November 1994

Axopatch 200A
PATCH CLAMP

THEORY AND OPERATION

QUESTIONS? Call (415) 571-9400

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VERIFICATION

THIS INSTRUMENT IS EXTENSIVELY TESTED AND THOROUGHLY CALIBRATED BEFORE LEAVING THE FACTORY. NEVERTHELESS, RESEARCHERS SHOULD INDEPENDENTLY VERIFY THE BASIC ACCURACY OF THE CONTROLS USING RESISTOR/CAPACITOR MODELS OF THEIR ELECTRODES AND CELL MEMBRANES.

DISCLAIMER

THIS EQUIPMENT IS NOT INTENDED TO BE USED AND SHOULD NOT BE USED IN HUMAN EXPERIMENTATION OR APPLIED TO HUMANS IN ANY WAY.

LATE INFORMATION

Please check the back of this manual. If there are pages in yellow, these should be read first. They contain errata and information that became available too late for inclusion in the body of the manual.

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INTRODUCTION

The Axopatch 200A is a new generation tight-seal patch clamp for single-channel and whole-cell voltage and current clamping. Emphasizing state-of-the-art technology, the Axopatch 200A achieves superb performance: its noise in single-channel recording is the lowest of any available patch clamp; its series-resistance compensation in whole-cell mode is the most effective of any existing patch clamp.

The Axopatch 200A achieves its ultra-low noise performance by implementing a capacitive feedback technique for single-channel recording. The technology was developed by Axon Instruments engineers over a period of several years, with extensive design and test contributions from scientific consultants.

The capacitive feedback technique is complicated. The headstage output has to be periodically reset every time the voltage across the capacitor increases towards the limits of the power supply of the instrument. Much of the circuitry of the Axopatch 200A is devoted to eliminating the transients introduced by these resets. In most circumstances, the transient elimination is so good that the resets will not affect the recording and the benefits of the capacitor feedback will be available without penalty.

For whole-cell recording, the noise benefits of the capacitive feedback technique are swamped by the noise sources of the electrode and the membrane capacitance. Since the noise benefits cannot be realized, and at the same time the frequency of resets is high because of the larger average currents, the Axopatch 200A uses the traditional resistive-feedback technique for whole-cell recording.

Patch clamping is a powerful technique that permits the direct observations of the behavior of the small ionic currents that flow through a single ion channel protein. To extract the most from the technique, meticulous attention to detail is required. We have designed the Axopatch 200A with great care and we are confident that it is an excellent tool. But the benefits of this instrument will not be realized if the user is not well versed in its operation and at the same time knowledgeable about the experimental techniques. Therefore, we have written this manual with two goals in mind. The first is to explain the operation of the instrument and many of the principles that underlie its design. The second is to provide the user with a guide to experimental techniques that our customers and scientific consultants have found useful during many years of experience in patch clamping.

We hope that you find this manual to be a useful laboratory companion. We aim to revise it periodically and we look forward to receiving any suggestions that you might have for its improvement.

NOTE

The Axopatch 200A is supplied with the U-type headstage(s). This type of headstage only connects with "U" (universal) type adapters, pipette holders and model cells. The U-type design offers several advantages and these are detailed in the section of the manual entitled Holders. The non-U-type headstages can still be used with the Axopatch 200A, but these headstages offer less advantages than their U-type counterpart.

Because Axon Instruments sells replacement pipette holders, adapters and model cells in two varieties, the U-type and the non-U-type, please specify the complete name of the product.

FUNCTIONAL CHECKOUT

When you receive your Axopatch 200A, you should first run a functional checkout to ensure the proper functioning of the instrument. All units are burned-in and thoroughly tested at the factory before shipping. If you observe any damage caused by shipping or if you encounter problems with the functional checkout, please call the factory.

Startup Procedure

For the initial checkout, the Axopatch 200A should be situated on a benchtop away from other equipment. Do not install in a rack until the checkout is complete. Make sure that the power is OFF. An oscilloscope is the only other piece of equipment required for these tests. A large sheet of aluminum foil is needed.

1. The only connections to the Axopatch 200A should be: a) the power cable, b) the headstage, c) a cable from the SCALED OUTPUT BNC to one channel of the oscilloscope.

   Take care to prevent static discharge near the headstage input connector.

   Turn the power on.

2. Set the front panel controls of the Axopatch 200A as follows:

PIPETTE OFFSET:	About 5.0
ZAP	0.5 ms
PIPETTE CAPACITANCE COMP.:	Minimum (fully counterclockwise)
SERIES RESISTANCE COMP. % PREDICTION:	0 %, OFF
SERIES RESISTANCE COMP. % CORRECTION:	0 %, OFF
SERIES RESISTANCE COMP. LAG:	1 μs
WHOLE CELL CAP.:	0 pF, OFF
SERIES RESISTANCE:	0 MΩ
HOLDING COMMAND:	0 mV, OFF
SEAL TEST:	OFF
METER:	Set switch to I
MODE:	V-CLAMP
CONFIG.:	WHOLE CELL
OUTPUT GAIN:	$\alpha = 10$
LOWPASS BESSEL FILTER:	5 kHz
LEAK SUBTRACTION:	T MΩ, OFF

Test the noise:

3. Shield the headstage with a large sheet of aluminum foil. Wrap the foil loosely and completely around the headstage. Leave room at the headstage input for the model cell in the next test. Connect the foil shield to the ground input of the headstage (gold-plated 2 mm socket at the rear of the probe) using a clip-lead. The easiest way to do this is to connect the clip-lead directly to the foil and to the 2 mm pin inserted into the headstage ground socket. (Ground is available from the gold-plated 2 mm socket at the rear of the probe, from the yellow 4 mm socket on the rear-panel of the main unit, or from the BNC shields.)

4. Turn the meter switch to I_{RMS}. Note the reading on the rms noise display for the two headstage configurations (toggle the configuration between the PATCH and WHOLE CELL positions). The expected values under optimal conditions are:

   CV 201AU (1 pF capacitor, 500 MΩ resistor)

PATCH	≈ .070 pA rms
WHOLE CELL	≈ 0.50 pA rms

   CV 202AU (1 pF capacitor, 50 MΩ resistor)

PATCH	≈ .070 pA rms
WHOLE CELL	≈ 1.30 pA rms

   I_{RMS} is shown on the panel meter always in a 5 kHz bandwidth using a Butterworth filter that is independent of the front-panel lowpass Bessel filter. The front-panel gain does not affect this reading.

   If the observed values are more than twice the expected values or if the meter is blanked due to exceeding its range, then check to see that the foil shield is correctly grounded and that all controls are in the positions noted in 2 above. Also check on the screen of the oscilloscope for 60 Hz interference or other noise pickup.

Test the PIPETTE CAPACITANCE COMPENSATION and FILTERS:

6. Set the oscilloscope gain to 0.5 V/div, trigger to line, and the sweep speed to 2 ms/div. Turn on the SEAL TEST switch. You should see oppositely going capacitance transients at about 8 ms intervals (about 10 ms for 50 Hz line frequency). Turn the FAST MAG and FAST τ controls and verify that the capacitance transients change their size and amplitude. Do the same with the SLOW MAG and SLOW τ controls. Switch from PATCH to WHOLE CELL and repeat the above procedures. Switch between the 1, 2, 5, 10, and 50 kHz positions on the LOWPASS BESSEL FILTER control and verify that the capacitance transient signals seen on the oscilloscope screen are affected.

   Turn off SEAL TEST.

Test WHOLE CELL and PATCH configurations:

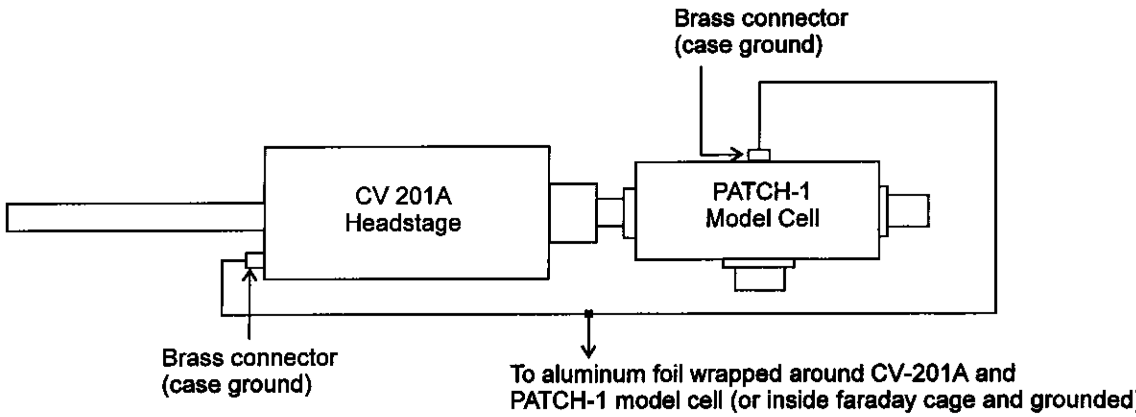


Figure 0: Connections for testing Axopatch 200A and PATCH-1 model cell

7. Remove the foil shield temporarily. Connect the black lead (2 mm pin at each end) to the PATCH-1U model cell ground (2 mm socket at central position) then connect this lead to the ground input of the headstage.

8. Connect the model cell BATH connection to the headstage input. Insert the Teflon collet of the model cell into the collar of the headstage (the fit will be snug). Make sure that the collet is inserted all the way so that the connector pin is fully inserted into the headstage input jack.

9. Shield the headstage and model cell with the foil. Reconnect the foil shield to ground.

10. Place the CONFIG. switch in the WHOLE CELL position. Turn the panel meter switch to the I position. Zero the panel meter using the PIPETTE OFFSET knob. If the meter cannot be easily zeroed within ±10 pA or if it constantly drifts, then the foil shield is probably not correctly connected to ground.

11. Turn the panel meter switch to V_{HOLD}/I_{HOLD} and set the HOLDING COMMAND switch to "+". Turn the HOLDING COMMAND potentiometer until the meter reads 10 mV. Turn the panel meter switch back to I.

    This clamps 10 mV across the 10 MΩ resistor in the model cell BATH position. The panel meter should read the correct current (1 nA) within error limits (2% for the meter, 1% for the resistor and 1% for the HOLDING COMMAND generator plus errors for zeroing, etc.).

12. Turn the HOLDING COMMAND switch OFF. Place the CONFIG. switch in the PATCH position. Zero the panel meter using the PIPETTE OFFSET knob.

    Switch the HOLDING COMMAND to "+". This again clamps 10 mV across the 10 MΩ resistor in the model cell. The panel meter should read 1 nA.

    At this point the INTEGRATOR RESET LED should be illuminated continuously because of the large number of resets needed to pass a continuous current of 1 nA.

Test I-CLAMP and TRACK (I=0):

13. Set mode switch to TRACK and turn the panel meter to V_{TRACK}. The meter should read 0 mV. Turn the PIPETTE OFFSET control one full turn clockwise. Meter should read +50 mV. This is the voltage applied by the TRACK circuit to keep the current at zero.

14. Turn the PIPETTE OFFSET control counterclockwise until the meter again reads 0 mV.

15. Switch mode to I-CLAMP NORMAL. Switch meter to V_{HOLD}/I_{HOLD}. Rotate HOLDING COMMAND control until meter reads 1.0 nA. Turn meter switch to V_m. Meter should read +10 mV. Confirm that you get the same reading when I-CLAMP FAST mode is chosen. You may need to adjust the FAST PIPETTE CAPACITANCE COMPENSATION to remove oscillations.

Test SERIES RESISTANCE and WHOLE-CELL CAPACITANCE COMPENSATION:

16. Set CONFIG. to WHOLE CELL and mode to V-CLAMP.

17. Again remove the foil from the headstage and unplug the model cell. Now connect the model cell in the CELL position to the headstage input connector and reattach the foil making sure it is grounded as before. Turn on the SEAL TEST switch. Again you should see capacitance transients on the oscilloscope screen but now they should be larger and longer lasting. Turn on the WHOLE CELL CAP. switch. Using the SERIES RESISTANCE and WHOLE CELL CAP. controls, observe a change in shape and size of the capacitance transients. The capacity transient must be nulled to read correct values of the membrane capacitance (C_m) and the series resistance (R_s) from these front panel controls.

18. Without completely nullifying the transient, turn on the % PREDICTION control and rotate it clockwise. Observe the capacitance transient getting larger and faster.

19. Now switch off the WHOLE CELL CAP. and turn the SERIES RESISTANCE control to about 10 MΩ. Set the LAG control to its maximum value and then turn on the % CORRECTION control. Rotate this to the right and again observe that the capacitance transients get larger and faster. Set the control to 100%. Rotate the LAG control counterclockwise and verify that the circuit will oscillate at lower LAG values.

Test ZAP:

20. Turn off SEAL TEST, PREDICTION & CORRECTION. Set the ZAP control to MANUAL and set the METER to I. While the ZAP button is depressed you should see a reading of approximately +2.6 nA.

21. Set the ZAP control to 0.5 ms. Depress the ZAP button several times. The OVLD light in the SCALED OUTPUT section should flash each time the button is pushed.

You have now verified that all the circuits are in working order.

USE OF THE PATCH CLAMP - A TUTORIAL

The purpose of this chapter is to ease you into the use of your Axopatch 200A. The controls have been carefully grouped for clarity. Many of them can be switched off and ignored until you become more familiar with the instrument and patch clamping.

Operation of the Axopatch 200A will be described initially in the context of a real experiment while using the PATCH-1U model cell supplied with the unit and illustrated in the *Model Cell* portion of the **REFERENCE SECTION: GENERAL INFORMATION**. An oscilloscope, a step generator, and some aluminum foil will be required.

Note that your headstage contains two feedback elements, a capacitor in the PATCH position and a resistor in the WHOLE CELL position. On each setting, the headstage gain is β mV/pA and this gain is multiplied by the setting on the rotary gain switch, α, to achieve the total output gain. In the CV 201AU headstage β is 1 in both PATCH and WHOLE CELL positions. In the CV 202AU headstage, β is 1 in PATCH position while it is 0.1 in WHOLE CELL position.

The PATCH-1U model cell emulates three experimental configurations:

BATH:	10 MΩ "electrode" resistor to ground.
PATCH:	10 GΩ "patch" resistor to ground. Approximately 5 pF stray capacitance to ground.
CELL:	10 MΩ "electrode" resistor. 500 MΩ "cell membrane" resistor in parallel with 33 pF "cell membrane" capacitor. Approximately 5 pF stray capacitance to ground.

Single Channel Recording (Model Cell)

Set the front panel controls of the Axopatch 200A as follows:

PIPETTE OFFSET:	About 5.0
PIPETTE CAPACITANCE COMP.	Minimum (fully counterclockwise)
ZAP:	0.5 ms
SERIES RESISTANCE COMP. % PREDICTION:	0 %, OFF
SERIES RESISTANCE COMP. % CORRECTION:	0 %, OFF
SERIES RESISTANCE COMP. LAG:	1 μs
WHOLE CELL CAPACITANCE:	0 pF, OFF
SERIES RESISTANCE:	0 MΩ
HOLDING COMMAND:	0 mV, OFF
SEAL TEST:	ON
METER:	Set switch to V_{TRACK}
MODE:	TRACK
CONFIG.:	PATCH
OUTPUT GAIN:	$\alpha = 10$
LOWPASS BESSEL FILTER:	5 kHz
LEAK SUBTRACTION:	∞ MΩ, OFF

Pipette Offset Adjustment

Install the PATCH-1U model cell in BATH position into your headstage connector as described in the **FUNCTIONAL CHECKOUT** section. Again, surround it with aluminum foil grounded to the headstage ground connector. Connect the SCALED OUTPUT BNC to one channel of your oscilloscope. Set the oscilloscope gain to 1 V/div and set its sweep speed to 2 ms/div. Select line synchronization.

Using The TRACK Mode

You will see on the oscilloscope a rectangular, somewhat drooping pulse lasting about 8 ms (10 ms for 50 Hz line frequency). This is the current driven through the 10 MΩ "electrode" in the model cell by the 5 mV SEAL TEST command. The total height of the pulse is about 5 volts. (Note: With a 10 MΩ resistance a 5 mV step will generate 0.5 V; the x10 gain provides a 10-fold amplification resulting in a 5 V output). While the SEAL TEST circuit puts out only a +5 mV signal, the current signal you see will be 2.5 volts above zero and 2.5 volts below. This is because the TRACK circuit keeps the total current at zero. It achieves this by supplying the appropriate command that, when summed with the 5 mV command, keeps the integral of the current at zero. What you see on the oscilloscope is comparable to what you would see immediately following the immersion of the pipette tip in the bathing solution surrounding your cells.

Now turn off the SEAL TEST and look at the value on the meter. Adjust the PIPETTE OFFSET control until the TRACK voltage is zeroed. At this point, the tracking circuit does not have to put out a voltage in addition to the pipette offset voltage to achieve zero current. This specifies the proper setting of the PIPETTE OFFSET control to zero junction potentials and electrode asymmetries.

Using The V-CLAMP Mode

Some investigators prefer not to use a tracking circuit but do their offset adjustments and sealing in V-CLAMP. The choice is a matter of personal preference. Both methods enable you to follow drifts in the electrode. In TRACK mode, drifts are observed as changing voltages on the panel meter when V_{TRACK} is selected. In V-CLAMP mode, drifts are observed as changing currents. To test this approach, change the OUTPUT GAIN to 5, switch to V-CLAMP mode, turn the meter switch to I, and again turn on the SEAL TEST. Now you will see a 2.5 V, about 8 ms (10 ms for 50 Hz line frequency) pulse that goes in the positive direction. Change the position of the PIPETTE OFFSET control and notice that the DC position of the rectangular pulse is altered as you do so. You could achieve rough zeroing of pipette offsets by simply adjusting the control until the rectangular pulse starts from zero on the oscilloscope screen. For more accurate adjustment, turn off the SEAL TEST and adjust the PIPETTE OFFSET control until the meter reads zero.

Adjustment Of Pipette Capacitance Compensation

Now connect the model cell in the PATCH position to the headstage connector and surround with grounded aluminum foil. With the panel meter set to I_{RMS}, the reading should be less than 0.200 pA rms. Return the meter setting to I.

Turn the gain switch (α) to 10 and turn on SEAL TEST. On the oscilloscope, you will see two capacitance transients, one near the beginning of the sweep and the other near the end. They will be of opposite polarity. This is comparable to what you would see immediately following a gigohm seal between your pipette and cell. Using the FAST MAG control, reduce the size of the capacitance transient as far as possible. Switch the gain to 500. Now use both the FAST MAG and FAST τ controls to minimize the transient. This is done iteratively. Turn the FAST MAG slightly and then readjust the FAST τ for minimum transient. Do this over and over again until you find the setting where the transient is finally minimized (Fig. 1). With some practice, you will be able to use the two controls simultaneously to compensate stray capacitance rapidly. With real electrodes, but probably not with your model cell, the capacitance transient will have more than one component. SLOW MAG and SLOW τ controls are provided for minimizing a second electrode component in real experiments.

Adjustment Of Leak Subtraction

With the model cell still in the PATCH position, turn off the SEAL TEST. The current should still read zero on the meter. Turn the HOLDING COMMAND switch to "+" and use the HOLDING COMMAND control to move the current substantially off zero. While looking at the oscilloscope screen, turn the LEAK SUBTRACTION control until the current is returned to zero. This circuit sums a scaled version of the command signal with the current. The scaling is determined by the LEAK SUBTRACTION control. Now change the HOLDING COMMAND switch from "+" to "-". If the LEAK SUBTRACTION circuit is properly adjusted, the current trace will stay on zero as the HOLDING COMMAND is switched back and forth between "+" and "-".

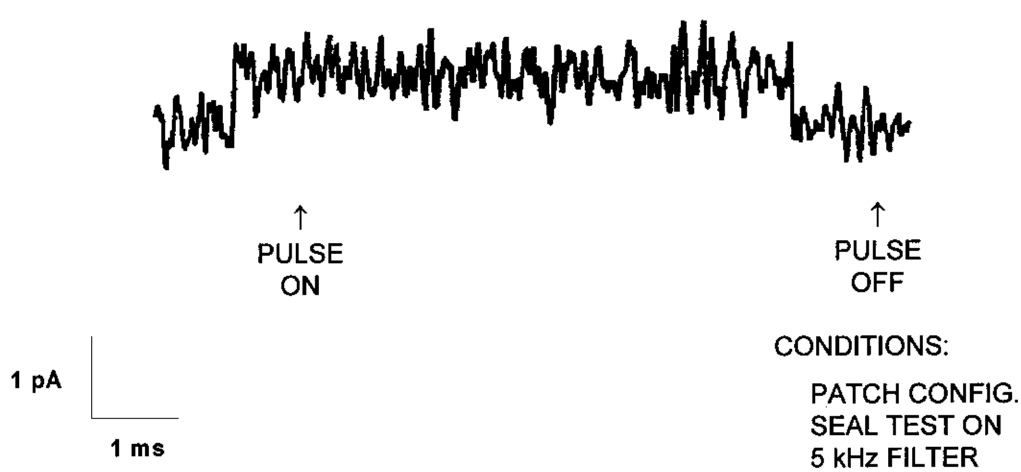


Figure 1. Electrode (Pipette) Capacitance Compensation

Whole-Cell Recording (Model Cell)

Set the front panel controls of the Axopatch 200A as follows:

PIPETTE OFFSET:	About 5.0
ZAP:	0.5 ms
SERIES RESISTANCE COMP. % PREDICTION:	0 %, OFF
SERIES RESISTANCE COMP. % CORRECTION:	0 %, OFF
SERIES RESISTANCE COMP. LAG:	1 µs
WHOLE CELL CAP.:	0 pF, OFF
SERIES RESISTANCE:	0 MΩ
HOLDING COMMAND:	0 mV, OFF
SEAL TEST:	OFF
METER:	Set switch to V_{TRACK}
MODE:	TRACK
CONFIG.:	WHOLE CELL
OUTPUT GAIN:	$\alpha = 1$
LOWPASS BESSEL FILTER:	5 kHz
LEAK SUBTRACTION:	∞ MΩ, OFF

Pipette Offset adjustment

These are done exactly as described in the *Single Channel Recording* section in the **TUTORIAL**. By the end of the pipette offset adjustment procedure a number of front panel controls will be changed from the above list. These include: MODE will be switched from TRACK to V-CLAMP, SEAL TEST will be ON, and OUTPUT GAIN will change from 1 to 5.

Whole-Cell Capacitance Compensation

There are a variety of approaches for adjusting the parameters to cancel whole-cell capacity transients. The approach we suggest here works well and is convenient for rapid and complete cancellation of whole-cell transients.

1. Select the whole-cell (resistive feedback) mode.

2. Attach the model cell to the headstage input in the CELL position. Insure that 60 Hz interference is sufficiently small. If necessary, this can be achieved by shielding with aluminum foil.

3. For a headstage with a 500 MΩ feedback resistor (CV 201AU), a gain setting of 0.5 is convenient; for a headstage with a 50 MΩ feedback resistor (CV 202AU) set the gain at 5.

4. Begin with the PIPETTE CAPACITANCE COMPENSATION controls fully off (counter-clockwise). Both WHOLE-CELL PARAMETERS should be off, *i.e.*, WHOLE CELL CAP. and SERIES RESISTANCE potentiometers should be fully turned counterclockwise, and the WHOLE CELL CAP. switch should be OFF. PREDICTION and CORRECTION should also be OFF.

5. Apply a square wave with a frequency of about 50 Hz and an amplitude of about 2 V to the EXT. COMMAND input. (Alternatively, if a pulse generator is not conveniently available you can use the SEAL TEST.) Trigger the scope from the external source. This will produce steps in the command potential of 40 mV amplitude and about 10 ms duration. Be sure that the HOLDING COMMAND switch is off and turn on the EXT. COMMAND switch. If you are using the SEAL TEST it will generate a 5 mV command instead of a 40 mV command. You should scale the amplitude values in the following paragraphs accordingly.

6. Initially select a 10 kHz or 50 kHz bandwidth since this will allow the fast capacity transient to be more readily distinguished from the slower whole-cell transient (Figure 2a). At this bandwidth use the FAST MAG and FAST τ and of the PIPETTE CAPACITANCE COMPENSATION to eliminate the fast capacity transient as previously described. The fast transient can be distinguished from the whole-cell transient by its more rapid time course. At a sweep speed of 50 or 100 μs per division and a vertical sensitivity of 0.5 or 1.0 V per division attempt to make the leading edge of the whole-cell transient look similar to Figure 2b.

7. Reduce the bandwidth to 5 kHz. At a slow sweep speed (*e.g.*, 5 ms/division) and high vertical resolution (*e.g.*, 20 mV/division) adjust the LEAK SUBTRACTION potentiometer (see *Adjustment of Leak Subtraction* above) to eliminate leak current from the 500 MΩ resistor simulating the membrane resistance in the model cell as in Figure 2c.

8. Turn the WHOLE CELL CAP. switch on. Reduce the vertical resolution to 1 V/div and increase the sweep speed to 1 ms/div. Using the WHOLE CELL CAP. and SERIES RESISTANCE controls simultaneously, minimize the capacity transients as in Figure 2d. There may be a small wiggle at the leading edge of the capacity transient due to minor misadjustment of the fast electrode compensation controls which can be removed by readjustment of those controls. Final adjustment should be done with a vertical resolution of about 50 mV/div. (Note: Be sure that the trace is flat at times beyond about 1 ms after its start. This is best accomplished with a sweep speed of 1 or 2 ms/div.) With a little practice it should be simple to reduce the capacity transient into the noise. The values of the series resistance and whole-cell capacitance determined in this way will be quite close to the correct values.

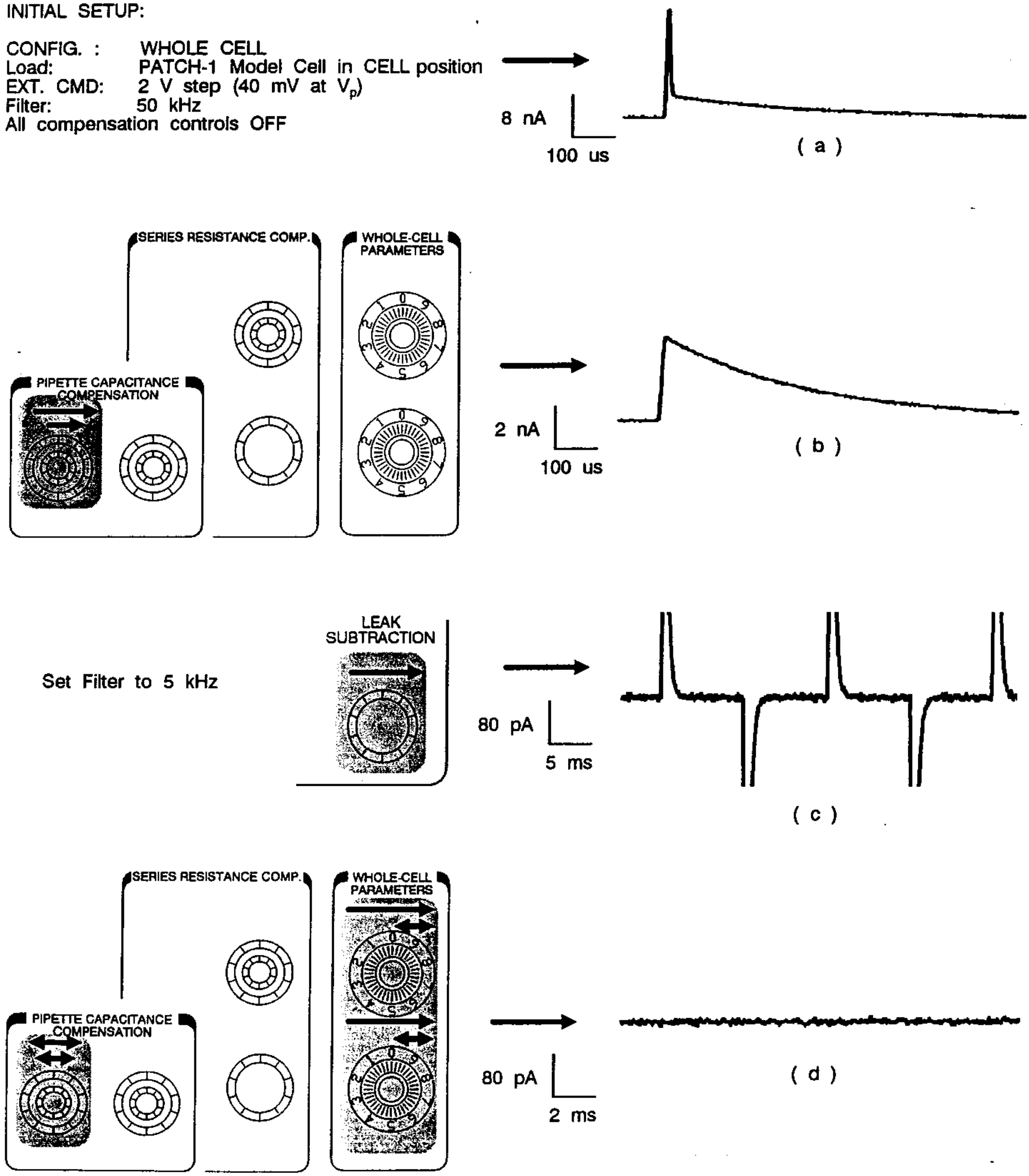


Figure 2. **WHOLE-CELL CAPACITANCE COMPENSATION**

Series Resistance Compensation

The Axopatch 200A is capable of series-resistance compensation equal to that of the Axopatch 200 and substantially better than has been possible with any other commercial patch clamp to date. In order to achieve the outstanding performance of the Axopatch 200A it is critical to set its controls properly. Because of the importance of this issue we go to great lengths here to describe in detail the methods for properly setting the instrument. We present both a brief method for experienced users and a more detailed method for those not yet skilled in patch clamping. (See **REFERENCE SECTION: PRINCIPLES OF OPERATION** for a discussion of the theory of whole-cell and series-resistance compensation).

Brief Method For Setting Series Resistance Compensation

At this point you have eliminated all capacitive current from the measured output. However, the cell membrane response to a step voltage command will still proceed with the time constant $R_S C_m$ of about 330 µs for the model cell (where R_S is the series resistance and C_m is the membrane capacitance). The Axopatch 200A uses dual controls to speed this response and to compensate for IR drops resulting from membrane current and series resistance, and for the filtering effect of the membrane capacitance and series resistance. These controls are labeled PREDICTION and CORRECTION. Non-ideal circuit characteristics require minor readjustments of WHOLE CELL CAP., SERIES RESISTANCE, and PIPETTE CAPACITANCE COMPENSATION potentiometers to achieve the best performance. The procedure here is intended to familiarize the user with these controls and with the necessary readjustments.

Users who are already skilled at adjusting both the fast electrode capacitance and whole-cell capacitance compensation circuits may prefer to set both PREDICTION and CORRECTION at the same time prior to readjusting the various controls to eliminate the final transient. Be aware that if you exceed the maximum achievable % PREDICTION you will produce unacceptable non-linearities (see Figure 3 and detailed explanation in the *Series Resistance* section in **PRINCIPLES OF OPERATION**).

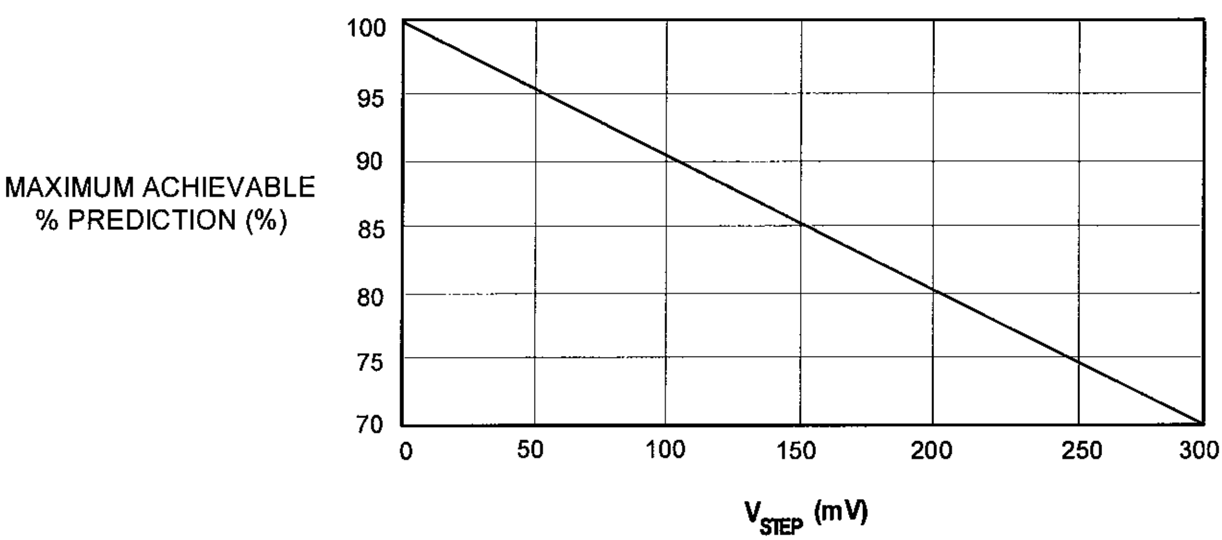
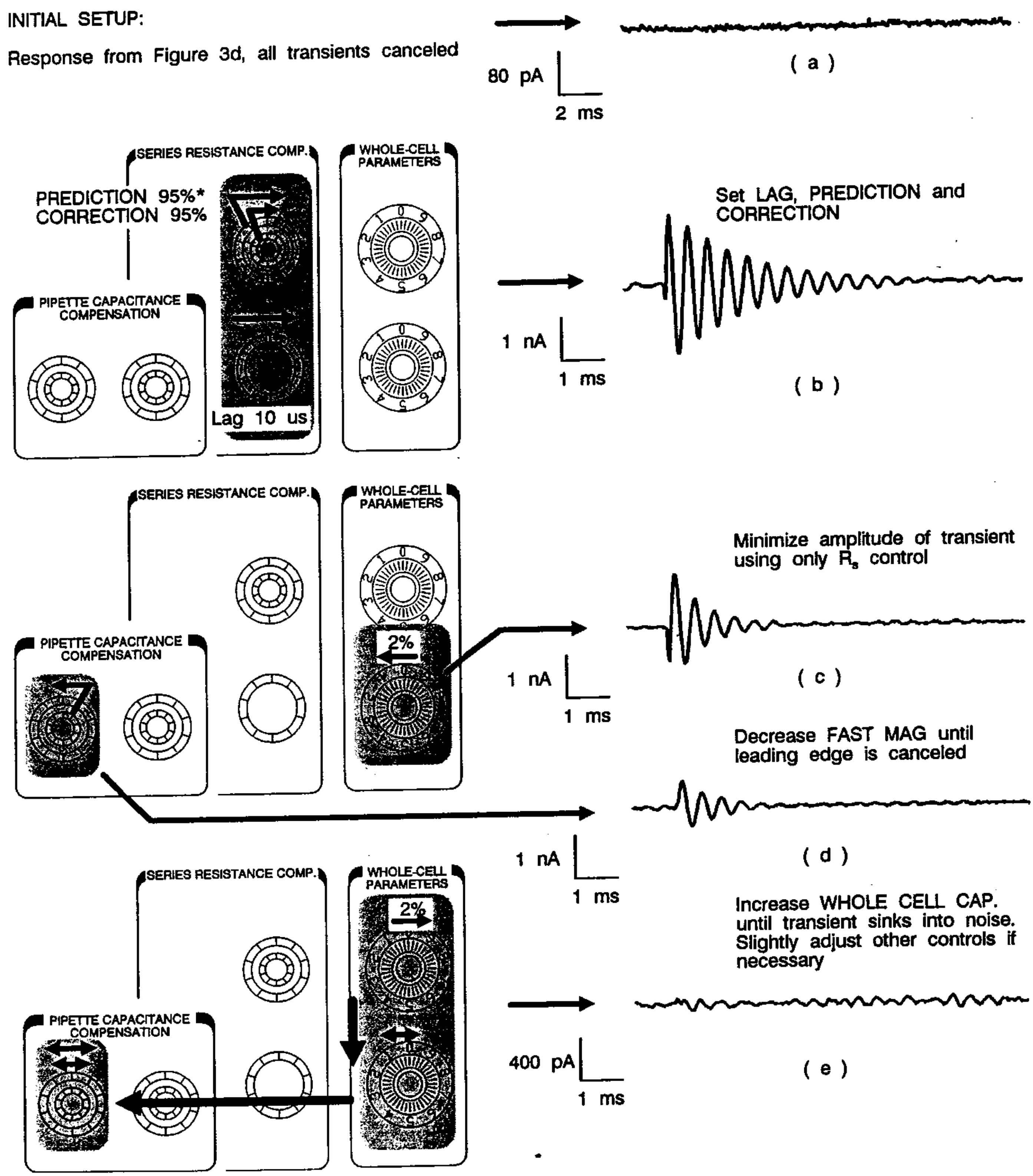


Figure 3. Maximum % PREDICTION as a Functioning Voltage Step

After adjusting the whole-cell capacitance as described above (Fig. 4a; same as Fig. 2d), advance the PREDICTION potentiometer setting to 95 or 96%. Without any other adjustments, set the LAG at 10 μs and advance the CORRECTION potentiometer setting to about 95%. A rather large transient should appear in the current at the beginning and end of the command step. Its peak-to-peak amplitude should be 2-4 nA and it should undergo several distinct "rings" requiring 2-4 ms to disappear into the noise (Fig. 4b). To completely eliminate this transient, begin by reducing the setting of the SERIES RESISTANCE control by about 2-3%. As you reduce this setting, the amplitude of the transient first decreases and then begins to increase. A distinct minimum exists and the desired setting of the SERIES RESISTANCE control is at this minimum (Fig. 4c).

Next, slightly decrease the FAST MAG setting. The fast leading edge transient should decrease along with the peak-to-peak magnitude of the overall transient. Stop when the leading edge transient disappears as in Figure 4d.

If all controls were set correctly from Figure 2d (or 4a), you should be able to completely obliterate the remaining transient with a small increase in the WHOLE-CELL CAP. setting. If the transient cannot be completely eliminated in this way, minor readjustments of the SERIES RESISTANCE control may be required, followed by further readjustments of FAST MAG and FAST τ. An iterative procedure works best. It should be possible to reach this high degree of compensation without any residual transient as shown in Figure 4e.



* % PREDICTION setting limited by V_{STEP}, see Fig. 2

Figure 4. Series Resistance Compensation, Brief Method

Detailed Method For Setting Series Resistance Compensation

New patch clamp users who are not yet skilled in setting all of the capacity compensation controls may find it impossible to achieve the exceptional series resistance compensation shown above. We, therefore, present here a detailed description of a method for setting the controls that ensures outstanding series resistance compensation. In this approach, PREDICTION and CORRECTION are set sequentially rather than concurrently as in the above section. Minor readjustments are then done to achieve the final result. We also provide the user with a detailed explanation of the series-resistance circuit operation.

If you are satisfied with your ability to set all of the capacity compensation controls, you can skip the following indented sections and resume at the *Current Clamp (Model Cell)* section.

> Note that PREDICTION is an open loop process, *i.e.*, it does not involve feedback, and instability is only possible if the internal circuitry that develops the prediction signals is pushed too far. Generally, the circuit is stable up to values of about 98%, but it can become non-linear, depending on the magnitude of V_{STEP} (Figure 3). If the PREDICTION potentiometer has been advanced too far it may not be noticeable in 5 kHz bandwidth until the current begins to oscillate. However, in 50 kHz bandwidth you will observe ringing developing in the transient as the PREDICTION percentage becomes too large. For best results you should start with whole-cell transients canceled as in Figure 5a (same as Fig. 2d). To begin, advance the PREDICTION potentiometer gradually up to a final value of 95 or 96%. A brief transient will emerge at the leading and trailing edge of the command potential step. The amplitude of this transient is typically about 500 $pA_{p\text{-}p}$[1] (in 5 kHz bandwidth) for a 40 mV step using the cell model (Fig. 5b). The residual transient at this stage is typically biphasic, with a total duration of about 200 μs in a bandwidth of 5 kHz, but it can be eliminated.
>
> To eliminate the residual transient that has resulted from the use of PREDICTION, small readjustments in the settings of the PIPETTE CAPACITANCE COMPENSATION FAST MAG control, the WHOLE CELL CAP., and SERIES RESISTANCE settings are needed. To begin, slightly reduce the setting of the FAST MAG control in PIPETTE CAPACITANCE COMPENSATION. The amplitude of the initial negative-going component of the residual transient will decrease. Continue to change the control setting until the waveform of the residual transient changes to a monophasic positive going response. At the point where the negative component has just been eliminated, you may observe a small wiggle at the leading edge of the residual transient. Continue to reduce the FAST MAG control until the leading edge of the transient is smooth and "S-shaped". This may lead to a small (5-10%) increase in the peak amplitude of the residual (positive-going) transient. Note that further reductions in the FAST MAG control will cause the residual transient to grow further in amplitude; stop when the leading edge is smooth. This is best accomplished at a sweep speed of 100-200 μs/div and a vertical sensitivity of about 100 mV/div, assuming a 40 mV command step (Fig. 5c).
>
> Reduce the value of the SERIES RESISTANCE control setting by about 2-3%. A notch should develop in the residual waveform and a slower positive-going component should develop at the trailing edge of the transient. Stop when the overall transient is centered around the baseline. A typical waveform at this point is shown in Figure 5d. If the initial direction of the waveform is negative, the FAST MAG may need to be decreased a little more.
>
> Slightly increase the setting of the WHOLE CELL CAP. potentiometer (1-2%) until the slow component of the residual transient disappears. The resulting waveform should be biphasic with positive-going initial component and a negative-going final component. In 5 kHz bandwidth its peak-to-peak amplitude should be about 100-200 pA and its total duration about 200 μs (Fig. 5e).

---

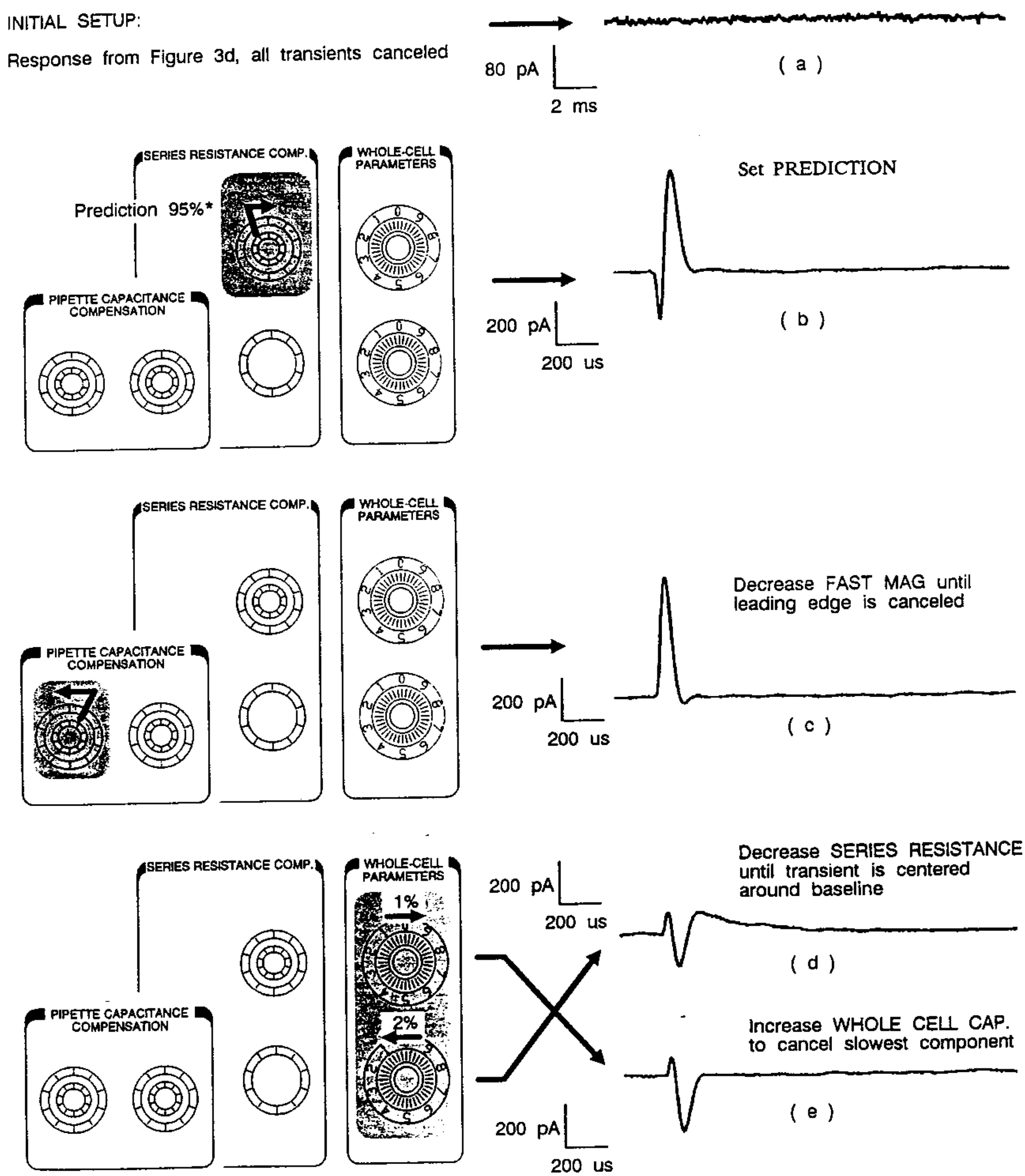
1 $pA_{p\text{-}p}$ - pA peak-to-peak

Adjust the FAST τ of the PIPETTE CAPACITANCE COMPENSATION until the residual transient seems to be minimized. Note that it will still be biphasic; keep the waveform as smooth as possible. The readjustment of FAST τ is usually not very large. Now readjust the FAST MAG control of PIPETTE CAPACITANCE COMPENSATION and minimize the final transient. A few iterative adjustments of the FAST MAG and FAST τ may be required to achieve best performance. If a reasonably fast (<200 µs) component remains that can not be compensated with the FAST MAG and FAST τ controls, it probably means that a very small readjustment of the SERIES RESISTANCE potentiometer is needed. At a slower sweep speed (*e.g.*, 1-2 ms/div) make sure that there is no slow component visible; if you observe a slow component you need to slightly readjust the WHOLE CELL CAP. potentiometer. With a little practice it should be possible to reduce the residual transient to an amplitude of about 10 pA so that it essentially disappears into the noise (Fig. 5f). You are now clamping the model cell membrane in about 30 µs and have completely eliminated all the capacity current from the output of the headstage. Similar performance can normally be achieved with real cells but the use of the slow component of the PIPETTE CAPACITANCE COMPENSATION is likely to also be required. Set the vertical sensitivity to 0.5 V/div and the sweep speed to 1-2 ms/div, and turn OFF and ON the WHOLE CELL CAP. switch (turning this switch OFF also disables PREDICTION but not CORRECTION). This is a striking demonstration of the improvement in performance that has been achieved by the adjustment procedure. Also note that in 5 kHz bandwidth turning ON and OFF this switch does not noticeably change the noise riding on the waveform.

Despite the fact that the membrane potential is now being changed extremely rapidly (about 30 µs) in response to a step command of potential, two important effects of series resistance (R_s) have **NOT** yet been eliminated. These are IR drops resulting from the flow of ionic currents (I) in the cell membrane and the filtering effect of the series resistance and cell membrane capacitance on measured membrane currents. An ionic current of 2 nA flowing across a series resistance of 10 MΩ will produce a 20 mV error in the true membrane potential relative to the command potential; this effect of series resistance is well known. It is far less well understood that series resistance in conjunction with membrane capacitance will have the effect of filtering the measured current with a one pole RC filter with a corner frequency given by $1/2\pi R_sC_m$. For R_s= 20 MΩ and C_m= 50 pF, this is only about 160 Hz.

The CORRECTION potentiometer is used to greatly reduce both of these errors. To set CORRECTION properly, first set the LAG potentiometer at about 10 µs. Without changing any of the settings previously established, advance the CORRECTION potentiometer gradually up to about 95%. Observe the current at a vertical gain of about 100 mV/div and a sweep speed of about 500 µs/div. As the CORRECTION percentage increases, a small transient will emerge at the leading and trailing edges of the command step. At the same time the noise in the measured current will increase. By the time the CORRECTION potentiometer setting has reached 95%, a ringing transient with a peak-to-peak amplitude of roughly 400 pA will have emerged (Fig. 5g). The transient should "ring" (*i.e.*, oscillate) and will reverse polarity 2 or 3 times before disappearing into the noise somewhat less than 1 ms (in 5 kHz bandwidth) after the beginning of the command step.

This residual transient is easily eliminated. Usually all that is required is to slightly (about 1%) increase the setting of the WHOLE CELL CAP. potentiometer setting to make the residual transient disappear into the noise (Fig. 5h). If this is not sufficient, very small readjustments of the SERIES RESISTANCE control and FAST MAG and FAST τ may also be required.



* % PREDICTION setting limited by V_{STEP}, see Fig. 2

Figure 5. Series Resistance Compensation, Detailed Method

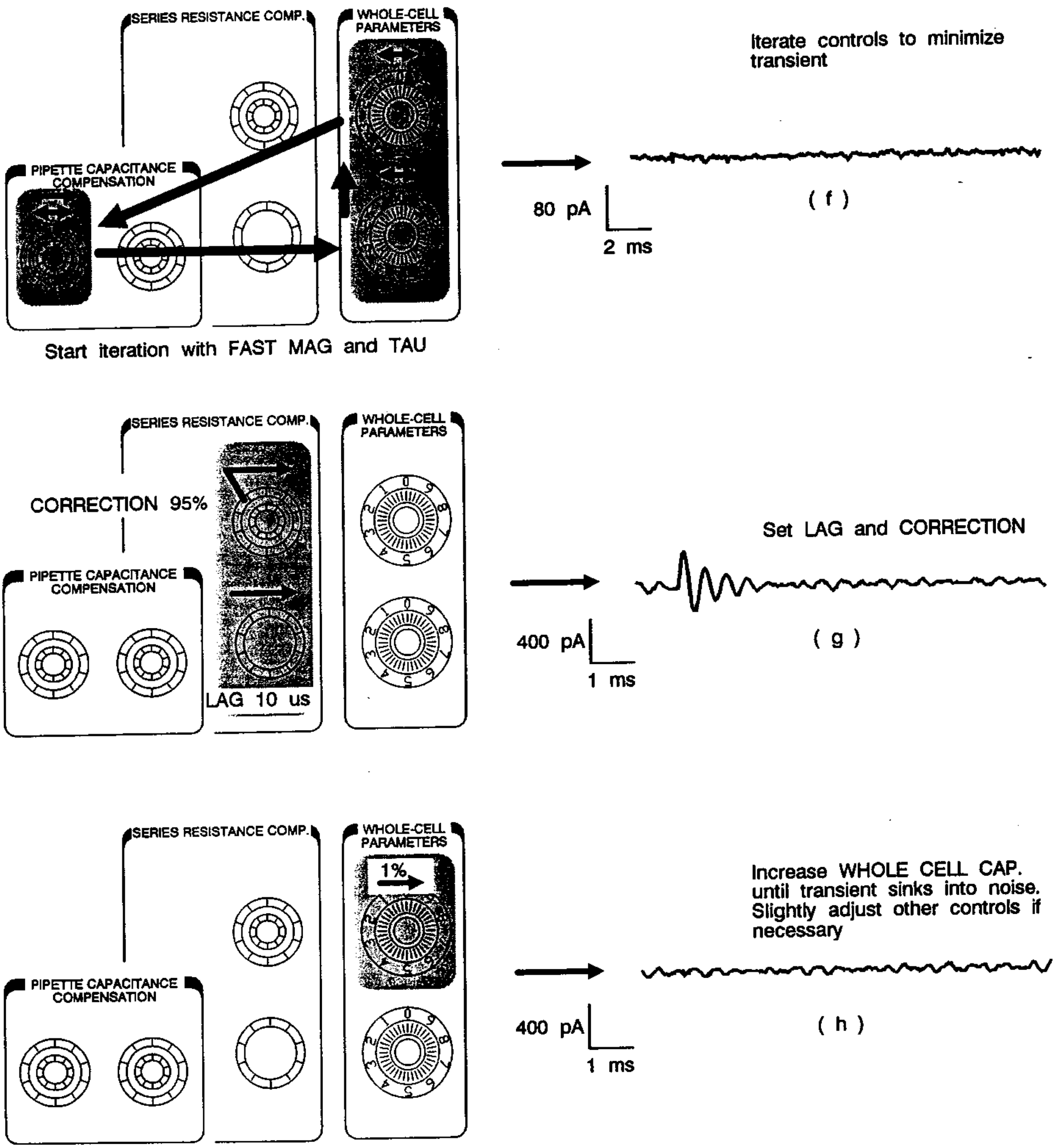


Figure 5. Series Resistance Compensation, Detailed Method (Cont.)

At this point, 95% of the approximately 10 MΩ series resistance has been compensated; the residual series resistance is 500 kΩ. An ionic current of 2 nA amplitude would now cause only a 1 mV error in the membrane potential relative to the command potential, *i.e.*, a 20-fold reduction from the situation prior to the use of CORRECTION. Moreover, the true membrane potential is established within about 30 µs after the start of the step command without overshoot or ringing. In addition, the bandwidth of current measurement has been increased from 480 Hz to about 9.6 kHz (of course the measurement bandwidth is still restricted to 5 kHz by the output filter). It is this increase in the bandwidth of current measurement that is responsible for the increased noise as the CORRECTION percentage is increased.

Turn on and off the WHOLE CELL CAP. switch and observe the improvement in performance. Set the vertical gain of the oscilloscope to 2 V/div and set the sweep speed to 1 or 2 ms/div. With the switch ON, the trace should be essentially flat. Recall that turning off this switch not only eliminates the correction signal applied to the 5.1 pF capacitor in the headstage used to compensate for whole-cell capacity transients, but also disables PREDICTION; however, CORRECTION is not disabled. With the WHOLE CELL CAP. switch turned off, series resistance is still compensated via positive feedback of the measured current. Turning off this switch will result in a large ringing capacity transient, with a peak-to-peak amplitude of more than 10 nA. At a higher vertical gain on the oscilloscope (*e.g.*, 200 mV/div), 7 or 8 discernible peaks can be observed in this transient before they disappear into the noise (about 2 ms following the beginning of the step command). The membrane potential will also ring severely and have a 1% settling time of nearly 2 ms. Turning the switch back ON completely eliminates the transient and results in a large improvement in stability: the true membrane potential changes smoothly without ringing to its new value in about 30 µs following the step command. Similar results can usually be achieved with real cells.

The percentage CORRECTION can be increased beyond 95%; 100% can often be achieved with the 10 µs LAG. However, with the parameters of the model cell, as CORRECTION is increased beyond about 95% the current record shows periodic noise (about 200 µs period; which is essentially the same as the ring frequency observed above) that may interfere with current measurement. This can be eliminated by increasing the LAG setting. However, increasing the LAG setting filters the signal used in CORRECTION (*e.g.*, 10 µs corresponds to a 16 kHz 1 pole RC filter, 20 µs corresponds to an 8 kHz filter, etc.). This oscillatory "noise" will also virtually disappear with the output filter set at 1 or 2 kHz. Similarly, also note that at the 95% level of CORRECTION the LAG control setting can be reduced to about 2-3 µs before the system becomes unstable. However, once again, noise will increase.

Current Clamp (Model Cell)

The usual mode of operation of a patch clamp is voltage clamp. In V-CLAMP mode the membrane potential is controlled and the current needed to maintain that potential is recorded. It is, however, often useful to allow the membrane potential to change while keeping the current constant. This can be done in I-CLAMP mode in which the membrane current is controlled (often at zero) while the membrane potential is recorded.

Current-clamp mode can be used in either the WHOLE CELL or PATCH configurations. However, it is most often used in the WHOLE CELL configuration. All time varying current command signals must be generated externally and brought in through the EXT. COMMAND BNCs. A DC holding current can be generated in I-CLAMP using the HOLDING COMMAND control. Do not try to use the SEAL TEST to generate current commands.

To obtain the most accurate cell parameter measurements while in I-CLAMP, it is important to have the PIPETTE CAPACITANCE COMPENSATION set correctly **before** entering I-CLAMP mode. This is because in I-CLAMP (for a first approximation) the pipette capacitance (C_p) appears to be in parallel with the membrane capacitance (C_m). If the PIPETTE CAPACITANCE COMPENSATION is set too low, the measurement of C_m will be too high and vise versa.

Typically, you will change to I-CLAMP mode after establishing whole-cell recording in V-CLAMP mode. Assuming you have just completed going through the whole-cell voltage clamp tutorial, you have already set the PIPETTE CAPACITANCE COMPENSATION controls to minimize fast transients. You may turn off CORRECTION, PREDICTION and WHOLE CELL CAP. if you wish; they will all be disabled automatically when you switch from V-CLAMP to TRACK (I=0) or I-CLAMP. Do not change the PIPETTE OFFSET control as that will lead to erroneous V_m measurements.

Switch the MODE to TRACK (I=0). You are now in a slow current clamp, but all commands are being ignored and the current is being clamped at zero. Now is the time to set the external command signal and holding current command desired when you go into I-CLAMP mode.

Set the external command for a 5 Hz, 100 mV_{p-p}[1] rectangular waveform (10 mV_{p-p} for a CV 202AU). This will cause a 200 pA_{p-p}[2] current to be forced through the model cell. Switch the MODE to I-CLAMP NORMAL. The signal at the SCALED OUTPUT is now V_m. Set the output gain to x10. There should be approximately 1 V_{p-p}[3] square waveform ($V_m \approx 100$ mV_{p-p}) with a time constant (τ) of 16.5 ms (± 15%, 10 - 90% rise time of 36.3 ms) that is identical to the τ of the model cell.

To observe the two different speeds of the I-CLAMP loop and at the same time confirm that the current clamp loop settles much faster than V_m of the model cell, connect an oscilloscope channel to the I OUTPUT BNC. Switch the rear panel slide switch near the BNC to 100 ßmV/pA ("up" position).

The observed current signal will have an amplitude of 20 V_{p-p} (2 V_{p-p} for CV 202AU). With the MODE switch set to I-CLAMP NORMAL the 10 - 90% rise time will be about 250 µs with about 10% overshoot. Switch the MODE to I-CLAMP FAST and the response will change to a rise time of about 35 µs with less than 10% overshoot (the internal rise time under these conditions is about 10 µs, the signal at the I OUTPUT BNC is filtered at 10 kHz and thus is limited to a rise time of 35 µs).

Single-Channel Recording (Real Cell)

Set the front panel controls of the Axopatch 200A as follows:

PIPETTE OFFSET:	About 5.0
ZAP:	0.5 ms
SERIES RESISTANCE COMP. % PREDICTION:	0 %, OFF
SERIES RESISTANCE COMP. % CORRECTION:	0 %, OFF
SERIES RESISTANCE COMP. LAG:	1 µs
WHOLE CELL CAP.:	0 pF, OFF
SERIES RESISTANCE:	0 MΩ

1 mV_{p-p} - mV peak-to-peak
2 pA_{p-p} - pA peak-to-peak
3 V_{p-p} - V peak-to-peak

HOLDING COMMAND:	0 mV, OFF
SEAL TEST:	ON
METER:	Set switch to V_{TRACK}
MODE:	TRACK
CONFIG.:	PATCH
OUTPUT GAIN:	$\alpha = 10$ or as desired
LOWPASS BESSEL FILTER:	1,2, or 5 kHz
LEAK SUBTRACTION:	∞ MΩ, OFF

Set the oscilloscope to line triggering. Switch off any external step generators.

Insert the pipette and its holder into the input connector of the patch clamp. Be sure to touch grounded metal before doing this to discharge any static charge that may have inadvertently built up on you or on the holder. Be sure to support the headstage with your other hand so that the micromanipulator will not have to absorb the force as you firmly insert the holder. Lower the pipette into the bath. Any voltage offset between the bath electrode and the patch electrode will show up as a non-zero tracking voltage on the meter. Adjust the PIPETTE OFFSET potentiometer until V_{TRACK} is zero. (Some investigators prefer not to use the tracking circuit but rather do their early adjustments and seal formation in voltage clamp mode. If you prefer this approach, place the MODE switch in the V-CLAMP position and the meter switch to I before placing the pipette tip into the bath. With the tip in the bath, adjust the PIPETTE OFFSET control until the current on the meter reads zero.)

Note: This is a good point to check the stability of your bath (ground) and patch (recording) electrodes. Drifting electrodes will cause a constantly changing V_{TRACK} voltage in TRACK mode whereas in V-CLAMP the current will continually drift off zero.

Switch the external command switch to the SEAL TEST position. A 5 mV positive-going rectangular pulse is applied to the patch clamp input. The pulse frequency is the same as the AC line frequency and the duty cycle is 50%. This will result in a square pulse of current whose amplitude depends on the pipette resistance as shown in Figure 6.

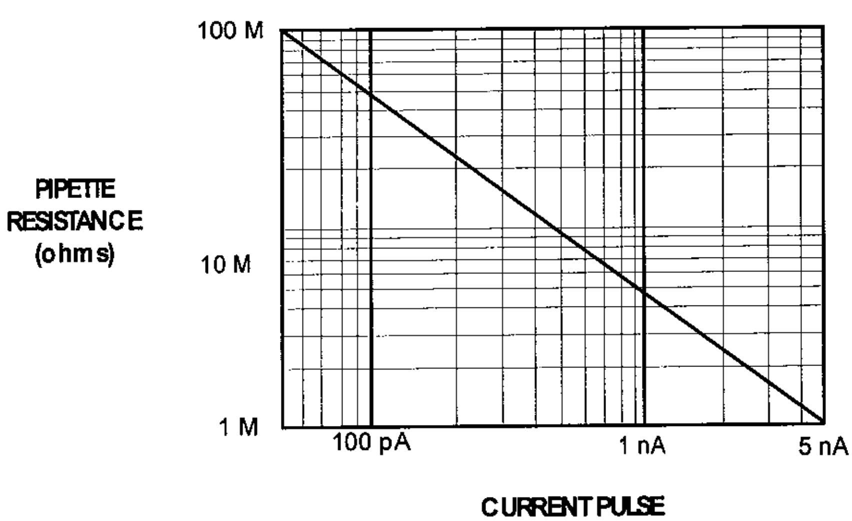


Figure 6. Pipette Resistance Measurement Using Seal Test

Lower the pipette until it just touches the cell. You will be able to tell when this happens because the resistance will increase as the pipette makes contact with the cell membrane, causing a decrease in the size of the current pulse (Fig. 7, three upper traces). Continue to lower the pipette until the fractional resistance increase is optimal to promote sealing to the cell. For many cells, a two-fold resistance increase is optimal (the size of the square pulse on the oscilloscope decreases in half) but you will have to determine the optimum for your particular cell type.

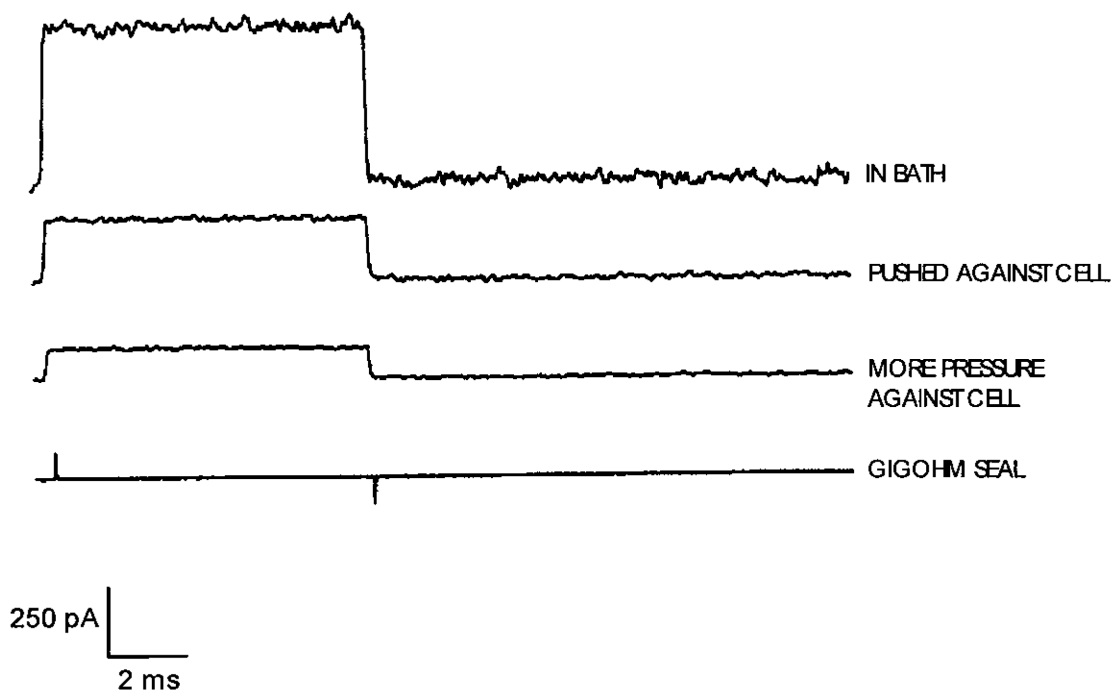


Figure 7. Change in Resistance While Forming a Seal

At this point, apply suction to the tubing connected to the pipette holder. Some investigators prefer to use mouth suction while others find that a 10 ml syringe works well. With the latter approach, it is best to start with the syringe plunger pulled back to a volume of 5-7 ml so that approximately this volume exists in the system before suction is applied. Pulling the syringe back by about 1 ml from this starting volume will reduce the pressure by 0.2 to 0.14 atmospheres. Since many other schemes of applying suction are used by investigators, we do not provide here an exhaustive description. As suction is applied, there should be a sudden increase in the resistance. Once a gigohm seal is established, the rectangular current pulse will disappear entirely and be replaced by capacitance transients in synchrony with the rising and falling edges of the command pulse (Figure 7, lowest trace).

If the seal was made using the tracking circuit, this circuit can now be turned off by switching to the V-CLAMP mode. Turn off the SEAL TEST switch and turn the meter switch to the I_{RMS} position. Check the noise level on the meter and decide if the quality of the seal and pipette coating are satisfactory. With the Axopatch 200A integrating headstage, proper pipette coating, low loss pipette glass, and proper grounding and shielding, it is possible to obtain noise levels in the 0.095-0.12 pA rms range if a high resistance seal is obtained. Forgetting to turn off the SEAL TEST signal will result in very high noise levels because of the noise contribution of the capacitance transients.

Turn the SEAL TEST signal back on and, if you plan to do pulsed experiments, proceed to cancel the capacitance transients. This is done using the fast and slow PIPETTE CAPACITANCE COMPENSATION circuits. First, turn the SLOW MAG control fully counterclockwise so that there will initially be no compensation for a slow component. Adjust the FAST MAG and FAST τ controls until the capacitance transient is minimized. (**Note:** with some practice you will be able to adjust MAG and τ simultaneously and decrease the time required to do the adjustments). If a slow component remains, minimize its size using the SLOW MAG and SLOW τ controls. Turn off the SEAL TEST switch and you are ready to record channel currents.

In some experiments, you may not require complicated pulse protocols and the application of a simple DC voltage to the Axopatch 200A command line is all that is required. For this, you can use the HOLDING COMMAND circuit included in the Axopatch 200A. Turn the meter switch to V_{HOLD}/I_{HOLD} and the meter will display the absolute value of the voltage set by the HOLDING COMMAND control. This is true even with the HOLDING COMMAND switch in the OFF position. This feature allows you to preset the voltage without delivering it to the preparation until you are ready. The voltage actually delivered to the preparation will be positive or negative depending on the position you choose for the HOLDING COMMAND switch (+ or -).

At this point, you should be seeing single-channel currents if channels exist in the patch. Adjust the OUTPUT GAIN rotary switch to a position appropriate for the size of the currents.

If you plan to use an external function generator or the output of a computer driven D/A converter to provide command potentials, the signal should be connected to the EXT. COMMAND INPUT BNC (front switched or rear-switched) on the back of the Axopatch 200A. This is switched with an attenuation of 20 mV/V when the EXT. COMMAND switch is set to the ON position.

When the HOLDING COMMAND is changed, the current baseline will move off zero current. This is due to the current flowing through the seal resistance. How much the trace moves will depend upon the magnitude of the seal resistance. For a large seal resistance, only small currents flow; but for low seal resistances, the currents can be large. For example, for a 1 GΩ seal and a 100 mV HOLDING COMMAND, the seal current will be 100 pA. If, for example, you have set the overall Axopatch 200A gain so that you can look at a 5 pA channel current taking up one division at 1 V/div on the oscilloscope screen, the application of this HOLDING COMMAND will cause the current trace to go right off the screen. If you were digitizing the current with an A/D converter, the baseline current would also exceed the range of some converters. The Axopatch 200A includes a leak subtraction circuit to alleviate this problem. With the HOLDING COMMAND applied, turn the LEAK SUBTRACTION control until the baseline current returns to zero. If the baseline current is really seal current, it should exhibit linear behavior. Once the LEAK SUBTRACTION is set properly it should be possible to toggle the HOLDING COMMAND switch between + and - and the baseline current should stay in the center of the oscilloscope screen. (**Note:** it is possible for patches to contain small channels or electrogenic transporters that do not produce discernible single-channel events. These will appear to be part of the seal current and may impart apparent non-linear behavior to the seal.)

The Axopatch 200A contains a 4-pole internal Bessel filter with five frequencies for filtering the current output. For many experiments, the 1, 2, 5, 10 and 50 kHz settings available will be sufficient. If other frequency cutoffs are required, they must be provided from an external filter. For this purpose, set the internal filter to 50 kHz and choose the desired bandwidth from your external filter. Note that the bandwidth achieved will be that of the cascaded filters.

Whole-Cell Recording (Real Cell)

Set the front panel controls of the Axopatch 200A as follows:

PIPETTE OFFSET:	About 5.0
ZAP:	0.5 ms
SERIES RESISTANCE COMP. % PREDICTION:	0 %, OFF
SERIES RESISTANCE COMP. % CORRECTION:	0 %, OFF
SERIES RESISTANCE COMP. LAG:	1 μs
WHOLE CELL CAP.:	0 pF, OFF
SERIES RESISTANCE:	0 MΩ
HOLDING COMMAND:	0 mV, OFF
SEAL TEST:	ON
METER:	Set switch to I
MODE:	TRACK
CONFIG.:	WHOLE CELL
OUTPUT GAIN:	α = 10 or as desired
LOWPASS BESSEL FILTER:	1,2, or 5 kHz
LEAK SUBTRACTION:	∞ MΩ, OFF

Insert the pipette and its holder into the input connector of the patch clamp. In this case, the pipette should usually contain a filling solution that is low in Ca^{2+} and the tip should be as large as possible to minimize the pipette resistance (the importance of low pipette resistance is obvious from the previous series resistance compensation discussion). At this point, proceed to insert the holder, lower the pipette into the bath and make a seal in the same manner as described in the preceding single-channel recording tutorial.

A pulse of strong suction is required to rupture the cell membrane. This can again be done by mouth suction or by a syringe. If you are using a 10 ml syringe, disconnect the suction line from the syringe end briefly and push the plunger all the way in. Reconnect the suction line. Draw back slightly on the plunger until a large capacitance transient suddenly appears. An example of this is shown in Figure 8. Again, many schemes of rupturing the cell have been used by investigators.

The Axopatch 200A contains a ZAP circuit to aid in breaking into the cell. This circuit delivers a pulse of 1.3 VDC volts for variable durations ranging from 0.5 to 50 ms. The user sets the duration which is optimal for the particular cells in use by adjusting the potentiometer that makes up the outer part of the control. When the center button on the control is depressed, the pulse is delivered. A successful result will again look like that in Figure 8. If the patch is not disrupted, the pulse duration can be increased and the pulse applied a second time, and so on. Some investigators have found that the application of moderate suction while the voltage pulse is given results in a higher incidence of successful patch disruption. The reappearance of the original rectangular pulse either means that you have lost the seal or that the cell does not have a large input resistance. It is not unusual for small cells to have an input resistance of several gigohms but with active conductances it might be as low as a few tens of megohms.

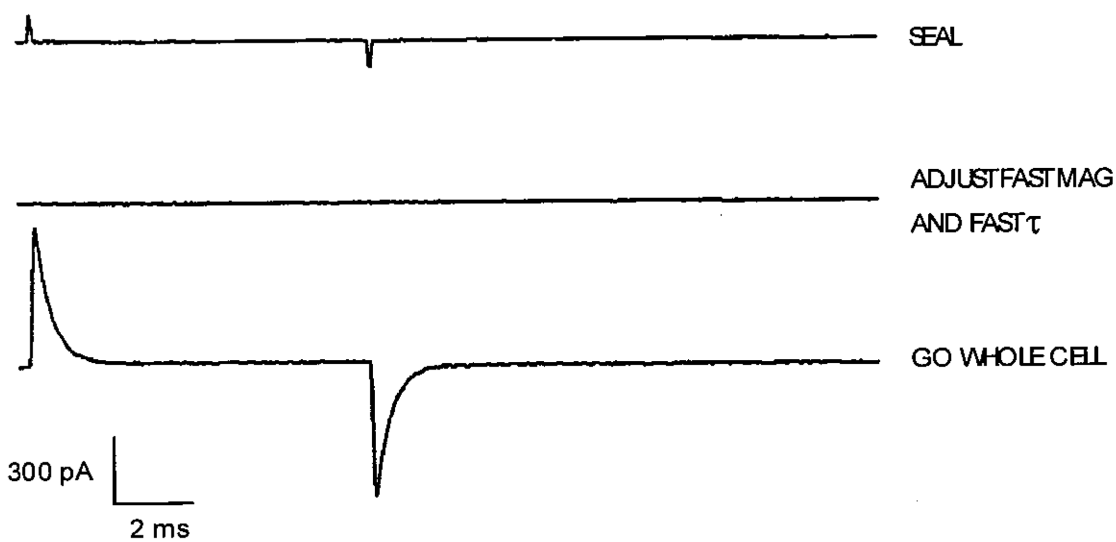


Figure 8. Going Whole-Cell: Capacity Transients Observed When Rupturing the Patch

Note: with some cells it has proven nearly impossible to go whole cell without loss of seal. If you have one of those cells, you might consider the PERFORATED PATCH technique. In this approach, the very tip of the pipette is filled with a normal filling solution and the rest of the pipette is backfilled with the same filling solution to which 120-150 µg/ml of Nystatin or Amphotericin B [from a stock solution of 30 mg/ml in DMSO] has been added. Over a 5-30 min. time period these polyene antibiotics form myriad tiny cation-selective, voltage-independent channels in the membrane patch. These channels allow small ions to equilibrate between the cell and the pipette allowing the cell to be voltage clamped through the open channels. Since substances as large as, or larger than, glucose will not permeate these channels, cell contents are not washed out as in standard whole-cell techniques. This is an advantage or a disadvantage, depending on the experiment. With this technique, a rise in whole-cell capacity transients will be observed as the antibiotic partitions into the cell as shown in Figure 9.

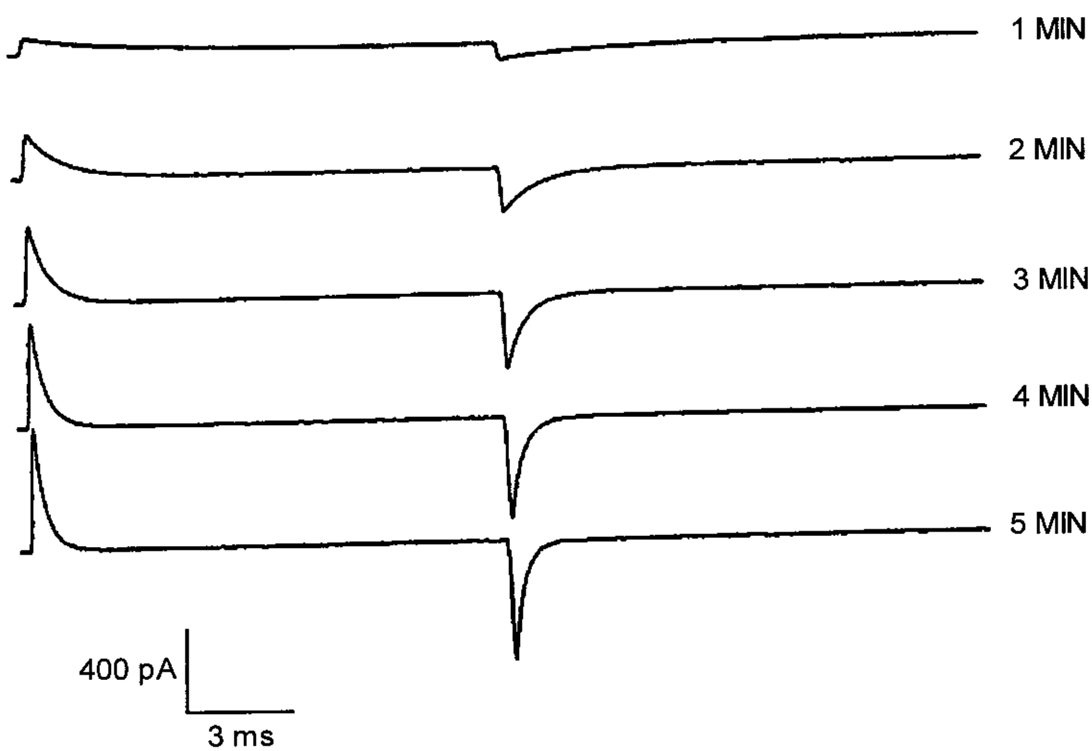


Figure 9. Going Whole-Cell: Capacity Transietns Observed During amphortericin Partitioning.

Now, turn off the SEAL TEST switch. The panel meter reads the output voltage of the tracking circuit that, at this time, represents the command voltage necessary to keep the whole-cell current zeroed. This is, by definition, the resting voltage of the cell. If you have made the seal and gone whole cell in V-CLAMP mode, you will see a DC shift in the current since the ionic gradients available to drive channel current will not be balanced by a resting voltage. Using the HOLDING COMMAND control with the HOLDING COMMAND switch set to (-), dial the current to zero either using the trace on the oscilloscope as a null indicator or by switching the meter switch to the I position and making the meter read zero. At this point, the reading on the HOLDING COMMAND control or on the meter set to V_{HOLD}/I_{HOLD} will be the membrane resting potential of the cell. Note that the membrane resting potential recorded under these conditions may not be the same as the resting potential in intact cells because the membrane resting potential depends on the ionic compositions of the pipette and the bath solutions.

Many investigators prefer to prevent the cell from experiencing this loss of HOLDING COMMAND as they go whole-cell since some excitable cells die quickly once depolarized. To avoid this, some researchers set the HOLDING COMMAND value at the anticipated resting potential of the cell. This will be applied to the cell interior at the instant they achieve the whole-cell configuration. If there is still a DC current shift (*i.e.*, their anticipated voltage was not quite correct), minor adjustments of the HOLDING COMMAND control can be made to zero the current.

Another possible scheme to go whole-cell without loss of resting potential is to disrupt the patch while in I-CLAMP mode. The current-clamp circuit is fast enough to keep the membrane current at zero and thus keep the cell at its resting potential during the patch disruption. It is important that no external command be applied while in current clamp; otherwise, cell voltage changes caused by the command will occur.

If you went whole-cell in either I-TRACK or I-CLAMP, set the desired HOLDING COMMAND and turn the MODE switch to V-CLAMP. Turn the SEAL TEST back on. Turn the WHOLE CELL CAP. switch to ON. You are now ready to adjust the whole-cell capacitance compensation. Using both the WHOLE CELL CAP. and SERIES RESISTANCE controls simultaneously, adjust them until the capacitance transient is minimized. It is also quite possible that the PIPETTE CAPACITANCE COMPENSATION will require slight readjustment at this time. (See *Whole-Cell Capacitance Compensation* in the *Whole-Cell Recording (Model Cell)* section of the **TUTORIAL**).

This procedure leads to unique settings of the SERIES RESISTANCE and WHOLE CELL CAP. controls corresponding to the electrode and cell being clamped. When the transient is minimized, the access resistance (pipette resistance and any resistive contribution from cell contents) and the cell capacitance can be read on the SERIES RESISTANCE and WHOLE CELL CAP. controls, respectively. Usually, the access resistance will be about three times that of the pipette alone. The whole-cell capacitance value can be used to estimate the total surface area of the cell assuming that 1 cm^2 of membrane has 1 μF capacitance.

If your currents are large, you will want to use series resistance compensation to increase the frequency response of the whole-cell clamp and to minimize the command voltage error due to the IR drop across the access resistance. The proper adjustment of these controls is discussed in the *Whole-Cell Recording (Model Cell)* section above.

The Axopatch 200A contains a 4-pole internal Bessel filter with five frequencies for filtering the current output. For many experiments, the 1, 2, 5, 10 and 50 kHz settings available will be sufficient. If other frequency cutoffs are required, they must be provided from an external filter. Again, be sure that you take into account that the final bandwidth is that of the cascaded internal and external filters.

We do not recommend the use of LEAK SUBTRACTION in the whole-cell configuration since with whole-cell recordings it is exceedingly difficult to determine the fraction of the leak current due to the seal vs. the fraction due to background currents which might have some dependence on voltage. Software packages like pCLAMP allow a user-specified after-the-fact leakage correction, which is a much safer procedure.

INTERFACING A COMPUTER TO THE AXOPATCH 200A

The Axopatch 200A has many features that allow extensive interactions with a laboratory computer. Some are inputs and some are outputs. This section describes the use of these features with a computerized patch clamp setup.

Most whole-cell experiments and many single-channel experiments require complex voltage-step protocols. These are best provided by D/A converters interfaced to a computer. For this purpose, Axon Instruments has developed pCLAMP, AxoTape, and AxoBASIC, software that works in conjunction with the Digidata 1200 and TL-1 interfaces. The output voltage from these D/A converters can be delivered either through the front-switched or the rear-switched EXT. COMMAND inputs. We recommend the front-switched EXT. COMMAND because it is often useful to be able to disconnect the computer input via the front panel.

The scaled and filtered current is also available either from a front panel or rear panel BNC (SCALED OUTPUT). One channel of the computer's A/D converter can be connected to this output for simultaneously sampling the current as the computer is delivering command voltages through its D/A converters. The I-OUTPUT is provided for users who choose to record at a high bandwidth and filter digitally at a later time. This BNC provides the current at a 10 kHz bandwidth and a rear switched gain of either ßmV/pA or 100 ßmV/pA, where ß is the headstage gain.

While you are busy conducting patch-clamp experiments it is very easy to forget to record the settings of switches that specify the size of the current, bandwidth, etc., making it impossible to analyze the currents. Therefore, the Axopatch 200A provides the majority of this information via telegraph outputs on the rear panel. By connecting these outputs to several channels of the computer's A/D converters, it is possible to have your software interrogate the outputs before recording data so as to automatically determine the important switch settings at the time the data is recorded. This information can be included with the data record so that analysis programs can read it and, thus, always have the correct information for analyzing the recording (*e.g.*, scaling).

These outputs include telegraphs for settings of the following switches: gain, filter (frequency), and mode. In addition, an output is available which is proportional to the whole-cell capacitance setting on the front panel WHOLE CELL CAP. potentiometer. (See *Output BNCs* in **REFERENCE SECTION: GENERAL INFORMATION** section for details of actual voltages and settings.)

For both single-channel and whole-cell experiments, there is a rear panel BLANK ACTIVATE INPUT. It holds the current at the level that exists at the time a positive TRANSISTOR TRANSISTOR LOGIC (TTL) pulse is applied to this input. The current is held at this level as long as the pulse is HI. This feature is particularly useful for blanking out the capacitance transient associated with the leading edge of a voltage command. One way to utilize it is by using a second channel of D/A converter or a digital output to activate this line either just before or simultaneously with the application of a voltage step to the EXT. COMMAND input. The duration of the blanking input would be that expected for the duration of the capacitance transient.

Two rear panel BNCs are provided specifically for use in single-channel recording with the integrating headstage. One is the FORCED RESET INPUT. A disadvantage of integrating headstages is that they must be reset periodically. The larger the current that flows, the more often resets occur. Although the reset glitches are small they may be bothersome to some single-channel detection schemes. In pulsed experiments it is possible to minimize the occurrence of these glitches by resetting the integrator immediately before applying a voltage step. For many kinds of channels this ensures that no reset glitches will occur during the brief recording period following the onset of the command. This is again done by using a second channel of D/A converter or a digital output to apply a TTL pulse to the FORCED RESET line just before applying the command step. To ensure that one does not unknowingly utilize data taken during a reset or an external blanking, a DATA NOT VALID OUTPUT is supplied to the user. This line produces a TTL HI (positive) during the time that the output is held via the sample and hold circuit either during reset or blanking. The user is free to implement some scheme to interrogate this line during data collection to determine precisely when the data is not valid.

LOW NOISE RECORDING TECHNIQUES

The PATCH configuration of the Axopatch 200A is capable of producing single-channel recordings with significantly lower noise than a standard resistive patch clamp because of the inherently low noise of the integrating headstage, particularly at low to moderate frequencies (below 10 kHz). To realize this performance the user must pay close attention to other sources of noise. This is because the total rms noise of a patch clamp recording is the square root of the sum of the individual squared rms noise sources. This means that any particular noise source that is large will dominate the total noise and make other noise sources insignificant. Therefore, all potentially contributing noise sources must be minimized. Specifically, the headstage, the pipette glass, the holder, and the seal contribute significantly even under circumstances where extraneous noise pickup from the environment is negligible. It is absolutely crucial that the entire preparation be properly shielded and hum from power supply, mains, and other sources be negligible, *i.e.*, <0.1 pA_{p-p}. (Actually, <0.01 pA_{p-p} is possible with some effort). In this section, we suggest some approaches to low-noise recording of single channels. While these same approaches are a good idea for whole-cell recording, they are less important there since in whole-cell recording the dominant noise source comes from the access resistance in series with the whole-cell capacitance.

Glass Type And Coating

The noise from pipette glass itself arises from the lossy characteristics of its walls[1]. Therefore, it is expected that glasses with the lowest inherent dielectric loss will have the lowest noise. Generally, the thicker the wall is, the lower the noise will be. These expectations have been largely born out by actual experiments. Table I presents the specifications from a large number of commercially available glasses that have been used for patch voltage clamping. Each of these glasses has been shown to be sealable to cell membranes in several different cells. Aluminosilicate glasses like Corning #1723 and high lead glasses like Corning #8161 are particularly noteworthy for their low inherent noise but have not found much acceptance for use in patch clamp studies. Aluminosilicate glasses are hard to pull because of their high softening temperature and some high lead glasses have been reported to modify channel currents. Since any glass may potentially modify channel currents, one must be aware of this fact and control for it regardless of the glass one uses. We recommend two glasses: Corning #7052 and quartz. Both have been successfully sealed to many different cell types. Quartz, with its significantly lower-loss factor, has produced the lowest noise recordings known to us. However, because of its extremely high-softening temperature, quartz requires a special puller like the P-2000 from Sutter Instrument Company.

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[1] When a sine voltage is applied across a perfect dielectric, the alternating current should be 90° out of phase with the voltage. The deviation from 90° is the "loss factor". The loss factor is related to the power dissipated in the dielectric. Since energy is lost in the dielectric, dielectrics (*e.g.*, glasses) are commonly referred to as "lossy".

Pipettes can be obtained from specialty glass houses like:

Clark Electromedical Instruments
P.O. Box 8, Pangbourne, Reading, RG8 7HU, England, (073) 573-888

Garner Glass
177 S. Indian Hill Road, Claremont, CA 91711, USA, (909) 624-5071

Jencons Scientific
Cherycourt Way Industrial Estate, Stanbridge Road, Leighton Buzzard Bedfordshire LU7 8UA, UK, (0525) 372-010

Sutter Instrument Company
40 Leveroni Court, Novato, CA 94949, USA, (415) 883-0128

Even if one uses electrically superior glasses, low noise will not be obtained unless the outer surface of the glass is coated with a hydrophobic substance, such as Dow Corning Sylgard #184. This substance prevents the bathing solution from creeping up the outer wall of the pipette glass. This is important since a thin film of solution on the outer surface of the glass produces a distributed resistance that interacts with the glass capacitance to produce a noise source that rises with frequency. Since it becomes the dominant noise source, it must be eliminated. While many other hydrophobic substances have been used, none, to the best of our knowledge, produces as low noise as does Sylgard #184. Sylgard also decreases the capacitance of the pipette wall and so reduces the lossiness of the wall as well. It has been shown experimentally that Sylgard will improve the noise of any glass but it will not turn a poor electrical glass into a good one. Low-loss glasses coated with Sylgard give significantly less noise than poor glasses coated with Sylgard. Obviously, the closer to the tip that the Sylgard can be painted the lower the noise.

TABLE I

Glass Electrical And Thermal Properties

Glass	Loss Factor	Log_{10} Volume Resistivity	Dielectric Constant	Softening Temp. °C	Description
7940	.0038	11.8	3.8	1580	Quartz (fused silica)
1724	.0066	13.8	6.6	926	Aluminosilicate
7070	.25	11.2	4.1	----	Low loss borosilicate
8161	.50	12.0	8.3	604	High lead
Sylgard	.58	13.0	2.9	----	#184 Coating cmpd.
7059	.584	13.1	5.8	844	Barium-borosilicate
7760	.79	9.4	4.5	780	Borosilicate
EG-6	.80	9.6	7.0	625	High lead
0120	.80	10.1	6.7	630	High lead
EG-16	.90	11.3	9.6	580	High lead
7040	1.00	9.6	4.8	700	Kovar seal borosilicate
KG-12	1.00	9.9	6.7	632	High lead
1723	1.00	13.5	6.3	910	Aluminosilicate
0010	1.07	8.9	6.7	625	High lead
7052	1.30	9.2	4.9	710	Kovar seal borosilicate
EN-1	1.30	9.0	5.1	716	Kovar seal borosilicate
7720	1.30	8.8	4.7	755	Tungsten seal borosilicate
7056	1.50	10.2	5.7	720	Kovar seal borosilicate
3320	1.50	8.6	4.9	780	Tungsten seal borosilicate
7050	1.60	8.8	4.9	705	Series seal borosilicate
KG-33	2.20	7.9	4.6	827	Kimax borosilicate
7740	2.60	8.1	5.1	820	Pyrex borosilicate
1720	2.70	11.4	7.2	915	Aluminosilicate
N-51A	3.70	7.2	5.9	785	Borosilicate
R-6	5.10	6.6	7.3	700	Soda lime
0080	6.50	6.4	7.2	695	Soda lime

The holders supplied with the Axopatch 200A are made of polycarbonate. Polycarbonate was experimentally found to produce the lowest noise among ten substances tested. It was only slightly better than polyethylene, polypropylene, and Teflon, but was much better than nylon, Plexiglass, and Delrin. Axon Instruments holders avoid metal and shielding, which are noise sources. Holders, however, do become a significant noise source if fluid gets into them. Therefore, great care must be taken in filling pipettes with solution. They should be filled only far enough from the tip so that the end of the internal reference pipette is immersed. Any solution that gets near the back of the pipette should be dried with dry air or nitrogen to keep it from getting into the holder. Holders that become contaminated with solution should be disassembled and sonicated in ethanol or pure deionized water, and allowed to dry thoroughly before being used again. It is also a good idea to periodically clean the holders by sonication even if no fluid has been observed in them.

The cleanliness of the holder can be checked before each attempt to make a seal. When the holder with a filled pipette has been inserted into the headstage connector, and the pipette tip is positioned just above the bathing solution, the rms current noise seen on the meter of the Axopatch 200A (meter switch in the I_{RMS} position) should not significantly exceed 0.08 pA.

Seal

The seal will usually be the dominant noise source if it is only a few gigaohms. Seal resistances in excess of 20 GΩ must be obtained if exceptionally low noise single-channel recordings are to be routinely achieved. Seal quality can be monitored by periodically observing the Axopatch 200A rms noise meter (I_{RMS}). The noise depends also on the depth of the pipette tip below the surface of the bathing solution since the effective pipette capacitance increases as the depth of immersion increases. The voltage noise of the headstage interacts with the pipette capacitance to produce a noise source that rises with frequency. With excised membrane patches lifted to just under the surface of the bathing solution, the integrating headstage can produce background noise as low as 0.083 pA rms in a 5 kHz bandwidth in membrane patches from several preparations, when used with quartz glass pipettes; 0.095-0.12 pA rms is routinely achievable. These numbers can serve as guidelines for what is potentially possible in your experiments.

Signal Generator

One last potential noise source to consider is the noise in the signal generator that provides the command. In the Axopatch 200A we have succeeded in minimizing this noise by heavily attenuating the external command. However, it is possible for this noise source to be significant, particularly if the command signal comes from a D/A converter.

REFERENCE SECTION: INSTRUMENT OPERATION

The major topics in this section are organized alphabetically.

Command Potentials

Command potentials can be obtained from two internal sources, HOLDING COMMAND and SEAL TEST, and from external sources via two rear panel BNCs.

Holding Command

In voltage clamp this control allows the user to apply a membrane holding potential of ±200 mV. In current clamp the control allows application of a holding current of ±2 nA with ß=1 or ±20 nA with ß=0.1. Turn the control fully counterclockwise for a zero command. Use the three position toggle switch to set the polarity or to switch off. This control is automatically switched OFF when TRACK (I = 0) mode is chosen.

Seal Test

The SEAL TEST command generator provides a convenient source of test pulses to be used during seal formation. The 5 mV step is gated by the internal line frequency oscillator; its duty cycle is between 40% and 60%.

Current Clamp

The Axopatch 200A can be used in current-clamp mode similarly to a conventional microelectrode amplifier. There are two current-clamp modes. I-CLAMP NORMAL for use with pipette resistances greater than 1 MΩ and I-CLAMP FAST for use with pipette resistances greater than 10 MΩ. The accuracy of the response can be improved by using the FAST and SLOW ELECTRODE CAPACITANCE COMPENSATION controls.

Membrane Potential

In voltage-clamp mode, V_m is simply equal to the command potential. In current-clamp mode, V_m is not clamped and automatically becomes the voltage needed to allow the commanded current to flow.

Note: To avoid introducing errors, you **should not** change the PIPETTE OFFSET control after you switch from V-CLAMP to I-CLAMP.

Whole-Cell Current Clamp

Current-clamp can be used from the very beginning of the experiment, or you can switch into current-clamp mode at any time (see *Current Clamp* section of the **TUTORIAL**). However, if whole-cell recording has been established, special care must be taken when switching between voltage-clamp and current-clamp modes because in voltage-clamp mode the EXT. COMMAND sensitivity is 20 mV/V and the HOLDING COMMAND sensitivity is 20 mV/turn. In current-clamp the EXT.

COMMAND sensitivity changes to 2 ÷ ß nA/V while the HOLDING COMMAND sensitivity changes to 200 ÷ ß pA/ turn.

There are at least two possible ways to achieve a well-behaved transition between voltage-clamp and current-clamp modes:

1) Dedicate one external source to voltage clamp and the other to current clamp. Connect each of them to one of the EXT. COMMAND BNCs on the rear panel. While in the I=0 mode, turn off the EXT. COMMAND corresponding to voltage clamp and turn on the current clamp EXT. COMMAND. Also, while in the I=0 mode, readjust (or turn OFF) to HOLDING COMMAND control for use in I-CLAMP.

2) Design your setup to be sensitive to the MODE TELEGRAPH BNC and have your program to automatically scale a single external command accordingly so that a smooth transition between modes is obtained (see *Telegraph Output* section for values).

Whole-Cell Parameters

SERIES RESISTANCE and WHOLE-CELL CAP. are disabled in current-clamp mode.

Capacitance Compensation

The speed of the pipette depends on the pipette resistance (R_p) and the pipette capacitance (C_p). As a first-order approximation, the pipette time constant depends on the product R_pC_p. The FAST and SLOW ELECTRODE CAPACITANCE COMPENSATION controls can be used to electronically reduce the effective value of C_p, thus reducing the pipette time constant. This is achieved by injecting a transient current into the headstage input to charge and discharge C_p during signal changes.

Limitations

Similar to current clamp (*i.e.*, voltage recording) in many other patch clamps, current clamp in the Axopatch 200A is not as fast or as stable as current clamp in a conventional amplifier such as the Axoclamp or the Axoprobe. This difference in current-clamp performance results from significant differences in the design of the headstages.

In a conventional current-clamp amplifier, the headstage is designed as a voltage follower. Current is injected through a resistor and the pipette voltage is continuously recorded. The headstage of a patch-clamp amplifier is designed as a current follower. The pipette voltage is controlled while the pipette current is measured. To simulate current clamp, a feedback circuit in the main unit automatically adjusts the pipette voltage to keep the pipette current at the desired value.

Like any feedback circuit, the stability is compromised if the open-loop gain is too high. When the headstage is grounded through a pipette, the voltage gain of the headstage is nearly equal the value of the feedback resistor divided by the value of the pipette resistance. In the WHOLE CELL mode and with a pipette resistance of 1 MΩ, this voltage gain is 500 (50 in the CV 202AU). In order to guarantee stability with pipette resistances as low as 1 MΩ, the current-clamp circuitry must be deliberately slowed down, compromising the response time for high-resistance pipettes. For low-resistance pipettes the main problem is stability. In the extreme case of a zero-resistance pipette (*i.e.*, a directly grounded input), the enormous voltage gain of the headstage guarantees instability. To compromise between the two conflicting requirements of speed and stability, the Axopatch 200A has been designed with a dual speed current clamp. For low-resistance pipettes (between 1 and 10 MΩ) the I-CLAMP NORMAL setting will guarantee that the loop will be stable. For higher resistance pipettes (above 10MΩ), I-CLAMP FAST setting will be stable and have a response many times faster than the I-CLAMP NORMAL setting. (It has been observed that in some cases the I-CLAMP FAST setting will still be stable with pipette resistances down to 3 MΩ).

Headstage

Offset Adjustment For Headstage

Headstage offsets drift slowly over time and, therefore, may require renulling every few weeks. To do this, allow the headstage to warm up for a minimum of 1 hour. In V-CLAMP mode, with PATCH mode selected and with open circuit input, adjust the rear panel PATCH HEADSTAGE OFFSET potentiometer for a zero current reading on the panel meter. Change to WHOLE CELL mode and adjust the rear panel WHOLE CELL HEADSTAGE OFFSET for zero current.

Frequency Boosting

The WHOLE CELL mode of the Axopatch 200A uses a resistor in the headstage feedback that requires frequency boosting (tuning) for optimal performance. The frequency boosting circuit is tuned at the factory and will usually not require readjustment in the field. If tuning is required, see **TUNING THE HEADSTAGE** section (page 83).

Case Ground Connector

The metal headstage case and the gold-plated 2 mm socket at the rear of the headstage are connected to ground. Use this ground for grounding the preparation.

No provision is made for driving a shield since using a driven shield around the pipette increases the high-frequency noise.

Mounting the Headstage

There are two mounting options provided with the Axopatch 200A headstage:

1) The simplest method that is often satisfactory is to grip the insulated mounting rod in a manipulator.

2) For maximum mechanical rigidity, the headstage can be mounted directly to some manipulators using the acrylic mounting plate located on the bottom of the headstage and mounting screws inserted through the four outside holes.

If you are not using the mounting rod, you can remove it by twisting it counterclockwise. If you are not using the mounting plate you can remove it by removing the four screws holding it onto the headstage case.

Cleaning

Wipe the headstage connector with a damp cloth to clean salt spills. Avoid spilling liquids on the headstage.

The Teflon input connector should be kept very clean. Effective cleaning can be done by spraying with alcohol or swabbing carefully with deionized water. If possible, avoid the use of Freon since it is thought to be detrimental to the environment.

Static Precautions

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

You should ***not*** switch off power to the Axopatch 200A when handling the headstage input since this will upset the thermal equilibrium.

Optical Pick-up

The Teflon input connector and the glass walls of the hybrid package inside the headstage are translucent. High intensity light can get through in sufficient strength to activate the input transistors inside the hybrid. Therefore, you should prevent bright light from falling on the input connector. If you notice line-frequency hum on the current record, it could be due to fluctuating light levels from a bright fluorescent light or equivalent. In general, low light levels are not a problem.

Acoustic Pick-up

Rare instances have been reported where the headstage was susceptible to low amplitude acoustic pick-up. The most troublesome being a situation where the audible hum of a nearby isolation transformer was being acoustically coupled to the input of the headstage. This was traced to the silver wire of the electrode and was solved by trimming off a fraction of the wire, thus changing its resonant frequency.

Tuning The Headstage

See **TUNING THE HEADSTAGE** section (page 83).

Holders

Features

The HL-U series holder provides for enhanced low-noise mechanically stable microelectrode recordings with or without suction. Because the new holder provides a universal fit for a very wide range of pipette diameters and will fit any of our redesigned headstages, it is named the HL-U.

The barrel of the holder is made out of polycarbonate for lowest noise. There are two different barrel lengths. The shorter barrel length contributes less to the operating noise and, therefore, is ideally suited for single channel patch clamp recordings. Although the longer barrel will contribute more to the operating noise, the increased length may provide the needed clearance between the headstage and other components in the experimental setup. Maintenance is simple because the holder can be fully disassembled for cleaning and parts replacement.

Mechanical stability of the pipette is assured in several ways. For example, as the pipette cap is closed, the cone washer is compressed on the pipette from the force applied to the front and back of the cone washer. The holder mates with the special threaded Teflon connector on U-type Axon Instruments headstages and is secured in place with a threaded collar.

The holder is designed to emerge along the long axis of the headstage. A right-angle adapter can be purchased if it is necessary for the holder to emerge at 90° from the headstage.

The HL-U holder is designed to be used with Axon Instruments amplifiers, and fit all U-type CV and HS series of headstages. These headstages have a *threaded* white Teflon collet. To minimize the added noise contributed by the holder in single-channel recording, the holder uses a small (1 mm) pin for the electrical connection and a large amount of insulating Teflon. This noise problem is peculiar to single-channel recording.

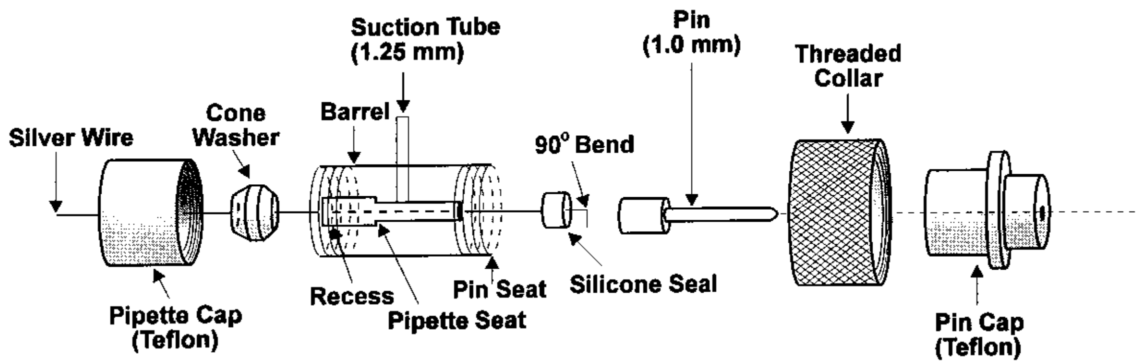

Figure 10. Exploded View of the Holder

Parts

The bore size of the HL-U accepts pipettes with an outer diameter (OD) of 1.0-1.7 mm. Pipettes are secured by a cone washer with an inner diameter (ID) that accommodates the pipette OD. Color-coding aids identification of the four sizes of cone washers: 1.0 mm (orange), 1.3 mm (clear), 1.5 mm (orange) and 1.7 mm (clear). Each HL-U is supplied with two barrel lengths, 16 mm and 28 mm.

It has been shown that a Ag/AgCl pellet offers no greater stability than properly chlorided silver wire. Moreover, the diameter of the Ag/AgCl (1 mm) restricts its use to pipettes with a large ID *i.e.*, > 1.1 mm. Therefore, the HL-U is supplied with 0.25 mm silver wire.

Spare components included with each holder are as follows: one 50 mm length of silver wire, 20 cone washers (5 of each size) and one 70 mm length of silicone tubing. Cut into 2 mm lengths, the silicone tubing will yield approximately 30 replacement silicone seals. Additional cone washers, silicone tubing, pins and silver wire can be purchased from Axon Instruments, as well as optional Ag/AgCl pellet assemblies.

Optional Ag/AgCl Pellets

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

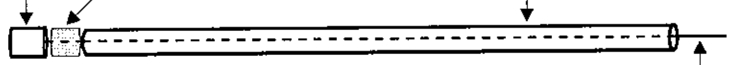

Figure 11. Ag/AgCl Pellet Assembly

Use

Insertion Of Pipette

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

To minimize cutting of the cone washer by the sharp back end of the pipette, you can smooth the pipette edges by rotating the back end of the pipette in a bunsen burner flame.

Cleaning

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

Filling Pipette

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

Silver Chloriding

It is up to you to chloride the end of this wire as required. Chloriding procedures are contained in many electrophysiology texts[1]. Typically the chlorided wire will need to be replaced or

[1] For easy-to-use recipes see Microelectrode Methods for Intracellular Recording and Ionophoresis, by R.D. Purves, London: Academic Press, 1981, p. 51.

The Axon Guide. Foster City, CA: Axon Instruments, Inc., 1993, p. 83.

rechlorided every few weeks. A simple, yet effective, chloriding procedure is to clean the silver wire down to the bare metal using fine sand paper and immerse the cleaned wire in CHLOROX bleach for about 20 minutes, until the wire is uniformly blackened. This provides a sufficient coat of AgCl to work reliably for several weeks as an internal reference pipette. Drifting or otherwise unstable offsets during experiments is suggestive of the need for rechloriding. The chlorided region should be long enough so that the pipetted solution does not come in contact with the bare silver wire.

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

The chlorided region should be long enough so that the pipette solution does not come in contact with the bare silver wire.

Replacing the Silver Wire

To replace the silver wire, insert the nonchlorided end through the hole of the silicone seal and bend the last 1 mm of wire over to an angle of 90°. Press the wire into the back of the barrel making sure that the silicone seal is flush with the back of the barrel. Slip the threaded collar over the back of the barrel. With the large end of the pin directed toward the bent-over wire screw the pin cap down firmly, but without excessive force. This assures good electrical contact. Screw the pin cap down firmly but without excessive force.

Glass Dimensions

Use the HL-U for pipettes with outside diameter (OD) of 1.0-1.7 mm. The optimal dimensions should match the inner diameter (ID) of the four sizes of cone washers, 1.1, 1.3, 1.5 and 1.7 mm. When the pipette OD falls between two sizes of cone washers, the larger size cone washer should be used. For instance, if the pipette OD is 1.6 mm, then use a cone washer with an ID of 1.7 mm.

Adapters

HLR-U right-angle adapters allow the HL-U series holder to emerge at 90° from the headstage. Use the HLR-U with the HL-U holder.

HLB-U BNC-to-Axon adapter allows conventional BNC-type holders to be used with Axon Instruments U-type headstages. Use the HLB-U with all U-type CV and HS headstages (*e.g.,* CV-4-1/100U and HS-2A-x1MGU). These headstages have a threaded white Teflon collet.

Input BNCs

BLANK ACTIVATE INPUT -- A positive TTL pulse delivered to this input causes the output current to be held at the value it had at the instant the pulse was activated and to hold it at this level for the duration of the pulse. This is useful, for example, to blank the capacitance transient associated with the leading edge of a voltage step.

EXTERNAL COMMAND INPUT (front panel switched) -- Applies external command signal divided by 50 to the command input of the Axopatch 200A when the front toggle switch is set to EXT. COMMAND.

EXTERNAL COMMAND INPUT (rear panel switched) -- Applies external command signal divided by 50 to the command input when the rear panel switch is engaged.

The two external command inputs are summed together inside the patch clamp.

FORCED RESET INPUT -- Causes the integrator and differentiator to reset on the positive edge of a TTL pulse. Used, for example, to force a reset just before applying a command to a cell or membrane patch. This increases the probability that no reset will occur during the brief recording period following the command.

SPEED TEST INPUT -- When switched on by the rear panel switch, the SPEED TEST injects a current into the headstage input through a 1 pF capacitor in the headstage. This is used for verifying the dynamic response of the headstage. Injected current waveform is the derivative of the voltage waveform applied at SPEED TEST input. For example, a 100 Hz 10 V_{p-p} triangle wave will inject a 1 nA_{p-p} square wave into the headstage input.

Output Section

All the controls in the Output Section affect only the signal on the Scaled Output.

Filter

The filter is a 4-pole lowpass Bessel filter. The attenuation of signals and noise above the -3 dB frequency is 80 dB/decade (24 dB/octave). The Bessel characteristic is suitable for patch and voltage clamping because it introduces < 1% overshoot.

All lowpass filters slow the rise time of the signal. For filters with < 10% overshoot the 10-90% rise time is:

$$t_r \approx \frac{0.35}{f_c}$$

where f_c is the -3 dB frequency in Hertz. For example, the 10-90 % rise time of a 1 kHz filter is approximately 350 µs.

Note: If you use an external filter: Some manufacturers specify the -3 dB frequency based on the phase response of the filter instead of its amplitude response, or based on a straight line approximation to the filter characteristics instead of the actual characteristics. You should check your external filter by checking t_r for a step signal applied to its input.

When a signal with 10-90% rise time t_1 is passed through a filter with 10-90% rise time t_2, the rise time of the output signal is approximately:

$$t_r \approx \sqrt{t_1^2 + t_2^2}$$

Output Gain (α)

There are ten output gain settings in a 1, 2, 5 ratio. These are: 0.5, 1, 2, 5, 10, 20, 50, 100, 200, and 500.

In patch clamp amplifier design, there are conflicting concerns when deciding where to place the Bessel filter and the gain amplifier in the signal pathway. On the one hand, to minimize the noise contribution of the circuitry of the Bessel filter itself, the gain amplifier should come first. On the other hand, if the gain amplifier comes first, the peaks of the amplified signal and noise might get clipped if the gain setting is high. After filtering, it is difficult to distinguish the clipped signals from biological channel currents.

The very effective compromise solution adopted in the Axopatch 200A is to put an initial gain of ten in front of the filter if the gain switch is set to 5 or more. The rest of the gain amplification is after the filter.

The overload (OVLD) light illuminates if either the input to the filter or the output of the final gain amplifier exceeds ±10 V for longer than the 100 μs. This signifies that the signal output is being clipped and has become non-linear.

The electronic gain internal to the Axopatch 200A output section is twice the α value shown on the gain switch. The reason is clear if you consider the output in the WHOLE CELL configuration. To achieve the best compromise between noise and dynamic range, the Axopatch 200A uses a 500 MΩ feedback resistor. The output of the current to voltage converter is, therefore, 0.5 mV/pA. To simplify the daily mental scaling task for the user, the headstage output is presented to the user as 1 mV/pA by including an additional two times (x2) gain. In PATCH mode, the output of the differentiator is also 0.5 mV/pA and, therefore, similar considerations apply.

Output BNCs

CELL CAPACITANCE TELEGRAPH — Puts out a voltage between 0 and 10 V that linearly specifies the whole-cell capacitance between 0 and 100 pF (between 0 and 1000 pF for CV 202AU). The voltage is 0 to -10 V if the WHOLE CELL capacitance switch is turned off. If the WHOLE CELL CAP. switch is ON but the control is overridden (*e.g.*, by current-clamp mode) the telegraph output will be between 0 and +10 V.

DATA NOT VALID OUTPUT — Puts out a positive TTL voltage while the current output is blanked either during reset or during external blanking via the blank activate input.

FREQUENCY TELEGRAPH — Provides a series of voltages that can be read by a computer to determine the setting on the filter switch. The voltages and their associated frequency settings are as follows:

Frequency (kHz):	1	2	5	10	50
Frequency Telegraph Voltage Output (V):	2	4	6	8	10

GAIN TELEGRAPH — Provides a series of voltages that can be read by a computer to determine the setting of the gain switch. The gain takes α and β factors into account.

I (mV/pA):	0.05[1]	0.1[1]	0.2[1]	0.5	1	2	5	10	20	50	100[2]	200[2]	500[2]
V_m (mV/mV):	N/A	N/A	N/A	0.5	1	2	5	10	20	50	100	200	500
Telegraph Output (V):	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5

These values have been chosen to fit in the range 0-10 V so that they can be read on A/D converter channels of most computers.

I OUTPUT — Current output filtered at 10 kHz (3-pole Bessel). Gain is chosen by rear panel switch to be βmV/pA or 100β mV/pA.

MODE TELEGRAPH OUTPUT — Provides a series of voltages that can be read by the computer to determine the setting on the MODE switch. The voltages and their associated switch settings are as follows:

Mode:	V-CLAMP	TRACK	I-CLAMP NORMAL	I-CLAMP FAST
Scaled Output:	I	I	V_m	V_m
Telegraph (volts):	6	4	2	1

SCALED OUTPUT -- Provides the current (filtered and scaled) in V-CLAMP and TRACK modes or the membrane voltage (filtered and scaled) in I-CLAMP modes. This BNC is duplicated on the front and back panels. On the front panel, two LEDs specify whether the output is current (I) or membrane voltage (V_m). The current gain is α ßmV/pA. The voltage gain is α mV/mV.

10 V_m OUTPUT -- Provides the membrane voltage multiplied by ten (x10) for use in current-clamp experiments.

In V-CLAMP mode, 10 V_m is ten times the *command* potential. If the pipette current is zero, or if the series resistance correction is 100%, the command potential is identical to the *actual* membrane potential. The actual membrane potential is given by the following equation:

$$\text{Actual Membrane Potential (mV)} = \frac{10\,V_m}{10} - \frac{(100 - \text{CORR})}{100}\, I\, R_s$$

where I = pipette current in nA
CORR = % correction set on SERIES RESISTANCE CORRECTION control
R_s = resistance in MΩ set on SERIES RESISTANCE control

In TRACK mode, 10 V_m is ten times the *actual* potential.

[1] Applicable to CV 202A only
[2] Applicable to CV 201A only

In I-CLAMP mode, if the current is zero, 10 V_m is ten times the *actual* membrane potential. If current is flowing in the pipette, 10 V_m includes the voltage drop across the pipette. The actual membrane potential is given by the following equation:

$$\text{Actual Membrane Potential (mV)} = \frac{10\,V_m}{10} - I\,R_s$$

where I = pipette current in nA
R_s = resistance in MΩ set on SERIES RESISTANCE control

Panel Meter

The main panel meter displays one of five signals. Selection is made by a rotary knob. The five signals are:

1) V_{TRACK}: The output of the automatic offset nulling circuit.

2) V_m: The membrane potential.

3) I_{RMS}: The rms current noise. The noise is measured in a 5 kHz bandwidth using a Butterworth filter that is independent of the front-panel lowpass Bessel filter. The front-panel gain does not affect this reading.

4) I: The pipette current. The reading is automatically scaled to suit the headstage gain. The operation is autoranging; that is, the decimal point and the units indicators automatically shift so that large DC currents can be displayed.

5) V_{HOLD}/I_{HOLD}: The value set on the HOLDING COMMAND potentiometer. When V_{HOLD}/I_{HOLD} is selected, it will show the absolute value of the HOLDING COMMAND even if the HOLDING COMMAND control is OFF.

Zap

In order to go from cell-attached patch clamping to whole-cell patch clamping it is necessary to rupture the patch. This is normally done by carefully controlled suction.

Another, easier to apply, technique for rupturing the patch is ZAP. ZAP works by applying a large hyperpolarizing voltage (1.3 V_{DC}) to the patch for a controlled duration. This often causes dielectric breakdown of the membrane.

Suggested Use

Apply a repetitive test command (*e.g.*, Seal Test). Start with Duration = 0.5 ms. Press Trigger to ZAP the membrane. Successful zapping is accompanied by an increase in the current noise and by large capacitance-charging current transients in response to the test command. Use the briefest Zap that will rupture the membrane. Too long a Zap could cause the seal resistance to deteriorate.

Power-Supply Voltage Selection & Fuse Changing

Supply Voltage

The Axopatch 200A can be directly connected to all international supply voltages. The input range is from 85 to 264 V_{AC}. No range switching is required. Alternatively, the instrument can be powered by a DC voltage of 110 - 340 V_{DC}. In the DC case, the SEAL TEST is inoperative.

Changing The Fuse

The Axopatch 200A uses a 2.0 A, 250 V slow acting 5 x 20 mm fuse.

Before changing the fuse investigate the reason for its failure.

To change the fuse:

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

REFERENCE SECTION: GENERAL INFORMATION

Grounding and Hum

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

The Axopatch 200A has inherently low hum levels (less than 0.005 pA_{p-p}). To take advantage of these low levels great care must be taken when incorporating the Axopatch 200A into a complete recording system. The following procedures should be followed.

1) **Ground the preparation bath only by directly connecting it to the gold ground connector on the back of the headstage.**

2) Place the Axopatch 200A in the rack in a position where it will not absorb radiation from adjacent equipment. A thick sheet of steel placed between the Axopatch 200A and the radiating equipment can effectively reduce induced hum.

3) Initially make only one connection to the Axopatch 200A -- from the SCALED OUTPUT to the oscilloscope output. After verifying that the hum levels are low, start increasing the complexity of the connections one lead at a time. Leads should not be draped near transformers located inside other equipment. In desperate circumstances, the continuity of the shield on an offending coaxial cable can be broken.

4) Try grounding auxiliary equipment from a ground distribution bus. This bus should be connected to the Axopatch 200A via the yellow banana (4 mm) socket on the rear panel. This socket is connected to the signal ground of the Axopatch 200A (*i.e.*, the outer conductors of all the BNC connectors). The signal ground in the Axopatch 200A is isolated from the chassis and power ground.

5) Experiment. While hum can be explained in theory (*e.g.*, direct pickup, earth loops), in practice the ultimate theory is the end result. Following the rules above is the best start. The final hum level can often be kept to less than 0.1 pA_{p-p}. One technique that should **not** be used to reduce hum is the delicate placement of cables so that a number of competing hum sources cancel out. Such a procedure is too prone to accidental alteration.

Finally, the Axopatch 200A can be run off DC power with no alteration of circuitry. (However, the SEAL TEST function is inoperative). The power requirements are 30 W at 110 to 340 V_{DC}. This could be a last resort option to sever the possibility of ground loops. Call the factory for details.

Model Cell

The PATCH-1U model cell (Fig. 12) can be used to assist with testing and setting up. The pipette is modeled by a 10 MΩ resistor, the cell is modeled by 500 MΩ in parallel with 33 pF (the membrane time constant is 16.5 ms), and the patch is modeled by a 10 GΩ resistor. The pipette capacitance is about 4-6 pF. The charging time constant is approximately 330 μs (10 MΩ x 33 pF).

The PATCH-1U model cell has been made without a switch to change the model between BATH, PATCH and CELL positions. This is because even the best switches have an enormous amount of leakage resistance and capacitance which increases the noise three to five times beyond what you can achieve with a good seal. Instead of switches, three separate plug positions have been provided and you can rotate the model cell into the position required. With this technique the noise contribution of the model cell is still somewhat more than can be achieved with a good seal, but the increase is less than 50%.

The PATCH-1U model cell can conveniently be used in conjunction with the tutorial at the front of this manual.

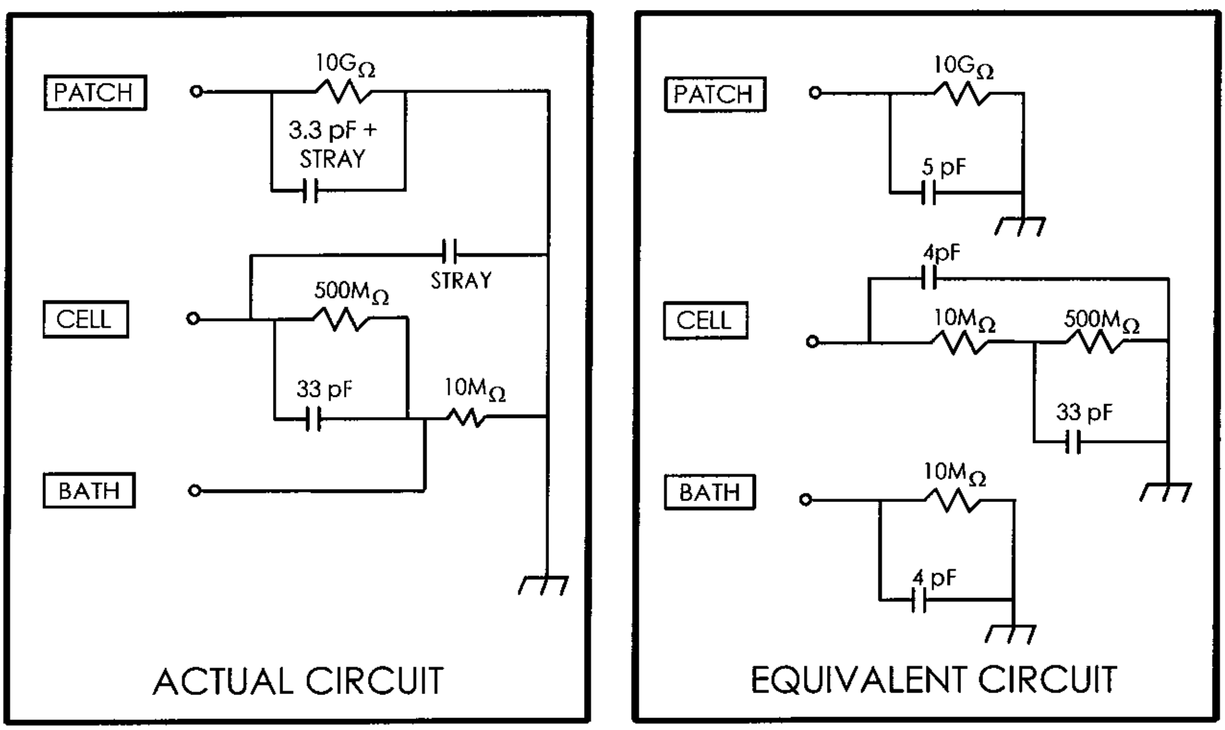

Figure 12. PATCH-1U Model Cell

Model Bilayer

The MCB-1U model bilayer contains a 10 kΩ resistor that models the pipette in series with a 100 pF capacitor that models the bilayer membrane (Fig. 13).

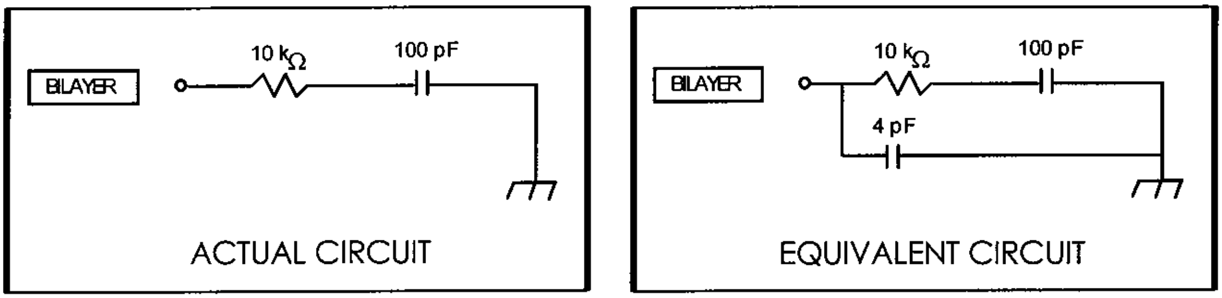

Figure 13. MCB-1U Bilayer Model

Power-Supply Glitches

The Axopatch 200A has been designed to minimize the effects of power-supply transients (glitches). Some power-supply glitches do, however, get through. These can cause transients to appear on the voltage and current outputs which may corrupt high-sensitivity recordings.

The only completely effective way to gain immunity from mains glitches is to eliminate them at the source. Most glitches are due to the switching on and off of other equipment and lights on the same power-supply circuit. Precautions to be taken include:

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

In most circumstances, occasional transients on the outputs are inconsequential and, therefore, no precautions have to be taken.

Ten-Turn Potentiometers

The ten-turn potentiometers used in the Axopatch 200A are high-quality wirewound types.

An inherent problem of wirewound potentiometers is that the wire elements tend to oxidize. When this happens, significant instrument noise becomes noticeable when the potentiometer is turned. This condition is easily cured. If noise is observed when a potentiometer is turned, the potentiometer manufacturer recommends to rapidly spin the knob 20-30 times between full clockwise and full counterclockwise. This clears the oxide off the element and restores noise-free operation.

---

1 RFI - Radio Frequency Interference

REFERENCE SECTION: PRINCIPLES OF OPERATION

Headstages

Principals of Operation

Patch-clamp headstages are current-to-voltage (I-V) converters. That is, the voltage output is proportional to the current input. In contrast, traditional microelectrode amplifier headstages are voltage followers in which the voltage output corresponds to the voltage input.

Resistor Feedback

The essential parts of a resistive headstage are shown in Figure 14:

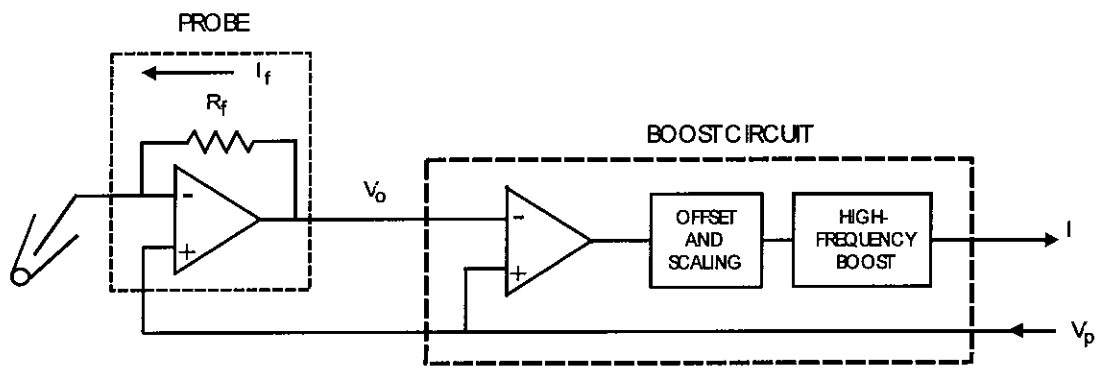

Figure 14. Resistive Headstage

For an ideal op amp[1] the pipette current is the same as the current through the feedback element (R_f). Since the op amp in the probe acts to keep the voltage at its two inputs equal to each other, we know that the potential at its negative input equals V_p (pipette potential). Thus, the voltage across R_f is V_o - V_p, which is calculated by the differential amplifier in the control box. Subsequent amplifiers are used to scale the gain and remove voltage offsets.

A fundamental problem of this circuit when used for patch clamping is that the output bandwidth of the probe is inherently low. To a first approximation, the bandwidth is set by the product of R_f and the stray capacitance across it. For example, if R_f is 500 MΩ and the stray capacitance is 0.5 pF, the bandwidth is about 600 Hz.

To overcome this limitation, the probe output is passed through a high-frequency boost circuit. The gain of this circuit is proportional to the frequency.

[1] op amp - operational amplifier

Capacitor Feedback

An alternative to measuring current across feedback resistors is to measure current as the rate of change of the voltage across a capacitor. Figure 15 shows the essential parts of an integrating headstage.

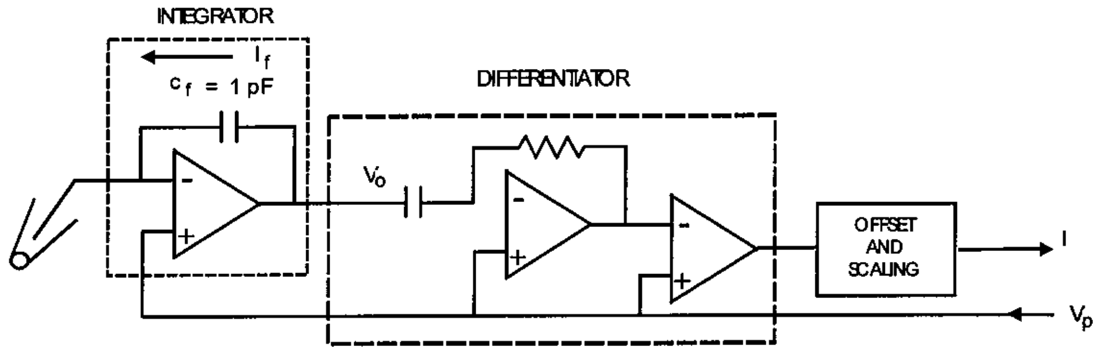

Figure 15. Capacitive Headstage

Nearly ideal capacitors exist whereas high-gigohm resistors found in patch clamp headstages possess intrinsic noise (in excess of thermal noise) and have limited bandwidth due to stray capacitance. The benefits of capacitors are taken advantage of in the PATCH configuration (capacitive feedback) of the Axopatch 200A headstage, which is designed for ultra-low noise single-channel recordings. The headstage measures the integral of the current which is subsequently differentiated to allow measurement of the current itself. Unlike the resistive headstage, the output of the capacitive headstage is fast. No boost circuit is required to increase its high-frequency response. The capacitor mode achieves a substantial reduction of noise and has much better linearity compared to resistive feedback headstages. This noise reduction is particularly significant in the frequency band of interest for single channel recordings (10 Hz - 10 kHz). In integrating headstages, the low-frequency asymptote of the noise depends on the gate current of the headstage input transistor rather than on the thermal noise of the feedback resistor. With the U430 transistors used in the Axopatch 200A, this low frequency noise can be substantially less than that of the 50 GΩ feedback resistor customarily used in resistive headstages. At the same time, the high frequency noise is less because the capacitor lacks the excess high frequency noise associated with gigohm value resistors.

While the integrating is quieter and more linear than resistive feedback headstages there is one disadvantage. The voltage across the feedback capacitor cannot ramp in one direction forever (the rate of change describes the current at the input). At some point the capacitor voltage will approach the supply limits and the integrator must be reset to start again near zero volts. Thus, the current record must be interrupted for 50 µs while the integrator and differentiator reset. The frequency of resets depends on the current that passes through the headstage. The larger the current the more frequent the resets.

When this reset occurs, a sample and hold circuit maintains the value of the current at the level it had just prior to the reset. It does this for the duration of the reset while the DATA NOT VALID line specifies that the reset is in progress. Following reset, the sample and hold is inactivated, the DATA NOT VALID line goes low and integration of the current again proceeds.

Figure 16 shows the signal pathway for the capacitor-feedback configuration. The output current of the capacitor-feedback headstage is normally connected through a switch to the output pre-filter amplifier, then to the lowpass filter and finally to the post-filter amplifier. The current signal also goes through a lowpass filter to a sample-and-hold amplifier. During reset, the switch shifts to the RESET position. Simultaneously, the sample-and-hold amplifier is switched to the hold mode so that the signal immediately before the reset transient occurs is presented to the output amplifiers.

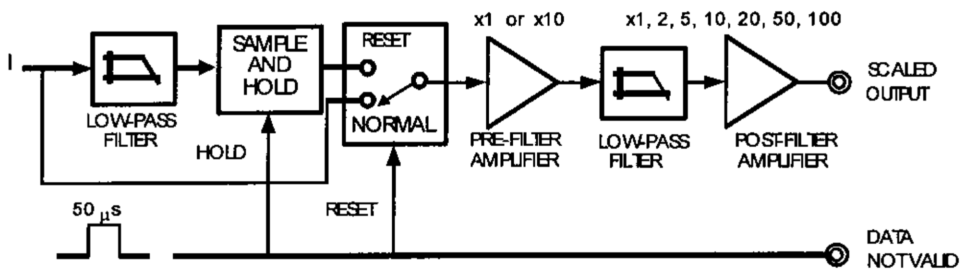

Figure 16. Signal Handling During Resets

The Axopatch 200A contains both a resistive and capacitive feedback elements. The capacitive element is selected using the front panel CONFIG. switch in the PATCH position, while the resistive headstage is engaged when the switch is in the WHOLE CELL position.

The capacitive feedback (PATCH) is recommended for single-channel recording because it offers the lowest noise. In such recordings, the average current is not more than a few pA and so the resets are infrequent.

In whole cell experiments, the average current is generally tens or hundreds of picoamps. Resets would occur very frequently if capacitive feedback was selected. Therefore, we recommend resistive feedback (WHOLE CELL) for these experiments.

If the Probe Output is Slow, How Can Voltage Clamping be Fast?

In resistive headstages (but not capacitive headstages) the current output of the current-to-voltage (I-V) converter in the probe is slow. The high-frequency boost occurs afterwards and cannot influence the events at the electrode. Thus, one might conclude that the voltage clamp of the pipette must also be slow.

In fact, despite the slow current output of the I-V converter, the voltage clamp of the pipette is rapid. The pipette is connected to the negative input (summing junction) of the op amp. The command potential is connected to the positive input of the op amp. The operation of the op amp in this configuration is to force the potential at the summing junction to rapidly follow the potential at the positive input. If the command potential is a step, the potential at the summing junction, and hence the pipette, is also a step. The current required to achieve this step is passed through the feedback resistor (R_f) and the associated stray feedback capacitance (C_{Rf}) of the I-V converter. The output of the I-V converter settles to the final value with time constant $R_f C_{Rf}$. This relatively slow settling occurs despite the fact that the step at the summing junction is fast.

In this discussion, we have carefully referred to the fact that it is the "pipette" that is rapidly voltage clamped. The membrane potential is voltage clamped to its final value much more slowly. To a reasonable approximation, the time constant for voltage clamping the membrane is R_pC_m, where R_p is the pipette resistance and C_m is the membrane capacitance.

What is Clamped During Voltage Clamping?

Voltage clamping is the intrinsic mode of operation of a patch clamp headstage. The series combination of the pipette and the patch/cell membrane is voltage clamped -- its voltage remains constant at a user-specified value (V_c), assuming that the membrane potential equals the command potential. This is true only if the current causes a negligible voltage drop across the pipette resistance.

Capacitance Compensation

Pipette Capacitance Compensation

The FAST and SLOW PIPETTE CAPACITANCE COMPENSATION controls are used to charge the pipette capacitance (C_p) during a voltage step. A simplified circuit of the fast and slow compensation circuitry is shown in the Figure 17.

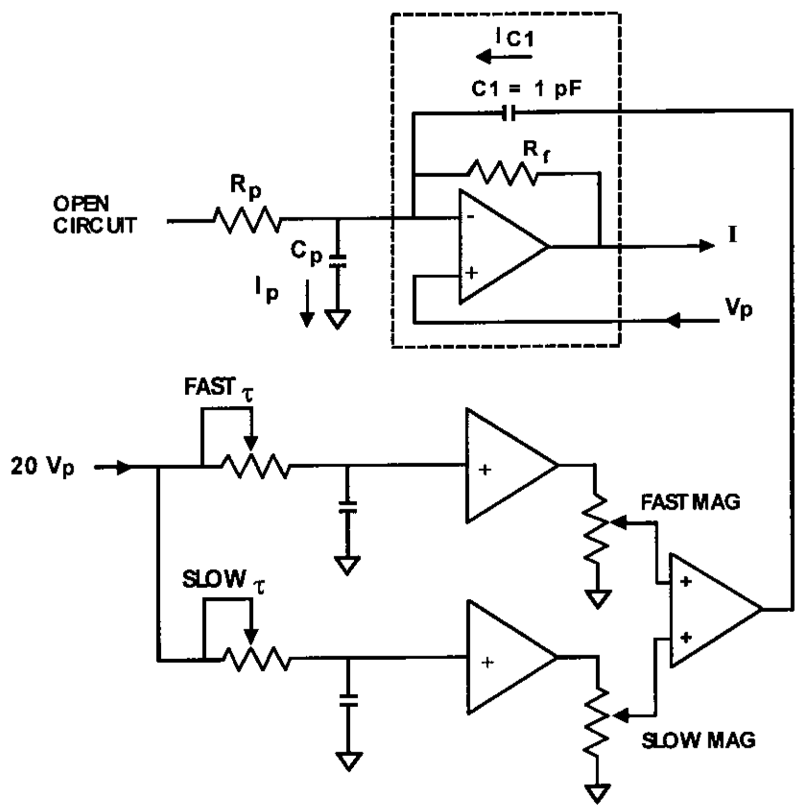

Figure 17. Pipette Capacitance Compensation Circuit

When the pipette command potential (V_p) changes, current I_p flows into C_p to charge it to the new potential. If no compensation is used, I_p is supplied by the feedback element (R_f) resulting in a large transient signal on the output (I).

By properly setting the fast and slow magnitude and τ controls, a current (I_{C1}) can be induced in capacitor C1 (connected to the headstage input) to exactly equal I_p. In this case no current needs be supplied by R_f, and there is no transient on the output.

The FAST controls compensate that part of C_p that can be represented by a lumped capacitance at the headstage input. This is the major part of C_p. A small amount of C_p can only be represented as a capacitor with a series resistance component. This takes longer to charge to its final value and is compensated by the SLOW controls.

Whole-Cell Capacitance Compensation

The SERIES RESISTANCE and WHOLE-CELL CAP. controls are used to charge the membrane capacitance (C_m). Figure 18 is a simplified circuit of these controls.

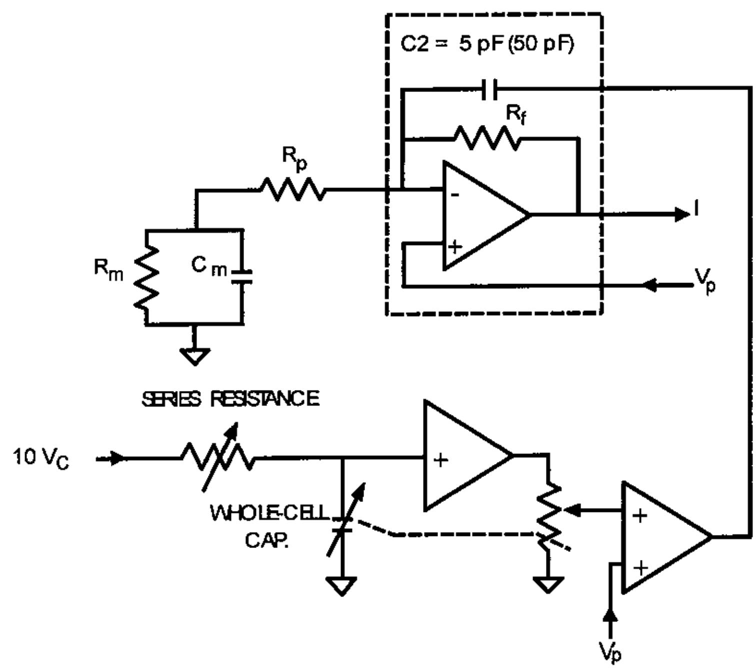

Figure 18. Whole-Cell Capacitance Compensation Circuit

Assume that the fast and slow electrode compensation controls have already been set to compensate for C_p. By appropriately adjusting the SERIES RESISTANCE and WHOLE CELL CAP. controls, the current injected through C2 will supply the transient membrane current (I_m). These adjustments do not alter the time constant for charging the membrane. Their function is to offload the burden of this task from the feedback resistor, R_f. In many cells, even a small command voltage (V_c) of a few tens of millivolts can require such a large current to charge the membrane that it cannot be supplied by R_f. The headstage output saturates for a few hundred microseconds or a few milliseconds, thus extending the total time necessary to charge the membrane. This saturation problem is eliminated by the appropriate adjustment of the SERIES RESISTANCE and WHOLE CELL CAP. controls. This adjustment is particularly important during series resistance correction (see *Series Resistance* section in **PRINCIPLES OF OPERATION**) since it increases the current-passing demands on R_f. By moving the pathway for charging the membrane capacitance from R_f to C2, the SERIES RESISTANCE circuitry can operate without causing the headstage input to saturate. The value of C2 is 5 pF in the CV 201AU headstage and 50 pF in the CV 202AU headstage.

The effect of transferring the current-passing burden from R_f to C2 is illustrated in Figure 19.

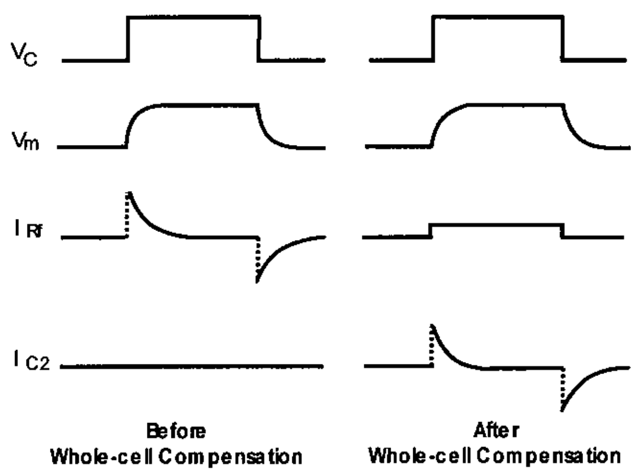

Figure 19. Using the Injection Capacitor to Charge the Membrane Capacitance

After perfect whole-cell compensation is applied, the current to charge the membrane capacitor is removed from the I_{Rf} trace and only the steady state current remains. All of the transient current appears in the I_{C2} trace. (The I_{C2} trace in the figure was recorded using an oscilloscope probe connected to the internal circuitry). The I and V_m outputs on the Axopatch 200A show the I_{Rf} and V_c trace illustrated in Figure 19. It is easy to mistakenly think that the time course for charging the membrane is very fast but this is clearly not the case. Use of an independent electrode in the cell would show that the cell charging rate is not affected by these adjustments.

Absolute Value

The absolute value of the membrane capacitance shows on the WHOLE CELL CAP. dial after the whole-cell current transient has been eliminated. This value may be used for estimating the surface area of the cell assuming that the capacitance of 1 cm^2 membrane is 1 μF. The setting of this control is available for computer acquisition on the CELL CAPACITANCE output telegraph on the rear panel of the Axopatch 200A.

Limitations

The measurement of series resistance (R_s) and cell capacitance (C_m) is accurate only if the membrane resistance (R_m) is significantly greater than R_s.

Series Resistance

In patch clamping, the term refers to the pipette resistance plus any other access resistances to the patch or cell.

The SERIES RESISTANCE and WHOLE CELL CAP. controls, along with % PREDICTION and % CORRECTION, are active in WHOLE CELL mode. In PATCH mode, only SERIES RESISTANCE and % CORRECTION are functional.

Absolute Value

The absolute range of the series resistance shows on the SERIES RESISTANCE dial after the whole-cell current transient has been eliminated. The range of the SERIES RESISTANCE values is 0 - 100 MΩ for both the CV 201AU and the CV 202AU headstages.

Correction, Prediction, and Lag

As discussed in the previous section (*Capacitance Compensation*), eliminating the current transient by setting the SERIES RESISTANCE and WHOLE CELL CAP. controls does not improve the speed of clamping the cell.

Uncompensated series resistance (R_S) has several effects on the fidelity of whole-cell voltage clamp measurements. In the absence of series resistance compensation, these are:

1. Following a step change in command potential, V_c, the actual cell membrane potential, V_m, will respond with an exponential time course ($V_m = V_c(1-e^{t/\tau_S})$) with a time constant given by $\tau_S = R_S C_m$, where C_m is the cell membrane capacitance. This time constant is 330 μs for the model cell provided with the Axopatch 200A (R_S = 10 MΩ, C_m = 33 pF). This means that the actual membrane potential response to a step voltage command will have a 10-90% risetime of more than 0.7 ms and will not settle to within 1% of its final value until about 1.5 ms after the start of a step command. It is typical that after achieving a whole-cell recording, the access (series) resistance is approximately twice as large as the original pipette resistance; a three-fold or higher resistance increase is not uncommon. Thus, series resistances of 20 MΩ or more may be encountered. With a 100 pF cell a series resistance of 20 MΩ will result in a membrane-charging time constant of 2 ms. Settling of the true membrane potential to within 1% of its final value will require nearly 10 ms after the start of a step command. Note that the uncompensated whole-cell capacity transient has the shape of the derivative of the true membrane potential and both will have the same time constant.

2. Uncompensated series resistance will also cause the membrane potential to deviate from the command potential when ionic membrane current, I_m , flows. The magnitude of this error is given by $R_S I_m$; *e.g.*, for R_S = 10 MΩ and I_m = 2 nA, a 20 mV error will result. In extreme situations in the presence of voltage gated channels, complete loss of control of membrane potential can occur.

3. The first two types of errors associated with series resistance described above are well known to most investigators. The third type of error is less commonly recognized. Series resistance in conjunction with membrane capacitance forms a one-pole RC filter with a corner (-3 dB) frequency given by $f=1/2\pi R_S C_m$ for the measurement of membrane currents. This filter will

distort currents regardless of their amplitude. For the parameters of the whole-cell model provided with the Axopatch 200A (R_S = 10 MΩ, C_m = 33 pF) this filter restricts true measurement bandwidth to 480 Hz without series resistance compensation. For a situation with R_S = 20 MΩ and C_m = 100 pF (as, for example, may be encountered with isolated cardiac myocytes), the actual bandwidth of current measurement is only about 80 Hz with no compensation for series resistance.

The Axopatch 200A uses a dual approach for the correction of the above errors associated with series resistance. In this regard, the performance of the Axopatch 200A is unparalleled by any other commercial patch clamp.

The approach taken to whole-cell capacity transient cancellation and series-resistance compensation in the Axopatch 200A involves the following front panel controls:

1. WHOLE-CELL PARAMETERS: WHOLE CELL CAP. potentiometer and its ON/OFF switch, and SERIES RESISTANCE potentiometer. These controls are used to cancel the whole-cell capacity transient. Their action is coordinated with series resistance compensation controls described below. Note that the WHOLE CELL CAP. switch must be ON to cancel whole-cell capacity transients. Turning this switch OFF disables the signal injected through the C2 capacitor in the headstage used to cancel the capacity transient (Fig. 18); it also disables the PREDICTION potentiometer (see below). With the switch ON (for now assume that PREDICTION is OFF), the signal injected through C2 capacitor has an amplitude that is determined by the setting of the WHOLE CELL CAP. control and a time constant that is determined by the setting of both the WHOLE CELL CAP. and SERIES RESISTANCE controls. Precise canceling the whole-cell capacity transient with these controls requires a unique setting in each case. These settings are accurate representations of R_S and C_m to within 2-3%. As will be described below, the use of PREDICTION will modify the time course of the signal applied to the capacitor C2.

2. SERIES RESISTANCE COMPENSATION: PREDICTION, CORRECTION, and LAG potentiometers. These controls are used to correct for the errors associated with series resistance.

3. PIPETTE CAPACITANCE COMPENSATION: FAST MAG and FAST τ, and SLOW MAG and SLOW τ. Note that when using series resistance compensation it is important that the fast capacity transient arising from stray and pipette capacitance be adequately canceled.

PREDICTION adds a transient signal to the command potential, speeding the rate at which the true membrane potential will change in response to a step voltage command. It is similar to the idea of SUPERCHARGING introduced by Armstrong and Chow (Armstrong, C.M. and Chow, R.H. (1987) Biophys. J. 52, 1333.). The signal added to the command is derived from the command input and from the setting of the WHOLE-CELL PARAMETERS (WHOLE CELL CAP. and SERIES RESISTANCE control settings). It enables the actual membrane potential to be a faithful replica of the command potential; *i.e.*, the effects of series resistance in distorting the command potential at the cell membrane are removed up to the percentage setting of the control (*e.g.*, a 98% setting means that, in effect, only 2% of the original series resistance remains in terms of command potential). PREDICTION works with the setting of the SERIES RESISTANCE potentiometer and the WHOLE CELL CAP. potentiometer that, in conjunction with the percentage set on the PREDICTION control, determine the magnitude and time constant of the compensating signal added to the command input.

Simultaneously, the signal applied to the C2 capacitor used to cancel whole-cell capacity transients is appropriately modified as the percent PREDICTION is increased. (The value of C2 is 5 pF in the CV 201AU headstage and 50 pF in the CV 202AU headstage).

For example, consider a whole-cell voltage clamp situation where R_s = 10 MΩ and C_m= 50 pF and the resting membrane resistance R_m is very large with respect to R_s. Assume that the SERIES RESISTANCE control is set at 10 MΩ and the WHOLE CELL CAP. control is set at 50 pF so that the whole-cell capacity transient is perfectly canceled. If the PREDICTION control is OFF (0%), the signal applied to the headstage 5 pF capacitor (50 pF in CV 202AU) in response to a step voltage command will have a time constant of 500 µs and an amplitude that is appropriate to cancel a whole-cell capacitance transient arising from these parameters (about 10 V_c). With 0% PREDICTION nothing is added to the command potential waveform. In response to a step voltage command the cell membrane potential will change to its new value with a time constant of 500 µs (R_sC_m). If the % PREDICTION control is advanced to 50%, a transient will be added to the command potential step, V_c, with a time constant of 250 µs and an amplitude equal to that of the command step itself. This will have the effect of changing the cell membrane potential in response to a step command with a time constant given by R_sC_m (1 - %PREDICTION/100); here this is 250 µs. More formally, the command potential with the PREDICTION signal included, V_{cp}, can be expressed in terms of the command input, V_c, by:

$$V_{cp}= V_c\,(1+s\tau_s)/(1+s\tau_{srp})$$

where $\tau_s = R_sC_m$, $\tau_{srp} = R_{srp}C_m$, where R_{srp} is the residual (uncompensated) series resistance in terms of PREDICTION given by $R_{srp} = R_s$ (1 - %PREDICTION/100), and, in the frequency domains = jw (w is the natural frequency, $w = 2\pi f$), or in the time domain s is the operator d/dt. Thus, $V_{cp} = V_c\,(1 + (R_s/R_{srp} - 1)e^{-t/\tau_{srp}})$.

Moreover, the membrane potential, V_m, is given by $V_m = V_{cp}/(1+s\tau_s) = V_c/(1+s\tau_{srp})$, or $V_m = V_c\,(1 - e^{-t/\tau_{srp}})$. Therefore, advancing the PREDICTION potentiometer setting to 80% gives R_{srp} of 2 MΩ and τ_{srp} of 100 µs. That is, the speed with which the membrane potential responds to a voltage command is improved 5 fold over that which is achieved with 0% PREDICTION. PREDICTION of 98% gives R_{srp} of 200 kΩ and τ_{srp} of 10 µs. The membrane potential will now respond to a step voltage command with a 10-90% risetime of about 22 µs and will settle to within 1% of its final value in less than 50 µs.

Saturation Effects

Note that the equation presented above for V_{cp} (*i.e.*, the command potential plus PREDICTION signal) can be used to define the maximum allowable %PREDICTION for a given size step voltage command (this limit should not be confused with limitations imposed by the stability of the PREDICTION circuit itself). The command plus PREDICTION signal is attenuated at the headstage by a 10:1 voltage divider. Since the circuitry in the Axopatch 200A mainframe will saturate at about ±11-12 V, V_{cp} is limited in absolute value to about 1.1 to 1.2 V. To be conservative, we will use 1.1 V in the following calculations:

The peak amplitude of V_{cp} for a step voltage command, V_c, is given by $V_c\,(R_s/R_{srp})$ which can be rewritten as V_c /(1 - %PREDICTION/100). So we may state the limitation on V_c as a function of % PREDICTION as:

$V_c \leq 1.1(1 - \%PREDICTION/100)$

or the limitation on % PREDICTION as a function of V_c as:

$\%\ PREDICTION \leq 100(1 - V_c/1.1)$

Thus, for example, if it is known that the maximum command step to be used in a particular experiment is 100 mV, PREDICTION may be set at 91% without fear of saturation of V_{cp}; this is true regardless of the value of R_s or C_m. In fact, this is a rather conservative estimate since it is derived on the assumption that the signal V_{cp} will instantly jump to its maximum value following a step voltage command. In fact, due to limitations in the speed of the PREDICTION circuitry, this over-estimates the maximum value of V_{cp}, particularly when % PREDICTION is large. In actual practice, PREDICTION can typically be set to about 94% for a 100 mV command step. Figure 3 shows maximum % PREDICTION as a function of voltage step.

As the PREDICTION potentiometer is advanced the signal applied to the 5 pF capacitor (C2) in the headstage is modified appropriately so that it will continue to cancel the whole-cell capacity transient despite the fact that the speed of this transient has increased. This is simply accomplished by reducing the time constant of this signal as % PREDICTION is increased. If the circuitry worked perfectly, and if the whole-cell capacity transient had been perfectly canceled with 0% PREDICTION, no transient would appear as % PREDICTION is increased up to the maximum allowable values. However, due to the complexity of this circuitry and a variety of non-ideal characteristics, cancellation of whole-cell capacity transients does not remain perfect as % PREDICTION is increased. The small residual transient that emerges can, however, be completely removed by small readjustments of the setting of the WHOLE CELL CAP., SERIES RESISTANCE, FAST MAG and FAST τ controls. A detailed description of the required procedure is provided in the *Series Resistance* section of the **TUTORIAL**.

It should be noted that PREDICTION will work for any command waveform, not just steps. This may be useful for capacitance measurements using phase sensitive techniques or lock-in amplifiers.

Although PREDICTION can greatly speed the response time of the true membrane potential with respect to the command potential and, thus, overcome one important effect of series resistance, it does not correct for the effects of series resistance associated with the flow of membrane ionic current (*i.e.*, IR drops and filtering effects described above). This is the role of the CORRECTION potentiometer. CORRECTION feeds back a portion of the measured membrane current; this signal is added to the command potential. The percentage set by the CORRECTION potentiometer refers to the setting of the SERIES RESISTANCE control of WHOLE-CELL PARAMETERS. For example, if the SERIES RESISTANCE control is set at 10 MΩ, a 90% setting of the CORRECTION control means that 9 MΩ of series resistance is compensated; the residual (uncompensated) series resistance in terms of CORRECTION, R_{src}, is 1 MΩ.

The LAG potentiometer is used to determine the time constant of a one-pole RC filter through which the CORRECTION signal is passed prior to being summed with V_c. The -3 dB bandwidth of this filter is given by $1/2\pi\tau_{LAG}$, where τ_{LAG} is the setting (in seconds) of the LAG control. For example, a LAG of 5 µs corresponds to filtering the CORRECTION signal at 32 kHz; 10 µs corresponds to 16 kHz, 20 µs corresponds to 8 kHz, etc. The LAG control is used to ensure stability when large amounts of CORRECTION are used. It is generally good practice to begin using CORRECTION with the LAG control set at 10-20 μs or more. However, once the desired level of CORRECTION has been achieved, it is usually possible (if desired) to significantly reduce the LAG setting; 5 μs is usually quite adequate for 90% CORRECTION.

Continuing with the example considered above, *i.e.*, a cell with R_s= 10 MΩ and C_m = 50 pF, a 90% CORRECTION setting will reduce voltage errors in the true membrane potential resulting from the flow of ionic current to 10% of the error present with 0% CORRECTION. For example, a 2 nA ionic current would produce a 20 mV error in V_m with 0% CORRECTION, whereas 90% CORRECTION will reduce this error to only 2 mV. At the same time, the use of CORRECTION will reduce the filtering effect of R_s and C_m on the measured current. With 0% CORRECTION the actual bandwidth of current measurement prior to any output filtering is limited to $1/2\pi R_s C_m$, which will be about 320 Hz in this example. As % CORRECTION is increased this "filter" moves to $1/2\pi R_{src} C_m$, so that for 90% CORRECTION the possible bandwidth for current measurement is increased to 3.2 kHz in this example. With 95% CORRECTION the possible bandwidth is increased to 6.4 kHz and with 98% it is further increased to 16 kHz (although the effects of LAG should not be forgotten).

If the capacity transient has been canceled prior to the use of CORRECTION (and for now assume that PREDICTION has already been set at 95%), then, in principle, there is no capacity current to feed back when CORRECTION is utilized. Note that the discussion here of capacity current should be distinguished from the discussions of the ionic current. Therefore, no transient should develop as CORRECTION is advanced. In practice, however, a small transient will emerge as % CORRECTION is increased. Again, this is due to non-ideal characteristics of the circuitry. Procedures for eliminating this transient by minor readjustments of SERIES RESISTANCE, WHOLE CELL CAP., FAST MAG and FAST τ controls are described in detail in the **TUTORIAL**.

There are many situations in which it will be desirable to have the % PREDICTION and the % CORRECTION controls set at different values. For example, for a 200 mV step command PREDICTION should be limited to about 80% (see Figure 3; however, somewhat higher values can often be used) to avoid saturation. However, it is usually possible to compensate series resistance up to 90 to 95% or more by use of the CORRECTION control. In other patch clamps the issue of saturation would limit the amount of compensation used for ionic currents; this is not true in the Axopatch 200A. On the other hand, in some cases it might be impossible to advance the CORRECTION percentage beyond about 70% without causing instability. Nevertheless, PREDICTION, which is inherently stable up to 98% or more, can be set to a value substantially higher than 70% (about 95%), thereby ensuring that the true transmembrane potential changes rapidly in response to the command potential even though a substantial series resistance remains uncompensated in terms of ionic currents.

Oscillations

One of the practical problems when using the % CORRECTION function of SERIES RESISTANCE compensation is that there is a great risk of oscillations because the CORRECTION circuitry is a form of positive feedback. The main cause of oscillations is the inability of the circuitry to distinguish between current that flows down the pipette and into the cell from current that flows through the stray capacitance of the pipette into the bath. The current that flows through the pipette resistance into the cell is the current that is intended to be compensated. The CORRECTION circuitry also tries to compensate for the current into the pipette capacitance. However, in this case there is no significant series resistance component to compensate, and the CORRECTION circuit will oscillate as soon as the CORRECTION control is advanced.

The tendency to oscillate depends, therefore, on the relative magnitude of the pipette resistance to the pipette capacitance and the degree of compensation of the pipette capacitance.

Using Lag to Prevent Oscillations

The tendency to oscillate can be reduced by limiting the bandwidth of the positive-feedback circuit. This is the function of the LAG control.

Limitations

Series-resistance compensation is an attempt to electronically reduce the effect of the pipette resistance. Because of practical limitations, it is never perfect. Even if 100% compensation could be used with stability, this would only apply to DC and medium-speed currents. Very fast currents cannot be fully corrected.

For best results, the cell membrane resistance should be many fold higher than the pipette resistance. This is normally the case for cells at rest carrying small drug-activated or synaptic currents. However, during voltage activation the cell membrane resistance could fall a hundredfold or more to values similar to or less than the series resistance. In these cases it is probable that:

1) There will be a significant error due to the voltage drop across the pipette. This error is not obvious to the user because the patch clamp controls the combined voltage drop across the pipette and the cell.

2) The setting of the SERIES RESISTANCE and WHOLE CELL CAP. compensation controls will become erroneous because it is based on the time constant to charge the membrane capacitance before the change in membrane resistance occurred. Since this time constant depends on the parallel value of membrane resistance and the pipette series resistance, this error could become substantial. The effect will be a larger transient at voltage levels that activate the fall of membrane resistance.

If the cell input resistance becomes comparable to, or less than, the pipette resistance, the whole-cell patch technique will probably not work. In this case, it would be preferable to use a discontinuous (chopped) single-electrode voltage clamp, such as the Axoclamp, that will give more accurate results.

Pipette Offset

The PIPETTE OFFSET control is used to compensate for the total offset of the liquid-liquid and liquid-metal junction potentials in the electrode and bath, and the offset of the probe input amplifier.

The PIPETTE OFFSET is a ten-turn potentiometer used to add up to ±250 mV to the pipette command potential (V_p). It is used at the beginning of each experiment to zero the pipette current (I) when the electrode first touches the solution. Since the pipette offset tends to change during and after seal formation, the PIPETTE OFFSET potentiometer may be used again to rezero I. A description of how to use the PIPETTE OFFSET is given in the *Pipette Offset Adjustment* section of the **TUTORIAL**.

In TRACK mode, V_p is continuously adjusted to keep I = 0 (or near zero) even though the pipette offset may be changing at a fairly rapid rate. TRACK mode is most often used during seal formation to stop the I trace from jumping into saturation.

I is severely distorted during TRACK mode; the effect is similar to AC coupling. The Axopatch 200A should **never** be left in TRACK mode once data is being recorded.

The rate at which TRACK returns I to zero depends on the pipette resistance. If you are applying a test pulse, you will find that for pipette resistance of 10 MΩ or lower you will see an obvious droop.

After a seal is formed the TRACK circuit becomes very slow in its efforts to keep I = 0.

Leak Subtraction

The passive membrane response to a voltage step consists typically of a transient and a steady-state component. It is often helpful to subtract these from the output so that only active responses are observed.

The transient component is eliminated by using the CAPACITANCE COMPENSATION controls as discussed in the *Capacitance Compensation* section above. The steady-state component is eliminated by the LEAK SUBTRACTION control. This circuit simply subtracts a scaled version of the command voltage from the current. The *Adjustment of Leak Subtraction* section in the TUTORIAL describes the use of this control.

Since both the CAPACITANCE COMPENSATION and LEAK SUBTRACTION controls are driven by the command voltage, the passive responses remain eliminated for all polarities and magnitudes of command.

When using the CV 202AU headstage the setting of the LEAK SUBTRACTION control is to be multiplied by β=0.1.

LEAK SUBTRACTION is disabled in Current Clamp and Track modes.

Zap

The conventional technique for rupturing a membrane patch to go to whole-cell recording is to apply a pulse of suction. Sometimes this technique damages the cell. **Zap** provides an alternative method. It applies a pulse of voltage across the patch that ruptures the patch, presumably by causing dielectric breakdown. A timing circuit lets you find a Zap duration that is most likely to achieve the desired result without damaging the seal.

Lipid Bilayers

Experimental Techniques

The integrating (PATCH) configuration is uniquely suited for performing experiments on lipid bilayer membranes. This is because the probe can be triggered at the onset of a voltage step so that it can quickly charge the membrane. Also, because the Axopatch 200A is stable while driving purely capacitive loads (up to 1000 pF), one has the ability of minimizing noise by minimizing access resistance.

To realize the full potential of the design, the user should externally initiate a reset at each voltage step applied to the membrane. This is accomplished by applying a positive going TTL pulse to the FORCED RESET INPUT BNC on the rear panel. Because of internal timing delays, the positive going edge of the reset pulse can occur simultaneously with the command step. The reset time is independent of the length of the reset pulse (it is factory set at 50 µs).

If it becomes necessary to blank the output for longer time periods, the BLANK ACTIVATE INPUT BNC should be used. When a HI logic level pulse is applied to this input, the output signal will be blanked for the duration of the pulse. In this circumstance, both FORCED RESET and BLANK ACTIVATE inputs could be tied together. For example, if a 1 ms pulse is applied to both BNCs, a normal reset will be initiated while blanking the output for 1ms.

The following is an explanation of the reset charging process:

When the Axopatch 200A probe is in the PATCH configuration, it has a 1 pF capacitor as its feedback element (Fig. 20).

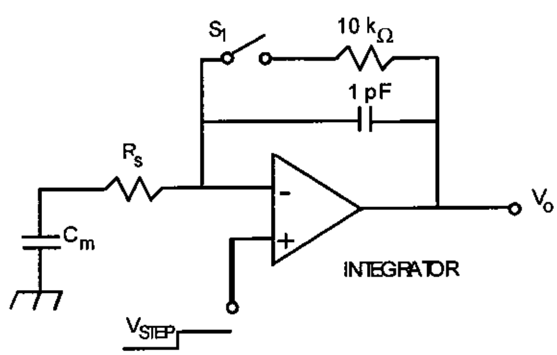

Figure 20. Integrator Driving Bilayer Model

When a voltage step, V_{step}, is applied to the command input, the output, V_O, of the integrator, will attempt to become:

$$V_O = -V_{step} \frac{C_m}{C_f}$$

where C_m is the membrane capacitance in pF and C_f is the feedback capacitor, the value of which is 1pF.

If $V_O \geq 10$ V (*e.g.*, 100 mV on 100 pF), a reset of the integrator is initiated internally; an external reset pulse is not needed. In the reset state, switch S_1 is closed and the 1 pF capacitor is shunted by the 10 kΩ reset resistor. This all happens within 2 μs from the time the integrator reaches + 10 V or - 10 V.

During the time the integrator is in reset (10 μs), it can pass up to 1 mA (10^6 nA) of current to charge the membrane. For example, if C_m = 100 pF and R_s = 10 kΩ, the charging time constant (τ) is 1μs. Therefore, the integrator will be in its reset mode for 10τ. This is long enough for the membrane to essentially reach its final value.

If the voltage step is small enough so that it does not cause a reset (V_O<10 V), it is recommend that a reset signal be applied externally. This is not required in order to help the membrane to charge faster, but to keep the succeeding circuitry (differentiator, gain blocks, etc.) from saturating and erroneously indicating a slowly charging membrane.

If you are not sure whether you need to apply an external signal, it is best to just include it. There is no conflict in having a reset initiated by both an external and internal signal.

Noise vs. Access Resistance

The Axopatch 200A is quite comfortable with loads of up to 1000 pF of pure capacitance (the maximum bandwidth decreases to about 20 kHz, no overshoot). This can be used to great advantage when doing bilayer experiments; the lower the access resistance, the lower the noise. While there will be some lower limit on the value of the access resistance, it will not be set by stability criteria of the instrument.

In bilayer applications, one is typically working with bandwidths below 1 kHz. In this region, the e_nC_{in} noise has not yet become the major contributor to the overall noise (where e_n is the voltage noise of the probe input FETs[1] and C_{in} is the capacitance of the input of the headstage, which is primarily the bilayer membrane capacitance). However, a resistance in series with the bilayer membrane capacitance produces voltage noise just as though the headstage had high intrinsic noise. If this resistance is large enough, then it becomes the major noise contributor.

Figure 21 shows peak-to-peak noise versus series resistance in a 1 kHz bandwidth, for a given bilayer membrane capacitance.

[1] FET - Field Effect Transistor

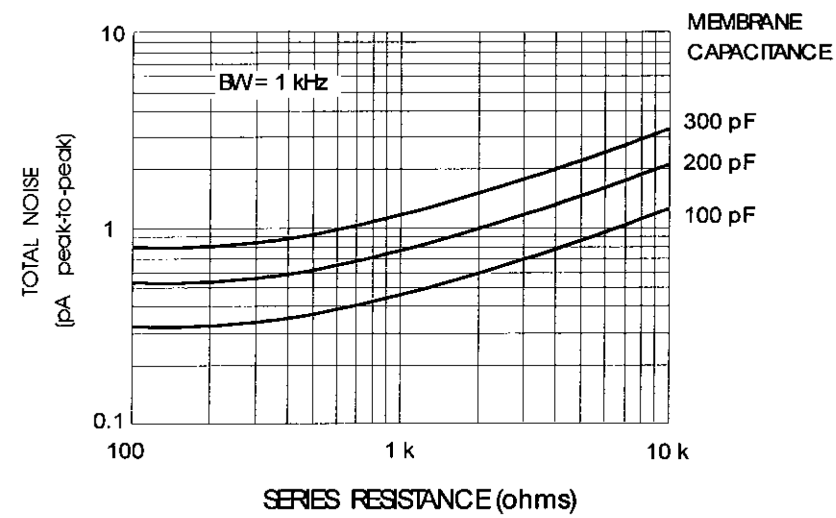

Figure 21. Typical Current Noise in Bilayer Experiments

Current and Voltage Conventions

The terminology used in this discussion applies to all amplifiers manufactured by Axon Instruments.

Positive Current

The flow of positive ions *out* of the headstage into the microelectrode and out of the microelectrode tip into the preparation is termed positive current.

Inward Current

Current that flows across the membrane, from the outside surface to the inside surface, is termed inward current.

Outward Current

Current that flows across the membrane, from the inside surface to the outside surface, is termed outward current.

Positive Potential

The term *positive potential* means a *positive* voltage at the headstage input with respect to ground.

Transmembrane Potential

The *transmembrane potential* (V_m) is the potential at the inside of the cell minus the potential at the outside. This term is applied equally to the whole-cell membrane and to membrane patches.

Depolarizing / Hyperpolarizing

The resting V_m value of most cells is negative. If a positive current flows into the cell, V_m initially becomes less negative. For example, V_m might shift from an initial resting value of -70 mV to a new value of -20 mV. Since the absolute magnitude of V_m is smaller, the current is said to *depolarize* the cell (*i.e.*, it reduces the "polarizing" voltage across the membrane). This convention is adhered to even if the current is so large that the absolute magnitude of V_m becomes larger. For example, a current that causes V_m to shift from -70 mV to +90 mV is still said to depolarize the cell. Stated simply, *depolarization* is a *positive* shift in V_m. Conversely, *hyperpolarization* is a *negative* shift in V_m.

Whole-Cell Voltage and Current Clamp

Depolarizing / Hyperpolarizing Commands

In whole-cell voltage clamping, a *positive* shift in the command voltage causes a positive shift in V_m and is said to be *depolarizing*. A *negative* shift in the command voltage causes a negative shift in V_m and is said to be *hyperpolarizing*.

Transmembrane Potential *vs.* Command Potential

In whole-cell voltage clamp, the command potential controls the voltage at the tip of the intracellular voltage-recording microelectrode. The transmembrane potential is thus equal to the command potential.

Inward / Outward Current

In a cell generating an action potential, depolarization is caused by a flow of positive sodium or calcium ions *into* the cell. That is, *depolarization* in this case is caused by an *inward* current.

During intracellular current clamping, a depolarizing current is a *positive* current out of the microelectrode tip into the interior of the cell. This current then passes through the membrane *out* of the cell into the bathing solution. Thus, in intracellular current clamping, a *depolarizing (positive)* current is an *outward* current.

An *inward* sodium current flows in some cells after a depolarizing voltage step. When the cell is voltage clamped, the sodium current is canceled by an equal and opposite current flowing into the headstage via the microelectrode. Thus it is a *negative* current. When two-electrode voltage clamping was first used in the early 1950's, the investigators chose to call the *negative* current that they measured a *depolarizing* current because it corresponded to the depolarizing sodium current. This choice, while based on sound logic, was unfortunate because it means that from the recording instrument's point of view, a negative current is *hyperpolarizing* in intracellular current-clamp experiments but *depolarizing* in voltage-clamp experiments.

To prevent confusion, Axon Instruments has decided to always use current and voltage conventions based on the instrument's perspective. That is, the current is always unambiguously defined with respect to the direction of flow into or out of the headstage. Some instrument designers have put switches into the instruments to reverse the current and even the command voltage polarities so that the researcher can switch the polarities depending on the type of experiment. This approach has been rejected by Axon Instruments because of the real danger that if the researcher forgets to move the switch to the preferred position, the data recorded on the computer could be wrongly interpreted. Axon Instruments believes that the data should be recorded unambiguously.

Patch Clamp

By design, the patch-clamp command voltage is positive if it increases the potential inside the micropipette. Whether it is hyperpolarizing or depolarizing depends upon whether the patch is "cell attached", "inside out" or "outside out". The patch-clamp pipette current is positive if it flows from the headstage through the tip of the micropipette into the patch membrane.

Cell-Attached Patch

The membrane patch is attached to the cell. The pipette is connected to the outside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become more negative, therefore it is *hyperpolarizing*. For example, if the intracellular potential is -70 mV with respect to 0 mV outside, the potential across the patch is also -70 mV. If the potential inside the pipette is then increased from 0 mV to +20 mV, the transmembrane potential of the patch hyperpolarizes from -70 mV to -90 mV.

From the examples it can be seen that the transmembrane patch potential is inversely proportional to the command potential, and shifted by the resting membrane potential (RMP) of the cell. A positive pipette current flows through the pipette, across the patch membrane into the cell. Therefore a *positive* current is *inward.*

Inside-Out Patch

The membrane patch is detached from the cell. The surface that was originally the inside surface is exposed to the bath solution. Now the potential on the inside surface is 0 mV (bath potential). The pipette is still connected to the outside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become more negative, therefore it is *hyperpolarizing*. For example, to approximate resting membrane conditions of V_m = -70 mV, the potential inside the pipette must be adjusted to +70 mV. If the potential inside the pipette is increased from +70 mV to +90 mV, the transmembrane potential of the patch hyperpolarizes from -70 mV to -90 mV.

From the example it can be seen that the transmembrane patch potential is inversely proportional to the command potential. A positive pipette current flows through the pipette, across the patch membrane from the outside surface to the inside surface. Therefore a *positive* current is *inward.*

Outside-Out Patch

The membrane patch is detached from the cell in such a way that the surface that was originally the outside surface remains exposed to the bath solution. The potential on the outside surface is 0 mV (bath potential). The pipette interior is connected to what was originally the inside surface of the membrane. A *positive* command voltage causes the transmembrane potential to become less negative, therefore it is *depolarizing*. For example, to approximate resting membrane conditions, assuming that V_m = -70 mV, the potential inside the pipette must be adjusted to

-70 mV. If the potential inside the pipette is then increased from -70 mV to -50 mV, the transmembrane potential of the patch depolarizes from -70 mV to -50 mV.

The membrane potential is directly proportional to the command potential. A positive pipette current flows through the pipette, across the patch membrane from the inside surface to the outside surface. Therefore a *positive* current is *outward.*

Summary

1) *Positive* current corresponds to:

Cell-attached patch	patch inward current
Inside-out patch	patch inward current
Outside-out patch	patch outward current
Whole-cell voltage clamp	outward membrane current
Whole-cell current clamp	outward membrane current

2) A *positive* shift in the command potential is:

Cell-attached patch	hyperpolarizing
Inside-out patch	hyperpolarizing
Outside-out patch	depolarizing
Whole-cell voltage clamp	depolarizing

3) The correspondence between the command potential (V_{CMD}) and the transmembrane potential (V_m) is:

Cell-attached patch	Vm = RMP - Vc
Inside-out patch	Vm = - Vc
Outside-out patch	Vm = Vc
Whole-cell voltage clamp	Vm = Vc

Trouble Shooting

It has been our experience at AXON INSTRUMENTS that the majority of troubles reported to us have been caused by faulty equipment connected to our instruments.

If you have a problem, please disconnect ***all*** instruments connected to the Axopatch 200A except for the headstage. Ideally, remove the Axopatch 200A from the rack. Work completely through the *Functional Checkout.* This can often uncover a problem that is in your set up. If the problem persists, please call us for assistance.

Another common problem is caused when dirt or corrosion build up in the headstage connector socket. This can cause unstable current and voltage offsets. It is important to keep the holders and the headstage input clean.

SPECIFICATIONS

Unless otherwise noted: $T_A = 20°C$, 1 hr warm-up time.

CV 201AU or CV 202AU Headstage

Construction: All critical components are in a sealed hybrid.

Configuration: High-speed low-noise current-to-voltage converter.

Headstage Gain (β): CV 201AU: 1 mV/pA in either PATCH or WHOLE CELL mode.
CV 202AU: 1 mV/pA in PATCH mode, 0.1 mV/pA in WHOLE CELL mode.

Note: If headstages are changed, a full re-calibration is required. See section B-1.

Feedback Element: CV 201AU: PATCH 1pF
WHOLE CELL 500 MΩ in parallel with 1pF
CV 202AU: PATCH 1pF
WHOLE CELL 50 MΩ in parallel with 1pF

Feedback Element Selection: FET[1] switches in hybrid enable remote selection of either a capacitor (PATCH mode) or a parallel combination of a capacitor and resistor (WHOLE CELL mode).

Tuning (WHOLE CELL mode only): Tuning circuit to idealize response of the feedback resistor is contained in the main instrument. Tuning is automatically bypassed when the capacitive feedback is selected.

Pipette-Capacitance-Compensation Injection Capacitor Value: 1 pF

Whole-Cell-Capacitance-Compensation Injection Capacitor Values:

PATCH mode: none (either model)

WHOLE CELL mode: CV 201AU: 5 pF
CV 202AU: 50 pF

Case: Case connected to ground. Case jack mates to 2 mm plugs.

Bandwidth: Test signal applied via SPEED TEST input; PATCH or WHOLE CELL mode:
Internal: 70 kHz
Max. External: 50 kHz (limited by output filter)

Capacitive Load Stability: 1000 pF, 0 Ω in series

1 FET - Field Effect Transistor

Maximum Instrument Noise: Measured with minimal external noise sources (*i.e.*, radiated line frequency noise, mechanical vibration), 8-pole Bessel filter.

		PATCH	WHOLE CELL CV 201AU	WHOLE CELL CV 202AU
Without holder:	Line freq. & harmonics	0.005 pAp-p	0.005 pAp-p	0.005 pAp-p
	0.1-100 Hz	0.075 pAp-p	0.50 pAp-p	1.60 pAp-p
	0.1-1 kHz	0.020 pA rms	0.20 pA rms	0.60 pA rms
	0.1-5 kHz	0.070 pA rms	0.50 pA rms	1.50 pA rms
	0.1-10 kHz	0.160 pA rms	1.00 pA rms	3.00 pA rms
With holder:	0.1-10 kHz	0.185 pA rms	1.00 pA rms	3.00 pA rms

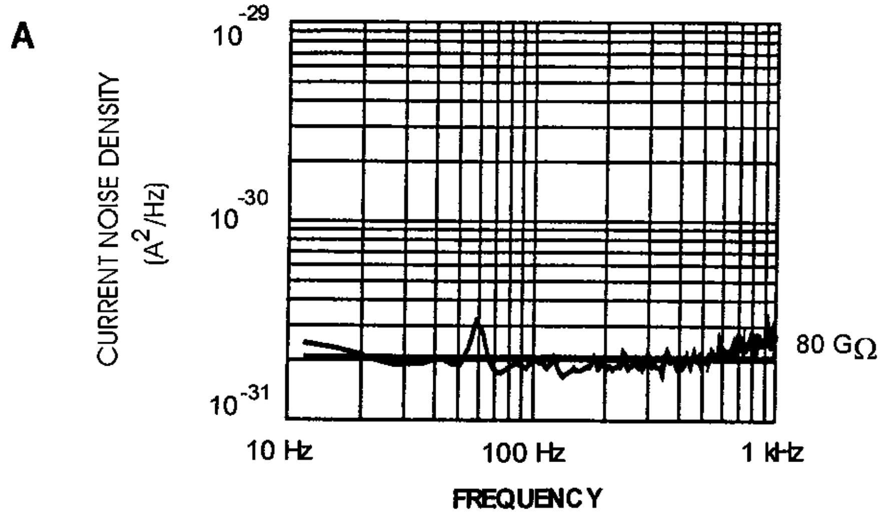

Figure 22A. Typical Low Frequency Current Noise Spectrum, Patch Mode

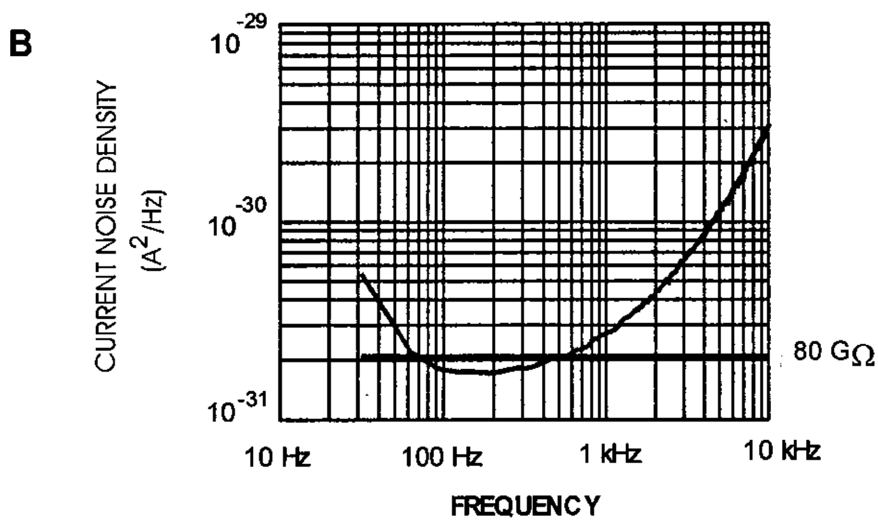

Figure 22B: Typical Broadband Current Noise Spectrum, Patch Mode

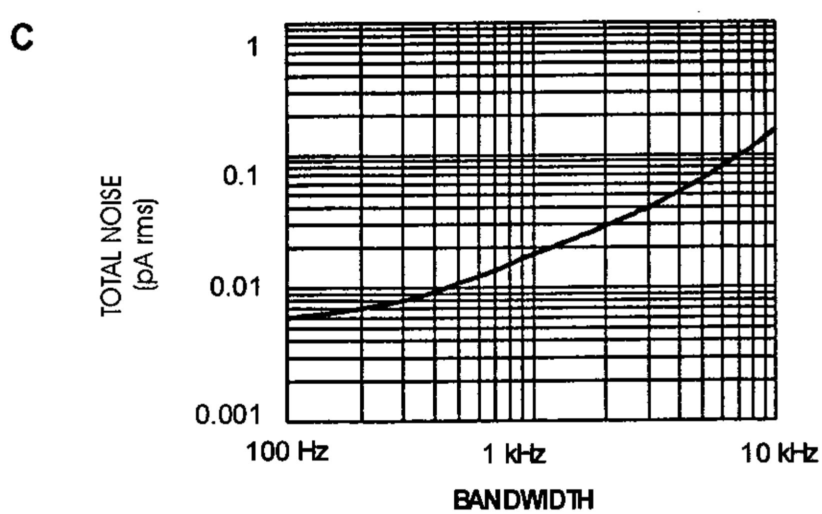

Figure 22C: Typical Total Current Noise as a Function of Bandwidth, Patch Mode

Reset Characteristics (Patch Mode only)

Total reset time: 50 μs ± 10%

this includes

integrator reset	10 μs
differentiator reset	30 μs
other overhead	10 μs

Time between resets (Tbr):

For DC currents:

$$T_{BR} = 10/(I_{DC} - I_{BIAS})$$

where I_{DC} and I_{BIAS} are in pA and T_{BR} is in seconds.

I_{BIAS} is typically 0.3 - 1.0 pA.

For transient currents:

A reset will occur if the headstage must deliver more than 10 pC of charge to the membrane. For example, a 60 mV step imposed on a 200 pF bilayer membrane will cause a reset (12 pC of charge needed) whereas a 40 mV step will not (8 pC of charge needed).

Reset transients in current waveform at Scaled Output (typical):

100 Hz bandwidth	± 0.25	pA
1 kHz	± 0.5	pA
10 kHz	± 2	pA

Current Clamp

The current clamp mode has two speed settings: I-CLAMP NORMAL and I-CLAMP FAST. I-CLAMP NORMAL is for use with electrode resistances greater than 1 MΩ. I-CLAMP FAST is for use with pipette resistances greater than 10 MΩ.

The speed of the current clamp depends on the MODE setting (NORMAL or FAST), the time constant of the cell and the pipette resistance.

R_p	R_m	C_m	10 - 90% rise time (overshoot) I-CLAMP NORMAL	10 - 90% rise time (overshoot) I-CLAMP FAST
1 MΩ	0 MΩ	0 pF	15 μs (10%)	N/A
1 MΩ	500 MΩ	33 pF	350 μs (0%)	N/A
10 MΩ	0 MΩ	0 pF	200 μs (20%)	20 μs (<1%)
10 MΩ	500 MΩ	33 pF	250 μs (10%)	10 μs (<1%)
50 MΩ	500 MΩ	33 pF	500 μs (30%)	150 μs (<1%)

HL-U Pipette Holder

HL-U holder mates to threaded Teflon input connector of the CV headstage. Post for suction tubing is 1 mm OD. HL-U holder accepts glass 1.0-1.7 mm OD. Supplied with silver wire. Optional HLR-U right-angle adapter and HLB-U BNC adapter are available.

Capacitance Compensation

1. Pipette Capacitance

Fast τ:	0.2- 5 μs
Fast Magnitude:	0 - 10 pF
Slow τ:	0.1 - 10 ms
Slow Magnitude:	0 - 1 pF

These controls are used to charge pipette capacitance. In I-CLAMP modes they act as a negative capacitance.

2. Whole-Cell Capacitance

CV 201AU:	0.3 - 100 pF
CV 202AU:	3 - 1000 pF

3. Series Resistance 0 - 100 MΩ

These controls are used to charge membrane capacitance in whole-cell voltage clamp. For PATCH mode, whole-cell capacitance is not operative. In I-CLAMP modes neither control is operative. The whole-cell capacitance control places an analog voltage proportional to setting on CELL CAPACITANCE TELEGRAPH OUTPUT. The range of the SERIES RESISTANCE values is 0 - 100 MΩ for both the CV 201AU and CV 202AU headstages.

Series Resistance Compensation

% Prediction: OFF, 0-100 %. Acts with WHOLE-CELL PARAMETERS to speed up charging of the membrane. Maximum achievable % PREDICTION is limited by magnitude of voltage step (see Figure 3).

% Correction: OFF, 0-100%. Acts with SERIES RESISTANCE setting to reduce series resistance errors and speed up response to ionic currents.

Lag: 1-100 μs. Cuts high-frequency response of series-resistance correction circuit to enable a higher CORRECTION setting.

Mode

V-Clamp: Pipette voltage is clamped.

I-Clamp Normal or Fast: Pipette current is clamped.
Normal mode stable for electrode resistances greater than 1 MΩ
Fast mode stable for electrode resistances greater than 10 MΩ

Track (I=0): Slow I-clamp to zero current.
Selected mode sets analog voltage on MODE TELEGRAPH OUTPUT.

Zap

Amplitude: +1.3 V_{DC} at pipette for chosen duration.

Duration: 0.5-50 ms or Manual. Triggered by front-panel pushbutton. In Manual position ZAP amplitude is maintained as long as pushbutton is depressed.

Command Potentials

Seal Test: 5 mV command at line frequency.

External Commands: Two separate BNC inputs, one front switched, one rear switched.

Sensitivity: 20 mV/V in V-CLAMP, 2 ÷ ß nA/V in I-CLAMP, disabled in TRACK (I=0).

Input impedance: 10 kΩ. Inputs may be connected in parallel to increase sensitivity.

Holding Command: Ten-turn potentiometer with dial. Polarity switch. Value can be previewed on meter.

V-CLAMP mode: ±200 mV
I-CLAMP modes: ±2 nA for ß=1, ±20 nA for ß=0.1
Disabled in TRACK (I=0) mode.

Pipette Offset:

Manual: ±250 mV. Ten-turn control with uncalibrated dial.

Track (I=0): ±200 mV. Nulling potential automatically adjusts to maintain zero pipette current.

RMS Noise

3.5 digit meter displays rms current noise in pA. Measurement bandwidth is 30 Hz to 5 kHz. Upper -3 dB frequency is set by 4-pole Butterworth filter.

Inputs

Forced Reset: Positive edge triggered. Initiates a reset of the integrator; has no control over the duration of reset.

Blank Activate: Causes SCALED OUTPUT and I OUTPUT to hold their initial value for the duration of the blanking pulse. Does not affect $10V_m$ output.

Speed Test: Injects current into headstage input through a 1 pF capacitor. Injected current waveform is the derivative of the voltage waveform applied at SPEED TEST input. For example, a 100 Hz 10 V_{p-p} triangle wave will inject a 1 nA_{p-p} square wave into the headstage input.

Signal Outputs

Scaled Output: Scaled and filtered by output control settings. Sample and hold pedestal compensation. Output is I ($\alpha\beta$ mV/pA) when in V-CLAMP or TRACK (I=0) mode. Output is V_m (α mV/mV) when in I-CLAMP mode. BNCs on front and rear panels are identical.

I: Pipette current. Rear switched gain of either ßmV/pA or 100 ßmV/pA; fixed filter: 10 kHz 3-pole Bessel. Output does not benefit from sample and hold pedestal compensation.

10Vm: Membrane potential at x10 gain. Junction potentials removed.

Output Controls

Output Gain(α): 10 values from 0.5 - 500. Affects SCALED OUTPUT only. Selected value sets analog voltage on GAIN TELEGRAPH OUTPUT for reading by computer.

Low Pass Bessel Filter: 4-pole lowpass Bessel filter with five settings: 1, 2, 5, 10 and 50 kHz. Selected value sets an analog voltage on FREQUENCY TELEGRAPH OUTPUT.

Leak Subtraction: Causes a signal proportional to the command to be subtracted from current record. Range: 100 ßMΩ to ∞.

Telegraph Outputs

Gain

Takes α and β gain factors into account

I (mV/pA):	0.05[1]	0.1[1]	0.2[1]	0.5	1	2	5	10	20	50	100[2]	200[2]	500[2]
V_m (mV/mV):	N/A	N/A	N/A	0.5	1	2	5	10	20	50	100	200	500
Telegraph Output (V):	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5

Frequency

Filter Setting (kHz):	1	2	5	10	50
Telegraph Output (V):	2	4	6	8	10

Mode

Mode:	V-CLAMP	TRACK	I-CLAMP NORMAL	I-CLAMP FAST
Scaled Output:	I	I	V_m	V_m
Telegraph (volts):	6	4	2	1

Cell Capacitance

Telegraph Output: 0 to +10 V, proportional to setting 0-100 pF (0-1000 pF for CV 202AU) when WHOLE CELL CAP. switch is in the ON position.

0 to -10 V, when WHOLE CELL CAP. switch is in the OFF position.

Data Not Valid

Output goes High during a reset in PATCH mode or for the duration of a BLANK ACTIVATE pulse in either PATCH or WHOLE CELL mode.

Panel Meter

3.5 digit meter displays TRACK potential (V_{TRACK}) in mV, membrane potential (V_m) in mV, current noise (I_{RMS}) in pA rms, membrane current (I) in pA or nA, or HOLDING COMMAND (V_{HOLD}/I_{HOLD}) in mV or nA. Meter has autoranging feature when membrane current (I) is chosen.

---

[1] Applicable to CV 202A and CV 202AU only
[2] Applicable to CV 201A and CV 201AU only

Grounding

Signal ground is isolated from chassis and power ground. Signal ground is available on rear panel.

Control Inputs

Above 3 V accepted as logic High. Below 2 V accepted as logic Low. Inputs protected to ±15 V.

Models

Unit is supplied with two model assemblies, the PATCH-1U and the MCB-1U.

PATCH-1U Model Cell

Emulates three experimental conditions

BATH: 10 MΩ electrode resistor to ground. 4 pF pipette capacitance.

CELL: 10 MΩ electrode resistor connected to a 500 MΩ// 33 pF cell. 4 pF pipette capacitance.

PATCH: 10 MΩ electrode connected to a 10 GΩ patch. 5 pF pipette capacitance.

MCB-1U Model Bilayer

Emulates a bilayer membrane. 10 kΩ resistor in series with a 100 pF capacitor.

Headstage Dimensions

Case is 2.25 x 1.14 x 0.87" (57.2 x 29.0 x 22.1 mm). Mounting rod is 4" (102 mm) long. Available mounting rod diameters (D) are 1/4, 5/16 or 3/8" (6.3, 7.9 or 9.5 mm). Specify required mounting-rod diameter with order. A removable polycarbonate mounting plate 2.45 x 1.94 x 0.25" (62 x 49 x 6.3 mm) is supplied. Cable length is 10 feet (3 m).

Cabinet Dimensions

3.5" (89 mm) high, 19" (483 mm) wide, 12.5" (317 mm) deep. Mounts in standard 19" rack. Handles included. Net weight 11.5 lbs (5.1 kg).

Supply Requirements

Line Voltage: 85 to 264 Vac (or 110 to 340 V_{DC}) Universal voltage input.

Line Frequency: 50-60 Hz.

Power: 30 W

Fuse: 0.5 A slow. 5 x 20 mm.

Line Filter: RFI filter is included.

Line cord: Shielded line cord is provided.

Accessories Provided

Theory and Operation Manual
HL-U Pipette Holder
Spare fuse
PATCH-1U Model Cell
MCB-1U Model Bilayer

REFERENCES

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflügers Archiv, 391, 85-100.

Purves, R.D. (1981). Microelectrode Methods for Intracellular Recording and Ionophonesis. London: Academic press.

Rae, J.L., Levis, R.A. (1984). Patch voltage clamp of lens epithelial cells: Theory and practice. Molec. Physiol, 6, 115-162.

Sakmann, B., Neher, E. (1983). Single-Channel Recording. New York: Plenum Press.

TUNING PROCEDURE FOR THE CV 201AU OR CV 202AU HEADSTAGE

This procedure should be carried out if the user has done any of the following:

(1) Purchased an additional headstage and needs to match it to the main unit.

(2) Has found the step response of the WHOLE CELL configuration is unacceptable. This should be verified by applying a high quality triangle voltage waveform to the SPEED TEST BNC. (A 10 $V_{p\text{-}p}$ 100 Hz triangle wave will inject a 1 $nA_{p\text{-}p}$ square wave into the headstage input).

(3) Has determined that the reset transients in PATCH mode are more than twice the typical values (see **SPECIFICATIONS**). This should be verified by warming up the unit for at least one hour and connecting the PATCH-1U model cell (PATCH position) as a load. Set the HOLDING COMMAND to + or - 200 mV to induce resets. Monitor the SCALED OUTPUT BNC and trigger your scope from the DATA NOT VALID BNC.

Equipment Required

Oscilloscope
High quality triangle waveform generator
100 Hz, 4-pole (or greater) Bessel filter (optional)
PATCH-1U model cell
Various BNC cables
Large piece of aluminum foil for shielding the headstage

Gain and Offset Trim

These trims are optional if you are just trimming your present headstage and not changing headstages.

Set CONFIG. on PATCH

Connect the headstage to PATCH-1U model cell in the BATH position

Carefully shield the headstage and model cell with foil. Ground foil to brass pin on headstage using suitable jumper.

Select I on FRONT PANEL METER

Use JUNCTION NULL to zero the current

Set HOLDING COMMAND to 100 mV

Switch HOLDING COMMAND between + and -

☐ Trim RT4 so that the difference on the meter is 20 nA (nominally ±10 nA if the current has been exactly zeroed)

Repeat procedure with CONFIG. set to WHOLE CELL trim RT5 instead of RT4.

Remove PATCH-1U model cell.

Use appropriate trim pots on back panel to zero the meter for both PATCH and WHOLE CELL CONFIG.

Set CONFIG. to WHOLE CELL

Set HOLDING COMMAND to 200 mV

Switch HOLDING COMMAND between + and -

☐ Trim RT14 until meter reads zero for both polarities

Frequency Tuning (Whole Cell Config.)

The appropriate chip must be installed at position U94 of the main board of the Axopatch 200A in order for the whole-cell mode to operate correctly. The CV201AU and CV202AU require different chips. An LF 357 must be used with a CV201AU and an LF 356 must be used with a CV202AU.

Set OUTPUT GAIN to x1 (x10 for CV 202AU)

Set FILTER to 50 kHz

Connect scope to SCALED OUTPUT BNC

Trigger scope from waveform generator

Set scope to 0.2 V/div, 1 ms/div

Apply a 10 V_{p-p} 100 Hz triangle wave to the SPEED TEST BNC

On the scope you should see about a 1 V_{p-p} square wave

If the square wave response is unacceptable in terms of rise time or linearity continue with procedure, otherwise move on to RESET TRANSIENT COMPENSATION.

Set FILTER to 10 kHz

☐ Turn RT27 and RT30 fully counterclockwise (CCW). They can be found near the headstage connector towards the rear of the board.

When the end of adjustment is reached you should hear barely audible clicks. If not, turn a total of about twenty rotations to ensure a fully CCW position.

☐ Adjust RT31 until you get a response which looks like either trace a or trace b of Figure 23.

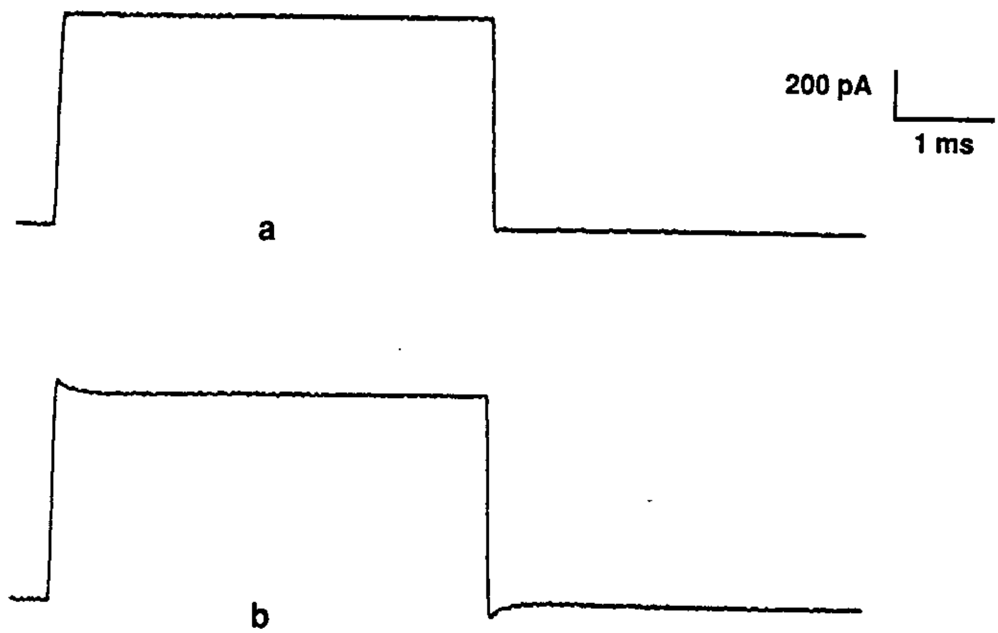

Figure 23. Frequency Tuning of Whole Cell Configuration

If the response looks like trace a, skip the next step and move on to trimming RT29. The trim pots you turned fully CCW will not be needed.

If the response looks like trace b (not quite flat after the step), perform the following step:

☐ Iterate between RT27 and RT30 until the desired response is achieved. This may also require a small readjustment of RT31 to make the waveform very flat.

Set scope to 10 µs/div

Set FILTER to 50 kHz.

☐ Trim RT29 for fastest rise time without overshoot. The 10 - 90% rise time should be about 6 - 8 µs.

Reset Transient Compensation

This is a fairly complicated procedure. The results rely on the patience and skill of the person who is doing the adjustments. Please confirm that the reset transients are indeed unacceptable before proceeding. Because of the consistency between units, you may not have to do these adjustments even if you are changing headstages.

Make sure the unit has been on continually for at least one hour.

Forced Reset

Remove PATCH-1U model cell

CONFIG. to PATCH

OUTPUT GAIN to x100

FILTER to 5 kHz

Scope to 0.2 V/div, 0.5 ms/div

Trigger scope from DATA NOT VALID BNC

Connect pulse generator to FORCED RESET BNC

Set pulse generator +5 V (> 100 µs duration), 100 Hz

Turn switch 1 of SW8 (near center of PC board) to OFF

Find reset transient on scope (see Figure 24a)

Turn switch 1 of SW8 to ON

☐ Trim RT22 (TAIL TAU) and RT23 (TAIL MAG) to minimize transient

Internal Reset

Remove pulse generator from Axopatch 200A

Connect PATCH-1U model cell in PATCH position to headstage

Set FILTER to 10 kHz

Adjust PIPETTE OFFSET for zero current

Set HOLDING COMMAND to 200 mV

Switch HOLDING COMMAND between + and -

☐ The INTEGRATOR RESET LED should be flashing about twice a second at either + or -200 mV.

If there is an obvious time difference between + and -, trim RT24 (CHG INJ) to balance it out

Set switches 3,4 and 5 of SW8 to OFF (internal reset compensation)

Find reset transient on scope. It should have about a 2-7 pA amplitude with an apparent decay time constant of roughly 0.5 ms (see Figure 24b). The transient should be riding on a DC level of + or - 20 pA and should change polarity with DC level.

Set scope to AC coupling 0.5 V/div, 1 ms/div

Set switch 3 of SW8 to ON

☐ Trim RT18 (DIAB-1 MAG) and RT19 (DIAB-1 TAU) to eliminate fastest portion of transient as in Figure 24c.

Switch HOLDING COMMAND between + and - and find a the best possible compromise. There will be some amount of unavoidable asymmetry.

Set FILTER to 1 kHz

Set scope to 0.1 V/div, 5 ms/div

Set switch 4 of SW8 to ON

☐ Trim RT20 (DIAB-2 MAG) and RT21 (DIAB-2 TAU) as above to remove the most significant portion of the reset transient. See Figure 24d. It may be necessary to re-trim RT18 and RT19 to optimize response.

If a slow tail ($\tau > 5$ ms) still remains, it can be compensated with RT25 (DIAB-3 MAG) and RT26 (DIAB-3 TAU). For the most accurate adjustment of these trims, a 100 Hz 4-pole (or greater) Bessel filter should be connected between the SCALED OUTPUT and the scope. The next steps assume the 100 Hz filter is installed.

Set scope to 20 mV/div, 50 ms/div

Set switch 5 of SW8 to ON

☐ Trim RT25 and RT26 to minimize slowest transient. As above, some re-trimming of RT20 and RT21 may be necessary to achieve optimum compensation. See Figure 24e.

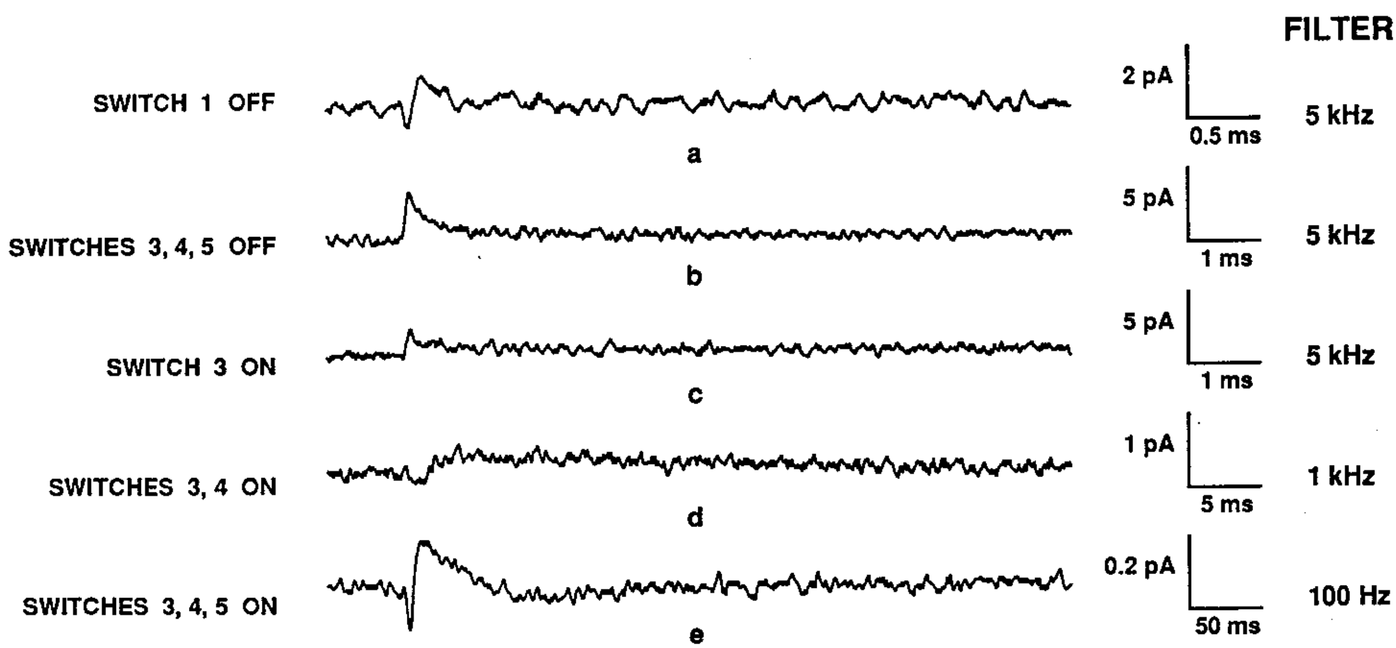

Figure 24. Reset Transient Compensation

For clarity, only one reset polarity is shown (positive DC current)

WARRANTY

We warrant every Axopatch 200A and every headstage to be free from defects in material and workmanship under normal use and service. For 12 months from the date of receipt, we will repair or replace without cost to the customer, any of these products that are defective and that are returned to our factory properly packaged with transportation charges prepaid. We will pay for the return shipping of the product to the customer.

Before returning products to our factory the customer must contact us to obtain a Return Merchandise Authorization number (RMA) and shipping instructions. Failure to do so will cause long delays and additional expense to customer. Complete a copy of the RMA form on the next page and return it with the product.

This warranty shall not apply to damage resulting from improper use, improper care, improper modification, connection to incompatible equipment, or to products which have been modified or integrated with other equipment in such a way as to increase the time or difficulty of servicing the product.

This warranty is in lieu of all other warranties, expressed or implied.

WARNING

Shipping the Axopatch 200A

The Axopatch 200A is a solidly built instrument designed to survive shipping around the world. However, in order to avoid damage during shipping, the Axopatch 200A must be properly packaged.

In general, the best way to package the Axopatch 200A is in the original factory carton. If this is no longer available, we recommend that you carefully wrap the Axopatch 200A in at least three inches (75 mm) of foam or "bubble-pack" sheeting. The wrapped Axopatch 200A should then be placed in a sturdy cardboard carton. Mark the outside of the box with the word FRAGILE and an arrow showing which way is up.

We do not recommend using loose foam pellets to protect the Axopatch 200A. If the carton is dropped by the shipper, there is a good chance that the Axopatch 200A will shift within the loose pellet packaging and be damaged.

If you need to ship your Axopatch 200A to another location, or back to the factory, and you do not have a means to adequately package it, Axon Instruments can ship the proper packaging material to you for a small fee. This may seem like an expense you would like to avoid, but it is inexpensive compared to the cost of repairing an instrument that has sustained shipping damage.

It is your responsibility to package the instrument properly before shipping. If it is not, and it is damaged, the shipper will not honor your claim for compensation.

RETURN MERCHANDISE AUTHORIZATION

RMA No. ______________________ **Date of RMA** ______________________

Shipping check list:

☐ 1. Package instrument with at least 3 inches of packing material all around.

☐ 2. Enclose a completed **copy** of this form.

☐ 3. Write RMA number on outside of package.

☐ 4. Pre-pay freight for door-to-door delivery.

Model ______________________ Serial No. ______________________

☐ In warranty ☐ Out of warranty

Customer's purchase order No. ______________________
(not required for warranty repair)

DESCRIPTION OF PROBLEM: ______________________

____

____

____

Customer's Shipping Address:

Name ______________________

____

____

____

____

Phone (____)______________________

Customer's Billing Address:

Name ______________________

____

____

____

____

Phone (____) ______________________

Send completed form with merchandise to:

Axon Instruments, Inc.
1101 Chess Drive
Foster City, CA 94404
U.S.A.

Write RMA number on outside of package.

CIRCUIT DIAGRAMS REQUEST FORM

All the information that you require for operation of the Axopatch 200A is included in the operator's manual. In the normal course of events, the Axopatch 200A does not require any routine maintenance. If, for some reason, the headstage is changed, the Axopatch 200A must be recalibrated. In anticipation of this, the recalibration procedures are described in the operator's manual, and circuit diagrams are not required.

Should you need the circuit diagrams for the Axopatch 200A, Axon Instruments will be pleased to supply them to you. However, we caution you that the Axopatch 200A is a sophisticated instrument and that service should only be undertaken by talented electronics experts.

To request a copy of the circuit diagrams and the parts lists, please complete the form at the bottom of this page and mail it to:

Axon Instruments, Inc.
Sales Department
1101 Chess Drive
Foster City, CA 94404
USA

This form must be completed in full and signed. Telephone orders will not be accepted.

Name of registered owner: ____________________

Department: ____________________

University/Institute: ____________________

Street address: ____________________

City: ____________ State __________ Zip Code __________ Country __________

Telephone: ____________________ Fax: ____________________

Model: Axopatch 200A Serial number ____________________

Declaration
Please send me the circuit diagrams and parts lists for the Axopatch 200A. I agree that I will only use the circuit diagrams and parts lists for service of the Axopatch 200A. I will not use them to create equivalent or competing products. If I transfer the circuit diagrams or copies thereof to someone who is assisting in the service of the Axopatch 200A, I will ask them to make the same undertaking that I am declaring herein.

Signature: ____________________ Date: __________

Name: ____________________ Title: __________

PROBLEMS AND SUGGESTIONS REPORT

Copy this form. Fill out and mail/fax to:

Scientific Applications
Axon Instruments, Inc.
1101 Chess Drive
Foster City, CA 94404, USA

Neatly print your name and address in the box below.

Phone: ____________________

Fax: ____________________

Product: ____________________

Version: __________ **Serial No.** __________

Computer Make __________ Model: __________

Monitor: ____________________

Memory size: ______ MB Operating System Vers: ____

Speed: __________ MHz

Disk size: __________ MB

Acquisition hardware: ____________________

Other Axon hardware: ____________________

Description: Please describe the problem or suggestion and how it can be reproduced. Enclose printouts, figures or data files if helpful. List any error messages and symptoms.

Date: ____________________

Use continuation pages if necessary.

Axon use only.
Date received: __________ answered: __________ closed: __________ ☐ Product Manager