2.0 V in 0.2 V steps
<b>I<sub>m</sub>/V<sub>m</sub> telegraph</b>	TTL logic 0=I clamp mode 1=V clamp mode
<b>Sync Out</b>	TTL signal for synchronization oscilloscope with test pulse
<b>OPERATING CONDITIONS:</b>	Equipment is intended to be operated in a controlled laboratory environment.  Temperature: 0-40 °C Altitude: sea level to 2000 m Relative humidity: 0-95%
<b>PHYSICAL DIMENSIONS</b>	
<b>Power Requirements</b>	110 - 130 or 220 - 250 VAC, 50/60 Hz
<b>Main Unit</b>	9 x 42 x 30 cm (H x W x D)
<b>Headstage:</b>	1.9 x 3.5 x 5.7 cm (H x W x L) with 1.8 m cable
<b>Headstage Mounting Rod:</b>	6.3 mm x 6.3 cm (Dia. x L)
<b>Shipping Weight:</b>	11.4 kg
<b>Warranty</b>	Three years, parts and labor

\* Axon pClamp and Instrutech Pulse.

**Accessories and replacement parts**

Model Number	Order Number	Description
<b>Headstages:</b> When ordering additional or replacement headstages, please reference the serial number of your <b>PC-505B</b> .		
<b>LC-201</b>	<b>64-0004</b>	50 G $\Omega$ /500 M $\Omega$ feedback resistors
<b>HC-202</b>	<b>64-0005</b>	50 G $\Omega$ /50 M $\Omega$ feedback resistors
<b>HB-205</b>	<b>64-0006</b>	50 G $\Omega$ /500 M $\Omega$ feedback resistors, modified for bilayer
Standard cable length is 1.8 meters. Lengths up to 3.05 meters (10') are available.		
<b>Electrode Holders:</b> One electrode holder is supplied with each headstage. The standard model is <b>QSW-AxxP</b> (straight). Holder part numbers are completed by replacing the "xx" with 10x the OD (in mm) of the pipet glass to be used.		
<b>QSW-A10P</b>	<b>64-0821</b>	for 1.0 mm OD pipette glass
<b>QSW-A12P</b>	<b>64-0822</b>	for 1.2 mm OD pipette glass
<b>QSW-A15P</b>	<b>64-0823</b>	for 1.5 mm OD pipette glass
<b>QSW-A17P</b>	<b>64-0978</b>	for 1.65 mm OD pipette glass
<b>QSW-A20P</b>	<b>64-0824</b>	for 2.0 mm OD pipette glass
Other style holders (such as 90°, 45° and microperfusion) are available. Contact our Sales Department for complete details on holders, capillary tubing and replacement parts.		

## Warranty and service

We recommend that all questions regarding service be referred to our Support Department.

Normal business hours are 8:30 AM to 5:30 PM (EST), Monday through Thursday and 8:30 AM to 5:00 PM on Friday. Our offices are located at 1125 Dixwell Avenue, Hamden, CT 06514, and we can be reached by phone at (800) 599-4203 or (203) 776-0664. Our fax number is (203) 776-1278. In addition, we can be reached by e-mail at [support@warneronline.com](mailto:support@warneronline.com) or through our Web page at <http://www.warneronline.com>.

### Warranty

The model **PC-505B** is covered by our Warranty to be free from defects in materials and workmanship for a period of three years from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover instrument failure or damage caused by physical abuse or electrical stress (inputs exceeding specified limits).

In the event that instrument repairs are necessary, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments, Inc.

This warranty is not extended to electrode holders since these items are considered disposable.

### Service notes

- A) If the instrument POWER light fails to light, check the fuse at the rear panel. If the fuse is found to be defective replace it with a 3AG 1/2 amp normal blow fuse (1/4 amp for facilities using 220-240 V line voltages). If the replacement fuse also fails, please call Warner Instruments for assistance.
- B) Occasionally, a knob on the front panel will loosen after long use. These are "collet" style knobs and are tightened with a screw located under the knob cap. To gain access to the adjustment screw, pry the cap off with a thin bladed screwdriver or similar tool.
- C) Should service be required, please contact the factory. The problem may often be corrected by our shipping a replacement part. Factory service, if required will be expedited to minimize the customer inconvenience.
- D) Instruments are inspected immediately upon receipt and the customer is notified if the repair is not covered by the warranty. Repairs can often be completed in 1-2 days from our receipt of the instrument.

If factory service is required, please observe the following instructions:

- 1) Package the instrument with at least 3 inches of cushioning on all sides. Use the original shipping carton if it is available.
- 2) Insure the shipment for its full value.
- 3) Include with the shipment an explanation of the problem experienced.

**IMPORTANT: CUSTOMERS OUTSIDE OF THE U.S.**

Please be sure to contact us before return shipping any goods. We will provide instructions so that the shipment will not be delayed or subject to unnecessary expense in clearing U.S. Customs.

**Recommended reading**

Sakmann and Neher (1983) Single-channel Recording, *Plenum Press*

Hamill, Marty, Neher, Sakmann, and Sigworth (1981) *Pflugers Archiv* **391**: 85-100.

Boulton, Baker, and Walz (1995) *Neuromethods 26: Patch-clamp Applications and Protocols*, *Humana Press*

Hille (2001) *Ion Channels of Excitable Membranes*, *Sinauer Press*

Kettenmann and Grantyn (*Editors*) (2001) *Practical Electrophysiological Methods*, *Wiley-Liss*

## Certifications

***Declaration of Conformity***  
***CE MARKING (EMC)***

**Application of Council Directive: 89/336/EEC**

Standards To Which Conformity  
Is Declared:

EN55022 Class A  
EN61000-3-2  
EN61000-3-3  
EN50082-1:1992  
EN61000-4-2  
EN61000-4-3  
ENV50204  
EN610000-4-4  
EN610000-4-8  
EN610000-4-11

Manufacturer's Name:

Warner Instruments, LLC

Manufacturer's Address:

1125 Dixwell Avenue  
Hamden, CT 06514  
Tel: (203) 776-0664

Equipment Description:

Instrument Amplifier

Equipment Class:

ITE-Class A

Model Numbers:

PC-505B

***I the undersigned, hereby declare that the equipment specified  
above, conforms to the above Directive(s) and Standard(s).***

Place: Hamden, Connecticut USA

Signature:



Full Name: Burton J. Warner

Position: President

***Declaration of Conformity***  
***CE MARKING (LVD)***

**Application of Council Directive: 73/23/EEC**

Standards To Which Conformity Is  
Declared:

EN61010-1:1993

Manufacturer's Name:

Warner Instruments, LLC

Manufacturer's Address:

1125 Dixwell Avenue  
Hamden, CT 06514  
Tel: (203) 776-0664

Equipment Description:

Instrument Amplifier  
Safety requirements for electrical  
equipment for measurement and  
laboratory use

Equipment Class:

Class I

Model Numbers:

PC-505B

***I the undersigned, hereby declare that the equipment specified  
above, conforms to the above Directive(s) and Standard(s).***

Place: Hamden, Connecticut USA

Signature:



Full Name: Burton J. Warner

Position: President

## WEEE/RoHS Compliance Statement

### EU Directives WEEE and RoHS

#### To Our Valued Customers:

Harvard Apparatus is committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for those Harvard Apparatus products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a "wheelie bin" symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of Harvard Apparatus' products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. Harvard Apparatus will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.



- Do Not Dispose Product with Municipal Waste.
- Special Collection/Disposal Required.

## Glossary

**A/D converter** – Analog to Digital converter. Computers are inherently digital while the voltage or current output from an amplifier is analog. Therefore, a signal must be first converted to a digitized form before a computer or its software can accept it. Desirable features in an A/D converter include rapid signal conversion, small-step resolution and low noise.

**analog** – Continuous or non-discrete. Often dynamically varying. *Compare to:* digital.

**bandwidth** – The range of frequencies a device is capable of processing with minimal distortion. A bandwidth of 1 Hz indicates that the device can faithfully process a signal occurring once per second (1 Hz). The larger the bandwidth, the faster the device.

**Bessel filter** – A device used to attenuate the high frequency components of a signal. The cutoff frequency of a filter is normally defined as the frequency at which the amplitude of the signal is attenuated by 3 dB. A higher order filter (i.e., 8-pole vs. 4-pole) will attenuate the high frequency components more rapidly. An 8-pole Bessel filter attenuates at 14 dB per octave.

**BNC connector** – A type of connector used to connect coaxial cables to high frequency electronic equipment.

**CAP COMP** – See: capacity compensation.

**capacitance** – A capacitor can be represented by a small break in a conducting pathway bounded by two parallel plates. The electric field generated across the space between the plates in the presence of an applied voltage maintains a charge density on each plate. The numerical measure of a capacitor's ability to maintain charge separation at a given potential is its capacitance. Capacitors effectively block DC currents while passing AC currents. Has units of Farad (F).

**capacity compensation** – The process wherein the current generated when charging a capacitor is subtracted (or compensated) from the output signal.

**channel conductance** – See: unitary channel conductance

**chassis ground** – A connection used to link the amplifier chassis to an external potential.

**circuit ground** – The potential to which all other potentials within the circuit are referenced. Also, a connection used to link the reference potential of the amplifier circuit to an externally defined potential.

**CMD IN** – Command Input. An external input into the amplifier allowing the application of user defined command voltages to the headstage. Connection is usually via BNC.

**command sensitivity** – Selectable scaling of CMD IN input. Attenuation values of CMD IN are  $\times 0.1$ ,  $\times 0.01$ , and  $\times 0.001$ .

**command voltage** – The voltage applied to the headstage resulting in a desired transmembrane potential in the system under study.

**control blocks** – Organization of controls on the amplifier into functional groups. Blocks are delineated by titled blue boundaries.



**current-voltage relationship** – A measure of the way in which the current varies as a function of the applied voltage. In an Ohmic device (obeys Ohm's law or  $V=IR$ ), this relationship is linear. An understanding of the current-voltage relationship of a channel yields information about that channel's function.

**depolarization** – A biological membrane in which charge separation has resulted in transmembrane voltage is termed 'polarized'. Electrically, depolarization refers to any action which tends to reduce the degree of polarization. Biophysically, a polarized membrane has a resting transmembrane potential between  $-40$  and  $-90$  mV, relative to the inside of the cell. An action which tends to increase the polarization (e.g., increase the transmembrane potential to, say,  $-100$  mV) is termed hyperpolarization, while depolarization refers to any action which decreases the transmembrane potential. (It should be noted that by this definition, a transmembrane potential of  $+100$  mV is still depolarized.)

**digital** – Quantized or discrete. Normally refers to information manipulated by a computer. All processes within a computer are discrete and are composed of 0's and 1's. The universe we interact with is functionally analog, therefore information we wish to manipulate with a computer must be digitized prior to use by the computer.

**DIN connector** – Deutsche Industrie Norm. A German standard for electronic and industrial products. DIN connectors can be 3 to 6 pin plugs with the same outer diameter and appearance.

**electrode** – One terminal of a voltage source which can either supply or collect current.

**electromagnetic** – From physics. An electric current induces a magnetic field and a changing magnetic field induces an electric current. Therefore, these two entities are related to each other and are combined into electromagnetism.

**electrophysiologist** – A scientist who combines the disciplines of physics, electrical engineering, and physiology to the study biological systems.

**Faraday cage** – A grounded conducting enclosure which shields its interior from external electric fields. Named after Michael Faraday, who first described it in 1875.

**gain** – The numerical value of the amplification of a signal by an amplifier. User selectable in the OUTPUTS block of the amplifier.

**gain telegraph** – A defined voltage dependent on the gain setting appearing at the associated BNC at the rear of the amplifier. Used to communicate the gain setting to external devices.

**ground loop** – A loop formed from multiple connections into the circuit ground plane by the same device. The flux of magnetic fields through this loop can induce small currents within the ground plane resulting in increased noise in the circuit. Careful consideration of the interconnection between several devices is often required to identify ground loops.

**headstage** – A low gain amplifier placed as close to the preparation as possible. Used to amplify small currents to a range sufficient for the main amplifier to accept.

**$I_m$**  – A measure of the current passed through an open channel in the presence of a driving force. Operationally, the current appearing at the  $I_m$  OUTPUT of the amplifier.

**intracellular** – Situated or occurring within a cell.

**junction potential** – A difference in conductivity between two dissimilar materials will appear as a small voltage when the two materials are brought into contact. This voltage is termed the junction potential.

**LED** – Light Emitting Diode. The red, green or yellow lighted indicators on the front of many devices. LED's are preferred indicator light sources due to their low power consumption.

**mean closed time** – The average length of time a gating channel will remain in the closed state.

**mean open time** – The average length of time a gating channel will remain in the open state.

**mini-jack** – A small plug on the headstage to which the electrodes are attached.

**model cell** – An electric circuit designed to model the electrical characteristics of a biological membrane.

**open probability** – The calculated probability of finding a channel open at time  $t$ , given that the channel is in a closed state at time  $t=0$ .

**oscilloscope** – A device used to monitor voltages within an electrical circuit.

**output current** – See  $I_m$

**OUTPUT SYNC** – A pulsed signal appearing at the OUTPUT SYNC BNC on the instrument rear panel. Used to synchronize the PULSE GENERATOR or CAP TEST signal to an external device such as an oscilloscope.

**periodic** – That which repeats itself at regular intervals.

**perfusate** – The solution being perfused.

**perfusion** – The exchange of one solution with another.

**plasma membrane** – The surface membrane of a cell. Contrast with an intracellular membrane which is a membrane contained entirely within the cell.

**potentiometer** – A single- or multi-turn dial used to make a continuously varying selection with a range. In its heart this is a variable resistor.

**pulse code modulator** – A device which converts an analog signal into a form acceptable for storage on VCR tape. Also converts data previously stored on VCR tape back into an analog signal.

**signal polarity** – Defined as the sign applied to a current generated through a membrane in the presence of an applied holding potential. The electrophysiological definition is determined by the membrane such that an outward directed current and a depolarizing potential are both positive.

**single channel** – Refers to a solitary channel protein functioning within a measurement milieu.

**step potential** – A functionally instantaneous change in potential from one value to another.

**time constant** – In a system governed by exponential kinetics this is the time required for a value to change to  $1/e$  of its initial value, where  $e=2.71828$  is the base of the natural logarithm.

**transient** – Momentary.

**transmembrane** – That which spans a membrane or is referred from one side of a membrane to the other.

**trim pot** – An adjustable variable resistor used for making fine adjustments to a circuit.

**TTL** – Transistor, Transistor Logic. Voltage ranges used to define an on or off state in binary devices. 0-0.8 V defines a logic 0 state and 2.4-5.0 V defines a logic 1 state.

**unitary channel conductance** – A measure of the ability of a channel to pass an ion from one side of the membrane to the other. An intrinsic property of a single channel which depends on the ionic species under consideration. Determined by measuring the current through an open channel in the presence of a driving force (transmembrane potential) at different potentials. Measurements made within the Ohmic range of the channel's response will graph as a straight line. The slope of this line when plotted as current (I) vs. potential (V) will yield the conductance (or inverse resistance) of the channel under these conditions.

**V<sub>m</sub> HOLD** – The transmembrane potential generated by the amplifier and applied to the headstage. This driving force appears in addition to any other driving forces which may be present.

**V<sub>c</sub>** – The user selected potential set in the COMMANDS APPLIED TO REFERENCE block of the amplifier.