

IR-183A & IR-283A

Intracellular Recording Amplifier Instruction Manual

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Warranty

Cygnus Technology, Inc. warrants to the original purchaser that its intracellular recording amplifier, model IR-183/IR-283, and the component parts thereof, will be free from defects in workmanship and material for a period of one year from the date of purchase.

During the warranty period, Cygnus Technology, Inc. will, without charge, repair or replace, at its option, defective product or component parts.

Exclusion: This warranty does not apply in the event of misuse or abuse of the product or as a result of unauthorized alterations or repair. This warranty is void if the serial number is altered, defaced or removed.

DISCLAIMER THIS EQUIPMENT IS NOT INTENDED TO BE USED, AND SHOULD NOT BE USED IN HUMAN EXPERIMENTATION, NOR SHOULD THIS INSTRUMENT BE APPLIED TO HUMANS IN ANY WAY. **Intracellular Recording Amplifier Adjustment Procedure**

Note: The IR183/283 is adjusted at the factory, this procedure should only be done if a known problem exists or the headstage is changed. An oscilloscope and a 10megaOhm resistor are required.

Step 1:

- 1.The power switch must be off (red power light off).
- 2.Connect the headstage.

3. Put a 10MegaOhm resistor between the input connector and the ground terminal.
4. Keep all switches at "off" or "Gnd" (Ground) position and turn all dials to the zero position. All dials should be at the minimum position (knobs completely counterclockwise).
5. Turn on the oscilloscope and set the gain to 10mV/div.
6. Remove the top cover of the amplifier with Phillips screwdriver.
7. Check that the line voltage setting is correct, turn on the amplifier power switch and wait 10 minutes.

Step 2: (Circuit board adjustment)

Caution!!

Do not touch any line voltages!! Do not disturb any wires.

1. Connect the oscilloscope to V-out.
2. Turn Balance (bridge balance) dial to zero.
3. Flip Balance switch back and forth from off to X5 position.
4. If the DC potential change occurs, minimize the difference with Trim Pot VR5
5. Adjust V-out to zero with Trim Pot VR4.
6. Turn Bridge Balance to maximum.
7. Flip Balance switch from "off" to X1.
8. Minimize difference, if any, with Trim Pot VR3.

Caution!!

Do not touch the amplifier inputs without keeping the Input Switch at ground position. The amplifier input is grounded for protection when the Input Switch is in the "Gnd" position. When the Input Switch is in the "on" position, however, the input FET-amplifier is very easily damaged by high voltage and static electricity discharge.. The warranty does not cover input amplifier damage due to high voltage or static electricity discharge.

9. Turn on Input Switch. If V-Out shifts, adjust this to zero with Trim Pot VR2. (Trim Pot VR2 is for current offset adjustment.)

10. Connect I-Monitor to the oscilloscope.
11. Adjust this output to zero with Trim Pot VR-1.
12. Turn off Input Switch

Step 3 (Circuit board adjustment [Applies to Model IR-283 only])

Repeat Steps 1 through 12 for Channel 2.

Step 4

1. Turn off power switch
2. Put back the top cover of control box.

Operation:

Front Panel:

Connect headstage(s) and a remote switch. The headstages of the two channel model are labeled with numbers. Each amplifier channel is specifically calibrated with one headstage. It is not necessary to connect a headstage which is not in use.

Rear Panel:

(One set of inputs and outputs are located on the front control panel of the IR-183.) Voltage outputs(s), I-Monitor(s) to oscilloscope, and I-Command(s) to pulse generator(s). A pulse generator with high voltage output cannot be connected to the 10nA/volt Command In BNC terminal(s). Maximum input voltage of this terminal(s) is +/- 15volts. The 2 nA/volt Command In terminal(s) accepts up to +/- 75 volts maximum. Keep Aux. terminals open.

1. Connect an indifferent electrode wire to the headstage ground.
2. Input switch(s) should be in "off" position(s).
3. Turn on power switch.
4. Set oscilloscope to 100 mV/div. and DC input mode.
5. Mount a micro-electrode to the headstage and advance it into your preparation.
6. Turn input switch to "On".....

Caution!!

Input switch should be in "ground" position if the input is not connected via a load to ground.

Electrode Tip Potential Adjustment:

1. Ground the oscilloscope input, set it to DC mode, and adjust the beam to the center of the oscilloscope.

2. Set the input switch to "on". Adjust trace to the zero position on the screen using the Position potentiometer. The number on the position dial is the electrode tip potential. If the oscilloscope trace does not appear on the screen, the LED trace finder will help you to find it (Beam finder).

3. To locate the trace onto the screen, flip the position Switch in the direction of the LED lamp which is lit (up or down).

4. Adjust the position dial in the direction of the lit LED to move the beam onto the screen (clockwise or counterclockwise direction). If the opposite LED lamp lights, the beam has been move too far.

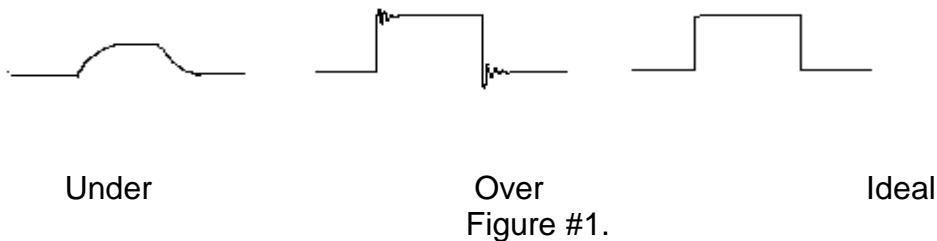
Capacitance Neutralization:

1. Turn on the calibration pulse generator.

*Note: The calibration pulse generator should be placed between the headstage ground and indifferent electrode.

2. Turn on the C-Neutralizing switch.

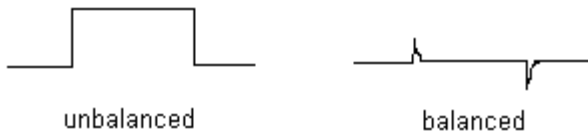
3. By adjusting the coarse and fine C-neutralizing controls, try to obtain a clean square wave calibration pulse. See Figure #1



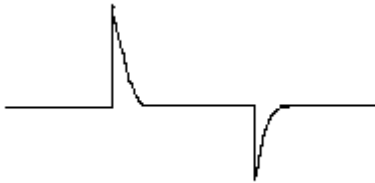
Capacity compensation needs to be increased when advancing the electrode because the electrode capacitance increases as more of it's length enters the bath solution..

Electrode Resistance:

1. Turn on the pulse generator. The pulse generator should not have any DC offset voltage.
2. Make +.5nA, 20ms pulse. (0.5 nA is equal to 50 mV at the I-Monitor.)
3. Flip the Bridge Balance switch to x1 or x5 and turn the dial until the top of the pulse is at the base line level on V-out.

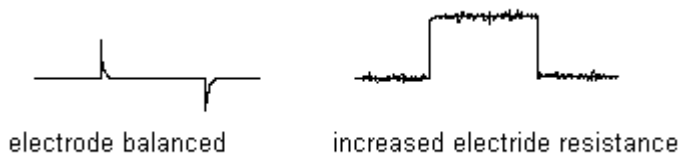


4. Read the dial, this is the electrode resistance value.
You may see transient spikes resembling this:



Turn on the Transient Suppression switch. Adjust the control to minimize the transient noise height.

When advancing the electrode, increased electrode resistance can often be observed. One of the reasons is that the electrode may be touching the cell membrane. This is a good time to activate the Cell Penetration System.

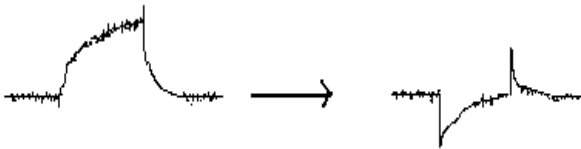


Intracellular Bridge Balance Method

If you need to readjust electrode resistance after the penetration of a cell, use the short pulse bridge balance method (See J. Neurophysiol. 41, 1662-1675, 1978.):

1. Generate a 0.5nA 1ms positive current pulse.
2. The electrode capacitance should be neutralized properly.
3. Adjust the peak potential of the charging voltage change to the baseline with the bridge balance.

The logic behind this is that the electrode time constant is so short that the potential charged is almost maximum during 1ms, but almost zero for the potential charging membrane. Therefore, the adjustment done at 3 is for the electrode resistance unbalanced



Cell Penetration System

An Audio monitor is very helpful with this procedure.

Turn on Cell Penetration Switch and select mode of penetration.

A. High Voltage Mode (HV Mode): This mode is most useful with very high resistance electrodes. Generally, a negative polarity is more effective, so use less intensity. If you are using a dye electrode, polarity must be considered to prevent spilling dye when penetration is attempted. Use the opposite polarity of the dye's charge, or try to use the AC mode. One hit of this HV-mode can inject HRP into the cell without perfect cell penetration.

B. I-Mode: This mode is milder than HV-mode. Control of penetration is easier. Before use of this mode, increase capacitance neutralization a little until you can hear the electrode white noise from your audio monitor. This slight increase of capacitance neutralization will give you a much higher chance of cell penetration success. Negative polarity is more effective in this mode also.

C. AC Oscillation mode (AC-mode): Use your audio monitor to hear the AC tone. Higher frequencies have less intensity than low ones. If the cell diameter is about 15 microns, use the oscillation sound of "hee" (more than 4kHz) or "pee" (2-3 kHz), not "bee" (less than 800 Hz) sound. "Bee..." sound has too much power, so the cell is destroyed easily. Dye spill is less with this mode.

* Total energy can be changed not only by changing intensity, but also by duration.

1. When using HV- or I-mode, start with positive low intensity or short duration.

When using AC-mode, start with high frequency oscillation and shortest duration.

* Too much energy destroys the cell.

2. Make a +0.5nA, 20ms pulse and center it on your oscilloscope. Null the bridge balance.

3. Advance the electrode very slowly until more than 5mV of bridge unbalance occurs in the direction of increasing resistance

4. Push the remote switch. If your electrode has penetrated the cell, you should see a hyperpolarizing potential shift. Even if the resting potential is as low as -30mV, do not give up.

5. If you see a negative potential shift, quickly turn on polarization switch to negative and turn the dial, until the resting potential reaches -70 mV or until it stops firing spikes. (Hyperpolarizing DC current injection.)

Too much hyperpolarization will kill the cell, Do not hyperpolarize beyond -90mV.

6) Increase the current pulse height. If you see action potentials in the voltage recording then the electrode is in the cell.

1) Quickly turn off the current pulse. Give the cell a chance to recover. Wait until

the resting potential begins to increase.

- 2) If the Resting potential begins to increase, reduce the hyperpolarizing DC current. If you are lucky, you may be able to bring the holding current all the way to zero. If not, begin the experiment anyway.
- 3) To protect the cell from accidental injury it is a good idea to turn off the cell penetration system when not in use.
- 4) If you have difficulty catching cells, try to improve the electrode. A good electrode will show a resistance change and an increase in baseline white noise when the electrode is in contact with the cell membrane.

Dye Injection:

- 1) Select injection polarity according to the charge of the dye molecule. Note, only ionic substances can be delivered electrophoretically.
- 2) Select the injection intensity.
 - In the case of Horseradish Peroxidase (HRP), adjust the electrode solution PH to 7.1 with Tris buffer at body temperature and use positive current. Maximum intensity of injection current (nA) could be the same number as the cell diameter in microns, e.g. 10nA for a 10 micron cell for 10 seconds is adequate in many cases.
 - Caution! The PH of Tris buffer can be temperature dependent, be sure to adjust at the preparation's temperature.
 - In the case of Lucifer Yellow, use negative current. Maximum intensity of current (nA) could be set to a tenth of the cell diameter in microns when the dye is dissolved in distilled water, e.g. -2nA for a 20 micron cell.
 - In order to reduce electrode resistance, 1M lithium chloride can be added to a Lucifer Yellow medium. Lucifer Yellow will precipitate by adding K-acetate. In this case you could be able to apply more current for injection.

Caution, large currents can irreversibly block an electrode.

- 3) Choose injection channel.
- 4) Turn off any holding current (Polarize) in the selected channel.
- 1) Turn on the injection switch. This will activate an approximately 2Hz constant current square wave.

Electrode Decoupler (Model IR-283A only)

Theta tube and twisted pair electrodes usually show some signs of common resistance and interchannel capacitance. If a pulse current is injected from one of the channels the other channel will measure a DC (resistive) and AC (capacitive) coupled potential. The "decouple" controls can be used to minimize these artifacts.

The DC decouple control subtracts a scaled version of the other channel electrode current command from the voltage output. The AC decouple control functions similarly but with an AC coupled version of the current command.

Grounding

The amplifier and headstage cases are connected to safety ground through the 3 prong power connector. The signal ground is floated from case ground through a 10 Ohm resistor to help eliminate ground loops. It is suggested that the preparation be grounded only through the ground jack on the headstage.

NOTE: The standard headstage input is a BNC type connector **BUT** the outer conductor is a driven shield and should not be connected to anything other than a shield.