OPERATING MANUAL

PC-501A

PATCH/WHOLE CELL CLAMP

CONTENTS

Section	Page
1.0 Description	1
2.0 Specifications	2
3.0 Operating Controls	4
3.1 Definitions and Sign Conventions	4
4.0 Installation and Familiarization	10
5.0 Electrode Holders: Care and Use	13
6.0 Patch and Whole Cell Recording	15
6.5 Current Clamp	18
6.6 Whole Cell	19
Table 1 Check lists	20
Table 2 Current Calibration	21-23
Table 3 Resistances Rp, Rs	21-23
7.0 Bilayer Recording	24
8.0 Replacement Probe Calibration	25
9.0 Replacement Parts	26
10.0 Service and Warranty	27
Appendix	
Fig. 3 PC-501A Panel and Headstage	28
Fig. 4 Patch Lab Basic Setup	29

WARNER INSTRUMENT CORP.
1125 Dixwell Avenue • Hamden, CT 06514
Tel: 203-776-0664 800-599-4203 Fax: 203-776-1278

1.0 DESCRIPTION

1.1 Model PC-501A is a versatile patch clamp amplifier designed for single channel, whole-cell, and bilayer studies, with a selection of four headstage probes to accomodate the special requirements of each case.
Principal features include:
•Voltage clamp and Current clamp modes, with independently selectable Vhold and Ihold.
•Noise levels down to 0.06 pA rms.
•Bandwidth to 10kHz; Internal 4-pole Bessel filter.
•Built-in Test Generator.
•Automatic Junction Potential compensation.
•Three-speed Current clamp speed control.
•Three-range Capacitance compensation.
•Leakage Resistance and Series Resistance compensation.
•Adjustable duration Zap circuit for whole cell membrane penetration.
•Variable output gain with rear-panel gain telegraph.
•Front and rear panel controls and connectors organized and color coded for easy understanding and convenient operation. **Section 3.0**

1.2 The HEADSTAGE, or PROBE, is a high-impedance solid state current-to-voltage converter. As such, **STRICT HANDLING PRECAUTIONS are necessary to avoid damage by static discharge. Section 4.3.**
The headstage housing is a metal enclosure that serves as a driven shield, with an insulated metal rod for mounting to a micromanipulator. A 6' (1.8m) cable with 8-pin plug connects the headstage to the PROBE jack on the front panel. The headstage input terminal is a 1mm jack connector that plugs into a 1mm pin on the electrode holder. Pin jacks on the side of the headstage provide for grounded and/or driven shield applications. **Section 4.3.d.**

Headstage Types:	Application	Features
5101-10G	Patch recording	10 GΩ headstage. Currents up to ± 1 nA.
5101-01G	Whole cell studies	1 GΩ headstage. Currents up to ± 10 nA.
5101-100M	Whole cell studies Large cells	100 MΩ headstage. Currents up to ± 100 nA.
5101-10GB	Bilayer studies	10 GΩ headstage. Currents to ± 1 nA. Handles bilayer capacitance up to 250 pF

1.3 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 eletrolyte solution in the micropipet. **Section 5.0, Fig.1.**
Holders are machined from polycarbonate to minimize electrical noise, and are custom bored to accommodate a specified size of pipet electrode glass. The micropipet is secured with a rubber gasket and polycarbonate screw cap matching the O.D. of the pipet glass. A 1mm pin, pressed against the silver wire, plugs the holder onto the headstage. A 1/16' O.D. port is provided for applying suction to seal the pipet tip to the cell membrane.

The standard holder used with each headstage is type QSW-AxxP (straight body) where "xx" specifies the glass O.D. in mm. **Section 5.1.** (Note: holder is ordered separately from instrument.)

2.0 SPECIFICATIONS

NOISE, referred to headstage input:
For 10GΩ headstage with input open, measured with an eight-pole Bessel filter:

Bandwidth	RMS NOISE
1.0 kHz	0.06 pA rms
5.0 kHz	0.2 PA rms
10 kHz	0.4 pA rms

HI FREQ BOOST:
Enables calibrated bandwidths to 10 kHz.
Front panel adjustments to calibrate 1G and 10G headstages using SPEED TEST.
See **Section 7.0**

COMMAND INPUT: CMD IN
± 10 Volt maximum external command voltage at the CMD IN , Input Z=20 KΩ in parallel with 20 pF.

External command voltages are attenuated to X0.1, X0.01, or X0.001 by the COMMAND SENSITIVITY switch.
The **actual command voltage** at the headstage is the attenuated voltage, so that actual command voltage ranges are:
±1.0V, ±100mV, and ±10mV.

ZAP:
Internal +1.5 Volt pulse at the headstage, duration adjustable from 0.1 to 10 msec. Unaffected by COMMAND SENSITIVITY attenuator.

TEST PULSE:
Internal 100 Hz, 1.0V peak-to-peak square wave, attenuated to 100mV, 10mV, or 1.0mV by COMMAND SENSITIVITY switch.
<u>TEST PULSE ON disengages the CMD IN terminal.</u>

BANDWIDTHS: Im OUTPUT
Low Pass 4-pole Bessel filter,
HI FREQ BOOST ON:
Seven -3dB cutoff frequencies:
0.1, 0.2, 0.5, 1, 2, 5 kHz with FILTER switch ACTIVE,
10 kHz with FILTER BYPASS.

VOLTAGE CLAMP MODE:
HOLDING VOLTAGE:
Adjustable 0 to ±240 mV with 10 turn control.
(Max METER reading is ±199.9 mV)
Unaffected by COMMAND SENSITIVITY attenuator.

JUNCTION ZERO:
Adjustable to ±100 mV with 10 turn control.
Unaffected by COMMAND SENSITIVITY attenuator.

CURRENT CLAMP MODE:
External command voltage at CMD IN terminal, attenuated by COMMAND SENSITIVITY, determines CLAMP CURRENT as follows:

<u>COMMAND SENSITIVITY</u>	<u>CURRENT/ Volt at CMD IN</u>
X.001	10 pA/V
X.01	100 pA/V
X.1	1nA/V

Currents are independent of headstage in use.

HOLDING CURRENT:
Adjustable 0 to ±2nA with 10 turn control.
(Max METER reading is ±199.9 mV)
Unaffected by COMMAND SENSITIVITY attenuator.

CAPACITANCE COMPENSATION, CAP COMP:
Three ranges, AMPLITUDE and TIME CONSTANT adjustments:

FAST	10 turn	0-5 μS,
MEDIUM	1 tunn	0-2 ms,
SLOW	1 turn	0-20 ms,

SERIES RESISTANCE COMPENSATION, SERIES R COMP:
0 to 100 MΩ with 10 turn digital dial.

LEAK SUBTRACTION, HEAD STAGE:
Adjustable 0 to full headstage resistance, all four headstage types.

OUTPUTS.
Im: Membrance current.

HEADSTAGE	OUTPUT GAIN
10 GΩ	10 to 1000mV/pA
1 GΩ	1 to 100mV/pA
0.1 GΩ	0.1 to 10mV/pA

Approx. output resistance 47

ΣVc X10:
10 times the sum of all voltage clamp commands, in Volts.
Approx. output resistance 47 Ω

Vm x10: (reads only in current clamp mode)
10 times the membrane potential, in Volts.
Approx. output resistance 47 Ω

Im Output Low Pass Filter:
4 pole Bessel, -3dB frequencies at 0.1, 0.2, 0.5, 1, 2 and 5kHz.
BYPASS enables full 10kHz bandwidth.

GAIN TELEGRAPH OUTPUT, rear panel:
DC voltages at 0.2 V intervals to indicate 7 Im OUTPUT GAIN settings 1 to 100 mV/pA and the headstage probe in use:

PROBE	DC Volts
10 GΩ	3.0 to 4.2
1 GΩ	1.6 to 2.8
0.1 GΩ	0.2 to 1.4

Output resistance: 1kΩ / V out.

SYNC OUTPUT, rear panel:
±10 V, 1ms pulses, alternating, 100 Hz with TEST PULSE or SPEED TEST ON.
Output resistance: 150Ω

PANEL METER: 3 1/2 digit LCD.
Provides DC, or time-averaged readout.

Voltage settings	Range
Vc+h In, Vc, Rm:	±199.9 mV.

A reading of 1.0 = 1.0 mV, micropipet-bath.

Current settings	Range
Im:	± 1999 pA.

A reading of 001 = 1pA, micropipet into bath.

HEADSTAGES:

5101-10G	10 GΩ, current to 1 1nA
5101-01G	1.0 GΩ, current to 1 10nA
5101-100M	100 MΩ, current to 1 100nA
5101-10GB	10 GΩ, current to 1nA, bilayer capacitance to 250pF.

ELECTRODE HOLDERS: Polycarbonate.
QSW-AxxP (straight) bored for specified diameter of pipet glass, where "xx" = 10x diameter in mm.

QSW-A12P specifies 1.2mm O.D. glass
Suction port accepts 1/16" I.D. plastic tubing.

POWER REQUIREMENTS:
100 130 VAC or 220 - 240 VAC, 50/60 Hz

Physical Dimensions:

	H	W	D
Control Unit	5.1/4"	16 3/4"	10"
	13.3cm	42.5cm,	25cm
Headstage	2 1/4"	1 1/8"	1"
	5.7cm	2.9cm	2.5cm

Detachable mounting rod, 1/4" (6.2mm) dia. x 2.5" (10cm) long. Cable length 6' (1.8m).

Weight: Nominal 8 lbs. (3.6 kg)
Shipping 25 lbs. (11.4 kg)

3.0 OPERATING CONTROLS: Locations and functions.

Refer to Section 2.0 SPECIFICATIONS for further details.

The front panel of the control unit is divided into 12 sections, each outlined in blue and clearly labelled to indicate its functions and use.

Controls and settings for CURRENT CLAMP operation, and POWER on-off, are color-coded red.
Those for VOLTAGE CLAMP and general operations are color-coded black.

3.1 Definitions of front panel nomenclature and conventions used on the PC-501A.

Physical quantities:

Im Membrane current.

Sign convention: Im 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 Im.

Vm Membrane potential.

Sign convention: 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 Vm.

Front Panel labels and abbreviations:

CMD IN	External command voltage input terminal. In COMMANDS section.
CMR	Common mode rejection. In PROBE SELECT section.
h	HOLDING voltage or current. In COMMANDS section.
Vc	External command voltage applied to the CMD IN terminal.
Vc + h IN	METER switch setting. External command voltage plus HOLDING voltage.
ΣVc	METER switch setting. The sum of all command and compensation voltages: Vc + h + JUNCTION + AUTO ZERO + SERIES R COMP.
ΣVc X10	10 times ΣVc. OUTPUT terminal.

The sign convention for all command voltages is as for Vm.

3.2 COMMANDS section, top to bottom:

HOLDING VOLTAGE and CURRENT controls with -OFF+ switches. These controls provide independent holding potential and holding current settings, within the ranges ±200mV and ±2.0nA respectively, and are not attenuated by the COMMAND SENSITIVITY switch. This allows switching between VOLTAGE CLAMP and CURRENT CLAMP MODE without having to readjust the HOLDING settings.
However, when switching clamp modes, switch the unselected mode's HOLDING switch OFF. If both are on, the two command signals become additive.

To set the HOLDING VOLTAGE or HOLDING CURRENT using the METER, switch the METER to Vc+h, switch COMMAND SENSITIVITY OFF, and switch on the desired HOLDING command. The METER reads holding VOLTAGE in mV, or holding CURRENT in 10's of pA, (i.e., 10pA/mV), so a current reading of 01.5 = 15 pA.

See Section 3.4 CLAMP MODE, ZERO CURRENT for further details.

3.2 COMMANDS section, continued:

COMMAND SENSITIVITY OFF X.001 X.01 X.1 switch attenuates externally generated command voltages conected to the CMD IN BNC input terminal, and also the internally generated TEST PULSE signal, by the factors indicated. When switched OFF, it disconnects both the external signal and the TEST PULSE.

TEST PULSE OFF ON switch. The TEST PULSE is an internally generated 100 Hz, 1.0V peak-to-peak square wave provided for (**1**) adjusting the capacitance compensation (CAP COMP) controls; (**2**) measuring the pipet resistance in the bath; and (**3**) monitoring the formation of the gigohm seal at the electrode tip. When switched **ON**, it disengages the **CMD IN** terminal, so the external input can remain connected while watching the effects of the test pulse.

CMD IN BNC terminal is a differential input terminal for command voltages from an external source, e.g. signal generator or computer. The center pin and sleeve are the (+) and (-) inputs respectively. The sleeve is not grounded internally. The applied voltage is attenuated by the COMMAND SENSITIVITY switch, in both VOLTAGE CLAMP and CURRENT CLAMP modes.
In CURRENT CLAMP mode, the red 1V/nA label below the BNC terminal means 1nA/V of input voltage after attenuation by the COMMAND SENSITIVITY switch. CMD IN is completely disengaged when TEST PULSE is ON, and is disengaged from the headstage circuits when COMMAND SENSITIVITY is OFF, and when CLAMP MODE is set on ZERO CURRENT.

ZAP generates an internal +1.5 V pulse to the headstage.
The pulse duration may be adjusted from 0.1 to 10 milliseconds as indicated.
The pulse begins when the yellow ZAP button is released.

3.3 ZERO section

These controls compensate electrode potentials, liquid junction potentials and any other offset voltages present, in order to establish a zero baseline reference potential, by setting Im to zero.
This is ordinarily done with all COMMAND voltages off, immediately after lowering the micropipet tip into the bath for the first time.
Set the METER to Im, and adjust to 000 current as follows:

The 10-turn JUNCTION ±100 mV knob zeros Im manually, with immediate response.

With the AUTO switch ON, the pilot light is on, and the pushbutton zeros the current automatically. The settling time is fast with MΩ pipet resistances, but takes several seconds if used with GΩ resistances. For complete compensation, hold the button in until Im on the METER reads 000.

<u>With **AUTO ON**, the manual JUNCTION control remains active.</u>
The AUTO button will then zero the manual setting, any HOLDING potential, the time-averaged TEST PULSE, COMMAND voltage, or any noisy signal.

Switching AUTO OFF restores the uncompensated current and voltages.
To rezero, repeat either zeroing procedure.

3.4 CLAMP MODE section

In **VOLTAGE CLAMP mode** (upper knob), all COMMANDS are active, including the HOLDING CURRENT.
Command voltage settings and operation are described in section 3.2.
The range of voltage clamp potentials at the preparation is ±1 V.
The HOLDING VOLTAGE Vh setting for VOLTAGE CLAMP is not affected by switching between voltage and current clamp modes, and is independent of the holding current. In VOLTAGE CLAMP mode, the HOLDING CURRENT switch should ordinarily be switched **OFF**.

ZERO CURRENT (upper knob) is a transition mode between VOLTAGE CLAMP and CURRENT CLAMP.
It disengages all COMMANDS and functions from the headstage and the preparation, except CAP COMP, HI FREQ BOOST, and ZAP. Its main uses are:
1) To protect the preparation when switching between VOLTAGE and CURRENT CLAMP MODES;
2) To **preset the HOLDING VOLTAGE** before switching to VOLTAGE CLAMP, or to **preset the HOLDING CURRENT** before switching to CURRENT CLAMP.
Use the COMMAND controls, and monitor **Vc+h IN** on the METER as described in section 3.2.

In **CURRENT CLAMP mode** (upper knob), all COMMANDS including TEST PULSE are again active.
The clamp current range at the preparation is limited by the ±10V maximum input and the headstage resistor, as follows:
±1nA with 10GΩ headstage, ±10nA with 1.0GΩ headstage, ±100nA with 100MΩ headstage.
In CURRENT CLAMP mode, the HOLDING VOLTAGE switch should ordinarily be switched **OFF**.

Three **CURRENT CLAMP RESPONSE** speeds, **FAST, NORMAL and SLOW** (lower knob) are used to adjust the response time of the curent clamp to the characteritics of the preparation, with a trade-off between speed and feedback stability. These settings interact with **CAP COMP** and the **Low pass bessel filter.**

3.5 CAP COMP (capacitance compensation) section

The three pairs of controls, FAST, MEDIUM and SLOW, are used to adjust the AMPLITUDE (gain) and TIME CONSTANT (tau) of three auxiliary amplifiers that compensate for capacitative currents due to pipette, membrane, and stray capacitances, by applying an appropriate capacitative countercurrent to the headstage feedback resistor.

When properly adjusted the six controls have two important functions:

(1) to make the voltage clamp or current clamp waveform at the cell membrane approximate the command signal waveform as closely as possible; and

(2) to avoid large current transients that can occur with fast step commands (e.g. the TEST PULSE), which if uncompensated can drive the headstage amplifier into saturation. When that happens, several ms of data may be lost while the headstage recovers from saturation.

3.6 HEADSTAGE section

The **LEAK SUBTRACTION** knob compensates current leaking to the bath through the seal resistance Rs between the pipet electrode tip and the membrane patch. This is important for "leaky" seals (Rs around 1GΩ), to provide a stable current baseline independent of the waveform of the command voltage. It can usually be ignored with "tight" seals (Rs 10-100 GΩ). Fully clounterclockwise (0) switches it off.

3.7 PROBE SELECT section

The headstage cable is plugged into the PROBE connector.
The PROBE SELECT switch must be set to the resistance of the headstage in use.
The CLIPPING light will come on to indicate an overload condition when the headstage input voltage exceeds ±10 volts.
The CMR common mode rejection screwdriver control is factory-set and should need adjustment only when using a headstage not originally supplied with the main unit.

3.8 SERIES R COMP section

Series resistance compensation is used in whole cell recording, to compensate the error voltage produced by the current through the resistances in series of the pipet electrode, cytoplasm, and bath.
It is switched **OFF** for **single channel** recording, since the series resistances are negligible compared with cell membrane and single chanel resistances.

3.9 HIGH FREQ. BOOST section

HIGH FREQUENCY BOOST precisely compensates the end-to-end capacitance of the headstage feedback resistor, to enable the PC-501A's calibrated bandwidths. The boost switch is left **ON** except when viewing signals with a bandwidth less than 150 Hz.

SPEED TEST is used to readjust the boost trimmers, normally only required when replacing a headstage. These adjustments are factory set for the headstages originally supplied. Frequency boost adjustment is not required for the 100 megohm headstage.
GAIN TRIM adjustments are also factory-set for any headstages supplied with the main unit and will need readjustments only for replacement headstages.

3.10 METER section

The 4 METER switch settings interact with other controls, as noted

Vc+h IN = Vc + h, where **Vc** = the CMD IN voltage after attenation by COMMAND SENSITIVITY, and **h** = the internal HOLDING voltage.
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.
Any command signal **Vc (t)** with high frequency components, e.g. a pulse or periodic waveform, will be read as its DC time average, and added to h. Slowly varying signals will produce varying meter readings which are probably not very useful.
In VOLTAGE CLAMP MODE the meter reading Vc+h is in mV. Full scale range is ±199.9 mV.
In CURRENT CLAMP MODE the reading in mV is proportional to the command current, 10 pA/mV, so that COMMAND CURRENT in pA=10 X meter reading. Full scale range is ±1999 nA.

ΣVc = the sum of all command voltages: (Vc+h) + (JUNCTION and AUTO ZERO) + (SERIES R).
It does not include LEAK SUBTRACTION. Full scale range is ±199.9 mV.

Vm = the transmembrane potential when in CURRENT CLAMP MODE only. Full scale range is 1199.9mV.

Im = the transmembrane current when in VOLTAGE CLAMP MODE. Full scale range is ±1999 pA.

3.11 OUTPUT section

This section contains:

The 7-step Im GAIN mV-pA switch, and 3 gain multiplier indicator lights (LED's).
The gain multiplier corresponds to the type of headstage in use, and is set by the PROBE SELECT switch.

The **Low pass 4 pole bessel filter** switches, -3dB Frequency Hz, and FILTER/BYPASS.
With FILTER ACTIVE, the -3dB switch provides 6 low pass cutoff frequencies 0.1, 0.2, .05, 1, 2 and 5 kHz.
FILTER BYPASS disengages the -3dB switch and provides the full 10 kHz bandwidth.

The 3 BNC output terminals for: VOLTAGE CLAMP CURRENT **Im**, the summed command voltages **VcX10**, and the CURRENT CLAMP transmembrane potential **VmX10** (in red).
The BNC Terminal sleeves are connected to CIRCUIT ground and are insulated from the chassis.

Im OUTPUT, GAIN and FILTER are active in both VOLTAGE CLAMP and CURRENT CLAMP modes.
The GAIN and FILTER switches affect only the Im OUTPUT signal.
The ΣVcX10 and VmX10 signals, with fixed gain of 10, are not affected.
The VmX10 output is active only in CURRENT CLAMP mode.

The **Im GAIN mV/pA** selector switch setting, multiplied by the **gain multiplier** indicated by the lit LED, has 2 important uses:

1) It sets the magnitude of the voltage signal appearing at the Im OUTPUT Terminal. This is useful for recording a wide range of currents. Use the GAIN mV/pA switch for this, **NOT** the gain multiplier.

2) Its reciprocal **converts the output signal in volts, to Im in amperes.**

The available gain ranges and currents per volt out for 3 headstage types are:

Headstage	**Gain mV/pA X multiplier**	**Im, A/V**
10 GΩ	10mV/pA to 1000mV/pA	100pA/Vto 1pA/V
1 GΩ	1mV/pA to 100mV/pA	1 nA/V to 10 pA/V
100 MΩ	0.1mV/pA to 10mV/pA	10nA/Vto 100pA/V

Tables 2A, 2B and 2C (Pgs 19-21) are convenient shortcuts for Im in amperes.

3.12 The REAR PANEL Contains:

CIRCUIT and CHASSIS GROUNDS, the PATCH/BILAYER switch, BNC terminals for GAIN TELEGRAPH output, SYNC output, and SPEED TEST IN.
The BNC sleeves are connected to CIRCUIT ground, and are insulated from the chassis.

CIRCUIT and CHASSIS GROUNDS connectors are binding posts with a shorting link. For most recording situations, the shorting link should be disconnected so that the two grounds are isolated. However, there are occasions where 60 Hz noise is reduced when the two grounds are joined. Therefore, it is best to try both connected and unconnected to determine which is best for each particular experiment.

The PATCH/BILAYER switch is placed in the PATCH position for all single channel and whole cell applications.
It is switched to BILAYER only when a modified headstage (5101-10GB) is used.

GAIN TELEGRAPH OUTPUT provides a dc signal for computer analysis programs.
The output signal indicates the gain switch selection and has a different range for each of the headstages.

OUTPUT GAIN	**PROBE SELECT**		
mV/pA	**10G**	**1G**	**0.1G**
1	3.0V	1.6V	0.2V
2	3.2V	1.8V	0.4V
5	3.4V	2.0V	0.6V
10	3.6V	2.2V	0.8V
20	3.8V	2.4V	1.0V
50	4.0V	2.6V	1.2V
100	4.2V	2.8V	1.4V

SYNC OUT provides a pulse output for synchronizing an oscilloscope sweep trigger.
The sync pulse is derived from the internal generator which provides the TEST PULSE and SPEED TEST signals.

SPEED TEST input is provided for applying an externally generated speed test triangle wave, in situations where computer control of this signal is required.

4.0 INSTALLATION & FIRST-TIME FAMILIARIZATION

Refer to Figures 3 and 4 at the end of the manual for set-up information.

4.1 POWER LINE VOLTAGE REQUIREMENTS for the PC-501A are specified on the serial number nameplate attached to the chassis rear. They are wired for either 100-130 VAC or 220-240 VAC,50/60 Hz. Check to be sure the PC-501A is wired for the line voltage to be used.

4.2 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-501A chassis to the power recepticle ground, and insures safe operation of this equipment.
However, if interference due to ground loops among items of apparatus is a problem, use a two-prong adapter to isolate the PC-501A chassis from the power line ground. **In this case, a separate ground wire MUST be used to securely connect the rear panel CHASSIS GROUND terminal of the PC-501A to a proper earth ground.**

4.3 HEADSTAGE PRECAUTIONS:

THE PC-501A HEADSTAGE IS A HIGH-IMPEDANCE STATIC-SENSITIVE DEVICE, AS NOTED ON THE PROTECTIVE ENVELOPE IN WHICH IT IS SHIPPED. IT MAY BE SERIOUSLY DAMAGED BY STATIC DISCHARGE OR INADVERTENT GROUNDING.
THESE PRECAUTIONS MUST BE FOLLOWED TO INSURE PROPER OPERATION:

a. ALWAYS DISCHARGE THE STATIC ELECTRICITY FROM YOUR BODY BEFORE HANDLING THE HEADSTAGE.
Your body, with a capacitance of around 100-200 pF to ground, can pick up enough static charge by touching the face of a video monitor, handling styrofoam, walking across a dry carpet, wearing polyester clothing, etc, to raise your electric potential by as much as 10 kV above or below ground. To discharge:

EITHER: lightly moisten a finger and firmly grab any SECURELY-GROUNDED part of the setup, OR: wear a GROUNDED WRIST STRAP, available from any electronics store.

b. NEVER GROUND THE HEADSTAGE INPUT CONNECTOR PIN.

c. NEVER APPLY ANY LOW-IMPEDANCE SIGNAL SOURCE DIRECTLY TO THE INPUT CONNECTOR PIN.

d. The headstage case is driven (isolated from ground) at the command potential when in operation.
NEVER LET THE HEADSTAGE PROBE CASE COME INTO CONTACT WITH ANYTHING GROUNDED WHEN THE POWER IS ON.

Two 1mm **pin jacks** on the side of the headstage provide for grounded and/ or driven shield applications.
The rear **pin jack** is insulated from the case by a black collar, and is **at circuit ground potential.** It can be used to ground the shielded enclosure described in Section 4.5, the reference electrode in the bath, and any other grounded shielding within easy reach.

The **uninsulated pin jack** toward the front is at the **command potential,** and can be used to drive any additional guard shielding such as foil covering or conductive paint on the pipet electrodes. In that case,
INSURE THAT THE DRIVEN GUARD SHIELDING NEVER TOUCHES GROUND.

4.4 INITIAL SETTINGS. These are identical for all Headstage Types except as noted.

Front panel controls	5101-10G	1.0G	0.1G	10GB
POWER:	OFF (O)			
COMMAND HOLDING VOLTAGE:	OFF			
COMMAND HOLDING CURRENT:	OFF			
COMMAND SENSITIVITY:	OFF			
TEST PULSE:	OFF			
AUTO ZERO:	OFF			
CLAMP MODE SWITCH:	VOLTAGE			
CAP COMP CONTROLS:	Fully CCW (counter clockwise)			
LEAK SUBTRACTION:	0 (zero)			
SERIES RESISTANCE:	OFF			
PROBE SELECT	**10G**	**1.0G**	**0.1G**	**10G**
HIGH FREQUENCY BOOST:	ON			
SPEED TEST:	OFF			
METER SWITCH:	Im			
Im OUTPUT GAIN SWITCH:	1mV/pA			
FILTER:	BYPASS			
Im OUTPUT terminal:	Connect to oscilloscope input, and to computer Im input if in use.			
Rear panel				
PATCH/BILAYER switch	**PATCH**	**PATCH**	**PATCH**	**BILAYER**
SYNC OUTPUT terminal:	Connect to oscilloscope external trigger input.			
GAIN TELEGRAPH OUTPUT:	Connect to computer gain monitor if in use.			

4.5 HEADSTAGE PREPARATION.

With proper handling precautions (Sect. 4.3), plug the headstage cable connector into the front panel PROBE receptacle, and connect the Im OUTPUT terminal to an oscilloscope. The headstage probe normally needs a grounded enclosure in order to shield it from 60Hz interference. Satisfactory shields can be made from a loosely fitting cardboard tube a few cm longer than the headstage case, wrapped on the outside with aluminum foil; or with a copper screen cylinder of similar size, with insulation between the screen and the probe case. Insulating tape stuck onto the long edges of the case, or pieces of split plastic tubing held in place with tape or rubber bands work well.

DO NOT USE STYROFOAM.

Ground the shield to the insulated circuit ground jack on the side of the case, using a 1mm pin connector provided with the headstage, and slide the probe into the shield so that the input pin is approximately centered on the axis of the cylinder.

4.6 POWERING UP AND INITIAL CHECKS.

Turn the POWER switch from OFF (**0**) to ON (**I**). Im should read zero volts both on the PC-501A METER, and at the Im OUTPUT with the oscilloscope.

Prepare a 10 MΩ resistor grounded at one end by a few inches of flexible hookup wire, with any suitable 2mm pin connector at the other end. This resistor simulates a pipet electrode in a grounded bath.

Next, with proper handling precautions, carefully slide the headstage far enough from the shielded enclosure to expose the input pin, hold the 10MΩ resistor by the grounded end and connect it to the input pin.

Slide probe and resistor back into the enclosure, and keep the 10 M resistor in place for the next tests.

4.7 JUNCTION ZERO.

Recheck the Im OUTPUT and readjust to zero with either the manual JUNCTION ZERO or the AUTO ZERO. For AUTO ZERO operation, turn the AUTO zero switch on, press the AUTO zero push button and hold until Im reads zero. With a 10 MΩ resistor, zero is attained almost instantly. With several G it can take a few seconds.

To read the offset potential that it took to bring Im to zero, you can switch the METER to ΣVc, or you can monitor the ΣVcX10 OUTPUT with the oscilloscope and divide by 10.

4.8 TEST PULSE AND RESISTANCE CHECK.

With the 10 MΩ resistor in place, switch the TEST PULSE switch ON, and switch the COMMAND SENSITIVITY switch to X.001.
To synchronize the oscilloscope trace with the test pulse, connect the SYNC OUT signal from the rear of the PC-501A to the external trigger input on the oscilloscope, and set the scope for external trigger.
Switch the PC-501A FILTER BYPASS/ACTIVE switch to ACTIVE and set the filter to 5 kHz, or lower if the noise on the Im OUTPUT signal makes it difficult to read the current.

The TEST PULSE ON switch disconnects any external voltage connected to the CMD IN BNC input connector, and applies a 100Hz 1V peak-to-peak square wave to the COMMAND SENSITIVITY attenuator. The attenuated square wave is applied as the command voltage to the headstage input pin, with peak-to-peak amplitude equal to 1V X COMMAND SENSITIVITY, as you can verify by monitoring the ΣVc X10 OUTPUT with the oscilloscope. In this case the command voltage is 1 mV peak-to-peak.

To measure the "unknown" resistance of the 10 MΩ resistor, monitor the resulting peak-to-peak current through it at the Im OUTPUT terminal, and calculate the resistance by Ohm's Law. Tables 2 and 3 are provided to facilitate the calculation during the preoccupations and distractions of an experiment.

4.9 CAPACITANCE COMPENSATION.

Keep the TEST PULSE ON, and with proper handling precautions, remove the 10 MΩ resistor from the input pin, and reposition the probe within the shield. With the resistor removed, the input resistance is virtually infinite, simulating a perfect pipet seal to a membrane patch.
On the oscilloscope the Im OUTPUT signal should now read zero except for capacitive current transients occuring at 5 ms intervals, on each step of the test pulse square wave. The meter will also read Im zero.
If the transient spikes aren't clearly visible, you can: (1) increase the COMMAND SENSITIVITY to X0.1 (100mV pk-pk); (2) increase the Im OUTPUT GAIN; (3) increase the filter-3dB Frequency; (4) increase the oscilloscope beam intensity; (5) increase the oscilloscope input sensitivity setting.

To compensate the capacitance, adjust the CAP COMP controls to minimize the transients.
The FAST and MEDIUM AMPLITUDE controls will probably have the greatest effect, followed by the respective TIME CONSTANTS. With larger amplitude test pulses and higher filter frequencies, minimizing transients becomes more precise, and also more difficult.

Resistance check and capacitance compensation may also be done with an external signal generator or computer-generated waveform connected to the CMD IN terminal, in place of the internal test pulse.

4.10 MODEL CELL units available from Warner Instruments Corporation provide convenient and more accurate cell and membrane simulations for system tests. These are designed for each headstage type:

Patch Cell	Model Cell MC-10G	for 5101-10G headstage
Small Whole Cell	Model Cell MC-10G	for 5101-01G
Large Whole Cell	Model Cell MC-100M	for 5101-100M
Bilayer	Model Cell MC-10GB	for 5101-10GB

To attach the model cell to the headstage:
With proper handling precautions, connect the wire from the model cell to the insulated ground jack on the headstage, to eliminate the need for shielding the headstage.
With proper handling precautions again, now plug the input of the Model Cell directly to the headstage input jack.
When finished testing, remove the Model Cell in reverse order.

5.0 ELECTRODE HOLDER USE AND CARE

5.1 The standard holder supplied with PC-501A is the QSW-AxxP (straight) as shown in Fig. 1. It uses a 0.010" diameter, 99.99% pure silver wire-the actual electrode- to couple the signal from the micropipet 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 that secures the micropipet.
A 2mm O.D. port on the side of type QSW-AxxP 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 O.D. in mm. For example, QSW-A15P specifies 1.5mm O.D. pipets.

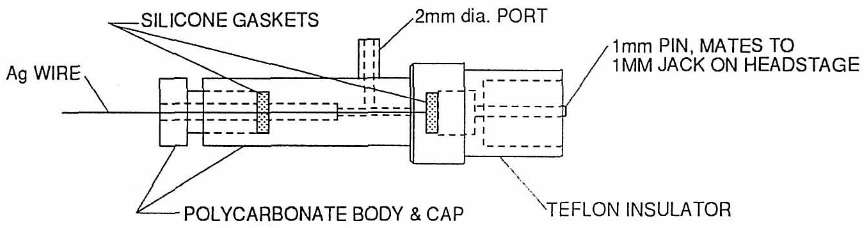


Fig.1 QSW-AxxP electrode holder

5.2 CHLORIDING THE SILVER WIRE

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.

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 6V 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 1mA/cm2 for about 1 minute or until adequately plated. For a 2cm length of 0.01" wire, this is about 150μA. 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 convenient.
The electrolyte solution can be saved and reused indefinitely.

Chloriding chemically: Immerse the Ag wire in Chlorox® or a sodium hypochlorite solution until the wire is uniformly light grey, about 15s to 1min. Rinse well with tap water, then with distilled.

5.3 CARE & 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.

Fill pipets with only enough electrolyte to cover several mm of the AgCl coating when inserted into the holder. This minimizes both 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; and especially on the pin jack, which could damage the headstage. Should this happen, disassemble the holder as appropriate, rinse thoroughly with distilled water, dry thoroughly, and reassemble.

5.0 ELECTRODE HOLDER USE AND CARE continued.

5.4 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" I.D. plastic tubing, or a 50mm x10 mm I.D. plastic vial with a small nylon set screw.

5.5 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. Replacing is necessary when the wire becomes hopelessly kinked or badly jammed into the holder, as by carelessly inserting into pipets.

To replace the wire, unscrew the pin connector, and remove the rubber seal with blunt forceps. 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 5mm extends on the pin connector side, and bend this end 90° at the seal. 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, being careful that the seal sits squarely in the hole, and the wire doesn't jam in the holder on its way through.

5.6 REFERENCE ELECTRODES

A reference electrode in the recording chamber maintains the bath at circuit ground potential, the 0mV reference potential for all measurements. It is also the return path for currents from the pipet electrode. A variety of Ag-AgCl reference electrode types are available from Warner Instrument Corp.

A simple reference electrode can be made from a silver wire somewhat thicker than the one in the pipet electrode holder. The end going into the bath should be chlorided as in Sect. 5.2., so that the Ag-AgCl coat can be completely submerged, and no bare Ag is wet. The free end is connected to circuit ground. Any bare Ag surface that could contact the bath solution should be insulated with a waterproof coating of epoxy cement, insulating varnish, or similar durable waterproof coating. This precaution provides a stable baseline so long as the bathing solution is not changed.

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

6.0 SETUP and OPERATIONS for Exised Patch, Cell-Attached Patch, and Whole Cell Recording.

Sections 6.1-6.4 describe PC-501A operations in detail for VOLTAGE CLAMPING excised or cell-attached membrane patches using the 5101-10GΩ headstage.
Refer to **Sections 3,4, and 5, and Tables 1, 2, and 3** for further details as necessary.

Section 6.5 describes changes to make for CURRENT CLAMPING with the 10GΩ headstage.

Section 6.6 describes changes for WHOLE CELL RECORDING with the 5101-01G or 5101-100M headstage.
Table 1 is a check list of PC-501A and oscilloscope connections and settings for 3 main stages in a VOLTAGE CLAMP experiment.
Tables 2A, B and C give oscilloscope calibrations for Im OUTPUT current with various combinations of gain settings on the PC-501A and oscilloscope, for 10GΩ, 1GΩ, 0.1GΩ headstages, respectively.
Table 3 gives a quick method for calculating pipet and seal resistances with the oscilloscope.

RECOMMENDED READING: Sakmann & Neher Single-Channel Recording, Plenum Press, 1983
Hamill, Marty, Neher, Sakmann, and Sigworth (1981) Pflugers Archiv 391: 85-100.

6.1 VOLTAGE CLAMPING: PRELIMINARY SETUP

This section assumes that the following items are in place:

1) oscilloscope and recording system, e.g. computer, tape recorder, CD recorder etc.
2) air table or equivalent shockmounting e.g. a heavy baseplate standing on tire inner tubes
3) micromanipulator with hydraulic microdrive
4) inverted microscope
5) recording dish or chamber
6) Ag-AgCl reference electrode with or without KCl-agar salt bridge
7) electrode holder with flexible tubing attached to the side port (Section 5.1)
8) prepared patch pipets (Section 5.3)
9) solutions for bath and pipets
10) cells

6.1.1 Initial settings and connections on the PC-501A: With POWER OFF, set the front and rear panel controls and connections as in Section 4.4, except set the Im OUTPUT FILTER ACTIVE, 1kHz (Table 1, initial).
Prepare the headstage as in Section 4.5, mount it on the micromanipulator, set the appropriate PROBE SELECT, and turn POWER ON.

6.1.2 Electrodes:
Position the Ag-AgCl reference electrode or KCl-agar salt bridge into the recording chamber, and connect the Ag wire to the ground pin on the headstage case. If using a bare Ag-AgCl reference electrode, make sure that all of the exposed AgCl surface is completely submerged, and that any bare Ag wire is insulated from the bath solution. Epoxy cement or waterproof varnish is adequate. These precautions help to stabilize the reference potential.

6.1.3 Insert a properly filled micropipet into the electrode holder (Section 5.3), and gently but firmly insert the holder onto the headstage. It should be possible to do this without removing the grounded shield covering the headstage.

6.2 IN THE BATH

6.2.1 Just before lowering the pipet tip into the bath, carefully aspirate the surface of the bathing solution with a small flexible tube connected to a vacuum line, or wipe it quickly with a small clean of lens tissue. Then apply **continuous gentle air pressure** (about 1-2 kPa or 10-20 cm of water) to the side port of th electrode holder to prevent any remaining debris from fouling the pipet tip, and lower the pipet into the bath.
Now change the **PC-501A and oscilloscope settings** as listed in **Table 1, in Bath.**
Keep the pipet tip in the bath and maintain the pressure in the electrode holder, as you proceed with the following steps, in order:

6.2.2 Zeroing the junction potential: ZERO CONTROLS (Section 3.3)
On entering the bath, the junction potential between the pipet electrode and reference electrode will usually cause an appreciable current through the pipet, indicated by the panel METER Im reading in pA, and on the oscilloscope from the Im OUTPUT terminal. To establish the **zero current baseline,** set the current to zero as described in **Section 3.3.** After zeroing the current, the magnitude of the junction potential can be read from the meter (Vc+h) or on the oscilloscope from the Vcx10 output.
IMPORTANT ! DO NOT CHANGE THIS ZERO SETTING AGAIN until you have to take a new pipet. If you change it after forming a gigaseal, you will not know the true transmembrane potential. The zero baseline condition should remain stable as long as the AUTO ON-OFF switch in ON. If not, (a) Make sure the reference electrode is properly submerged; (b) Re-Zero Im; (c) if Im still drifts, which is unlikely, check for some error in the setup.

6.2.3 CAPACITANCE COMPENSATION: (Section 3.5)
Set the COMMAND SENSITIVITY Switch to X.001, and turn the TEST PULSE switch ON, to activate the 100 Hz, 1mV peak-to-peak square wave. Adjust the oscilloscope and Im OUT GAIN pA/V settings to clearly display 1 or 2 complete cycles of the 100 Hz current signal from the Im OUTPUT terminal. Consult Table 2 for useful combinations of settings.
Use the 3 pairs of CAP COMP controls to square up the Im trace. The FAST set (0-5µs) squares up the leading edge of the square wave. The MEDIUM and SLOW pairs compensate the flatness. Because the controls interact, some trial and error is necessary. Compensation is optimal when the square wave has as sharp rise and fall as possible, flat top and bottom, and minimum overshoot.
Risetime also improves with higher frequency FILTER settings, which however, introduce a tradeoff between faster response and greater noise.

6.2.4 PIPET RESISTANCE Rp:
Measuring pipet resistance is the routine method for determining the pipet's condition before attempting to record data. Useful pipets for patch recording have resistances typically in the range 1 to 10 MΩ.
Those with much higher resistances are likely to be blocked with debris from poorly filtered pipet or bath solutions, or to have over-polished, constricted tips. Those with much lower resistance are probably broken. In either case, discard and take a new pipet, and start over at Section 6.1.3.

Measure PIPET RESISTANCE with the pipet in the bath, and the 100 Hz 1mV TEST PULSE ON. Read the peak-to-peak amplitude on the oscilloscope, of the test current I from the Im OUT terminal. For convenience, express the current I in nA or PA with the aid of TABLE 2, and calculate Rp in MΩ with the aid of Table 3.

If you prefer, you can determine the current I from the Im Gain mV/pA and oscilloscope Volts/division settings, and calculate Rp by Ohm's law.

If the 1mV TEST PULSE produces a very small current the pipet is probably blocked, but if you want to estimate its resistance anyway, e.g. to estimate its tip diameter, you can increase COMMAND SENSITIVITY to X.01 for 10mV, or X.1 for 100mV peak-to-peak test pulse amplitudes.

6.3 ON THE PATCH

6.3.1 FORMING THE GIGASEAL. This operation requires a well shock-mounted, vibration-free benchtop. Keep the 100Hz 1mV TEST PULSE ON, and advance the pipet tip to contact your chosen cell. When the pipet begins to dimple the cell membrane, the test current should begin to decrease. When that happens, release the applied positive pressure in the electrode holder, then apply and hold **gentle suction** (2-3 kPa or less), and watch the test current trace closely for 10-30 seconds.

When the seal forms, the test current decreases quickly, then the trace goes flat, indicating no measurable current at this oscilloscope sensitivity. At this point, carefully release the suction, and **change the settings** on the **PC-501A and Oscilloscope settings** as listed in **Table 1, ON PATCH.**

If the seal doesn't form within about 30 seconds, try alternately releasing and reapplying suction for about 10-30 seconds each. If the seal appears to be forming, continue alternating suction, or advance the pipet very slightly against the cell. If the seal hasn't formed within about 5-10 min, it probably will not. Take a new pipet and start again at **Section 6.1.3.** You can't re-use the same pipet because by now the tip is too dirty to form a seal.

6.3.2 MEASURE SEAL RESISTANCE Rs: You can do this either before or after excising the patch. Keep the 100Hz 1mV TEST PULSE ON, measure the peak-to-peak "leak" current **I** on the oscilloscope with the aid of **Table 2,** and calculate Rs by Ohm's law (R=V/I), or with the aid of Table 3 to keep track of the units.
A good **Gigaseal** resistance is typically in the range 1 to 10 GΩ, or considerably greater.

6.3.3 LEAK SUBTRACTION: If Rs is only a few GΩ, it may be important to compensate the leak current, so that the current baseline doesn't reflect the shape of the command voltage waveform. With the TEST PULSE still ON, turn LEAK SUBTRACTION clockwise until the Im trace on the oscilloscope becomes flat except for residual capacitance transients. Or, with the TEST PULSE OFF, run the HOLDING VOLTAGE up and down over a range that doesn't evoke channel currents, and adjust LEAK SUBTRACTION so that the current trace on the oscilloscope remains fixed, and doesn't follow the voltage trace.
If Rs is very high, you can probably get away without leak compensation. If so, turn the knob to 0 (off).

6.3.4 EXCISED PATCH. Watch with the microscope as you excise the patch. Quickly move the pipet back with the hydraulic microdrive, then up and away from the cell with the fine vertical control. 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 now over. If the cell remains attached to the pipet, try jiggling the micromanipulator controls, or tap gently on the micromanipulator, or bring the cell and pipet up briefly into the air, for about 1 second.
If the patch has not yet separated from the cell, you can proceed with cell-attached patch recording instead.

6.3.5 CELL-ATTACHED PATCH. If the cell moves freely with the pipet, raise the pipet and cell from the bottom of the dish, but keep submerged. If the cell is not free to move, take care to avoid vibration while recording.

6.4 VOLTAGE CLAMP RECORDING: Set the PC-501A and Oscilloscope controls as **Table 1, ON PATCH.**
VOLTAGE CLAMP waveforms are ordinarily applied to the CMD IN terminal as **externally generated test protocols,** with or without an internally preset HOLDING VOLTAGE.
TO preset the internal HOLDING VOLTAGE Vh to a fixed value before beginning to record, or to reset it to another fixed value at any time during the experiment, proceed as follows: (**Sections 3.2 & 3.4**)

1) Set CLAMP MODE to **ZERO CURRENT**. HOLDING commands will be disengaged from the headstage;
2) Set the METER to Vc+h IN;
3) Set the COMMANDS HOLDING VOLTAGE switch to - or +, and **switch HOLDING CURRENT OFF;**
4) Set the control knob to give the desired VOLTAGE reading on the meter;
5) Switch the HOLDING VOLTAGE OFF until you are ready to use it;
6) Return to VOLTAGE CLAMP MODE.

The HOLDING VOLTAGE control can also be used alone as a **quick check for voltage-gated channel activity.** Turn COMMAND SENSITIVITY OFF to disengage external inputs, and vary Vh in either direction.

6.5 CURRENT CLAMPING: Used mainly with whole-cell recording (**Section 6.6**)
CURRENT CLAMP MODE activates all commands including those marked in red Follow the initial setup procedures as for VOLTAGE CLAMP, **Sect. 6.1 through 6.4.3.** Only a few of the settings in **Table 1 On Patch** need to be changed, and all of the oscilloscope calibrations in **Tables 2A, 2B and 2C** remain valid.

6.5.1 Switching from VOLTAGE CLAMP to CURRENT CLAMP MODE:

1) **First, switch** to **ZERO CURRENT MODE.**
2) **Turn OFF** the **HOLDING VOLTAGE** and all other 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. Although command signals are disengaged in ZERO CURRENT MODE, they become active immediately on switching out of ZERO CURRENT MODE.
3) **DO NOT press ZAP**. It remains active in ZERO CURRENT MODE and can break the patch.
4) **DO NOT CHANGE** the **CAP COMP** or **LEAK SUBTRACTION** setting from those made while in VOLTAGE CLAMP MODE. They are probably still valid.
<u>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.</u>
5) Switch CURRENT CLAMP RESPONSE to **SLOW** now, to minimize those risks.
6) **PRESET the HOLDING CURRENT, if desired**, as detailed in (**Section 3.4**). In brief:
Select Vc+hIN on the meter, set the HOLDING CURRENT switch to + or - and adjust the CURRENT knob to the desired current. The current in pA is10 times the Vc+hIN meter reading. Then switch the HOLDING CURRENT switch OFF until you are ready to use it.
7) Now switch CLAMP MODE from ZERO CURRENT to CURRENT CLAMP.

6.5.2 CURRENT CLAMP RESPONSE TIME, FAST, NORMAL, SLOW.
The feedback stability of the PC-501A in current clamp mode varies with the resistance and capacitance of the cell preparation; the capacitance compensation settings established in voltage clamp mode; and the series resistance compensation, if used.
To set the CURRENT CLAMP RESPONSE TIME, turn COMMAND SENSITIVITY to X.001 or higher if necessary, turn TEST PULSE ON, and monitor the Vm X10 OUTPUT signal on the oscilloscope.
Select FAST, NORMAL, or SLOW for the fastest setting with no overshoot and no oscillation.

6.5.3 CURRENT CLAMP RECORDING. Commands can now be applied with either the preset holding current and externally generated test protocol into the CMD IN terminal, or manually with the HOLDING CURRENT control knob alone. Command currents can be monitored at the Im OUTPUT terminal, with oscilloscope calibrations as in Tables 2A, 2B and 2C.

6.5.4 EXTERNAL COMMAND CURRENTS. Currents produced by an external signalwhenoperating in CURRENT CLAMP mode are dependent on the COMMAND SENSITIVITY selected. A 1 Volt signal will produce the currents shown below. Currents are not effected by the headstage in use.
Polarity: Positive command voltage produces a positive (cation-outward) current from the pipet.

COMMAND SENSITIVITY	CURRENT OUTPUT per Volt at CMD IN
x 0.001	10 pA
x 0.01	100 pA
x 0.1	1 nA

6.6 WHOLE-CELL RECORDING with the 5101-01G or 5101-100M Headstage.

6.6.1 THE INITAL SETUP is essentially the same as for cell-attached patch recording in either VOLTAGE or CURRENT CLAMP MODE, but with the appropriate PROBE SELECT setting.

6.6.2 RUPTURING THE MEMBRANE PATCH:

Form a gigaseal, keep the cell attached, and keep the 100 Hz TEST PULSE ON. Then use the **ZAP** button and its **DURATION** knob to rupture the membrane patch. Start with 0.1 ms duration, then increase it on successive tries as necessary.

Rupture is signalled by a sudden large **increase** in test pulse CURRENT when in VOLTAGE CLAMP MODE, or a sudden **decrease** in test pulse VOLTAGE at the VmX10 terminal when in CURRENT CLAMP MODE.

6.6.3 SERIES RESISTANCE COMPENSATION

In single channel recording, the pipet electrode (access) resistance **Rp** is negligible compared to the patch and seal resistances. However, in whole cell recording the electrode resistance becomes a significant factor..

In CURRENT CLAMP mode, an error voltage drop ImRp is generated by current through the electrode. SERIES R COMP (10M /turn) cancels the error voltage by feeding back a portion of the output current.

1) If you have already measured the pipet resistance (**Section 6.2.4 PIPET RESISTANCE Rp**), just set the SERIES R COMP dial to Rp; e.g. for Rp=4.5MΩ the dial should read 045.

2) If the access resistance is unknown, set the SERIES R COMP fully counterclockwise (to 0), turn on the TEST PULSE, or apply an external square pulse to CMD IN, and observe the Im output on the oscilloscope. The leading and trailing edge of the waveform should appear to have a fast and slow component(see sketch). Adjust the SERIES R COMP control clockwise from zero to eliminate the fast portion.

If the fast component is not discernable, advance the SERIES R COMP control clockwise until the output is just on the verge of oscillating. Then back off the control 1/8 turn or until the output is stable.

Table 1 Check List of Patch Amplifier and Oscilloscope Settings. (** means no change)

PC-501A Settings	Initial	In Bath	On Patch
POWER	ON	**	**
ZERO: AUTO switch	OFF	ON	Don't change!**
AUTO pushbutton	**	push to zero Im	Don't change!**
JUNCTION knob	about 5 turns from either end	optionally, adjust for zero Im	Don't change!**
CLAMP MODE switch	VOLTAGE	ZERO CURRENT to preset Vh, or**	VOLTAGE
COMMANDS: HOLDING switches	OFF	**	VOLTAGE +or- CURRENT OFF
COMMANDS: HOLDING knobs	set to midrange 5 turns from end	**	ON
COMMAND SENSITIVITY switch	x0.001 (1 mV pk-pk)	**	x0.1 (100 mV pk-pk)
TEST PULSE	ON	OFF to zero Im ON to measure Rp	ON to measure Rs OFF to clamp a patch
SERIES R COMP switch	OFF	**	OFF for patch ON for whole cell
HIGH FREQ BOOST switch	ON	**	**
SPEED TEST switch	OFF	**	**
PROBE SELECT	per headstage used	**	**
METER	Vc	Im	Vc +h IN
OUTPUT GAIN mv/pA switch	1 (x10) = 10 mV/pA	**	10 (x10) = 100 mV/pA or Table 2
FILTER switch	1kHz	1 or 3 kHz or ad lib	1 or 3 kHz or ad lib
CAP COMP 6 knobs	all 6 fully ccw	adjust for best square test wave	readjust for best square test wave
Oscilloscope Settings			
TIME BASE: TRIGGERING	AUTO AC	**	**
SOURCE	EXTERNAL	**	**
LEVEL control	to synchronize TEST square wave	readjust if necessary	**
Sweep Speed	2 ms/div	**	adjust as necessary
VOLTAGE beam: Sensitivity	1 mV/div	**	adjust as necessary
PC-501A OUTPUT Terminal	Vc x 10	**	**
AC/DC selector	AC	AC or DC	DC
BANDWIDTH LIMIT if present	10 kHz ON	**	**
CURRENT beam: Sensitivity	0.2 V/div	**	1V/div or Table 2
PC-501a OUTPUT terminal	Im	**	**
AC/DC selector	DC	**	**
BANDWIDTH LIMIT if present	10 kHz ON	**	**

Table 2A PC-501A OSCILLOSCOPE CALIBRATIONS for 10 GΩ Headstage

Vertical columns give current per oscilloscope division in the units indicated

Oscilloscope settings for CURRENT	OUTPUT GAIN mV/pA setting with x10 multiplier						
	1	2	5	10	20	50	100
5 V/div	500 pA/div	250 pA/div	100 pA/div	50 pA/div	25 pA/div	10 pA/div	5 pA/div
2	200	100	40	20	10	4	2
1	100	50	20	10	5	2	1
0.5	50	25	10	5	2.5	1	0.5
0.2	20	10	4	2	1	0.4	0.2
0.1	10	5	2	1	0.5	0.2	0.1
50 mV/div	5	2.5	1	0.5	0.25	0.1	0.05
20	2	1	0.4	0.2	0.1	0.04	
10	1	0.5	0.2	0.1	0.05		
5	0.5	0.25	0.1	0.05			
2	0.2	0.1	0.04		Below noise level		
1	0.1	0.05					

Table 3 Quick resistance calculation using TEST PULSE

For the COMMAND SENSITIVITY setting in use, divide the corresponding TEST PULSE voltage by the peak-to-peak test current **I** to get the resistance in MΩ or GΩ.

COMMAND SENSITIVITY	TEST PULSE peak-to-peak	Pipet Resistance R_p	Seal Resistance R_s
x.001	1 mV	$\frac{1\ mV}{I}$ in MΩ	$\frac{1\ mV}{I}$ in GΩ
x.01	10 mV	$\frac{10\ mV}{I}$ in MΩ	$\frac{10\ mV}{I}$ in GΩ
x.1	100 mV	$\frac{100\ mV}{I}$ in MΩ	$\frac{100\ mV}{I}$ in GΩ

Table 2B PC-501A OSCILLOSCOPE CALIBRATIONS for 1.0 GΩ Headstage

Vertical columns give current per oscilloscope division in the units indicated

Oscilloscope settings for CURRENT	OUTPUT GAIN mV/pA setting with x1.0 multiplier						
	1	2	5	10	20	50	100
5 V/div	5 nA/div	2.5 nA/div	1 nA/div	500 pA/div	250 pA/div	100 pA/div	50 pA/div
2	2 nA/div	1 nA/div	400 pA/div	200	100	40	20
1	1nA/div	500 pA/div	200	100	50	20	10
0.5	500 pA/div	250	100	50	25	10	5
0.2	200	100	40	20	10	4	2
0.1	100	50	20	10	5	2	1
50 mV/div	50	25	10	5	2.5	1	0.5
20	20	10	4	2	1	0.4	0.2
10	10	5	2	1	0.5	0.2	0.1
5	5	2.5	1	0.5	0.25	0.1	0.05
2	2	1	0.4	0.2	0.1	0.04	
1	1	0.5	0.2	0.1	0.05	Below noise level	

Table 3 Quick resistance calculation using TEST PULSE

For the COMMAND SENSITIVITY setting in use, divide the corresponding TEST PULSE voltage by the peak-to-peak test current I to get the resistance in MΩ or GΩ.

COMMAND SENSITIVITY	TEST PULSE peak-to-peak	Pipet Resistance R_p	Seal Resistance R_s
x.001	1 mV	$\frac{1\ \text{mV}}{I}$ in MΩ	$\frac{1\ \text{mV}}{I}$ in GΩ
x.01	10 mV	$\frac{10\ \text{mV}}{I}$ in MΩ	$\frac{10\ \text{mV}}{I}$ in GΩ
x.1	100 mV	$\frac{100\ \text{mV}}{I}$ in MΩ	$\frac{100\ \text{mV}}{I}$ in GΩ

Table 2C PC-501A OSCILLOSCOPE CALIBRATIONS for 0.1 GΩ Headstage

Vertical columns give current per oscilloscope division in the units indicated

Oscilloscope settings for CURRENT	OUTPUT GAIN mV/pA setting with x10 multiplier						
	1	2	5	10	20	50	100
5 V/div	50 nA/div	25 nA/div	10 nA/div	5 nA/div	2.5 nA/div	1 nA/div	500 pA/div
2	20	10	4	2	1	400 pA/div	200
1	10	5	2	1	500 pA/div	200	100
0.5	5	2.5	1	500 pA/div	250	100	50
0.2	2	1	400 pA/div	200	100	40	20
0.1	1	500 pA/div	200	100	50	20	10
50 mV/div	500 pA/div	250	100	50	25	10	5
20	200	100	40	20	10	4	2
10	100	50	20	10	5	2	1
5	50	25	10	5	2	1	0.5
2	20	10	4	2	1	0.4	0.2
10	10	5	2	1	0.5	0.2	0.1

Table 3 Quick resistance calculation using TEST PULSE

For the COMMAND SENSITIVITY setting in use, divide the corresponding TEST PULSE voltage by the peak-to-peak test current **I** to get the resistance in MΩ or GΩ.

COMMAND SENSITIVITY	TEST PULSE peak-to-peak	Pipet Resistance R_p	Seal Resistance R_s
x.001	1 mV	$\frac{1\text{ mV}}{I}$ in MΩ	$\frac{1\text{ mV}}{I}$ in GΩ
x.01	10 mV	$\frac{10\text{ mV}}{I}$ in MΩ	$\frac{10\text{ mV}}{I}$ in GΩ
x.1	100 mV	$\frac{100\text{ mV}}{I}$ in MΩ	$\frac{100\text{ mV}}{I}$ in GΩ

7.0 BILAYER RECORDING with PC-501A

Bilayer recording is done with the 5101-10GB headstage.
This is a 10 GΩ headstage modified to allow for compensating the relatively large capacitance of the bilayer membrane, up to approximately 250 pF.
The noise level for this headstage is about 10% greater than for the standard 10 GΩ headstage, due to the increased capacitance compensation.

7.1 THE RECORDING CHAMBER

Bilayers are usually formed inside a chamber, such as the Warner Instrument BCH-13 and BCH-22. Figure 1 depicts such a chamber with connections to the headstage.

Because of the high impedence of the bilayer, the whole assembly must be shielded from interference to obtain low-noise recording. A grounded copper screen box large enough to accomodate the headstage and chamber is usually all that is required. A bench size Faraday cage is cumbersome and usually not particularly effective.

7.2 SETTING UP:

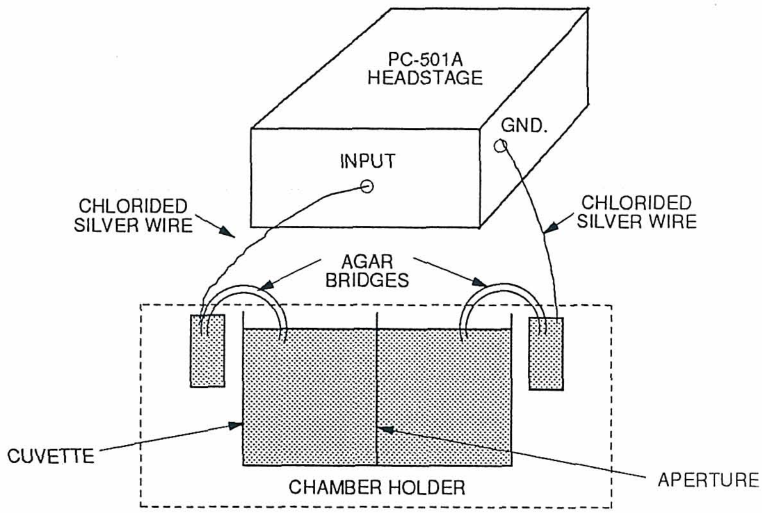
OBSERVE HANDLING PRECAUTIONS, Section 4.3, when handling the headstage and when connecting and removing the silver wire INPUT and GND electrodes.

Chloride the silver electrodes by one of the techniques described in **Section 5.2**

Plug the 5101-10GB headstage into the PROBE receptacle, turn the PROBE SELECT knob to the 10G position, and switch the rear panel **PATCH/BILAYER** switch to **BILAYER.**

7.3 CONNECTING TO THE BILAYER CHAMBER

The headstage silver-silver chloride electrodes should be connected via agar bridges (see figure 2) to the two bath compartments
of the bilayer chamber.
Chloride the silver wires (**see section 5.2**) and connect each one from the headstage into separate wells containing 3 M KC1. These wells should be adjacent to the baths so that short agar bridges can be used to complete the circuit



NOTE: The polarity of the command signal appearing at the headstage INPUT terminal is the same as the polarity presented to the CMD IN.

Fig.2 Connecting to a bilayer chamber

RECOMMENDED READING

C. MILLER, ed. (1986) Ion Channel Reconstitution. Plenum Press, New York
In particular, Chapter 5, "How to set up a bilayer system", covers many important aspects of the subject.

8.0 REPLACEMENT PROBE CALIBRATION.

Headstage probes supplied with the PC-501A at time of order have been calibrated for that unit, identified by its serial number on the rear panel. When purchasing additional probes, or using probes calibrated for other PC-501A's, these probes have to be recalibrated for the PC-501A on which they are going to be used.
FOLLOW PROPER HEADSTAGE HANDLING PROCEDURES THROUGHOUT. Section 4.3.

EQUIPMENT REQUIRED

1. An oscilloscope with differential input; both + and - input terminals are required.
2. To perform **Step 1 - gain adjust**, the following resistors are required:

For a 10GΩ probe	1 GΩ 1%
For a 1GΩ probe	100 MΩ 1%
For a 100MΩ probe	10 MΩ 1%

Handle 1 GΩ and 100 MΩ resistors by the leads only- fingerprints can shunt their resistance.

3. A screwdriver small enough to adjust the GAIN TRIM and SPEED TEST pots in the HI FREQ BOOST block.

SETUP:

1. Plug in the probe and shield it as described in Section 4.5, Headstage Preparation.
2. Connect the shield to the insulated 1mm ground jack on the side of the probe housing, or to the CIRCUIT GROUND terminal on the rear panel of the instrument.
3. Prepare the appropriate resistor listed above in EQUIPMENT REQUIRED, by grounding one end by a flexible hookup wire, and with any suitable 2mm pin connector on the other end.
Keep the connections as short as possible. Don't connect it to the headstage yet.
4. Turn the instrument on and connect Im OUTPUT to the oscilloscope.
Allow the instrument to warm up and stabilize for a minimum of one hour.
5. Set the filter for 5kHz and observe the output waveform.
6. Move the probe back and forth inside the grounded shield to obtain a minimum of 60Hz interference.
The waveform should appear as a straight line.

STEP 1 - GAIN ADJUST

Hold the resistor by its grounded end and connect it to the probe input pin. Slide the headstage and resistor well into the shield, and again adjust its position for minimum 60Hz interference. Placing a grounded piece of aluminum foil near the open end of the shield may help.

Turn the TEST PULSE switch ON and the COMMAND SENSITIVITY switch to X.1.
Connect Im OUTPUT to one terminal of the differential oscilloscope input, and set Im GAIN to 10mV/pA.
Connect the ΣVcX10 OUTPUT to the other oscilloscope input. Use the CAP COMP controls as necessary to square up the waveform.
In the HI FREQ BOOST block, adjust the GAIN TRIM potentiometer for the probe being calibrated, to null the test pulse square wave to a flat line on the scope.
That completes the gain adjustment.
Turn the TEST PULSE switch to OFF, and disconnect the ΣVcX10 OUTPUT from the scope.

STEP 2 - SPEED TEST

Speed test adjustments are required for 10G and 1G probes only.
Turn the SPEED TEST switch on, and set the FILTER/ACTIVE switch to BYPASS.
Monitor the unfiltered Im OUTPUT, and adjust the appropriate potentiometer for an optimum square wave.
The probe is now calibrated for use with the main unit.

9.0 REPLACEMENT PARTS

9.1 PROBES

When ordering additional or replacement probes, please reference the serial number of your PC-501A.
The 4 probe order numbers are:

5101-10G	10 GΩ feedback resistor
5101-01G	1 GΩ feedback resistor
5101-100M	100 MΩ feedback resistor
5101-10GB	10 GΩ feedback resistor, modified for bilayer

Standard cable length is 1.93 meters (6'). If a longer length is required, we can supply up to 3.05 meters (10').

9.2 ELECTRODE HOLDERS

Electrode holdres are NOT supplied with each probe orderd. They must be ordered separately. The standard type is:

Model QSW-AxxP (straight)

Holder part numbers are completed by replacing the "xx" with 10X the O.D. in mm of the pipet glass to be used: 10 for 1.0mm, 12 for 1.2mm, etc.

Other style holders are also available from Warner Instrument Corp., such as right angle, 45° and microperfusion types.
Request our Microelectrode Accessories catalog for complete details on holders, chambers, glass capillary tubing and holder replacement parts.

9.3 PLACING YOUR ORDER

Contact our sales department by mail, phone or fax:

Warner Instrument Corporation
1125 Dixwell Avenue
Hamden, Connecticut 06514 USA

Tel: (800) 599-4203
Fax: (203) 776-1278

10.0 SERVICE AND WARRANTY

10.1 WARRANTY

Warner Instrument Corporation warrents that this equipment shall be free from defects in materials and workmanship for a period of three years from date of purchase. If a failure occurs in this period, we will either repair or replace the faulty component or parts upon receipt. This warranty does not apply to instruments subjected to physical abuse or electrical stress(inputs exceeding specifications) or instruments modified without our authorization. Shipping charges to Warner Instrument Corporation are the responsibility of the customer. Return shipment charges (surface rate) will be paid by us. This warranty is not extended to electrode holders since these items are considered disposables.

10.1 SERVICE

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. 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. Our service department hours are:

Monday through Friday- 8:00 am to 4:00 pm Eastern Time

Telephone: (203)-776-0664 (800)-599-4203 (toll free in the US and Canada)

Fax: (203) 776-1278

10.2 RETURN TO FACTORY

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

A) Package the instrument with at least 3 inches of cushioning on all sides. Use the original shipping carton if it is available.

B) Insure the shipment for its full value.

C) 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.

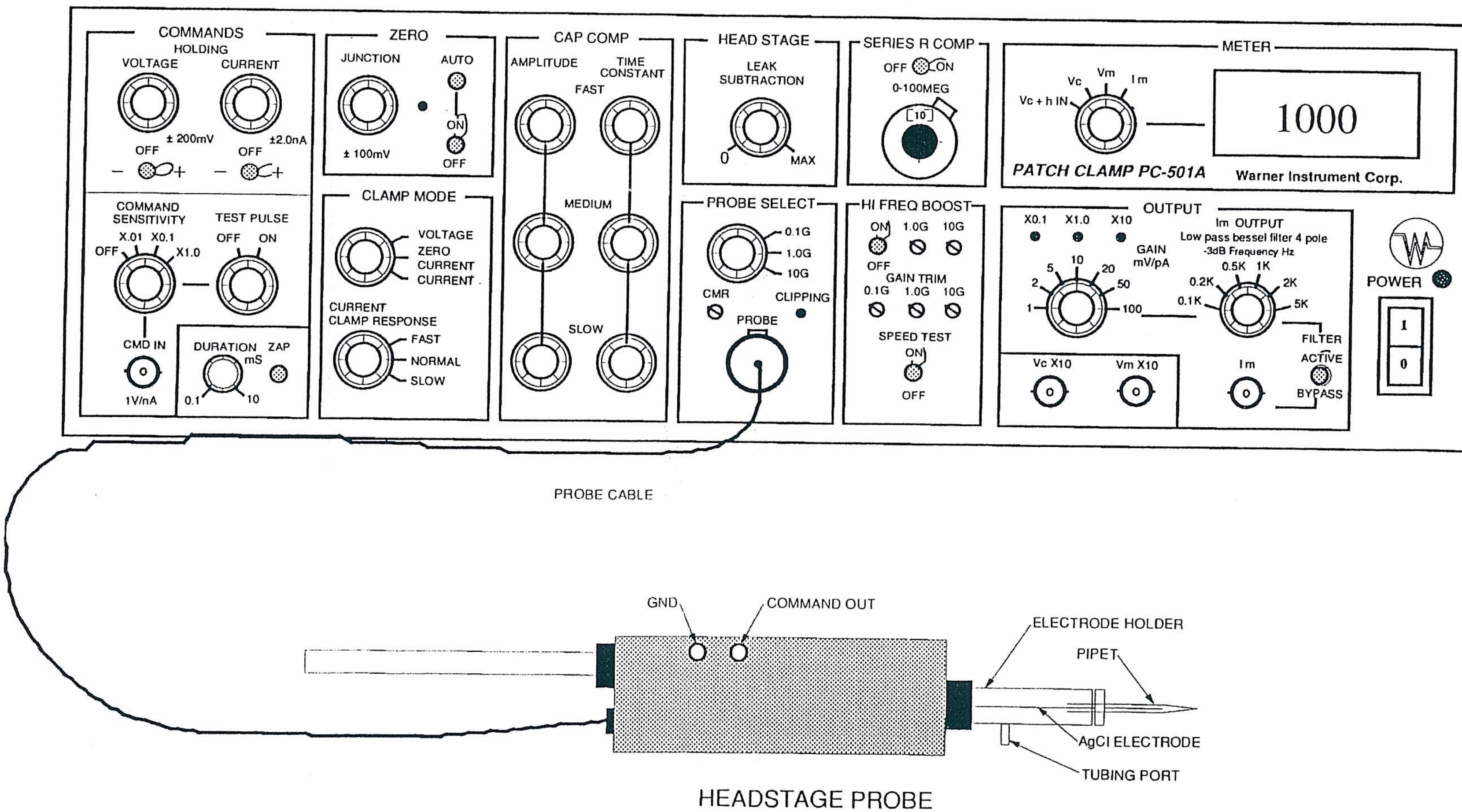


Fig. 3 PC-501A Panel and Headstage

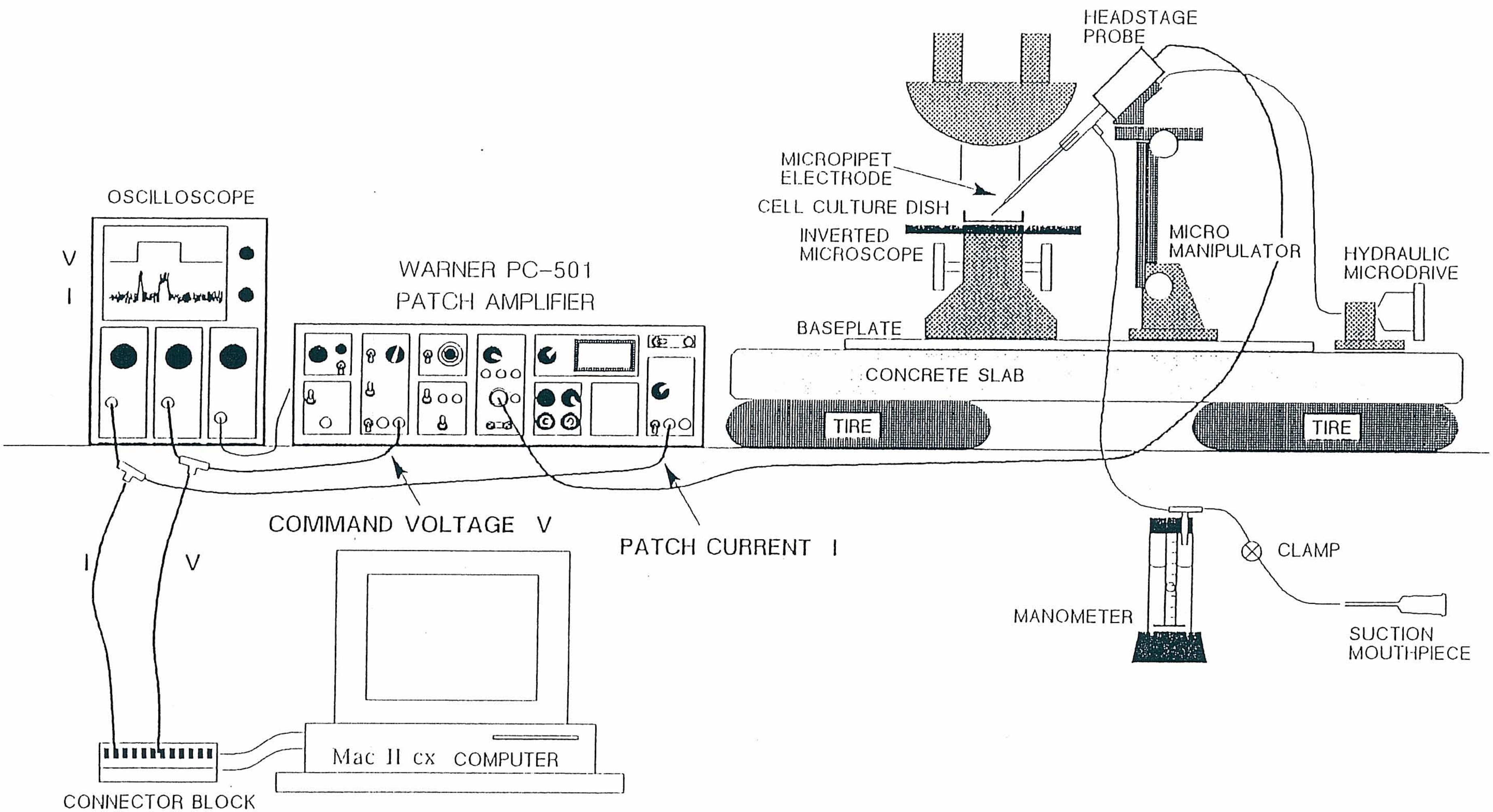


Fig. 4 Patch Lab Basic Setup