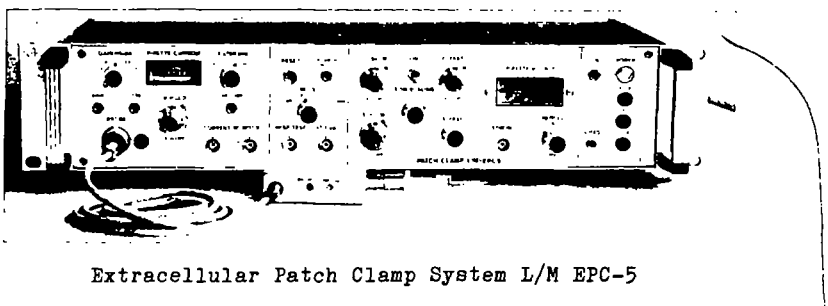
List-electronic

OPERATION MANUAL
EXTRACELLULAR PATCH CLAMP SYSTEM „L/M-EPC-5"



Extracellular Patch Clamp System L/M EPC-5

The EPC-5 is an extremely low noise current-measuring instrument designed for electrophysiological recording using patch pipettes. It is designed especially to make use of the various cell-attached and cell-free recording configurations that are possible with "giga-seals" (1). It is the lowest-noise picoampere recording system available with its bandwidth capabilities.

The EPC-5 consists of a controller, which is housed in a rack-mountable cabinet, and a probe, which contains the head-stage preamplifier and is designed to be mounted near the experimental preparation, for example directly on a micromanipulator. Each controller has been calibrated at the factory for use with its accompanying probe; because of variations in the characteristics of individual probes, part of the calibration procedure must be repeated when probes are exchanged. Controllers are available for use with either 110 or 220 volt, AC power; the rated voltage is marked on the rear panel.

Accessories that are presently available from List Electronic for the EPC-5 include pipette holders and a "model circuit" for testing the patch-clamp setup.

(Pat.pend.)

Reference:

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

I. Controls and Connectors

A. Controls on the Patch Clamp controller.

PIPETTE CURRENT meter. Monitors the pipette current. Full scale corresponds to approximately 1 volt at the CURRENT MONITOR outputs.

V-HOLD potentiometer. Adjusts pipette holding potential (Voltage Clamp mode) or the holding current (Current Clamp mode with command on). The setting 5.00 corresponds to zero holding potential in the VC mode; 10.00 (full scale) is +200 mV, while 0.00 corresponds to -200 mV. In CC mode the full-scale range in pA is -1V to +1V divided by the Gain setting. For example, at 10 mV/pA, 10.00 corresponds to +100 pA while 0.00 corresponds to -100 pA.

GAIN (mV/pA) switch. This switch selects the scaling of the CURRENT MONITOR output signal. It also sets the scaling of command signals in Current Clamp mode.

FILTER (kHz) switch. This switch selects the cutoff frequency (-3dB; 2-pole Bessel response) of the internal filter for the CURRENT MONITOR signal.

SIGNAL GND jack. This banana jack, which is next to the PROBE input connector, is a high-quality signal ground connection that can be used to ground other parts of the experimental setup as necessary.

CURRENT MONITOR outputs. These two connectors carry the same signal, which is proportional to pipette current. A positive voltage corresponds to current flowing out of the pipette.

MODE (SEARCH/VC/CC/CC+COMM) switch. This switch selects the operating mode.

In SEARCH, a slow "tracking" feedback loop adjusts the pipette potential to keep the average current near zero. This mode is designed to be used while establishing a pipette-membrane seal; voltage pulses can be applied to the pipette from the 10 x STIM input to monitor the seal resistance.

VC is the voltage-clamp mode: the pipette potential is controlled by the STIM signal and the V-HOLD potentiometer.

CC is the current clamp mode: high-speed feedback is used to control the pipette potential, keeping the pipette current at zero. This setting is useful for determining the resting potential in whole-cell recordings. It also allows the experimenter to reset the stimulus and V-HOLD settings when switching between VC and CC+COMM. Note: the current-clamp feedback dynamics depend on the FILTER and GAIN settings. A FILTER setting of 1 kHz is recommended to avoid oscillation.

CC+COMM is the current clamp mode with command on: the pipette current, instead of being held at zero, is determined by the V-HOLD setting and the 10 x STIM IN signal.

RESET BUTTON. Speeds up the feedback loop in SEARCH mode to rapidly bring the CURRENT MONITOR signal to near zero.

RESPONSE TEST input. This input is provided for test purposes. A signal applied here is attenuated by a factor of 10 and coupled to the probe input through a small capacitance (approx. 0.16 pF). The transient responses of the CURRENT-MONITOR signal can be checked by applying a triangle wave to this input and observing the square-wave signal at the CURRENT MONITOR connectors.

10 x VP output. This is the pipette potential monitor signal, scaled up by a factor of 10.

STIM SCALING switch (-1, 0, .01, .1, .5, 1). This selects the scaling of the 10 x STIM input signal. When it is set at 1, the 10 x STIM input signal is divided by 10 before being applied to the pipette potential; the other settings scale accordingly. The .01 position is useful for providing test pulses in the SEARCH mode to measure the pipette and initial seal resistance; at this setting the 10 x STIM IN voltage is divided by 1000, so a 100 mV pulse applied there results in 100 uV at the pipette. With a 10 megohm pipette resistance this pulse would result in 10 pA of pipette current.

C-SLOW, C-FAST. These potentiometers adjust the amplitude of capacitive-current cancellation. Full scale on each potentiometer correspondes to approximately 10 pF. C-SLOW is intended to cancel a capactive component that has an appreciable series resistance (for example, the cell membrane capacitance in a whole-cell recording configuration) while C-FAST is intended to cancel the fast transient due to the pipette capacitance.

C-SLOW ON switch. This switch allows the slow capacitance component to be switched on and off.

Tau-SLOW, Tau-FAST. These controls set the time constants of the two capacitance cancellation signals. Tau-SLOW has a range of approx. 30 us to 10 ms, with a calibration of 1 ms per turn. Tau-FAST has a range of 0.5 to 3 us and is used to obtain the best cancellation of the pipette capacitance transient.

PIPETTE VOLTAGE display. This LCD display shows the same voltage that is present (scaled by a factor of 10) at the 10 x VP output connector. It reads the pipette potential, shifted by the adjustable offset Vp-NULL (see below).

10 X STIM input. This input serves as the command input for the VC and CC modes. In VC, the voltage appled is scaled by 0.1 times the STIM SCALING setting and applied to the pipette; in CC, the voltage-to-current scaling is the STIM SCALING divided by the GAIN switch setting. For example, with the Gain at 10 mV/pA and STIM SCALING at 1, a 1 V Command results in 100 pA of pipette current. In the VC mode the V-HOLD knob adds a DC offset equivalent to 2 V (full scale) at the Command Input, while in the CC+COMM mode the V-HOLD knob adds the equivalent of 1 V.

VP-NULL adjustment. This potentiometer adjusts an offset voltage (20 mV full scale) that is added to the pipette potential but is not indicated on the PIPETTE VOLTAGE display and is not added to the 10 x VP output. It is intended to compensate for electrode offset potentials.

Power connections. When the Patch Clamp is powered from the AC line, up to 100 mA at ±15 V can be drawn from the banana jacks. Alternatively, the patch clamp can be powered from an external DC power supply by applying ±15 to ±20 V to these jacks; the current drain is approximately 80 mA.

B. Connectors on the probe.

INPUT. The center pin of this BNC connector is the pipette signal input; the shield, as well as the entire case of the probe, is not grounded, but is driven with the reference voltage. A leakage path from the case to ground will cause no damage, but will cause the pipette potential to be in error.

REF output. The reference voltage is available here for additional driven shielding. The impedance is approximately 90 ohms. NOTE: this output should not be used to drive any impedance lower than 10 kohm; otherwise, an error in the pipette potential will result.

GND connector. This is the high-quality ground connection that should be used to ground the bath electrode and metal surfaces in the vicinity of the pipette and holder. Ground is sensed at this connector for determining the pipette potential.

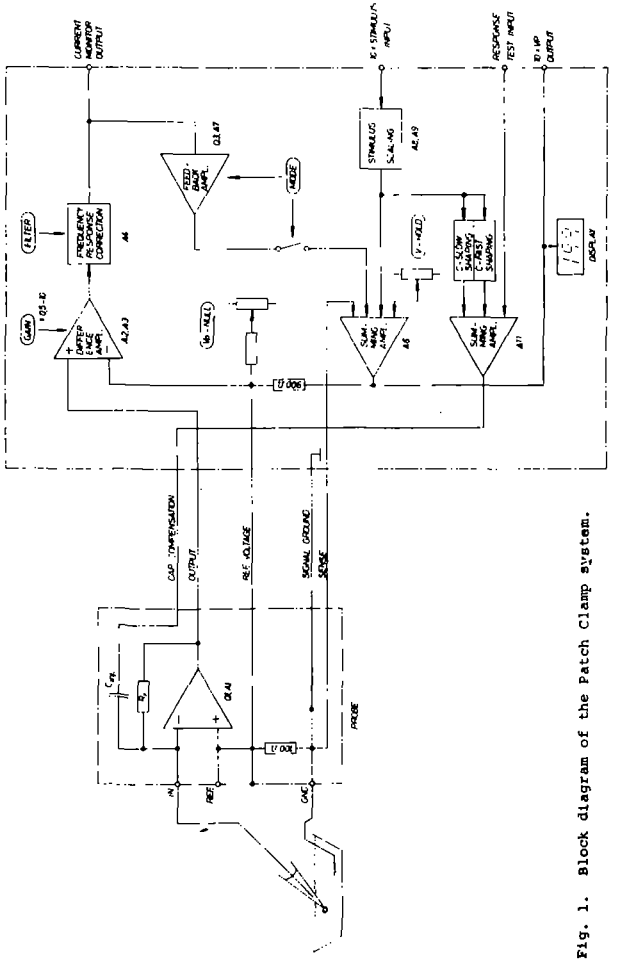


Fig. 1. Block diagram of the Patch Clamp system.

II. Circuit Description

Figure 1 shows a simplified diagram of the circuitry of the Patch Clamp. It consists mainly of a current-to-voltage converter (A1) in the probe, which measures the pipette current, and circuitry in the controller for amplifying and filtering the current signal and for controlling the pipette potential.

A. Voltage-Clamp mode

The operational amplifier A1-Q1 in the probe maintains the pipette potential very close to a reference potential V_{ref} by maintaining a current equal to the pipette current in the 10 gigaohm feedback resistor R_f. The voltage difference between the output of A1 and V_{ref} is proportional to the pipette current; it is amplified by a differential amplifier (A2, A3), and the frequency response is corrected by A4 for the effect of the stray capacitance of R_f.

The reference potential V_{ref} is a sum of several signals. Contributions from the V-HOLD potentiometer and the 10 x STIM input are summed in A6 along with the ground sense signal from the GND connector in the probe. This sum is available at the 10 x VP voltage monitor output. It is then divided by 10 and a small offset is added from the VP-NULL potentiometer to form the V_{ref} signal. The case of the probe is driven with V_{ref}.

A small capacitor C_{inj} (capacitance approximately 0.16 pF) is used to inject current into the input circuitry. Shaping networks and a summing amplifier allow charge to be injected to cancel the large capacitive currents that flow when voltage steps are applied at the 10 x STIM input. Currents can also be injected for test purposes using the RESPONSE TEST input. The injected current is proportional to the time derivative of the applied voltage. For example, a triangle-wave input results in a square-wave current; the signal at the CURRENT MONITOR output then shows the step response of the patch-clamp system.

B. SEARCH mode

The main difference between this mode and Voltage Clamp is that feedback from the pipette current to the pipette voltage is applied through the amplifier A7. It acts to vary V_{ref} to force the average pipette current to be zero. The time constant of the feedback varies proportionally to the resistance at the probe input

and inversely to the gain setting; with the GAIN at 100 mV/pA and an open pipette with a resistance of 6 megohms the time constant is 1 sec.

C. Current Clamp modes

For current-clamp operation the speed of the feedback through A7 is increased by several orders of magnitude. The feedback loop gain depends on the GAIN and FILTER settings; at some setting combinations oscillations may occur. The feedback characteristic has been designed for the 1 kHz filter setting, and the Current Clamp is most stable at the lower GAIN settings. In the CC+COMMAND mode the stimulus and V-HOLD signals are summed with the current monitor signal at A7 to bring the pipette current to a nonzero level set by these signals. In the current-clamp modes the capacitance-transient cancellation circuitry is disabled.

III. Setting up the patch clamp

In setting up to do patch clamp recordings, special attention should be given to shielding the pipette and holder and grounding the conducting surfaces near the pipette.

A. Shielded pipette holder. A shielded version of the pipette holder is available; this holder and a properly grounded microscope typically provide sufficient shielding from 50-60 Hz interference. The shielded holder however introduces more intrinsic noise than the unshielded one, especially when its internal surfaces become wetted. The difference in background noise level between the two holder types is roughly a factor of 1.5 to 2.

B. Grounding the microscope. In most cases the patch clamp is used in conjunction with a microscope; it and its stage typically constitute the conducting surfaces nearest the pipette and holder.

1. Make sure there is electrical continuity among the various parts of the microscope, especially the objective and condenser, which are usually the parts nearest the pipette. Electrically floating surfaces can act as "antennas", picking up line-frequency signals and coupling them to the pipette.

2. Make sure the lamp housing is also grounded. It is usually not necessary to supply DC power to the lamp provided that the cable to the lamp is shielded and that this shield is grounded at the microscope.

C. External shielding. Especially when an unshielded pipette holder is used, some external shielding is usually necessary. A table-top Faraday cage with a closeable front can provide this. If the pipette holder is somewhat exposed, a grounded screen near it may help.

D. Grounding practice. Avoiding ground loops is important for the patch clamp because extremely small errors in ground potential can be important noise sources. For example, a 10 uV ground difference between the bath electrode and the pipette causes a large

disturbance when the pipette resistance is being monitored with 100 uV pulses. As another example, a 1 mV ground error on the microscope can cause it to couple 0.3 pA of line-frequency interference into the pipette, assuming 1 pF of coupling capacitance.

1. The GND connector on the probe is the high-quality, sensed ground line that should be used to ground the bath electrode. It can also be used to ground other parts of the setup (e.g. the microscope) provided no large ground currents are expected. Note: in some situations the bath electrode must also be grounded to other apparatus (for example, to a microelectrode recording system). To avoid ground loops currents, a jumper wire in the probe (indicated by the dashed line in Fig. 1) can be cut. In this case the probe's GND connector becomes a sense input line only; the ground return must be provided separately, through the other apparatus.

2. For grounding of large surfaces such as a Faraday cage, the SIG GND connnector can be used. If a central grounding point is established on the entire system, it should be at the SIG GND (Signal Ground) connnector since this is the patch-clamp's signal ground.

E. Mounting the probe. For low-noise measurements, the probe must be mounted very close to the experimental preparation.. The probe is supplied with a plastic mounting plate for attachment to a micromanipulator or other mounting surface. The metal case must be kept insulated from any grounded objects, since it is driven with the reference potential.

IV. Other necessary equipment

We mention here some of the other equipment that is needed for making patch-clamp recordings.

Electrodes

A. Pipette electrode. An electrode for the pipette can be made simply from a chlorided silver wire. The wire can be protected from scratching by enclosing it in a piece of teflon tubing, into which notches have been cut to allow fluid access. The end of the wire is soldered to a BNC connnector pin for making contact with the probe input connector.

B. Pipette holder. Two types of pipette holder are available from List Electronic. The shielded holder is less sensitive to power-line pickup, but introduces considerably more random noise. The unshielded holder is recommended for all situations where sufficient shielding (e.g. a Faraday cage) is already present. If you choose to make your own holder, you should design it with the following criteria in mind:

1. It should provide an air-tight seal for the pipette and electrode, to allow suction to be supplied to the pipette

2. It should hold the pipette firmly so that suction causes no observable pipette movement (e.g. < 1 um).

3. Internal surfaces should be relatively hydrophobic to avoid formation of noise-generating conductive films of solution.

4. Total capacitance should be low (e.g. < 5 pF).

C. Bath electrode. A silver-silver chloride wire or pellet electrode can make an acceptable bath electrode. Ag^+ ions are lethal for some cell types, so a salt bridge may be necessary.

Stimulus generator

A stimulator or pulse generator is required to provide test pulses to measure the pipette resistance; the same device can be used to apply voltage jumps to the patch membrane.

A. Voltage Jumps. Typical pulse amplitudes at the 10X STIM INPUT are in the range 0.1 to 2 V, corresponding to patch potentials of 10 to 200 mV (in the STIM SCALING position 1). A division by 10 of the STIM IN signal has been built in because of high sensitivity of the patch clamp to noise in the stimulus: the division by 10 also divides the noise by 10. For critical work the stimulator's noise level should nevertheless be quite low: a noise voltage of about 15 uV in the band 1-10 kHz (1.5 uV at the pipette) can add noticeably to the probe amplifier's high-frequency noise level under typical experimental conditions. (Proper adjustment of C-FAST and Tau-FAST can cancel most of this injected noise, however.)

V. Pipette fabrication.

Only a brief description of pipette fabrication is given here; a more detailed description is given in Reference 1.

A. Glass capillaries. Standard hematocrit capillaries have suitable properties for general use; also thin-walled microelectrode capillaries (e.g. Kimax) are suitable. Thick-walled Pyrex capillaries (e.g. Jencon's HC-10) have better electrical properties for low-noise recording, but form unstable seals that might not allow cell-free recording.

B. Pulling. The goal of the pulling procedure is to produce pipettes with an opening diameter of about 0.5 um with as steep a taper as possible near the tip. A two-stage pulling procedure with a standard microelectrode puller is suitable; in the first stage the capillary is pulled 7-10 mm, yielding an outside diameter 200 um at its narrowest point. The length of the first pull, and especially the heat setting for the second pull, control the tip diameter of the final pipette. For reproduceability, mechanical stops and a regulated current supply on the puller are recommended.

C. Coating. Coating the pipette with an insulating layer greatly improves the noise level of giga-seal recordings. The pipette is coated as close as possible to the tip withoug touching it, and the coating should extend far enough up the shank of the pipette so that the glass surface will not become wetted. Suitable coating materials include "Q-Dope" and Sylgard.

D. Heat polishing. Polishing is not always necessary for establishing a giga-seal, but it reduces the likelihood of cell damage by the pipette tip. Heat polishing can be done under high magnification (e.g. 500x) in a microforge, or under a microscope using a small platinum-iridium heating wire. The pipette is brought near the heated wire until a rounding and darkening of the tip is visible.

E. Pipettes are best used a few hours after fabrication. If necessary, they can be cleaned by immersing the tip in methanol

while applying pressure to the interior. Pipettes can be filled by sucking a small amount of solution through the tip, and then back-filling. The filling solution should be filtered.

Recording Configuration	Outward membrane current	Positive membrane potential	Stimulus polarity to give depolarization
Cell attached Inside-out patch	- at CURRENT MONITOR output	- on PIPETTE VOLTAGE display	- stimulus
Outside-out patch Whole-cell recording	+ at CURRENT MONITOR output	+ on PIPETTE VOLTAGE display	+ stimulus

Fig. 2. Polarities of signals for various patch configurations. The rule: positive pipette potentials, and current flowing out of the pipette, are taken to be positive.

VI. Using the patch clamp

We give here a brief description of the techniques for establishing a seal and recording from a membrane patch or from an entire small cell. More details can be found in Ref. 1.

A. Forming a seal.

1. Initial setup. Apply test pulses to the 10x STIM IN connector (e.g. 100mV, 10 ms pulses) and set the STIM SCALING to .01. Set the FILTER bandwidth to 1 kHZ and set up to observe the CURRENT MONITOR signal on an oscilloscope, with its sensitivity and the GAIN setting chosen to give approximately 10 pA per division on the oscilloscope screen. Set the MODE to SEARCH.

2. Before the pipette is inserted into the bath, the current trace should be flat except for small capacitive pulses due to the stray capacitance of the pipette and holder.

3. When the pipette tip enters the bath the current trace may go off scale; it can be brought to zero by pushing the RESET button. The trace will be much noisier, and from the current response I to the test pulse the pipette resistance can be calculated according to the equation I = 100 pA/R (megohms). Note: if there is no change in the trace upon entering the bath check for an open circuit, for example: (1) a bubble in the pipette; (2) faulty connection to the probe input; (3) bath electrode not connected.

4. To null the electrode offset, push the RESET button while turning the VP-NULL knob to obtain a zero reading on the PIPETTE VOLTAGE meter.

5. When the pipette is pushed against a cell, the current pulses should become smaller to reflect the increasing seal resistance. Suction increases the resistance; if a giga-seal forms, the noise in the trace will disappear, and the trace will be similar to that obtained outside the bath (step 2 above).

6. To verify gigaseal formation, increase the STIM SCALING to .1. The trace should still appear essentially flat except for the capacitive spikes. The scaling is now 1 pA/R (gigohms).

B. Cell-attached recording.

1. VC mode. Switch the MODE to VC (voltage clamp). The pipette voltage will now become the level set by the V-HOLD potentiometer. (It is a good idea to start out with it set to mid-scale, which corresponds to zero pipette voltage!) If no voltage jumps are required, turn the stimulus off to avoid introducing artifacts. Set the GAIN and FILTER controls as desired. Caution: the SEARCH and VC modes are functionally very similar; however, if the mode is not switched to VC, the pipette potential will drift slowly with time.

2. Transient cancellation. If voltage jumps are to be applied, leave the STIM SCALING at .1 to make a coarse adjustment of the C-Fast and Tau-Fast controls before switching to the full-sized stimulus. Adjust these controls to minimize the size of the capacitive spikes. Then switch STIM SCALING to 1 and repeat the adjustments. Usulally there is a slower (100 us to 1 ms) capacitive component present as well. To cancel it, at least partially, turn on the C-SLOW switch and adjust C-SLOW and Tau-SLOW. Note: The C-SLOW knob adjusts the area, rather than the amplitude, of an exponentially-decaying component whose time constant is set by Tau-SLOW; the amplitude of the component therefore changes with the Tau-SLOW setting.

3. Polarities. For this cell-attached patch configuration, positive pipette voltages correspond to a hyperpolarization of the patch membrane, and inward membrane currents appear as positive signals at the CURRENT MONITOR outputs (see Fig.2). Positive pulses applied to the 10x STIM INPUT will result in depolarizations when the STIM SCALING is set to -1.

C. Whole-cell recording

1. After a giga-seal is formed, the patch membrane can be broken by additional suction. Electrical access to the cell interior is indicated by a sudden increase in the capacitive transients from the test pulse, and, depending on the cells input resistance, a shift in the current level. If the fast capacitance cancellation was set as described above before breaking the patch, then all of the additional capacitance transient will be due to the cell capacitance; its time constant will be the product of the cell capacitance and the access resistance.

2. Additional suction can sometimes lower the access resistance; this is seen as an increase in amplitude and decrease in the time constant of the capacitance transients, when observed at a sufficiently wide bandwidth (e.g. 3 or 10 kHZ FILTER settings) If C-SLOW and Tau-SLOW are used to cancel the transients, the approximate cell capacitance and access time constant can be read from the dials.

3. Polarities. Because the pipette now has access to the cell interior, the PIPETTE VOLTAGE meter reads the cell membrane potential, and the CURRENT MONITOR follows the usual convention of an outward current being positive.

4. Voltage Clamp recording can be performed as before; notice that in this case the stimulus scaling is the same as the membrane potential change.

5. A distortion of the time-course of the CURRENT MONITOR signal occurs when the probe amplifier saturates at high pipette current levels. Saturation occurs at approximately 1 nA for constant currents; short pulses (< 1 ms) of current can exceed this level somewhat without causing saturation, provided that the CURRENT MONITOR signal does not exceed 10 volts peak.

D. Whole-cell recording under current clamp. Two current-clamp modes are provided: CC, which holds the pipette current at zero, and CC+COMM, which allows the STIM IN signal and the V-HOLD potentiometer to set the pipette current. The functions and scaling of the CURRENT MONITOR and 10x Vp signals are the same as in the VC mode: the 10 x Vp output can be used to monitor membrane potential, while the CURRENT MONITOR serves as a stimulus monitor.

1. The current-clamp feedback may oscillate at certain FILTER AND GAIN settings. It is most stable with a FILTER setting of 1 kHz. The GAIN switch affects the feedback gain, giving the fastest and most accurate response, but some danger of oscillation, at the highest settings. The GAIN switch also affects the stimulus scaling in current clamp (see the description of the front panel controls).

2. To enter current clamp, switch the MODE to CC. The Pipette Voltage display now shows the cell's resting potential. Because the stimulus and holding current are disconnected in this mode, they can now be set up before entering the CC+COMM mode. The

V-Hold potentiometer sets the holding current, with full scale being ±1 volt divided by the GAIN setting, e.g. ±100 pA at 10 mV/pA, or ±10 pA at the 100mV/pA setting. The center position (5.00) corresponds to zero current.

E. Model Circuit. Fig. 3 shows a suggested model circuit for verifying the operation of the Patch Clamp. A switch selects between a 10 megohm resistance, modeling an open pipette, and a 10 gigaohm resistor, corresponding to a giga-seal. The capacitor models the pipette capacitance and can be used to check the capacitaince transient cancellation. Current-clamp operation can also be checked using the 10 gigaohm switch setting.

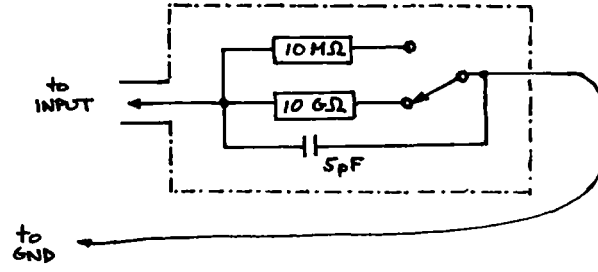


Fig. 3 Diagram of a model circuit. The circuit should be built in a shielded box.

VI. Low-noise recording

A. Background noise. In recording with "conventional" seals with resistances below 100 megohms, thermal noise in the seal conductance itself is the predominant noise source. For seal resistances above 10 gigaohms, however, this thermal noise is usually negligible in comparison to the contributions from other noise sources. Below we have listed some measures for reducing the background noise in gigaseal recording, beginning with the most important and ending with suggestions for those who want to reduce the noise variance by the very last factor of two.

1. Coating the pipette. The thin film of solution that forms on the glass surface represents a very serious noise source. This film generates thermal noise which is coupled capacitively to the interior of the pipette. A hydrophobic coating (e.g. Sylgard) prevents the formation of this film. The coating should have very low dielectric loss. It helps to coat the pipette as close as possible to the tip and to make the coating thick, since this reduces the capacitance between the pipette interior and the bath.

2. Keep the pipette holder clean and dry. Films of solution inside the holder can also couple thermal noise current into the pipette. Care should therefore be taken not to overfill pipettes to avoid solution leaking out. For critical work the holder can be rinsed with methanol and dried with a nitrogen stream before inserting each pipette. With the unshielded (plastic) pipette holder this noise source is not as serious because its lower capacitance to ground causes less noise current to be coupled to the pipette as well.

3. Use a low bathing solution level. This reduces the capacitance and the coupling of the glass dielectric noise to the pipette interior. A thick pipette coating makes the solution level less critical.

4. Use thick-walled capillaries, and a glass type with a low dielectric loss, for the pipettes. Pyrex glass has lower dielectric loss than the soft hematocrit capillary glass, for example.

5. The pipette shape is relatively uncritical for general gigaseal recording, but for critical work the tips should be relativley blunt to reduce the access resistance. This resistance

R_{acc} is a source of thermal noise whose contribution to the variance is proportional to $R_{acc} C_{tip}^2$ where C_{tip} is the capacitance at the electrode tip. Thus, thick-walled pipettes are advantageous for this noise source as well.

6. The capacitance of the pipette and holder increases the high-frequency noise resulting from the voltage noise of the probe amplifier. The effect of this capacitance is not large, with more than 10 pF being required to double the intrinsic high-frequency noise level of the probe. The plastic pipette holder introduces a capacitance of only about 3 pF.

B. Filtering. A gain in the signal-to-noise ratio of about 1.5 can result from using an external, higher-order Bessel filter for the CURRENT MONITOR signal. The built-in, 2-pole filter has a cut off slope of -12 db/octave, which is relatively shallow in view of the rising (about +6 db/octave) noise spectral density above 1 kHz. A higher-order filter provides a sharper cutoff asymptote while giving about the same rise time in response to a step input. Bessel filters have a monotonic step response, which is usually desirable for single-channel recording.

VII. Test and Calibration Procedures

The procedures for calibrating and testing the EPC-5 are listed here for reference. Under normal use recalibration should not be necessary unless components are replaced; the only exception to this might be the probe-related adjustments (sections ii and iii), which must be made individually for each probe. These probe adjustments should also be checked whenever components inside the probe are moved in any way, because of the large effects of stray capacitances.

i. Internal adjustments

A. Supply voltages. First adjust T13 and T14 to give +15.5 V at the outputs of the power supply board. Then adjust trimmers T11 and T12 to give +12.00 V at the positive supply line (pin 7 of any op amp) and -12.00 V at the negative supply line (pin 4 of any op amp).

B. A8 offset adjustment. Short the 10 x STIM IN input to ground and adjust T8 to bring the output of A8 (TP 37) to within 0.5 mV of zero.

C. A9 offset adjustment. Set STIM SCALING to 0. Adjust T9 for zero (withing 0.5 mV) at the output of A9 (TP 36).

The following adjustments D-F should be made with MODE set at VC.

D. V-HOLD scaling. Connect a DVM to the 10 x VP output. Adjust T5 to give +2.00 V when V-HOLD is at maximum (10.00) and -2.00 V when V-HOLD is set at zero. Readjust the supply voltage trimmers T- or T- if necessary to remove any offset larger than 20 mV.

E. PIPETTE VOLTAGE display scaling. Adjust the trimpot on the back of the LCD display circuit board to read 1/10 of the voltage at 10 x VP (e.g. +190 when 10 x VP has 1.90 V).

F. 10 x STIM IN scaling. Adjust V-HOLD to bring the voltage at 10 x VP to zero. Set STIM SCALING to 1 and connect a calibrated voltage source to 10 x STIM IN. Adjust T6 to make the voltage at 10 x VP equal to that applied to 10 x STIM IN. Check the scaling at each of the other STIM SCALING positions: it should agree within

2% to the labeled values.

G. A7 offset. Connect a 10 to 20-megohm resistor from the probe input to ground. Set GAIN to 5 mV/pA, FILTER to 1 kHz and MODE to CC. Adjust T7 to bring the voltage at the CURRENT MONITOR output to zero within 0.5 mV.

ii. Front-panel adjustments

Set V-HOLD to 5.00, VP-NULL to 0, GAIN 10 mV/pA, FILTER to 0.3 kHz, STIM SCALING to 0, and MODE to VC. Connect a known resistance of at least 10 megohms between the input of the probe and a calibrated millivolt source.

A. GAIN trimmer. Adjust the VP-NULL knob to bring the PIPETTE CURRENT meter near the center of its scale. Set up to inject 100 pA through the calibrated resistor (e.g. 2 mV through 20 megohms) and measure the CURRENT MONITOR signal with a DVM. Adjust the GAIN trimmer until a 100 pA change in input current causes a 1 V (within 1%) change in voltage at the output. Check that full scale on the PIPETTE CURRENT meter corresponds to approx. 1 V output. Check the gain at the other GAIN switch settings using smaller input currents as necessary.

B. OFFSET trimmer. Set the GAIN switch to 100 mV/pA and leave the probe input open. Adjust the OFFSET trimmer to bring the voltage at the CURRENT MONITOR output to zero (within 10 mV).

C. HF COMP trimmer. Leave the probe input open and set the FILTER to 3 kHz, GAIN at 10 mV/pA. Apply a 1-10 V, approx. 30 Hz triangle wave to the Response Test input, and monitor the waveform at the CURRENT MONITOR output with an oscilloscope. Note: a high-quality function generator must be used to provide the triangle wave; slight distortion of the triangle wave can cause severe distortion of the square-wave at the CURRENT MONITOR output.

1. Adjust the HF COMP trimpot for minimum distortion (flat top and bottom) of the square-wave output.

2. Note the size of the deviation from a square-wave shape: the deviation should be less than 1.5% of the peak to peak amplitude.

3. Check the waveform at the other Bandwidth settings--the top and bottom of the waveform should remain flat.

Note: the preceding three adjustments depend on the particular probe, and should be repeated if another probe is used. Adjustment C should be repeated if the feedback resistor in the probe is moved in any way because the circuitry is very sensitive to stray capacitances.

D. SEARCH NULL trimmer. Connect a 20 megohm resistor from the probe input to ground. Set GAIN to 100 mV/pA, FILTER to 0.3 kHz, MODE to SEARCH, and push the RESET button to bring the PIPETTE CURRENT on scale. Adjust the SEARCH NULL trimmer to bring the voltage at the CURRENT MONITOR output to zero, within 50 mV. Check the tracking speed by changing the VP-NULL setting slightly; the PIPETTE CURRENT meter should show a return to zero with a time constant of about 6 sec at 100 mV/pA GAIN.

iii. Capacitance cancellation adjustment

A. Compensating capacitor in probe: Set the GAIN to 10 mV/pA, FILTER to 3 kHz, STIM SCALING to 1, C-SLOW switch off. Leave the probe input open. Apply approx. 50 ms, 1 V pulses or square wave to STIM IN and monitor the signal at the CURRENT MONITOR output with an oscilloscope.

1. Adjust C-FAST and Tau-FAST for the minimum amplitude of spikes. This usually occurs at a setting near 1.00 on the C-FAST knob.

2. Turn the C-SLOW and Tau-SLOW knobs to maximum and turn on C-SLOW switch. The CURRENT MONITOR output should show exponentially decaying pulses with a time constant of about 10 ms and an initial amplitude of about 1 V. Adjust the capacitor C- in the proble to bring the amplitude within 10% of 1 V if necessary. (The capacitor can be adjusted by bending the insulated wire next to the lead of the 10 Gigohm resistor. The HF COMP trimmer may need readjustment after this step.)

B. C-SLOW and C-FAST check.

1. As Tau-SLOW is turned down, the pulses should become larger and shorter; at a setting of 1.00 the pulses should be about 1 V in amplitude when the STIM SCALING is reduced to .1, and should show a decay time-constant of about 1 ms. Check that the amplitude of the pulses scales scale proportionally to the C-SLOW setting.

2. Check the C-FAST calibration by turning off the C-SLOW switch, and readjusting C-FAST and Tau-FAST for best cancellation of the fast spike. Note the C-FAST setting, which should be in the range of 1.00-3.00. Then connect a known capacitor (for example 5 pF) between the probe input and ground, and readjust C-FAST for best cancellation. The difference in reading should correspond to a scale factor of 1 turn per pF, within 20%.

iv. Functional checks

A. Reference voltage. With MODE at VC, set V-HOLD to bring the PIPETTE VOLTAGE reading to 0. Connect a DVM to the REF output on the probe.

1. VP-NULL. Check that turning the VP-NULL knob varies the voltage over approximately -20 to +20 mV.

2. REF scaling. Set VP-NULL to bring the REF voltage to zero. Check that the voltage at the REF output agrees with the PIPETTE VOLTAGE display within 1% as V-HOLD is varied.

B. Common-mode rejection check: With the input open, GAIN at 10 mV/pA, monitor the CURRENT MONITOR voltage with a DVM while turning the V-HOLD knob. Varying V-HOLD from minimum to maximum should cause the CURRENT MONITOR output voltage to vary less than 4 mV.

C. Current clamp check: Connect a 20 megohm resistor from the probe input to ground; set the MODE to CC+COMM, STIM SCALING to 1, GAIN to 10 mV/pA, FILTER to 1 kHz, C-FAST at 0, C-SLOW switch off. Apply approx. 2.5 V, 20 ms pulses or a square wave to STIM IN. Pulses of the same size should be present at the CURRENT MONITOR output. Similar pulses but 1/50 as large should be present at the 10 x VP connector.

D. Noise check. Set the MODE to VC, GAIN to 100 mV/pA, and FILTER to 1 kHz. Connect a high-quality (e.g. silver mica) 5 pF capacitor between the probe input and the GND jack on the probe. Use a DVM to measure the noise voltage at the CURRENT MONITOR output, making sure that the probe is shielded to avoid 50 Hz pickup.

1. At the 1 kHz setting the noise voltage should be less than 8.0 mV, as measured with a true-RMS, AC voltmeter.

2. At the 3 kHz FILTER setting the noise voltage should be less than 35 mV RMS.

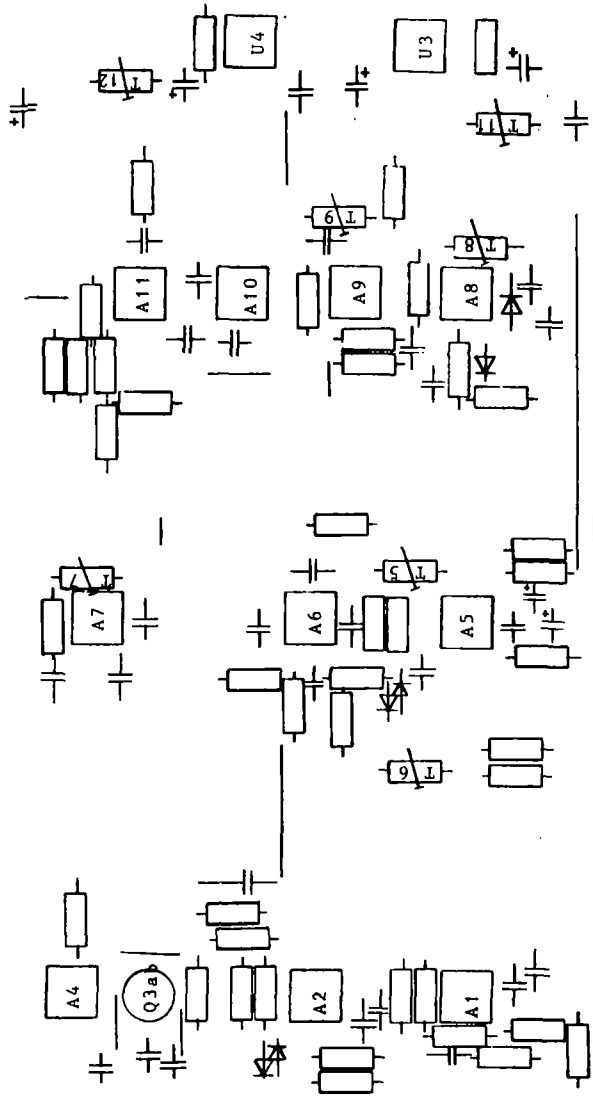


Fig. 4 Op-amp and trimmer locations.