Warner Instruments
Bilayer Clamp Amplifier
Model BC-535

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The Warner **BC-535** Bilayer Clamp Amplifier is a resistive-feedback voltage clamp amplifier designed specifically for applications using planar lipid bilayer membranes. The unique circuitry and dedicated design of this amplifier allows Warner to present an instrument of broad capability and superior quality at a cost significantly below that of our competitors.

The operational range of the **BC-535** has been enhanced by the introduction of dual feedback-resistor circuitry within the headstage. This enhancement allows the amplifier to comfortably pass currents of up to 2 nA while preserving the sub-pA sensitivity of the instrument. In addition, the range of the digital hold control has been extended to 400 mV for internally generated commands and the amplifier supports up to 1 V at the external command input, for a sum capability of 1400 mV hold potential.

The remaining functionality of the **BC-353** is built on the renown capabilities of the **BC-525D** and includes *junction potential auto-zeroing*, a unique *multi-step, digital hold potential circuit*, *audio monitoring of membrane formation*, and *direct readout of the membrane capacitance*.

Features of the **BC-353** include

- ✓ Dedicated design for bilayer applications
- ✓ Digital, multi-step hold potential control
- ✓ Hold potentials to ±1400 mV
- ✓ Currents to ±2000 pA
- ✓ Input offset with Auto-Zero
- ✓ Direct membrane capacitance measurement
- ✓ Low-pass 4-pole Bessel filter
- ✓ Audio output
- ✓ Capacitance compensation circuitry

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 reduce 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 NON-UNDERLINED SMALL CAPS.
- Finally, references to individual control settings are specified in *italic type*.
- Special comments and warnings are presented in highlighted text.

Any other formatting should be apparent from context.

Device panel abbreviations

The **BC-353** has several abbreviations on the front panel. They are listed here for quick reference. In addition, these and other terms are collected and included in a Glossary at the back of this manual.

Term	Meaning	Section
V_c	command voltage	METER, OUTPUTS
I_m	output current	METER, OUTPUTS
CMD IN	commend in	
CAP TEST	capacitance test	METER
CAP COMP	capacitance compensation	CAP COMP

CONTROL DESCRIPTION

The instrument front panel is divided into six control blocks titled HOLD, OFFSET, METER, OUTPUTS, CAP COMP, and POWER. The instrument rear panel has BNC connectors for the GAIN and FILTER TELEGRAPHS, I_M OUTPUT, CAP SYNC, MEMBRANE CAPACITANCE, and EXTERNAL COMMAND IN. A 9-pin DIN connector (for the headstage), a 15 pin D connector, binding posts for CIRCUIT and CHASSIS GROUND, and a SPEAKER OUTPUT are also located on the rear panel.

Front panel

Hold

The HOLD block contains a meter and controls for the application of internal or external V_m HOLD commands.

The appropriate membrane holding potential is achieved by summing the selected HOLD voltages (internal plus external) with the INPUT OFFSET voltage which results in a corrected transmembrane voltage. An LED indicates COMMANDS APPLIED to the headstage.

The *internal* HOLD control is comprised of a digital circuit providing discrete adjustment of the command potential. Two toggle switches directly below the COMMANDS APPLIED meter are used to step the applied command by *± 10* or *± 1 mV*, respectively. A black push button is used to quickly swap the polarity of the applied holding potential. The maximum range for this control is *± 400 mV*.

The internally generated HOLD command can be disabled by selecting the *off* position on the ON/OFF TOGGLE SWITCH to the right of the meter.

Note: The METER will still display the programmed hold voltage when the ON/OFF TOGGLE SWITCH is selected to *off*. However, the programmed command will not be applied and the COMMANDS APPLIED LED will remain *unlit*. This feature allows the user to select or change the holding potential without applying it to the membrane.

The V_c x 10 OUTPUT monitors the voltage command applied to the headstage multiplied by 10. This output reports the sum of potentials from V_m HOLD, CMD IN, and PULSE GENERATOR. Connection is made via BNC's located on both the front and rear panels of the amplifier.

The I_m OUTPUT reports the membrane current modified by amplifier gain and/or internal filtering. I_m output BNC's are located on both the front and rear panels of the instrument.

External commands are applied to the amplifier via the COMMAND INPUT BNC connectors located on both the front and rear panels. The FRONT/REAR TOGGLE SWITCH either disables all external input or selects the location for command inputs. Selectable attenuation values are *x0.1, x0.01,* or *x0.001*. Externally generated COMMAND INPUTS are summed with the internally generated HOLD voltage.

Offset

The OFFSET block contains the INPUT OFFSET and the AUTO-ZERO controls.

The INPUT OFFSET section is comprised of a rotary potentiometer with *low/high* LED's, the AUTO-ZERO pushbutton, the UNLOCK toggle, and an ACTIVE LED. This section is used to compensate for junction potentials produced by dissimilar solutions or other electrode potential differences.

The OFFSET circuit (AUTO-ZERO and OFFSET CONTROL) must be armed prior to use. This is achieved by use of the UNLOCK TOGGLE. This toggle is of the momentary-on style and is operated by an upward movement. When the circuit is armed the ACTIVE LED will be *lit*. Offset adjustments can then be easily achieved by use of the AUTO-ZERO control or ROTARY POTENTIOMETER. The circuit can be disarmed by a second movement of the UNLOCK TOGGLE.

The AUTO-ZERO control provides the most direct means for setting the junction potential. When armed, pressing the pushbutton initiates a cycle wherein the amplifier searches for and sets the offset potential. The offset circuit is automatically disarmed at the completion of the cycle. Cycle time is approximately 1 s.

The ROTARY POTENTIOMETER is used to provide manual adjustment of up to ±120 mV at the headstage input. Manual adjustment is only available when the offset circuit is armed. Fine adjustment of the rotary control can be achieved by *pressing the control in* while turning. *Low/high* LED's are provided to indicate which direction the manual offset control should be adjusted to achieve a null junction potential setting. The offset circuit must be manually disarmed when using this control.

In both cases, the applied OFFSET potential can be monitored on the METER by selecting OFFSET in the METER block.

Meter

The METER block contains a 3.5 digit LED METER and a four position switch for selecting OFFSET, CAP TEST, current output (I_m), or voltage command (ΣV_c).

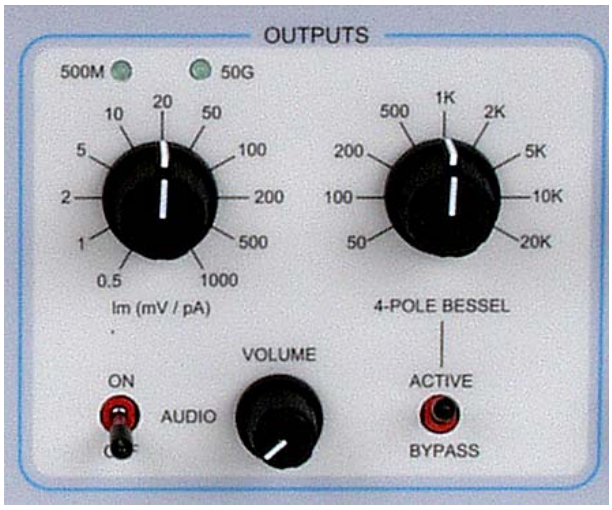
Selection of OFFSET displays the potential applied to the headstage via the MANUAL or AUTO-ZERO control located in the OFFSET block. Offset potential is displayed in units of pA. Alternatively, this display indicates the potential required to bring I_m to zero when the command input is set to zero.

Selection of CAP TEST places the instrument into *capacitance test* mode. This useful mode dynamically tests and reports the membrane capacitance. Capacitance values are reported on the meter in units of pF. A rear panel BNC also reports the calculated membrane capacitance whenever CAP TEST is selected. Reported units are 1 mV/pF.

Selection of I_m displays the value of the DC current presented at the I_m OUTPUT BNC. The meter is capable of displaying currents up to ±1999 pA.

Selection of ΣV_c displays the sum of all command voltages (V_m HOLD and COMMAND INPUT) applied to the headstage. The meter is capable of displaying command voltages up to ±1999 mV. The meter displays DC values and will average AC signals or pulses.

Outputs



The OUTPUTS block contains controls for selecting the I_m GAIN of the amplifier and signal filtering using the built-in 4-pole Bessel filter. This block also contains the audio output controls.

Amplifier gain is selected via an 11 position selector switch. Gain settings are from *0.5* to *1000 mV/pA* in 1-2-5 steps. For transmission to external devices, the selected gain setting appears as a defined voltage at the GAIN TELEGRAPH BNC on the instrument rear panel.

Internal filtering of the I_m signal is selected via a 9 position selector switch. Filter settings are from *50 Hz* to *20 kHz* in 1-2-5 steps. A BYPASS TOGGLE switch bypasses the 4-pole Bessel filter and presents the full bandwidth (75 kHz) of the amplifier at the I_m OUTPUT. Filtering is applied post-gain. For transmission to external devices, the selected filter setting appears as a defined voltage at the FILTER TELEGRAPH BNC on the instrument rear panel.

The AUDIO section is comprised of an *on/off* toggle and *volume* control. Audio output is useful during membrane formation to monitor the successful application of lipids. An open hole prior to membrane formation produces a characteristic low frequency sound while the same aperture with membrane produces a different characteristically higher frequency sound. The pitch of the signal is keyed to the membrane capacitance and will increase as the capacitance increases allowing non-visual monitoring of membrane 'thinning'.

Capacitance compensation

The capacitance compensation circuit allows for cancellation of large currents (capacity currents) generated when a step potential is applied to the bilayer membrane.

The CAP COMP block contains controls for the adjustment of AMPLITUDE and TIME CONSTANT for both FAST (0-10 μs) and SLOW (0-10 ms) components of the current. The adjustment is made in pairs, that is, the FAST pair (AMPLITUDE and TIME CONSTANT) is first adjusted to minimize the transient, followed by adjustment of the SLOW pair. Each pair is adjusted in turn as many times as required to completely minimize the transient.

The AMPLITUDE control for the FAST component is a ten turn potentiometer with a counting dial and can be used to provide a

reading of the capacitance in pF. The dial is calibrated to 50 pF/turn.

Power

Immediately adjacent to the CAP COMP block is the master power switch for the **BC-535**. An LED indicates power on status.

Rear panel

The instrument rear panel has BNC connectors for GAIN and FILTER TELEGRAPHS, I_M OUTPUT, CAP SYNC, EXTERNAL RESET IN, and EXTERNAL COMMAND IN. A 9-pin DIN connector (for the headstage), a 15 pin I/O INTERFACE, binding posts for CIRCUIT and CHASSIS GROUND, and a SPEAKER OUTPUT are also located on the rear panel.

The photo below shows the various attachment points on the instrument rear panel. Connections are described right-to-left.

Headstage

The HEADSTAGE is housed in a small aluminum enclosure and connects to the amplifier via a 1.8 meter cable. A 9-pin DIN connector is provided for this attachment.

Note: When routing the headstage cable from your Faraday cage to the instrument, we recommend intertwining the headstage and ground cables to minimize ground loops.

Circuit and chassis grounds

CIRCUIT and CHASSIS GROUND binding posts are provided at the rear of the amplifier to allow modification of instrument grounding.

The CHASSIS GROUND binding post is internally connected to the green-wire ground of the power plug. Therefore the instrument does not normally require a separate ground. However, it may become necessary to independently ground the chassis of the **BC-535** when it is used as a freestanding device and not incorporated into a rack.

The CIRCUIT GROUND binding post allows external connection to the internal ground circuitry of the amplifier. This post is used to provide a common circuit ground point for all active components (Faraday cage and contents, SUNS*tir*-3 assembly, temperature controller, etc.) within the bilayer rig, thus preventing ground loops.

In general, the internal circuitry of the **BC-535** maintains a virtual ground and is not normally not connected to chassis ground. However, when necessary, the CIRCUIT GROUND binding post can be used to tie the circuit and chassis grounds to a common potential. This is the default configuration when the instrument is shipped from the factory.

Note: We recommend separating the circuit and chassis grounds by disconnecting the bridging bar between the associated ground posts. Loosen the posts and slide the bridge to one side.

Gain Telegraph

The GAIN TELEGRAPH is a stepped voltage output designed to communicate the instrument gain setting to your acquisition software. DC voltages are stepped from 0.0 V to 5.5 V, in steps of 500 mV. GAIN TELEGRAPH voltage outputs for the associated amplifier I_m GAIN are specified below. (I_m GAIN settings are selectable in the front panel OUTPUTS block, *see* page 8)

I_m Gain (mV/pA)	Gain Telegraph (V)
standby	0.0
0.5	0.5
1	1.0
2	1.5
5	2.0
10	2.5
20	3.0
50	3.5
100	4.0
200	4.5
500	5.0
1000	5.5

Filter Telegraph

The FILTER TELEGRAPH is a stepped voltage output designed to communicate the instrument filter cutoff frequency setting to your acquisition software. DC voltages are stepped from 0.5 V to 5.0 V, in steps of 500 mV and are specified below. (FILTER settings are selectable in the front panel OUTPUTS block, *see* page 8)

Filter Frequency (Hz)	Filter Telegraph (V)
50	0.5
100	1.0
200	1.5
500	2.0
1k	2.5
2k	3.0
5k	3.5
10k	4.0
20k	4.5
bypass	5.0

Im output

The I_m OUTPUT signal present on the instrument front panel is mirrored on this rear panel BNC. Use of this output rather than the front panel BNC can unclutter your work environment.

External Command In

External COMMAND IN signals can be input via BNC connectors on either the instrument front panel or the instrument rear panel. Input location is selectable by the *front/rear* COMMAND INPUT toggle switch located in the HOLD control block on the instrument front panel. Use of the rear input can unclutter your work environment.

Capacitance Output

The calculated membrane capacitance is output on this BNC when the instrument is in CAP TEST mode. Switching the METER selector switch to *CAP TEST* activates the CAP TEST circuit. This feature is useful for recording the calculated membrane capacitance into a chart recorder or data acquisition system. Capacitance output values are 1 mV/pF.

Cap Sync Out

This signal is used to synchronize an oscilloscope or other device with the **BC-535** when using the CAP TEST function. The SYNC OUT signal is keyed to the peak of the triangular wave for CAP TEST which corresponds to the leading edge of the resulting square wave. The SYNC OUT signal is a standard TTL square wave and is 100 μs in duration.

External speaker

A standard ¼" RCA jack is provided for attachment to an external speaker for use in environments where the ambient noise exceeds the volume capabilities of the internal speaker.

ADDITIONAL INFORMATION

Headstage connections

The HEADSTAGE is housed in a small aluminum enclosure and connects to the amplifier via a 1.8 meter cable. Electrode connections are made to two 1 mm mini-jacks marked INPUT and REF (reference). A third mini-jack (GND; circuit ground) is located on the side of the headstage for connecting to shields or grounding equipment.

The ground connection on the headstage merits specific discussion. The headstage case is internally connected to the command potential (INPUT electrode) of the headstage. As a result, the headstage does not require a separate ground. However, the isolated grounding jack on the headstage is provided as a means to ground a small Faraday cage through the headstage if the user desires.

Notes:

1. If the Faraday cage is grounded through the headstage (not recommended), then ***do not*** run a separate ground connection from the Faraday cage to any other ground point.
2. Do not connect the ground on the headstage to either the *input* or *ref* electrode as this will disable the amplifier.

Model membrane

The **BC-535** is shipped with a model membrane, the **MC-1**, which can be used to test the performance of the amplifier. The **MC-1** contains a 100 pF capacitor connected in parallel with a 1 GΩ resistor. The precision of this resistor is ± 5%.

The **MC-1** connects to the two 1 mm mini-jacks on the headstage marked INPUT and REF. The green grounding wire on the **MC-1** is attached to the isolated grounding jack on the headstage.

SETUP

For those with little experience in bilayer work, we suggest a review of *Ion Channel Reconstitution* edited by C. Miller, Plenum Press, New York, 1986. In particular, Chapter 5, "How To Set Up A Bilayer System", covers many important aspects of the subject. Several other pertinent references are included in the appendix at the back of this manual.

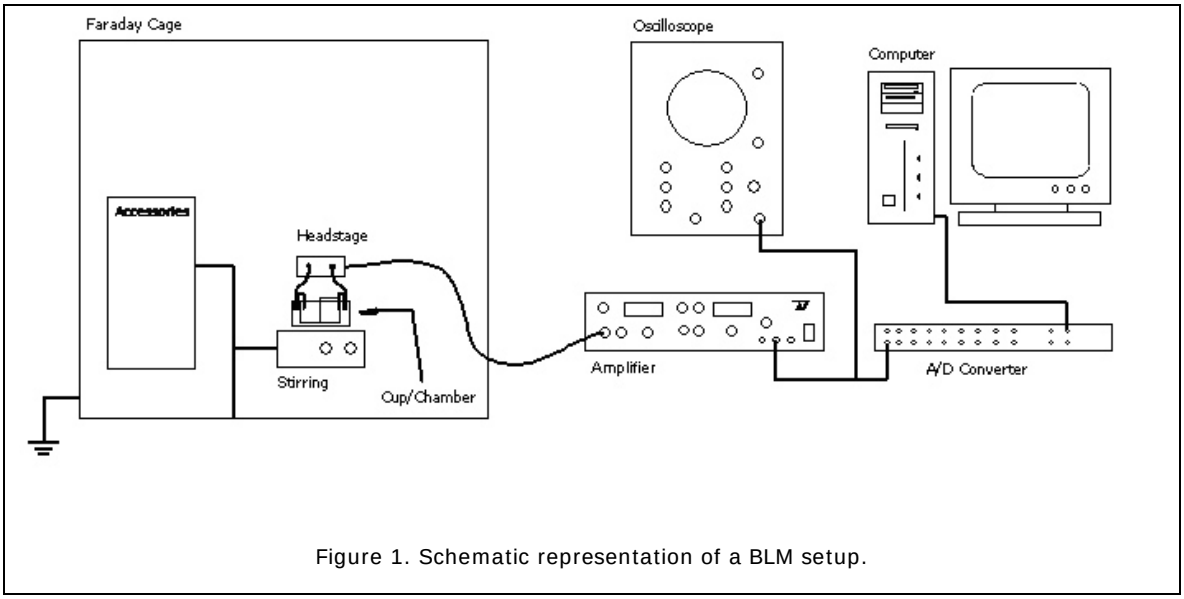


Figure 1. Schematic representation of a BLM setup.

Basic design

A planar lipid bilayer (BLM) workstation, used to record currents through actively gating, ion conducting single channels, is a complex apparatus requiring several components working in concert. These components include a means to support the lipid membrane, high gain amplification, shielding of electromagnetic interference, shielding of mechanical vibration, mechanisms for stirring and changing solutions, signal filtering, data acquisition analysis, and a means to archive acquired data.

A schematic representation of a basic BLM layout is shown in Figure 1. Warner Instruments provides all components used in the assembly of a BLM workstation, including Faraday cages, vibration isolation tables, a dedicated bilayer clamp amplifier, high quality signal filtering devices, illumination and stirring mechanisms, cups and chambers, and perfusion apparatus.

The components listed above may be assembled in various ways to achieve a working system. Regardless of the configuration used, care must be taken in the design of a BLM workstation to minimize both mechanical and electrical noise sources since single channel currents are often only a few pA in magnitude. In this section we describe the basic design of a BLM workstation.

Faraday cage

A Faraday cage is an enclosure designed to shield the sensitive electronics in the headstage from electromagnetic interference generated by noise sources in the vicinity of the apparatus. These sources include exterior lighting, nearby instrumentation and electrical wiring. The cage can be fabricated from any conducting material and is grounded. While the design of the **BC-535** facilitates

grounding the Faraday cage through the headstage (*see* **Headstage connections**, page 11), we do not recommend this procedure, but instead suggest that the cage be grounded through the amplifier circuit ground.

Several Faraday cage designs are available. The most common commercial design is that of a copper or aluminum wire mesh supported on an aluminum frame. This frame attaches to the conducting top of a floor-standing vibration isolation table which completes the cage enclosure. Entry is through large front panel doors. This design is most often used in conjunction with patch clamp setups since the large enclosure can house a microscope as well as several other devices.

An option exclusively presented by Warner Instruments consists of a Faraday cage with an enclosed vibration isolation table. This unique combination is specifically designed with the bilayer user in mind. The assembly requires little lab space, rests comfortably on a sturdy work surface, and actively isolates the tabletop from the cage enclosure. The cage is easily assembled and has several design features simplifying bilayer work.

Regardless of the Faraday cage employed, the headstage and membrane support system (e.g. cups and chambers) are contained within the cage which acts as the electromagnetic shield. Other devices such as a perfusion system or stirring apparatus may also be housed within the cage, but some investigators place these components on the outside (with proper grounding) to reduce their noise contribution.

Vibration isolation

The isolation and damping of mechanical noise is critical to increasing the signal to noise ratio of a BLM workstation. The significance of this becomes apparent when one considers that the acoustic coupling of normal speech to the buffers on each side of the bilayer is large enough to present a significant capacitance current artifact in the data.

Several approaches have been employed to eliminate large amplitude mechanical vibrations in an experimental setup. These include specially designed vibration isolation tables or optical benches. These floor standing benches employ a heavy table top resting on pneumatic supports. Alternatively, investigators have placed heavy concrete slabs (commonly referred to as balance tables) or large steel sheets on partially inflated inner tubes or tennis balls. We recommend the use of a high quality commercial table since these devices provide more long term stability and more effectively damp vibrational noise inputs.

Another, more subtle, source of noise in electrophysiological recording systems is associated with vibration of the headstage. This movement can produce a rapidly fluctuating stray capacitance which appears as increased noise in the amplifier output. This effect can be minimized by shock mounting the headstage to its support. Since it is advantageous to keep the associated moment arm as small as possible, the headstage should be directly mounted to its support rather than through a long connecting rod. Warner Instruments has developed the **HST-1** headstage holder system expressly for this purpose.

Membrane support

The general approach to the formation of a planar lipid bilayer membrane involves spanning lipids across a small hole or aperture in a membrane support. A cocktail of lipids, usually suspended in a

solvent such as decane, is manually painted or drawn across the aperture. Excess lipids drain away from the aperture and under hydrophobic pressure the remaining lipids orient themselves into a molecular bilayer.

Planar lipid bilayer membranes are routinely generated on a variety of supports including cups made from polystyrene, polysulfone, Teflon, or Delrin. Teflon sheets, Pasteur pipette tips, or plastic septa have also been used. These supports are either custom fabricated for the desired application or are purchased from commercial sources. Currently, the most commonly used system for supporting artificial bilayer membranes is the cup/chamber design. Warner Instruments manufactures cups and chambers in several combinations of material, cup volume and aperture size. These may be viewed in our catalog or at our website under the model numbers **BCH-13**, **BCH-22** and **BCH-P**.

The geometry of the aperture is important to the stability of the supported membrane. If the hole diameter is too large then the membrane formed will be electrically noisy and mechanically fragile. A smaller hole diameter reduces electrical noise and is mechanically more robust, however, the probability that a vesicle will fuse to the membrane is inversely proportional to the membrane size.

The simplest aperture geometry is that of a tubular channel drilled through the supporting septa. This geometry has the advantage of being easy to manufacture and maintain. It is generally assumed that the membrane formed is maintained at one end of the bore. This is the design employed by Warner cups.

Another aperture geometry commonly used is that of a conical hole with the small end of the hole supporting the bilayer membrane. This geometry, often employed on custom made cups, is generally formed by melting a small bubble (or boss) in the cup wall from the inside using a heated piece of pointed metal, and then shaving the outside boss away with a razor until a hole of the desired diameter is achieved.

Based on the above discussion, it is clear that the choice of hole size and geometry represents a trade-off between membrane noise, fragility, and the probability of vesicle fusion. The best hole for a particular application is usually determined empirically.

Amplification

A high-quality amplifier is an absolute requirement for recording single channel currents. The amplifier must be capable of resolving currents as low as 1 pA with very little added noise. While several manufacturers today produce amplifiers of high-quality, the greatest degree of variation between instruments is in the feature set. Warner Instruments is the only manufacturer to produce a dedicated bilayer clamp amplifier, and it's performance and feature set have been optimized for bilayer work.

Filtering

Filtering of the amplifier output is essential for resolving discrete channel fluctuations from the large amplitude high frequency noise present in the signal. Properly applied filtering is important since over-filtering of the data will obscure or modify channel gating events (a condition to be avoided!), while an under-filtered signal will not clearly resolve single channel events. The **BC-535** provides a built-in 4-pole Bessel filter which can be used to select filtering from *50 Hz* to *20 kHz*, or can be bypassed.

Optionally, many researchers filter their data using an external device. These devices are normally of the low-pass 8-pole Bessel design. While Butterworth filters have steeper frequency cutoff characteristics, they are less commonly used since they tend to overshooting a rapidly varying signal thus introducing an artifact into the data. In general, it is better to slightly under-filter the data being acquired in real-time since additional filtering can be performed later in the analysis software.

Warner Instruments provides a number of filtering devices which can be used in conjunction with the **BC-535** to achieve a high degree of filter resolution. We recommend the use of a high quality 8-pole Bessel filter such as the **LPF-8**.

Acquisition hardware and software

Since the analysis of single channel data is statistical in nature, a large number of channel events is required to produce significant results. This condition naturally lends itself to the use of a computer. However, since computers function digitally, the analog signal from the amplifier must first be digitized by an analog to digital (A/D) converter prior to analysis. Many A/D converters are bundled with software which emulates a chart recorder or oscilloscope to aid in data acquisition.

Since single channel gating kinetics can range from sub-ms open times to gating transitions lasting several seconds or more, the desired characteristics of a high-quality A/D converter include rapid response times, high signal resolution and low noise.

Data analysis

Once the data has been acquired and stored, it must be analyzed for its biophysical characteristics. Since the volume of data collected is often exceedingly large, analysis is usually performed by dedicated software programs. The single most popular program for this purpose is pClamp (Molecular Devices, Sunnyvale, CA). However, several competing software packages are available commercially or on the Internet. In addition, many investigators have written their own programs to address their specific needs.

Data archival

The ability to easily archive and retrieve data is an important component of a BLM workstation. During the course of an average experiment, a large volume of data is collected for later analysis. Several devices are available for data storage. These devices include, but are not limited to: VCR tape (requires a signal converter or pulse code modulator), DAT tape, portable or removable hard drives, Zip or Jazz drives, CD-RW, or the newer DVD-RW technology.

An important advantage of most of these archival systems is that they allow selective access to previously recorded data for subsequent analysis. The choice of the proper system will depend upon the needs of the researcher, the financial resources available, and the type of data acquired (fast or slow channel kinetics resulting in large or small file sizes).

Stirring

Stirring of solutions in the recording chamber is important for the production of reproducible results, especially following the addition of agonists or antagonists. Additionally, stirring is thought to facilitate the fusion of vesicles to the bilayer membrane, presumably by vibrating the membrane or by continuously introducing new vesicles to the bilayer. Ideally, the stirring process should produce

sufficiently little mechanical noise such that one is able to make recordings while simultaneously stirring.

Warner Instruments has developed a unique stirplate specifically designed for the planar lipid bilayer. This stirplate, the **SPIN-2**, provides separate rotating dipoles for each side (*cis* and *trans*) of the cup/chamber and its noise-free operation allows data recording while stirring.

Perfusion

Exchange of solutions (termed perfusion) normally occurs following incorporation of a channel to the bilayer membrane, when experimental conditions require an alteration in ionic conditions, or to remove a previously added compound.

Under ideal circumstances a good perfusion system is capable of exchanging solutions in the recording chamber without interrupting the recording process or rupturing the membrane. However, most researchers do not attempt to make recordings while perfusing since this is likely to result in a broken membrane.

Several techniques for solution exchange are available. These include gravity feed, pump driven devices, or manually-applied pressure driven systems. In general, fresh solution is added to the bottom of the recording chamber while the perfusate is removed from the top. Warner Instruments has developed the **BPS-2**, an easily assembled 'traditional' perfusion system, which integrates well with our Bilayer Workstation.

Oscilloscope

While many investigators use software emulated display devices coupled to their acquisition hardware to view data during acquisition, others rely on dedicated instrumentation for this purpose. These dedicated instruments include chart recorders and oscilloscopes.

The primary advantage of an oscilloscope over a chart recorder is one of speed. A chart recorder, however, produces a permanent record that is lacking in an oscilloscope. Software emulation can model either of these hardware devices. Regardless of whether the investigator uses a chart recorder, an oscilloscope, or a software emulated device, the data is previewed during acquisition and is stored for subsequent analysis.

INITIAL TEST

This section describes the basic setup for incorporating the amplifier and headstage into the BLM workstation. Procedures for testing the performance of the **BC-535** immediately follow.

Amplifier setup

The headstage connects to the amplifier via a 1.8 meter cable. It is a good idea to route the cable through a short shield (anti-wave guide) prior to its entering the Faraday cage. The presence of the shield will have no effect on the signal received by the amplifier but can help reduce the amount of electromagnetic interference entering the Faraday cage through the opening.

Since movement of the headstage can appear as a fluctuating stray capacitance at the headstage input, the headstage should be rigidly mounted to a fixed support. The headstage is shipped mounted to a platform that can be attached to various holders or micro-manipulators. In addition, the headstage should be placed as close to the preparation as possible to reduce noise due to increased input capacitance.

Overview

The procedures described here are provided to verify the functionality of the **BC-535**. These procedures should be performed when you first acquire the amplifier and can also be used re-assess the performance of the amplifier at a later time.

To perform these tests you will need:

- **BC-535** Bilayer Clamp amplifier
- included headstage
- included model membrane
- Faraday cage
- an oscilloscope (storage scope if available)
- 2 BNC connector cables

Initial conditions

Verify that the amplifier is disconnected and the power switch is *off*.

Place the HEADSTAGE into the Faraday cage. If using a small (1 cu ft or less) Faraday cage, then connect the cage to the GROUNDING JACK (*green plug*) on the HEADSTAGE. If using a larger Faraday cage, then make a ground connection from the cage enclosure to the coupled CHASSIS/CIRCUIT GROUNDS on the rear of the **BC-535**.

Connect the headstage to the amplifier via the input on the rear panel. Connect the provided power cable from the amplifier to your line source. You are now ready to begin the functional check of the **BC-535**.

CAUTION: Connection of the **BC-535** to the wrong line voltage could result in severe damage to the instrument. Therefore, before connecting the amplifier to the power source, check the serial number label on the rear of the amplifier for its voltage rating. Prior to turning on the **BC-535** for the first time, verify that the line voltage is correct for the instrument. If the instrument voltage rating is incorrect for your area, contact our Service Department.

Set all controls on the instrument to the values specified below.

Control block	Control	Setting
HOLD	OPERATE TOGGLE	*standby*
	COMMANDS APPLIED TOGGLE	*off*
	VOLTAGE UP/DOWN TOGGLES	*adjust until meter reads 0*
	COMMAND INPUT FRONT/REAR TOGGLE	*off*
	COMMAND INPUT ATTENUATION TOGGLE	*x0.01*
OFFSET	AUTOZERO	*inactive* (ACTIVE LED is *off)*
METER	SELECTOR SWITCH	ΣV_c
OUTPUTS	GAIN SELECTOR	*10 mV/pA*
	FILTER SELECTOR	*5 kHz*
	FILTER TOGGLE	*active*
	AUDIO TOGGLE	*off*
CAP COMP	ALL CONTROLS	Set to *zero*

Set the oscilloscope to the following settings:

- Connect V_cx10 OUTPUT to the input of your oscilloscope.
- time base to 5 ms/div
- voltage base to 0.2 V/div
- auto trigger
- DC coupling on the input channel

Turn on both the **BC-535** and the oscilloscope.

Hold voltage test

- Connect the model membrane (**MC-1**) to the headstage inputs and the green wire from the model membrane to the headstage grounding jack. Insure that the Faraday cage remains grounded, either through the headstage grounding jack or to the amplifier circuit grounding post.
- Switch the STANDBY/OPERATE switch to *operate*.
- Switch the SELECTOR SWITCH in the METER block to *offset.*
- *Activate* the OFFSET CONTROLS in the OFFSET block by moving the UNLOCK toggle to the *unlock* position. The ACTIVE LED will *light* and the toggle switch will return to its original position.
- Adjust the MANUAL INPUT OFFSET control (the twiddle-knob under the LOW/HIGH LEDS in the OFFSET block) until the meter in the METER block reads *0 mV*.

- *Deactivate* the OFFSET CONTROLS by moving the UNLOCK toggle to the *unlock* position. The ACTIVE LED will go *off* and the toggle will return to its original position. The offset reading on the METER in the METER block will not change.
- Adjust the position of the trace on the oscilloscope to a convenient reference line.
- Switch the SELECTOR SWITCH in the METER block to ΣV_c.
- Using the VOLTAGE UP/DOWN TOGGLE switches in the HOLD block, adjust hold potential until the meter reads *20 mV*.
- Switch the COMMANDS APPLIED toggle in the HOLD block to *on*. The COMMANDS APPLIED LED will light.
- Notice that the displayed voltage on the **oscilloscope** moves from zero to 0.2 V (one division upward).
- Notice that the METER also shows *20 mV*. This reading is correct since the V_c X 10 OUTPUT is the applied V_m HOLD multiplied by 10 (e.g.: 20 mV x 10 = 0.2 V).
- Switch the amplifier to *standby*. Notice that the applied voltage appears at the headstage input even when the amplifier is in *standby mode* or when the headstage has open inputs (i.e. the oscilloscope trace doesn't change when amp is in standby).
- Make similar adjustments to the hold control to assure yourself that the amplifier performs as expected.
- Switch the COMMANDS APPLIED toggle in the HOLD block to *off*.
- Using the VOLTAGE UP/DOWN TOGGLE switches in the HOLD block, adjust hold potential until the meter reads *0 mV*.
- Place the amplifier into *standby*.

Input noise test without model membrane

- Remove the model membrane. Insure that the Faraday cage remains grounded, either through the headstage grounding jack or to the amplifier grounding posts.
- Move the BNC connection from the V_m X 10 OUTPUT to the I_m OUTPUT (in the OUTPUTS block) and monitor the I_m OUTPUT with the oscilloscope.
- Set the amplifier GAIN to *100 mV/pA* and the oscilloscope voltage base to 50 mV/div.
- Verify that the FILTER TOGGLE is *active* and switch the FILTER control to *1 kHz*.
- Verify that the COMMANDS APPLIED toggle in the HOLD block is set to *off*.
- Switch the STANDBY/OPERATE switch to *operate*. You should observe that the noise level decreases to no greater than 30 mV p-p. At a gain setting of 100 mV/pA this corresponds to a maximum noise level of 0.043 pA RMS.
- Place the amplifier into *standby*.

Input noise test with model membrane

- Connect the model membrane (**MC-1**) to the headstage inputs and the green wire from the model membrane to the headstage grounding jack. Insure that the Faraday cage remains grounded, either through the headstage grounding jack or to the amplifier grounding posts.
- Set the I_m GAIN on the amplifier to *5 mV/pA*.
- Set the oscilloscope voltage base to *10 mV/div*.
- Verify that the filter is set to *1 kHz*.
- Verify that the COMMANDS APPLIED toggle in the HOLD block is set to *off*.

- Switch the STANDBY/OPERATE switch to *operate*.
- You should observe a noise level of no greater than 6 mV p-p, equivalent to 0.171 pA RMS at this gain setting.
- Place the amplifier into *standby*.

Test instrument I_m output

- Switch the SELECTOR SWITCH in the METER block to I_m.
- Set the amplifier GAIN to *20 mV/pA*
- Set the oscilloscope voltage base to 0.5 V/div.
- Using the UP/DOWN toggle switches in the HOLD block, adjust the V_m HOLD until it reads *40 mV*.
- Set the COMMANDS APPLIED toggle to *on*. The COMMANDS APPLIED LED will light.
- Set that the STANDBY/OPERATE switch to *operate*.
- Verify that the METER reads *40 pA.*
- Verify that the oscilloscope reads 0.8 V (or 1.6 div), equivalent to 40 pA at this gain setting. (40 pA x 20 mV/pA = 800 mV on oscilloscope)
- Adjust the gain, oscilloscope, and holding potential settings to verify that the instrument is working properly.
- Set the COMMANDS APPLIED toggle to *off*.
- Using the UP/DOWN toggle switches in the HOLD block, adjust the V_m HOLD until it reads *0 mV*.
- Place the amplifier into *standby*.

Cap test

- With the model membrane still connected, set the oscilloscope voltage base to 50 mV/div.
- Set the I_m GAIN on the amplifier to *1 mV/pA*.
- Place the amplifier into *operate*.
- Activate CAP TEST mode by setting the METER SELECTOR SWITCH to *cap test*.
- The I_m OUTPUT will now be a 100 Hz square wave with p-p amplitude of approximately 100 mV. This corresponds to a 100 pF membrane at this gain setting.
- Verify that the meter reports the model membrane capacitance of 100 pF.
- Place the amplifier into *standby*.

Autozero

- Set the oscilloscope voltage base to *100 mV/div.*
- Set the instrument GAIN to *10 mV/pA.*
- Switch the STANDBY/OPERATE switch to *operate*.
- Activate the OFFSET CONTROLS by moving the OFFSET TOGGLE into the *unlock* position. The ACTIVE LED will *light* and the TOGGLE will return to its original position.
- Switch the METER SELECTOR to *offset* and verify that the offset potential is *0 mV* (viewed in the METER section). If not, adjust to 0 mV using the MANUAL OFFSET CONTROL.
- Adjust the oscilloscope signal to a convenient reference point.
- Using the MANUAL OFFSET CONTROL, increase the offset potential to *10 mV*.
  (**Note**: Fine adjustment of the offset setting can be achieved by *pushing in* the OFFSET CONTROL while turning.)

- Verify that the signal on the oscilloscope moved 1 div upwards. This corresponds to a *10 pA* offset at this gain setting.
- *Depress* the AUTO-ZERO pushbutton.
- The ACTIVE LED will *flash*, indicating operation of the AUTOZERO function.
- Verify that the I_m OUTPUT signal on the oscilloscope returns to *baseline*.
- Verify that the OFFSET reading on the METER returns to *0 mV* and that the ACTIVE LED is *off.*
- Switch the STANDBY/OPERATE switch to *standby*.

Capacity compensation

Function of the capacity compensation circuit can be checked by attaching an externally generated square wave to the FRONT COMMAND IN BNC. Application of the signal to the model membrane is achieved by switching the COMMAND INPUT TOGGLE to *front*.

- Apply a square wave to the model membrane and observe the resulting signal on the oscilloscope. You will see a large transient on the leading edge of both the upward and downward excursions of the signal. This transient (or spike) represent the large amplitude current associated with the charging of the membrane capacitance.
- Adjusting the CAP COMP FAST AMPLITUDE control *clockwise* will reduce the spike. Slowly rotate this the control until a minimum is observed (this should be at a reading of approximately 2.0 on the amplitude dial, indicating a 100 pF capacitor).
- If necessary, adjust the FAST TIME CONSTANT control (directly below the FAST AMPLITUDE control) to further minimize the amplitude of the spike.
- Additional adjustment of the SLOW CONTROLS may be necessary to completely minimize the transient.
- Continue adjusting both controls until the overshoot is removed.
- Set the OPERATE/STANDBY switch to *standby* and disconnect the model membrane.

This completes the instrument check out.

OPERATION

The general procedure is to first set up the bilayer chamber, add solutions, and make electrical contact. This is followed by adjusting the input offset and forming the bilayer membrane. The strategy for incorporating channel containing membrane vesicles to the bilayer membrane will depend on the system under study, but will normally proceed by adding vesicles or purified protein to one side of the membrane under the appropriate ionic and/or osmotic conditions. Once a channel has been successfully incorporated into the bilayer the solutions are perfused and initial experimental conditions established. At this point recording of data proceeds.

Setup of the bilayer chamber

Bilayer membranes are formed across an aperture in a septum which separates two chambers. The most common configuration is that of a cup (which supports the aperture) placed inside a holder. The interior of the cup represents one chamber while the interior of the holder is the other chamber. The cup wall is the septum. Electrical connections are made via agar salt bridges into each chamber. The whole assembly must be shielded from electrical and vibrational interference to obtain low noise recording of bilayer currents.

The aperture is prepared to accept lipids prior to membrane formation. This is achieved by 'coating' the hole with the lipid cocktail before adding solutions to the cup or chamber. Several techniques are employed to coat the hole prior to membrane formation. While the choice of technique will depend on your application, the materials at hand, and your ingenuity and training, once the hole has been coated, the cup is inserted into the chamber and both the cup and chamber filled with the appropriate solutions. Two methods are presented below.

One method to coat the hole uses a small (1-2 mm) ball formed on the end of a glass rod or Pasteur pipette with a Bunsen burner. The rod is dipped into the lipid mixture and a coating of lipids is applied to the outside rim of the hole. An advantage of this technique is that it is relatively easy to keep the glass rod, and hence the resulting membrane, free from contamination.

An alternative method is to insert several lipid-covered hairs from a Red Sable paintbrush through the aperture. The brush is then revolved in a small circle until the hole is uniformly coated with lipid. (Use a size 00 or 000 Red Sable artists dotting brush which has been trimmed to present 3-5 hairs of the same length. The brush is cleaned and dipped into the lipid cocktail before coating the hole.) As suggested above, a disadvantage to this technique is that the brush can easily become contaminated over time or through misuse.

The headstage leads should not be directly connected to the bathing solutions. Instead, leads are routed to wells containing a salt solution which are in turn connected to the solution baths via agar salt bridges. The salt bridge wells should ideally contain the same solution used in the formation of the salt bridge, usually 1 M KCl. In addition, these wells should be adjacent to the baths so that the agar bridges used to complete the circuit from well to bath are as short as possible. The supplied sliver-wire electrodes require chloride-plating prior to their first use and insertion into the salt bridge wells. (*See* **Chloriding electrodes**, Appendix.)

Input offset

Prior to forming a bilayer membrane, the resistance of the aperture is exceedingly low, as small as 1 kΩ. Consequently, an input voltage as small as 1 mV can induce a large currents exceeding 1 nA to flow. These currents are much larger than the pA currents typical in single channel recording and can overload the headstage.

Alternatively, a series resistance within the electrical pathway can introduce a bias in the voltage applied to the membrane resulting in a systematic offset in the data acquired. This offset will appear as a junction potential.

It is therefore important to adjust the instrument input offset potential to compensate for these conditions.

Input offset adjustment

The INPUT OFFSET control provides up to ±120 mV DC at the headstage to compensate for input errors and solution junction potentials. The **BC-535** has an AUTOZERO circuit which greatly simplifies adjustment of input offsets. Manual adjustment of the offset to zero can also be achieved using the associated rotary control and is aided by the input overload LED's marked *high* and *low*.

In any case, junction corrections are performed in the absence of a membrane.

If using the AUTOZERO circuit (recommended), then simply activate the circuit and press the AUTOZERO button. The amplifier will cycle through the setting, find the offset potential, and disarm the AUTOZERO circuit. The results of the calculation (the applied offset voltage) will be presented to the METER when the SELECTOR SWITCH is in the *offset* position.

If using the manual approach, the control should be advanced slowly since a small change in rotation will result in a large change in the current through the open aperture. When both LED's are off, the offset voltage will be near zero and the I_m current can be accurately read on the METER or from the I_m OUTPUT BNC. In general, it will be extremely difficult to set the I_m current precisely to zero. It is sufficient to adjust the input offset until both the *high* and *low* LED indicators are both unlit.

At this point the OFFSET METER reading will indicate the input to bath ground potential difference. If this potential difference is large (greater than 10 mV for normal Ringer solution), then it is advisable to clean and re-chloride the silver wire electrodes (*see* **Chloriding electrodes**, Appendix) and check the agar bridges for deterioration or bubble formation. The potential difference reading should be noted prior to forming the bilayer membrane and rechecked at the end of the recording session to determine stability of the electrodes.

Note: Do not make adjustments to the INPUT OFFSET control once a membrane has been formed as this will introduce a bias into your data.

Bilayer formation

Current through the open aperture will be quite high until lipid covers it. Therefore, by monitoring I_m in the presence of a small applied voltage, you can easily determine when the aperture is covered. The **BC-535** has an integral audio amplifier to aid in monitoring membrane formation. In general, there will be a dramatic drop in current as soon as lipid has filled the aperture and the audio signal will change accordingly.

To observe the formation of a membrane, set the HOLD TOGGLE (in the HOLD block) to the *off* position, place the OPERATE/STANDBY switch in the *operate* position, and turn the METER SELECTOR SWITCH to *cap test*. Prior to membrane formation, and if the aperture is not occluded (with a bubble, for example), the METER will report *0 pF*. In addition, the triangular wave generated by CAP TEST will induce a trans-aperture current which exceeds the operational range of the amplifier input. This results in the appearance of a full scale (10 V p-p) pseudo-square wave at the I_m OUTPUT.

During membrane formation, the initial covering of the aperture by lipid dramatically decreases the amplitude this square wave allowing you to observe the formation of the bilayer on an oscilloscope. Additionally, the audio signal will change character and the METER will begin to report an increasing capacitance. As the bilayer continues to form you will observe a time-dependent increase in the amplitude of the square wave on the oscilloscope representing an increase in membrane capacitance.

In all cases, the capacitance increase is proportional to the area of the forming membrane and so allows you to observe both the size and stability of the bilayer formed. (For additional discussion *see* **Membrane capacitance calculations**, Appendix.)

If you are using the brush technique, then the bilayer membrane is initially formed by painting lipids across the hole. This is achieved by dipping a clean brush in the lipid cocktail and drawing a thin lipid film across the open aperture (reminiscent of making bubbles when you were a kid). Alternatively, the membrane can be formed by momentarily occluding the hole with the end of the lipid-coated glass rod. In either case, the lipids will initially occlude the hole in a thick layer. After a short time (several seconds to a few minutes), excess lipids will drain away from the hole until a bilayer is formed. The area of the forming membrane can be monitored on an oscilloscope or on the meter. In general, several attempts of the above procedure may be necessary before a stable membrane is formed.

Once the membrane has formed and appears stable, CAP TEST should be turned off and the leak conductance of the membrane checked. A good membrane will have a conductance of less than 10 pS (i.e., 1pA/100mV).

Commands

Once a stable membrane is formed, the appropriate ionic and/or osmotic conditions are established and channel bearing vesicles are added. The system is monitored in the presence of a transmembrane holding potential for a vesicle 'fusion event'. Once a channel has incorporated into the bilayer membrane the solutions are quickly perfused to prevent further vesicle fusions and the appropriate experimental conditions established.

Command voltages to the bilayer membrane are effected by the V_m HOLD control, by an externally applied signal (CMD IN) or by a combination of the two. V_m HOLD provides a DC potential of either polarity up to 400 mV. External signals at CMD IN are attenuated by *x0.1*, *x0.01*, or *x0.001*. Therefore, a 10 V DC signal at CMD IN results in an applied voltage at the headstage of 1000, 100, or 10 mV, respectively, for attenuation settings of *x0.1*, *x0.01*, or *x0.001*.

APPENDIX

Theoretical considerations

Shielding

Proper shielding of all cabling and recording apparatus are important in maintaining a large signal-to-noise ratio. The necessity of a high quality Faraday cage to protect the headstage from stray input signals cannot be over emphasized. If the noise levels are still unacceptably high after shielding, it may be possible to further reduce noise by wrapping all wiring between the Faraday cage and amplifier in aluminum foil and grounding the foil cover. However, under normal circumstances this should not be necessary. If the user chooses to wrap wiring in foil, care should be taken to induce stray capacitance due to movement of the aluminum foil shield.

Grounding

Since a large signal-to-noise ratio is important in single channel recording, the effort to eliminate ground loops in the circuit wiring gains significance. If your Faraday cage encloses a number of devices (e.g., microscope, stirrers, stepper motors, etc.), then the most common procedure is to create a central grounding location within the cage to which all instrumentation is attached. This is most readily achieved by the formation of a "star ground" and is diagrammed in the upper figure on the next page (central node grounding scheme). Mount a solid brass bar with a number of attachment points to the inside of the Faraday cage and connect the cage and grounds of all devices within the cage to this bar. The bar is then connected via a 14-16 gauge braided copper wire to an external central ground point which acts as the absolute reference for all devices. A bar of this type is provided in Faraday cages supplied by Warner.

Note: If the Faraday cage is grounded to the star ground, then do not ground the cage to the headstage.

Alternatively, if you use a small Faraday cage which does not contain numerous devices, then you can greatly simplify the circuit wiring by connecting the Faraday cage directly to the headstage ground jack. This design is also diagrammed on the next page (common mode grounding scheme). The major differences between this scheme and the one described above is 1) the Faraday cage is grounded through the amplifier headstage and 2) the Faraday cage is not connected to the external ground.

Note: Do not connect the Faraday cage to any other ground point.

The choice of which configuration to use depends on the number of components available and the response of your system to noise inputs. In general, it is better to use the central node grounding scheme since any attached devices will tend to generate large induced currents in the ground plane which can overwhelm the headstage. Nevertheless, if your setup is very simple, then grounding the Faraday cage through the headstage can provide a simpler circuit with lower noise.

For either configuration, the ground for all external devices should be attached to a single external ground point. The **BC-535** has two grounding posts on the rear of the instrument, one for CIRCUIT GROUND and one for CHASSIS GROUND. The instrument is shipped with these two grounds

connected together, or bridged. We recommend severing this connection and only using the CIRCUIT GROUND.

The schemes shown below and described above indicate connection of the **BC-535** CHASSIS GROUND to an external ground point. However, it is often advantageous to try different ground configurations with the **BC-535** to determine which works best in your environment.

Note: We do not recommend connecting the **BC-535** CIRCUIT GROUND (directly or indirectly) to the oscilloscope chassis ground. Doing so will create a ground loop and increase noise levels within the data. This condition can be avoided by disconnecting the oscilloscope chassis ground from the common ground point when connecting the **BC-535** CIRCUIT GROUND to the external ground.

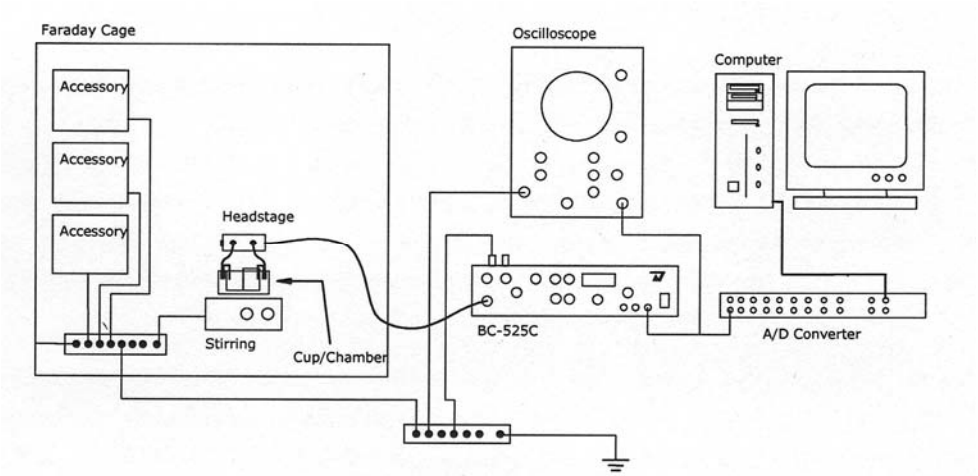


Figure 2a. Central node grounding scheme. Note the grounding block within the Faraday cage is connected to the external star ground point and the headstage is not externally grounded.

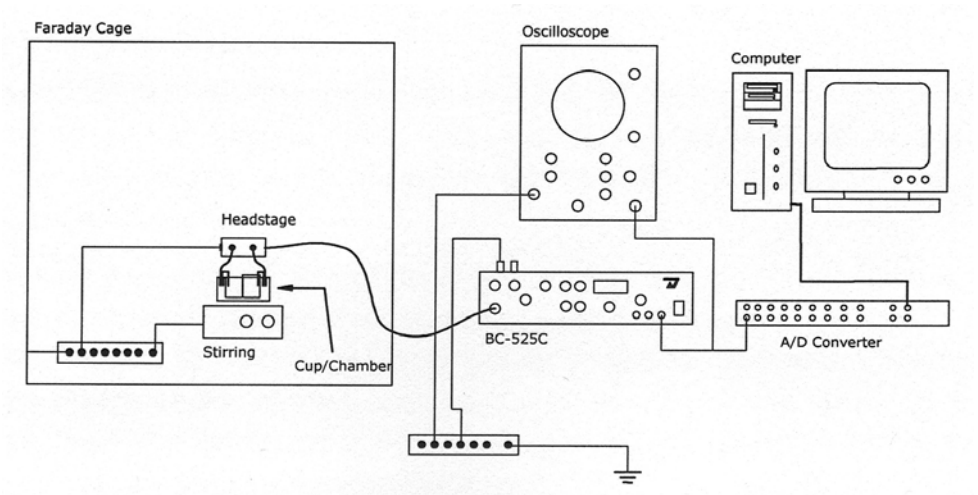


Figure 2b. Common mode grounding scheme. Note the grounding block within the Faraday cage is directly connected to the headstage. This mode only works for small cages containing few devices due to the ground currents involved.

Membrane capacitance calculations

It is possible to theoretically derive an equation to determine the size of the bilayer formed across the aperture. While this equation will probably not yield an exact result (most likely due to variation in the dielectric constant of your lipid mixture), it will give a reasonably approximate result.

Recall that we examine the formation of the bilayer by applying a triangular wave to the membrane and observing a square wave at the I_m output. The reason we see a square wave is that a capacitor returns the derivative of the applied voltage, as shown in the equations on the next page. However, under normal circumstances you will most likely dispense with a calculation and visually determine if the membrane size is appropriate by examining the amplitude of the square wave on the oscilloscope.

From physics, we know that the equation describing the capacitance of a parallel plate capacitor in the MKS system of units is

$$C = \varepsilon \frac{A}{d} \qquad (1)$$

where C is the capacitance (in Farads), ε is the dielectric constant of the material between the plates, A is the area of the plates and d is the plate separation (both in meters). Likewise, we know that that the steady-state charge on a capacitor can be expressed as

$$q = CV \qquad (2)$$

where q is the charge on one capacitor plate (in Coulombs) and V is the potential between the plates (in Volts). Equation (2) can be dynamically expressed by taking the time derivative of the charge, thus

$$i = C \frac{dV}{dt} \qquad (3)$$

where the current $i = \frac{dq}{dt}$ is defined as the time rate of change of the charge. Substituting equation (1) into equation (3) yields the general equation,

$$i = \varepsilon \frac{A}{d} \frac{dV}{dt}. \qquad (4)$$

Recall that a bilayer membrane is electronically represented as a capacitor, and that we monitor the forming bilayer through the application a triangular wave. Since a triangular wave, by definition, has a constant rate of change of applied voltage, $\frac{dV}{dt}$ is constant. Likewise, since ε is an intrinsic property of the lipid mixture, it is also constant.

Now consider the forming bilayer membrane. Once a sufficient quantity of lipids have drained away from the aperture, the remaining lipids begin to form a bilayer. Since the distance, *d*, separating both sides of the membrane (the plates of our hypothetical capacitor) is fixed by the length of the lipid tails, this term will also become constant. Therefore, the only remaining variable on the right side of equation (4) is the area, *A*, of the forming bilayer. Thus we can express our equation as

$$i = kA \qquad (5)$$

where *i* is the current appearing at the I_m output, *k* is a constant of proportionality, and *A* is the area of the forming membrane. It should be apparent from the preceding discussion that the magnitude of the current, and hence the amplitude of the resulting square wave, is linearly proportional to the area of the bilayer membrane.

Since the amplifier output is scaled to 1 mV/pF when the **BC-535** is in CAP TEST mode, application of the preceding discussion to the amplifier indicates that the measured capacitance of a membrane is simply the amplitude of the square wave (expressed in mV) divided by the instrument gain. For example, a 100 pF membrane would yield a 1000 mV square wave (p-p) when the amplifier gain is set to 10 mV/pA.

Suggested References

1. *Ion Channel Reconstitution* edited by C. Miller, Plenum Press, New York (1986). In particular, chapter 5, "How to set up a bilayer system" covers many important aspects on the subject.

2. *Single-Channel Recording* edited by B. Sackman and E. Neher, Plenum Press, New York (1985).

3. Reconstituting channels into planar membranes: a conceptual framework and methods for fusing vesicles to planar bilayer phospholipid membranes. F.S. Cohen and W.D. Niles, *Methods in Enzymology*, **220:**50-68 (1993)

4. Planar bilayer recording of ryanodine receptors of sarcoplasmic reticulum. R. Coronado, S. Kawano, C.J. Lee, C. Valdivia, and H.H. Valdivia, *Methods in Enzymology*, **207:**699-707 (1992)

Specifications

Noise:	Measured with 8 pole Bessel filter at specified cutoff frequency		
	Frequency range	Open input	100 pF at input
	DC to 1 kHz	0.060 pA RMS	0.82 pA RMS
	DC to 100 Hz	0.009 pA RMS	0.28 pA RMS
Bandwidth:	75 kHz		
Input commands:	HOLD:	Digital; 1 or 10 mV steps to ± 400 mV maximum	
	COMMAND IN:	Front and rear external input, 10 V/V (applied voltage is attenuated by 10/100/1000 at the command electrode)	
Junction zero:	AutoZero or manual adjust. Offset lockout feature. Cycle time 1.5 s. Correction to ± 120 mV		
Audio:	VCO with off switch and volume control. Internal speaker, and external speaker output.		
Capacitance test:	Triangle wave applied to command electrode. Derived membrane capacitance displayed on meter up to 1000 pF. Calibrated (1 mV/pF) square wave available at I_m output. Cap Sync (rear panel) synchronized with input triangle wave.		
Gain:	Membrane current gain selectable from 0.5 to 1000 mV/pA in 1-2-5 steps.		
Filter:	4-pole Bessel, selectable from 0.5 to 200 kHz in 1-2-5 steps, or bypassed for full amplifier bandwidth.		
Capacity compensation:	FAST (0-10 μs) and SLOW (0-10 ms) with adjustment of amplitude and time constant for each range. Maximum compensation 500 pF.		
Headstage:	Switching		
	Low current mode	50 GΩ feedback, 100 pA maximum current	
	High current mode	500 MΩ feedback, 2 nA maximum current	
I/O:	Front panel:		
	Command Input:	BNC input up to 10 V. Attenuated by 10, 100, or 1000.	
	I_m output:	Membrane current scaled by amplifier gain setting.	
	$V_c \times 10$ output:	Applied command voltage × 10	
	Rear panel:		
	I_m output:	Membrane current scaled by amplifier gain setting.	
	Cap Sync:	TTL compatible	
	Capacitance output:	Reports calculated membrane capacitance scaled to 1 mV/pF	
	Command Input:	BNC input up to 10 V. Attenuated by 10, 100, or 1000.	
	Gain telegraph:	Stepped DC voltage 0.5 to 5.5 V in 0.5 V steps for gain settings of 0.5 to 1000 mV/pA. Telegraph value of 0 V for standby mode.	
	Filter telegraph:	Stepped DC voltage 0.5 to 4.5 V in 0.5 V steps for filter settings of 0.05 to 20 kHz. Telegraph value of 5.0 V for full bandwidth (bypass) mode	
	External speaker:	Standard RCA jack	

Specifications (continued)

Digital meter:	3.5 digit LED	± 1999 mV full scale
	Junction offset:	± 120 mV full scale
	Cap Test:	0 to 1999 pF
	V_c :	± 1999 mV full scale
	I_m :	± 1999 pA full scale
Power:	100-125 or 220-240 VAC, 50/60 Hz	
Dimensions:	H × W × D	
	Case :	9 × 42 × 25 cm; (3.5 × 16.5 × 10 in)
	Headstage :	2.3 × 2.8 × 5.8 cm (0.9 × 1.1 × 2.25 in) 1.8 m connecting cable
Operating Conditions:	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%

Chloriding electrodes

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 Cl^- ions in solution. The chemical reaction is:

$$Cl^- + Ag \Leftrightarrow AgCl + e^-$$

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

The **BC-535** is shipped with two silver wires which must be chlorided prior to use. Over time, the AgCl coating on the wires will deteriorate. This will be most apparent as a gradual increase in the value of the junction potential seen at the beginning of each experiment. In addition, the electrodes may lose their purple-brown color. Once it has been determined that the electrodes require cleaning, the oxide should be removed and re-applied.

Techniques for chloriding silver wires

Before using Ag^+ wire as a current or voltage electrode, it must first be chlorided. New (previously unused) wire should be cleaned with ETOH before chloriding. Previously chlorided wire should be cleaned before re-chloriding.

Two methods for chloriding most commonly used are the plating techniques described below. These are soaking in household bleach or electroplating using a voltage source. As with a new wire, clean the wire with ETOH before proceeding to remove finger oils.

A) Soaking in bleach - This technique places a very useable, but relatively thin coating on the wire. Simply immerse the clean wire in full strength common household bleach (Clorox) for 5-15 minutes or until a purple-gray color is observed. Rinse and use.

B) Electroplating – While this technique requires more effort, it places a thicker and more uniform coating on the silver wire. Electroplating a silver wire with chloride is achieved by making the wire positive with respect to a solution containing NaCl (0.9%) or KCl (3M) and passing a current through the electrode at a rate of ~1 mA/cm^2 of surface area for 10-15 seconds or until adequately plated. A 1 cm length of 1 mm diameter wire will require approximately 0.3 mA. The color of a well plated wire will also be purple-gray. Periodic reversal of the polarity while plating the electrode tends to yield a more stable electrode.

When electroplating a previously plated wire, you may find that it does not plate evenly. Complete removal of the residual silver chloride is often necessary to effect a uniform coat. Before making the wire positive to the chloriding solution, reverse the polarity for 5-10 seconds to remove any remaining chloride that might be left in pits on the wire. Then proceed as described above.

Accessories and replacement parts

Description	Model No.	Order No.
Classic bilayer chamber, 1 ml working volume	**BCH-13A**	**64-0400**
Classic bilayer cups, 1 ml working volume. Available in various materials and aperture sizes. 150 um aperture size shown.	**CD13A-150** **CP13A-150** **CF13A-150**	**64-0410** **64-0404** **64-0416**
Classic bilayer chamber, 3 ml working volume	**BCH-22A**	**64-0401**
Classic bilayer cups, 3 ml working volume. Available in various materials and aperture sizes. 150 um aperture size shown.	**CD22A-150** **CP22A-150** **CF22A-150**	**64-0413** **64-0407** **64-0419**
Perfusion bilayer chamber, 1 ml working volume	**BCH-P**	**64-0423**
Perfusion bilayer cups, 1 ml working volume. Available in various materials and aperture sizes. 150 um aperture size shown.	**CD-P150** **CP-P150**	**64-0427** **64-0424**
A dynamically variable single channel simulator. 100 pF capacitance.	**CM-3/100**	**64-0027**
A non-variable version of the CM-3. 100 pF capacitance.	**CM-1/100**	**64-0025**
Bare silver wire, 1 mm pins with attached, 10 cm, 2 ea	**WA10-5**	**64-1327**

Warranty

The model **BC-535** 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.

Service

We recommend that all questions regarding service be referred to our Technical Support Department. Normal business hours are 8:30 AM to 5:00 PM (EST), Monday through 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** or through our Web site at **http://www.warneronline.com**.

Service notes

1. 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, call Warner Instruments for assistance.

2. 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.

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
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Instrument Amplifier
Equipment Class:	ITE-Class A
Model Numbers:	BC-535

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: Ralph Abate

Position: Managing Director

Declaration of Conformity

CE MARKING (LVD)

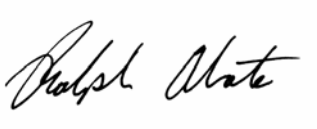
Application of Council Directive: 73/23/EEC

Standards To Which Conformity Is Declared:	EN61010-1:1993
Manufacturer's Name:	Warner Instruments
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:	BC-535

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: Ralph Abate

Position: Managing Director

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 strength of the signal is attenuated by 3 dB (10-fold decrease in power). 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.

BLM – Historically, Black Lipid Membrane from the effect of interference at the upper and lower faces of the thin film formed resulting in cancellation of all visible wavelengths. When the membrane thinned appropriately, it would 'disappear' or become black. Alternatively, Bilayer Lipid Membrane. Many researchers now observe membrane formation electrically and have altered the acronym to mean the molecular bilayer formed from the orientation of lipids such that their polar heads and hydrophobic fatty acid tails are in register. In an aqueous environment, the polar heads face away from the membrane leaving the hydrophobic domains within the bilayer.

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.

COMMAND IN – Also **CMD IN**. Command Input. An external input into the **BC-535** allowing the application of user defined command voltages to the headstage. Connection is via BNC.

command sensitivity – Selectable scaling of CMD IN input. Attenuation values of CMD IN are *x0.1*, *x0.01*, and *x0.001*.

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), 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 the effect 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 membrane – 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.

planar lipid bilayer – *See* BLM.

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 (PCM) – 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.

reset – An operation wherein the collected charge on the integrating capacitor in the headstage is dissipated, readying the system for further use.

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_m 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_c – The user selected potential set in the COMMANDS APPLIED TO REFERENCE block of the amplifier.