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Datum/Date: 1984

THE L / M - E P C - 7

PATCH CLAMP SYSTEM

(Patent)

U S E R S M A N U A L

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This is a description of the L / M - E P C 7 for useres who are generally familiar with patch clamp techniques. An introduction to basic recording procedures can be found in the L / M - E P C - 5 Users Manual (attached).

LIST - electronic

TABLE OF CONTENTS

I. General Description

A. Operating modes
   VC
   SEARCH
   TEST
   CC
   CC + COMM
B. Range changing
C. Current monitor signal processing
D. Transient cancellation facility (VC mode)
E. Series resistance compensation (VC mode)
F. Stimulus input and voltage monitor
G. Noise test facility

II. Setting up the EPC-7

A. Power
B. Static electricity
C. Initial checkout
D. Installing the probe

III. Theory of Operation

A. Current monitor pathway
B. Stimulus pathway
C. R_s compensation
D. Capacitive transient cancellation
E. Test circuit
F. Search and current clamp

IV. Adjustments

I. General Description

A. Operating modes

VC. This is the basic voltage-clamp mode for both single-channel and whole-cell recording. The pipette holding potential is set by the V-HOLD control, and pulses can be applied via the STIM IN connector, scaled according to the STIM SCALING switch.

SEARCH. This is the same as the VC mode, except that the output of an integrator is added to the pipette potential. The integrator acts to slowly bring the average pipette current to zero by offsetting the pipette potential up to ±130 mV. This mode is designed for use while the pipette is open and when a seal is being formed. The integrator counteracts electrode drifts: any uncompensated electrode offset appears on the V_{COMM} display and can be nulled using the V_p-OFFSET control. The RESET button speeds up the integrator to rapidly bring the pipette current to zero.

TEST. This mode is similar to the VC mode except that the signal applied to the STIM IN connector is converted to a current and applied directly to the probe input circuitry. The scaling is calibrated at 100 pA per volt applied, and the bandwidth is 0.5 Hz - 300 kHz. Square wave inputs of 20 Hz or higher frequency are injected wiht less than 1% distortion (i.e. droop) of the waveform. The TEST mode provides a simple way to check the gain and temporal response of the recording system.

CC. This mode is current clamp to zero current, i.e. the potential recording mode. The pipette

potential can be monitored at the V-MONITOR output. Input current can be nulled using the CC-NULL trim adjustment (range approx. ±10 pA).

CC + COMM. Here a commanded current can be applied to the pipette while its potential is being measured. The current is determined from the V-HOLD setting and the STIM IN signal, with a scaling, referred to the VC mode, of 1 pA:1 mV. For proper current clamp dynamics, the T_r switch should be set to 20 μs. The applied current can be monitored using the CURRENT MONITOR outputs (the scaling is correct only for the GAIN settings 0.5 to 20 mV/pA). In current clamp, the probe input capacitance is approximately 1.5 pF; no capacitance neutralization or series-resistance compensation is provided.

B. Range changing

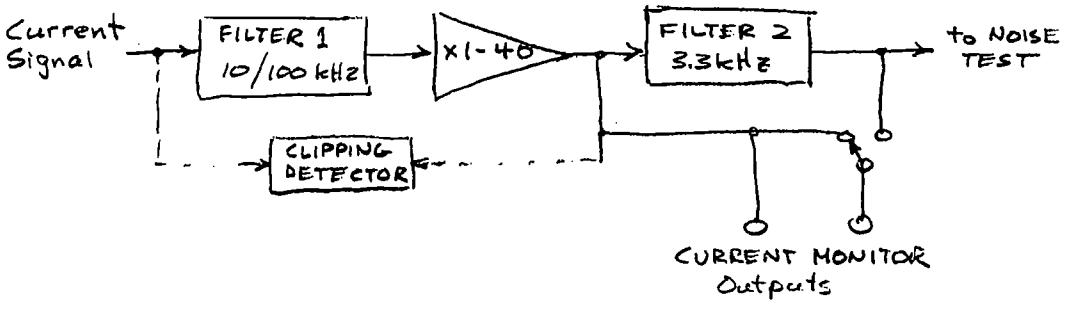
The probe has two current measuring resistors: 500 MΩ and 50 GΩ. Switching between these is accomplished automatically by the GAIN switch. Settings of 0.5 to 20 mV/pA select the low range (500 MΩ measuring resistor) and the higher settings select the high range.

In the low range the background noise is larger, but the full 100 kHz bandwidth is available, as are the special features of the 100 pF transient cancellation range, series resistance (R_s) compensation and the current-clamp modes. Currents up to about 25 nA can be recorded in the low range.

The high range is intended for single-channel recording. It has a very low noise level, but this is obtained at the expense of a maximum current limit of 250 pA. The maximum available bandwidth is about 60 kHz. The special features do not work properly in this range.

C. Current monitor signal processing

Two filters are available for the current monitor signal. Their locations in the signal pathway are diagrammed in simplified fashion below:



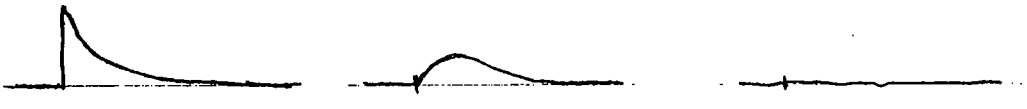
Filter 1 is a 3-pole active filter with switchable characteristics. Normally it has a bandwidth of 100 kHz (FULL position of the FILTER 1 switch) or 10 kHz, Bessel response. Its output, after amplification, is available at the left-hand CURRENT MONITOR output connector. The right-hand connector can receive either the same signal or the output of FILTER 2, a three-pole, 3.3 kHz Bessel filter. The intent of this arrangement is to allow a wideband signal to be sent to a recording device while simultaneously presenting a more filtered signal to an oscilloscope for observation during an experiment.

An additional circuit detects the saturation of any amplifier in the current signal pathway and indicates such "clipping" events with the front-panel LED. These events might otherwise go undetected if heavy filtering is in use. Pulse-stretching is employed, so that overdrives of $\geq$ 20 μs duration are visible.

The polarity of the CURRENT MONITOR signal is such that a positive voltage indicates a current flowing out of the pipette. This yields the normal polarity for recordings from outside-out patches and whole cells, in which outward current is positive.

D. Transient cancellation facility (VC mode)

The C-Fast and Tau-Fast controls have the same function as those on the EPC-5, namely for cancelling fast transients from capacitances up to 10 pF. The slow transient cancellation circuitry has the usual capacitance control (C-slow), but instead of a time-constant adjustment, a G-series adjustment is provided. This arrangement was chosen for two reasons. First, the G-series setting corresponds to the reciprocal of the series resistance and is used to adjust the R_s compensation circuitry as well. Second, this arrangement is often easier to use. While C-slow sets the <u>area</u> of the transient to be cancelled, G-series sets the <u>initial value</u>. For well-resolved transients, it is easiest to set G-series first, as sketched below:



Uncompensated transient | Set G-series for zero initial value | Adjust C-slow

The G-series knob has a normal range of 0.01 to 1.0 μS, corresponding to series resistances of 100 to 1 MΩ, respectively.

For cancelling whole-cell capacitive currents,

C-slow ranges of 0.2-10 and 2-100 pF are provided. In patch recording, slow transients arising from the pipette often need to be cancelled. These transients are smaller and slower, and a special C-slow range, labelled C_p, is provided. In this setting the C-slow control has a range of approximately 0.02-1.0 pF and the G series control 0.1-10 nS.

E. Series resistance compensation (VC Mode)

The R_s compensation circuitry is based on principles that have been described elsewhere*. The goal of compensation is to increase voltage-clamp fidelity and speed by cancelling the effect of a fraction α of the series resistance. The uncompensated clamping time constant τ_u is equal to $C_m R_s$ or, equivalently, C_m/G_s (C_m is the membrane capacitance, which appears as C-slow in practice). After compensation the time constant becomes

$$\tau_c = \tau_u (1 - \alpha) \qquad (1)$$

However, for stability α cannot approach too closely to unity, and is constrained by the speed of the current monitor circuitry. In the EPC-7 the characteristic time constant is about 1 μs, which yields the stability relation

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* F.J. Sigworth, "Electronic Design of the Patch Clamp," in *Single Channel Recording/*, ed. B. Sakmann and E. Neher (New York: Plenum, 1983).

$$\alpha \lesssim 1 - \sqrt{\frac{2\ \mu s}{\tau_u}} \qquad (2)$$

This means that when τ_u is small to start with, only a limited degree of compensation can be applied. If τ_u = 200 μs or larger, full 90% compensation can be applied.

In the EPC-7 the series resistance to be compensated is set by the G-series control. The two remaining controls are the speed switch and the %-COMPENSATION knob. At the SLOW setting, the switch slows down the feedback by a factor of four to make the adjustments less critical in the case of very slow recording situations (τ_u > 2 ms). The SLOW setting has to be used to avoid oscillation when the access resistance is very high (G-series < 0.05 μs).

The %-COMPENSATION control selects α. It should remain in the fully "off" position whenever R_s compensation is not in use. Its maximum setting is limited to 90% because of the following problem: when a voltage step of size ΔV is commanded, the pipette potential actually receives a step of size $\Delta V/(1-\alpha)$ due to the compensation effect. This magnification of the command thus necessitates a wide dynamic range for the pipette-potential and the transient-cancellation circuitry. The maximum pipette potential excursion in the EPC-7 is about $\pm$1.2 V, implying that 90% compensation can be used for steps up to about 120 mV in amplitude. Overload of amplifiers (obvious in practical use due to the loss of proper transient cancellation will occur if larger pulses are applied, unless the %-COMP setting is reduced.

The procedure for whole-cell voltage clamping using R_s compensation can be summarized as follows:

1. When the seal is first established, apply small (e.g. 1-10 mV) test pulses and adjust C-Fast and Tau-Fast for the smallest capacitance spikes.

2. After breaking into the cell, adjust G-series and C-slow for the smallest "slow" capacitive transients.

3. Turn up the %-COMP control. The settings of the transient controls all become more sensitive; readjust them if necessary for the smallest transients.

4. Start the recording.

During an experiment the G-SERIES setting occasionally needs readjustment as the access resistance changes. Strong Ca^{++} buffering of the pipette solution (e.g. 10mM EGTA) helps to prevent changes in access resistance due to resealing of the patch membrane.

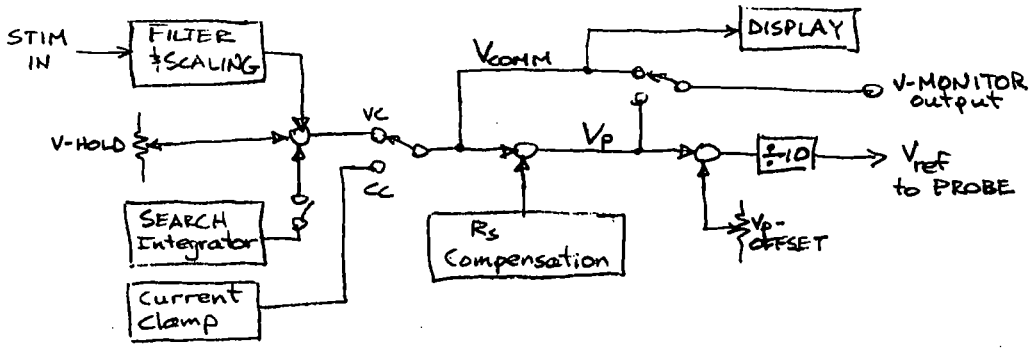
The approximate impulse response of the recording system can be observed by turning the C-SLOW RANGE switch to OFF and applying small command pulses. The capacitive transients then have the time course of the impulse response.

F. Stimulus input and voltage monitor

In the VC and CC+COMM modes the stimulus is first processed by a two-pole filter to "round off" step transitions in the stimulus voltage. This avoids nonlinear effects due to slew limiting in the internal circuitry, and reduces the amplitude of capacitive current transients under voltage clamp. The rise time

T_r of the filter is selectable.

Either of two voltage signals can be monitored, the command voltage V_{comm} or the pipette voltage V_p. These signals differ only when R_s compensation is in use. The derivation of the signals is diagrammed below.



V_{comm} is normally indicated on the LCD display.

In the current clamp modes the signals are also identical and serve as a voltage monitor.

In all cases V_p and V_{comm} do *not* include the offset from the V_p-OFFSET potentiometer, which is intended for cancelling electrode offsets. V_p-OFFSET is best adjusted in SEARCH mode, when the electrode is first placed in the bath, to bring the indicated V_{comm} to zero.

G. Noise test facility

When the NOISE TEST switch is pressed either downward or upward, the LCD display shows a measure of the noise level. The signal that is used is the output

of Filter 2 (3.3 kHz bandwidth), which is then passed through a three-pole, 300 Hz Butterworth high-pass filter and applied to a true-RMS measuring circuit. In the NOISE TEST mode the decimal point of the display vanishes, and the displayed value is in units of 0.1 mV RMS referred to the CURRENT MONITOR signal. When the GAIN is set to 100 mV/pA, the value is displayed directly in fA, RMS.

II. Setting up the EPC-7

A. Power

A switch on the rear panel of the controller selects between 110 and 220 volt operation. ***Make sure that the switch is in the proper position***, and that the correct fuse is installed, before applying power. Irreversible damage to the circuitry may otherwise occur.

B. Static electricity

The input circuitry of the probe can be damaged by static electricity. To avoid this, please observe the following rules:

1. Avoid touching the input terminal unnecessarily.
2. When it is necessary to touch the input (e.g. while inserting a pipette into the holder), ground yourself first by touching a grounded metal surface.

C. Initial checkout

The TEST mode allows one to easily check the basic current measuring circuitry of the EPC-7. Set the MODE switch to TEST, connect a function generator or stimulator to STIM IN, and connect the right-hand CURRENT MONITOR output to an oscilloscope. With the GAIN set to 10 mV/pA, any signal applied to the STIM IN connector should be reproduced with the same amplitude at the CURRENT MONITOR output. An applied signal amplitude of about 1 volt is appropriate. Please note

the following:

1. The probe input usually needs to be shielded from low-frequency noise. A simple way to do this is to cover the input connector with a cap made from aluminium foil.
2. The TEST mode current-injection system is intrinsically AC-coupled, which means that long (>30 ms) pulses may be distorted in shape. Also, large low-frequency inputs can cause the current-injection circuitry to saturate.

The action of the internal filters on the background noise level and the temporal response can be observed by switching in FILTER 1 and FILTER 2. An improved signal-to-noise ratio should be apparent when the GAIN is increased to 50 or greater (which selects the 50 GΩ measuring resistor). If the signal source is a sine-wave generator, the frequency response of the patch clamp can be verified directly.

NOTE: For critical response checking above 10 kHz in the TEST mode, set the STIM SCALING to zero and T_r to 20 μs. In the low gain range (0.5-20 mV/pA) the test circuitry distorts the frequency response above 50 kHz, so an external signal source should be used for very critical testing.

Further tests can be performed with the MC-7 Model Circuit, which is diagrammed below:

attachment to a matching plate or other surface. Later probes are supplied with both the flat mounting plate and an adapter for rod-mounting.

Because of the extreme sensitivity of the EPC-7, special care must be taken in grounding all surfaces that will be near the probe input to avoid coupling in line-frequency interference. A high-quality ground is available at the GND terminal of the probe; this is connected through the probe's cable directly to the SIGNAL GND in the controller. The reference voltage is also available at the probe; note, however, that the REF signal has a source resistance of about 90 Ω, and loading will cause the pipette potential to be in error.

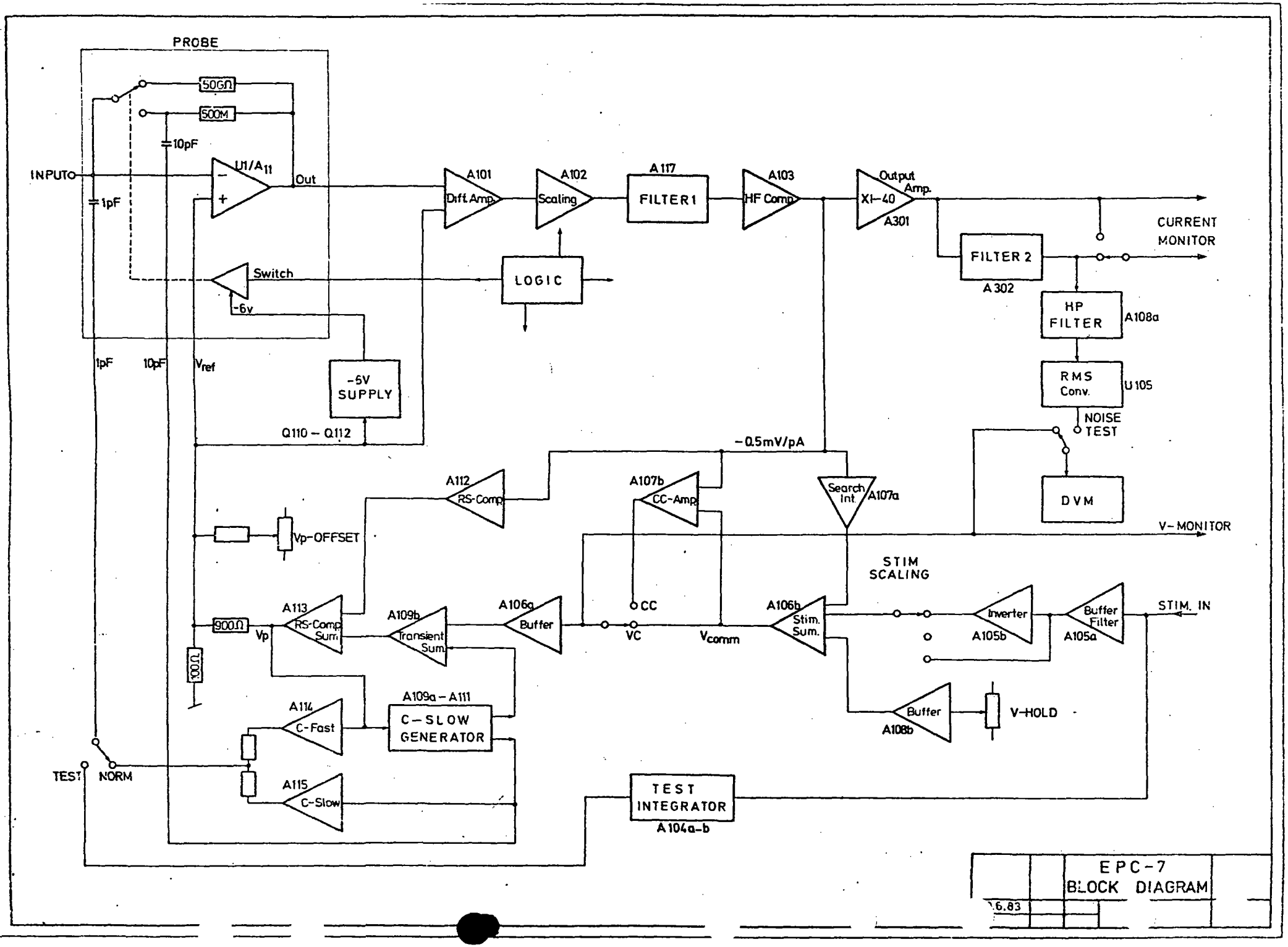
III. Theory of operation

The EPC-7 circuitry (see block diagram on the following page) consists of two main signal pathways, one for pipette current measurement and the other for setting the pipette potential. These pathways and other functions are described in this section.

A. Current monitor pathway

The basic current measurement is performed in the probe by an operational amplifier connected as a current-to-voltage converter. It forces the input voltage to follow the reference voltage V_{ref}, and the resulting current flows through a measuring resistor of either 500 MΩ (low range) or 50 GΩ (high range). The resistors are selected by logic circuitry controlled by the GAIN switch. All critical input circuit components, including the resistors, input transistors, and switching circuitry are contained in a hybrid integrated circuit. The hybrid also contains two current-injection capacitors which are used for transient cancellation and test functions.

The output from the probe is applied to a unity gain differential amplifier (A101) which subtracts the reference voltage V_{ref} to yield a voltage proportional to input current with a nominal scaling of 0.5 or 50 mV/pA. In the next stage (A102) the gain and offset of the signal is trimmed, and a small transient response correction can be applied (the main correction is made later, in A103).


PROBE
50GΩ
500M
10pF
INPUT0
1pF
U1/A11
Out
A101
Diff. Amp.
A102
Scaling
A117
FILTER 1
A103
HF Comp.
Output Amp.
X1–40
A301
CURRENT MONITOR
FILTER 2
A 302
HP FILTER
A108a
RMS Conv.
U105
NOISE TEST
DVM
Switch
-6v
LOGIC
1pF
10pF
Vref
-5V SUPPLY
Q110 – Q112
-0.5mV/pA
A112
RS-Comp.
A107b
CC-Amp.
Search Int.
A107a
V-MONITOR
Vp-OFFSET
STIM SCALING
A113
RS-Comp. Sum
900Ω
Vp
100Ω
A109b
Transient Sum.
A106a
Buffer
CC
VC
Vcomm
A106b
Stim. Sum.
Inverter
A105b
Buffer Filter
A105a
STIM. IN
A114
C-Fast
A109a – A111
C–SLOW GENERATOR
Buffer
A108b
V-HOLD
TEST
NORM
A115
C-Slow
TEST INTEGRATOR
A104a-b
EPC-7
BLOCK DIAGRAM
6.83

FILTER 1 is a two-pole, low-pass active filter stage. Normally it yields an overall 100 kHz or 10 kHz Bessel response for the recording system, as selected by the FILTER 1 switch. (A third pole for the 10 kHz response is provided in the output amplifier.) However, when R_s compensation is in use, its response is changed to an underdamped two-pole characteristic with natural frequencies of 30 kHz or 9 kHz, depending on whether FAST or SLOW compensation is selected.

The primary frequency response correction of the current signal is performed by A103. Trimmers can be adusted to match to match the characteristic time constants of this stage to the time constants τ_1 of measuring resistors in the probe. Above the frequencies determined by these time constants (about 100 μs for the 500 MΩ resistor, 1 ms for the 50 GΩ resistor), the gain of this stage increases proportionally with frequency up to limits of 50 kHz (in the high range) and 140 kHz (low range). The correction stage follows the Filter 1 to avoid amplifier saturation on high-frequency signals when filtering is in use.

The signal at the output of A103 has a flat frequency response and a scaling of -0.5 mV/pA in the low gain ranges. This signal is used by the Search, Current Clamp, and R_s compensation circuits. It is also scaled by a factor between 1 and 40 by the output amplifier (A301) to provide the low range gains of 0.5 to 20 mV/pA available at the CURRENT MONITOR output connectors. At the 50 mV/pA GAIN setting the probe resistor and other circuitry is switched to the high range, and the output amplifier's gain returns to 1. As the GAIN setting is further increased the output amplifier's gain is increased to a maximum of 20.

A 3.3 kHz, 3-pole Bessel filter (FILTER 2) follows the Output Amplifier. This filter has two purposes. First, it provides additional filtering for one of the

CURRENT MONITOR outputs. Second, it is part of the band-pass filtering of the signal for the Noise Test function. The remainder of the band-pass filter (a 3-pole, 300 Hz Butterworth high-pass response) is provided by A108, whose output is applied to the RMS-to-DC converter, U105. When the Noise Test mode is selected, the output of the converter is applied to the LCD display unit, scaled such that 0.1 mV RMS corresponds to one unit on the display.

B. Stimulus pathway

The pipette command potential V_{ref} is formed by summing contributions from the stimulus input, the V-HOLD potentiometer, the search integrator (in SEARCH mode) and R_s compensation (when in use). These signals are processed at 10 times their final scaling, and the resulting V_p signal is divided by ten and an offset from the V_p-OFFSET control is added to form V_{ref}.

The stimulus input signal is first buffered and filtered by A105a. The filter has two identical single time constants of either 0.5 or 7 μs which, with the filtering of V_{ref} itself, yields risetimes (10%-90%) of about 2 and 20 μs depending on the setting of the T_r switch. This filter has two purposes. First, the rounding-off of step inputs avoids slew-limiting and the accompanying nonlinear behavior in the succeeding signal processing stages. Secondly, a rounded stimulus produces smaller-amplitude capacitance currents that are easier to compensate.

After variable scaling, the stimulus is added together with the V-HOLD voltage and the output of the search integrator in A106b.

In the VC mode the stimulus is then fed directly to the buffer A106a, the output of which is called V_{COMM} and represents the commanded pipette voltage; V_{COMM} is

normally displayed on the LCD display (after being scaled down by ten to match the scaling of V_{ref}). The final two amplifier stages, A109a and A113, can be considered as two inverting, unity-gain stages. Their output is V_P, which differs from V_{COMM} only when R_s compensation is in use.

C. R_s compensation

Series resistance compensation for voltage clamp is performed in two stages in the EPC-7. The first stage is called prediction, in which the pipette potential is automatically changed in a way to rapidly charge the cell capacitance whenever a potential change is commanded. The second stage is correction. This is the conventional process in which the current monitor signal is appropriately scaled and added to the voltage command with the polarity of providing positive feedback.

The correction circuitry consists of the scaling amplifiers A112a and b, and the summing amplifier A113. The input is the -0.5 mV/pA current monitor signal. The gain of A112a-b is inversely proportional to the G-SERIES setting and proportional to %-COMP.

The prediction part of the compensation is provided by A109a and the C-SLOW generator. When the C-SLOW and G-SERIES controls are properly adjusted, the C-SLOW generator injects a current into the probe input to match the cell capacitance charging current. For proper R_s compensation a voltage equal to this current, times the series resistance value, must be added to the command voltage. In the EPC-7 a voltage is used from the C-SLOW generator which is proportional to the injected current. This voltage is scaled according to the reciprocal of the G-SERIES setting, and by another, tracking section of the %-COMP potentiometer.

D. Capacitive transient cancellation

The probe has two current-injection capacitors, both of which are contained in the hybrid circuit in the probe. One has a capacitance of 1 pF and is always connected to the input. It is used for both fast and slow transient cancellation, and for the TEST mode. The other 10 pF capacitor is switched in with the 500 MΩ measuring resistor and is therefore available only in the low gain range. It is used in the 100 pF C-SLOW range.

The C-fast circuitry imposes a scaled copy of the V_p signal on the 1 pF capacitor. The signal is filtered with a time constant (τ-FAST) that is variable over the range of about 0.2-1.2 μs to allow matching to the time constant of V_{ref} and short time constants in the pipette capacitance. It is scaled by the C-FAST potentiometer, amplified by A114, and applied to the injection capacitor through a resistive mixing network.

Slow capacitance transients to be cancelled are assumed to arise from a capacitance C_{slow} (typically the cell membrane capacitance) in series with a conductance G_s (i.e. the pipette access resistance). The current I_i induced in this combination by a step in voltage has an exponential relaxation with time constant C_s/G_s. To inject the proper cancelling current through a capacitor into the input, a voltage is required that is the integral of I_i. This injection voltage can be derived from the stimulus by filtering with the same time constant and scaling it proportionally to C_{slow}. The C-slow generator forms such a signal, along with a voltage proportional to the injected current for use by the R_s compensation-prediction operation.

The injection voltage is applied to the 1 pF capacitor through the scaling amplifier A115; the output is summed resistively with the C-FAST signal from

A114. When the SLOW RANGE is set to 100 pF, the injection voltage is also applied to the 10 pF injection capacitor, and the time constant of the C-SLOW generator is changed appropriately.

E. Test circuit

In the TEST mode the STIM IN signal is disconnected from the normal stimulus pathway by an analog switch. The signal is applied to the TEST integrator, A104. The integrator time constant is 10 ms, so that a 1 V input signal yields a 100 V/sec output. The output is applied through a second analog switch to the 1 pF injection capacitor in the probe; 100 V/sec yields a current of 100 pA. The test integrator has a second-order feedback loop to prevent DC offsets from driving the integrator into saturation.

F. Search and current clamp

Both the search and current clamp circuits act to vary V_{COMM} to keep the current monitor signal (actually the -0.5 mV/pA signal from A103) at zero or at a commanded level. In the search integrator, the main time constant was chosen to give an overall feedback time constant of about 1 s for a 10 MΩ resistance at the probe input. The RESET button speeds up the search integrator by a factor of 100.

The current clamp amplifier A107b acts to keep the pipette current at zero with a response time constant of about 10 μs. A small offset current (±8 pA) can be added by the CC-ZERO trimmer on the front panel to adjust the probe input current to zero. In the CC+COMM mode a voltage is added to the input circuit for commanding an injected current level. The output of A107b is attenuated (relative to V_{ref}) by a factor of

60, so that the voltage compliance at the probe input is about ±250 mV. In the CC modes the logic circuitry forces the low gain range and the 10 kHz Bessel characteristic for FILTER 1. It also disables both fast and slow capacitance cancellation.

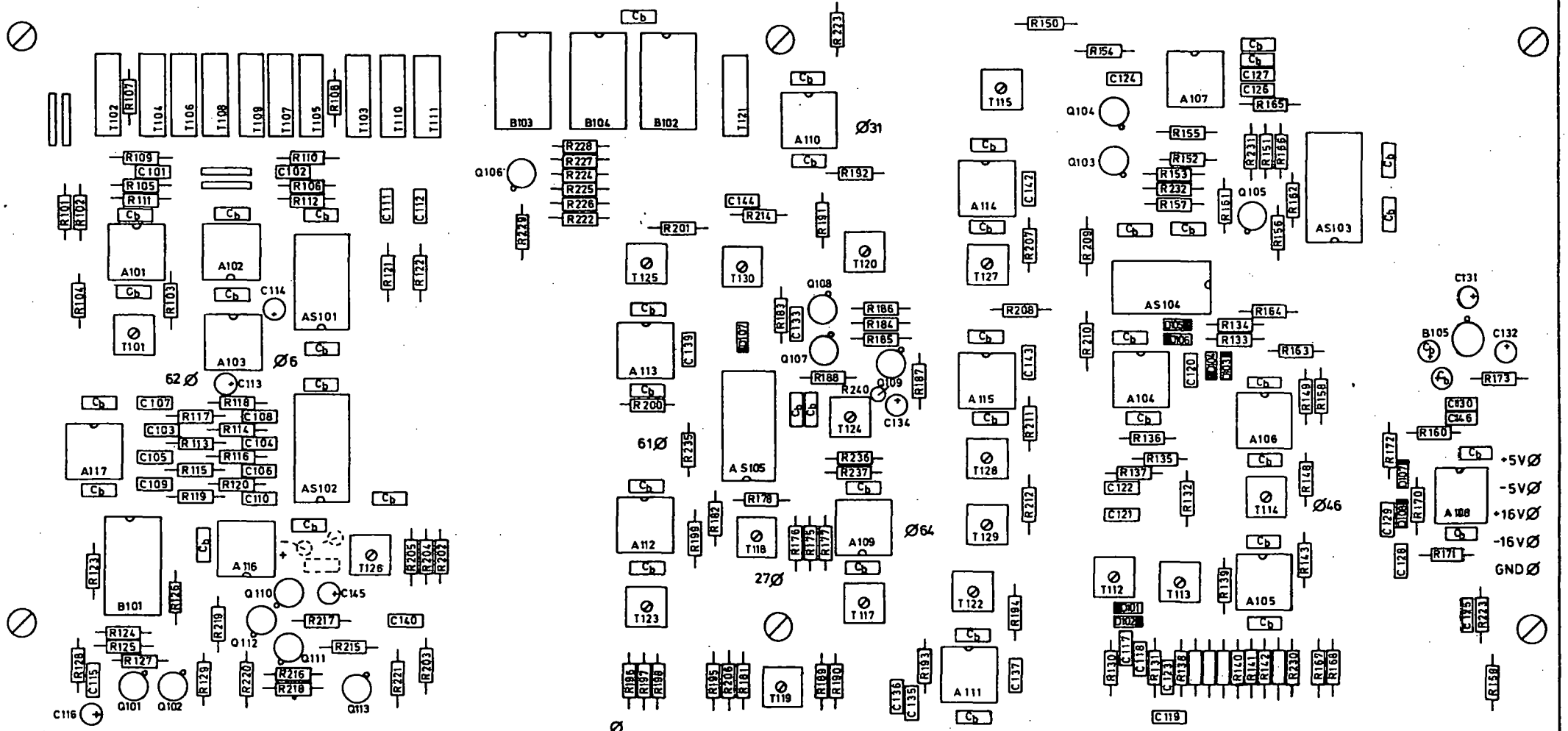
IV. Adjustments

The internal adjustments of the EPC-7 can be divided into three groups. One group consists of the single CC-zero trimmer on the front panel. It is used to trim the probe input current in special cases where a small bias current (<1 pA) might affect the measurement of membrane potentials. The second group consists of ten trimmers located at the left rear of the main circuit board in the controller (see the location diagram on the next page). These trimmers set the gain, offset, and frequency response trims for the current monitor pathway, and are used to match the characteristics of the 500 MΩ and 50 GΩ current-measuring resistors. Since the resistor characteristics may change with time, advanced users may want to touch up these trimmers, and for this reason they will be described here.

The remaining trimmers (some 22 of them) constitute the third group and should not need adjustment in normal use. They trim amplifier offsets, calibrate various functions and match the controller to the current-injection capacitors in the probe. Adjustment should be necessary only when components are replaced or when probes are exchanged. Since special fixtures are required, calibration is best performed at the factory, but the procedures are detailed in the *EPC-7 Service Manual*.

A. CC-ZERO trimmer

If the probe input is left open, any bias current will cause the pipette potential to drift when the MODE is switched from VC to CC. An 0.1 pA error causes a roughly 0.1 V/sec drift, since the input capacitance is



	Vers.	CONTROLLER EPC-7 COMPONENT LOCATIONS
83	7E	

about 1 pF. The V-MONITOR output gives the pipette potential scaled up by 10, so this output would slew at 1 V/sec. Adjust the trimmer to give the slowest drift.

B. Current monitor adjustments

The gain and transient response of the current monitor circuitry can be checked using the TEST mode as outlined earlier. Note, however, that this check depends on the accuracy of the oscilloscope and of the TEST circuitry, whose scaling is specified within 2%. At the factory, the gain is calibrated to within 1% by the method of injecting currents through a 10 MΩ resistor from a precision millivolt source. The offset can be adjusted simply by leaving the probe input open and adjusting for zero output at a CURRENT MONITOR connector. The trimmer locations are shown in the location diagram on the next page. The functions of the trimmmers are:

T102	High range gain
T104	High range offset
T106	High range aux. τ
T108	High range aux. amplitude
T109	Low range aux. amplitude
T107	Low range aux. τ
T105	Low range offset
T103	Low range gain
T110	High range frequency comp.
T111	Low range frequency comp.

The frequency comp. trimmers are the main adjustments for compensating the transient response.

Misadjustments result in under- or overshoots in the square-wave response having time constants of about 1 ms in the high range or about 100 μs in the low range. The aux. amplitude and aux. τ trimmers adjust an additional exponential component that can be used to correct for biphasic deviations in the square wave response. The amplitudes are zero when the wipers of T108 and T109 are to the rear (screws turned fully clockwise). Note: The aux. amplitude and aux τ trimmers interact strongly. It is wise to avoid making large changes in their settings.

EPC-7 USER'S MANUAL

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Table of Contents

Introducing the EPC-7 1

About this Manual 2
References

I. Description of the Probe and Controller 4
Probe
Controller

II. Operating Modes 13
Voltage-Clamp mode
Search mode
Current clamp modes
Test mode

III. Series-Resistance Compensation 18

IV. Setting up the EPC-7 24
Power
Static electricity
Initial checkout

V. The patch-clamp setup 27
Mounting the Probe
Grounding the microscope
External shielding
Connections to other instruments
Pipette holder and electrode
Bath electrode

VI. Patch-Pipette Lore 33
Glass capillaries
Pulling
Coating
Heat polishing
Use of pipettes

VII. Using the patch clamp 39
Forming a seal
Cell-attached recording
Whole-cell recording
Current-clamp recording

VIII. Techniques for low-noise recording 52
Patch recording technique
Noise in whole-cell recording

IX. Internal Adjustments 57
CC-ZERO trimmer
Current monitor adjustments

X. Circuit description 61
Current monitor pathway
Stimulus pathway
R_s compensation
Capacitive transient cancellation
Test circuit
Search and current clamp

Introducing the EPC-7

The letters EPC stand for *Extracellular Patch Clamp*, the method introduced by Neher and Sakmann (1976) for recording the currents in a small patch of membrane under voltage-clamp conditions. The EPC-7 represents roughly the seventh in the series of patch clamp designs in use in the Göttingen laboratories of Neher and Sakmann since their initial experiments. In the intervening years a number of technical advances have occurred, most notably the discovery of the "gigaseal" by E. Neher (1981), which have made the words *extracellular* and *patch* overly restrictive in describing this instrument. Intracellular recordings can be made with the same type of recording setup as used for patch recording from the cell surface, and cell-free membrane patches are also used. For lack of a better term and in view of the illustrious history of the patch clamp, however, we have retained the original name.

The versatility of the EPC-7 can best be appreciated by the variety of experiments that have been carried out with it. Besides high-resolution recordings of channels, it has been used in studies of exocytosis (by monitoring changes in cell membrane capacitance), whole-cell voltage-clamp and current-clamp studies, and recordings from artificial membranes. Technically, the EPC-7 is noteworthy for three special features, which are the range-changing capability of the head stage, the extremely wide bandwidth available from the current monitor circuitry, and the integrated transient cancellation and series-resistance compensation functions. Together these features mean that the single head stage suffices for both single-channel and whole-cell recordings, and that both kinds of recordings can be made with high time resolution and low noise.

About this Manual

This manual is designed to acquaint the user with the particular features and idiosyncrasies of the EPC-7 and to provide a general guide for setting up and using this instrument for experiments. It is assumed that the reader has some familiarity with patch-clamp techniques; should you be a newcomer to the field perhaps the best place to start would be the paper by Hamill *et al.*, where the basic gigaseal techniques are described. Also if you are a newcomer you should not feel compelled to read this manual straight through. For example Chapter I of this manual is designed as much to be a reference chapter as an introduction to the instrument, so that if you have never worked with a patch clamp before you might want to read Chapter II first, to get a clearer idea of what the EPC-7 does, before you read Chapter I. You could then skip Chapter III (mainly of interest to whole-cell clampers, and fairly advanced), reading Chapters IV - VII to get an overview of how to set up the patch clamp and use it. More experienced users might want to read straight through the manual, proceeding on to Chapter VIII, where details of low-noise technique are discussed. Those who are curious about the details of the circuitry can read Chapter X and, if desired, the more detailed *Circuit Description and Diagrams--EPC-7 Patch Clamp* which is available separately from the manufacturer.

Many users will want to read some of the more advanced and complete discussions of individual topics that are to be found in original articles and in the book *Single Channel Recording*, B. Sakmann and E. Neher, eds., Plenum Press, New York, 1983. We list here the articles that will be cited in this manual.

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Colquhoun, D. and F. J. Sigworth. Fitting and statistical analysis of single-channel records. Chapter 11.

Corey, D. P. and C. F. Stevens. Science and technology of patch-recording electrodes. Chapter 3.

Marty, A. and E. Neher. Tight-seal whole-cell Recording. Chapter 7.

Sigworth, F. J. Electronic design of the patch clamp. Chapter 1.

I. Description of the Probe and Controller

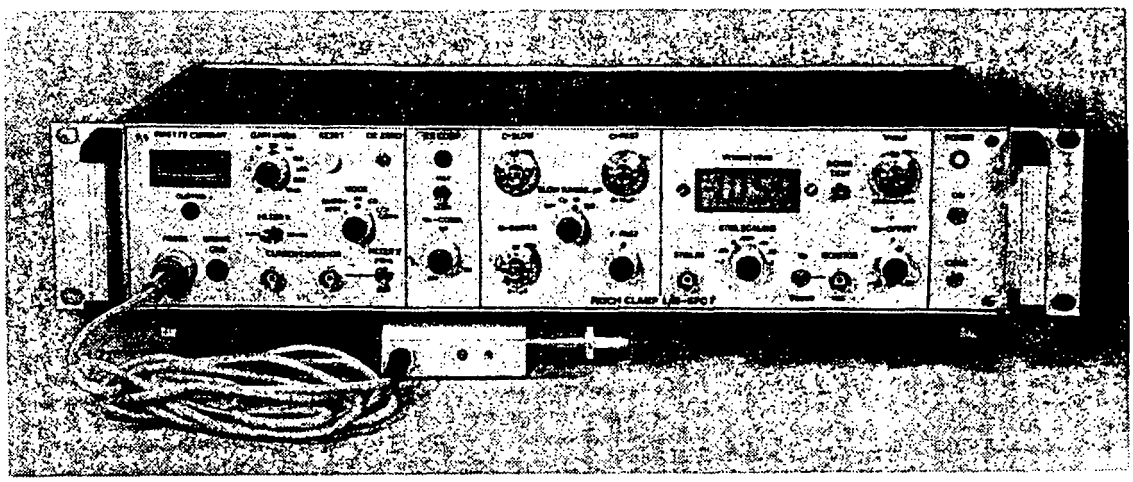
Probe. The probe, or "head stage" of the EPC-7 is contained in a small enclosure designed to be mounted on a micromanipulator and directly attached to the recording micropipette. It contains the sensitive amplifier that constitutes the current-to-voltage converter, as well as components for injecting test signals into that amplifier. On the probe are the following connectors:

1. **Input connector.** This is a Teflon-insulated BNC connector. The standard pipette holder plugs directly into this connector; the center pin is the amplifier input, and the shield is driven with the command potential V_p.

2. **REF output.** The red .04" pin jack carries the command potential V_p for driven shielding. Note: the source resistance is 90 Ω, and significant loading will attenuate the command voltage that controls the pipette potential. This means that you should not connect this signal to anything having an impedance below about 10 kΩ, or else the pipette potential will be smaller than indicated by the Controller.

3. **GND connector** The black pin jack carries a high quality ground signal which is useful for grounding the bath electrode and nearby shields without potential errors that would arise from ground loops. Unlike the EPC-5, this ground is not sensed, but is connected directly to the signal ground on the controller through the probe's cable. See Chapter V for more on grounding practices.

Controller. The main unit of the EPC-7 is the Controller, which contains the power supply, the signal processing electronics, and all of the controls. Essentially all of the calibration adjustments are



FRONT PANEL OF EPC-7 CONTROLLER

made in the controller, including those which depend on the properties of components in the Probe. For this reason, Probes can not be interchanged without a fairly extensive recalibration of the Controller.

The controls on the Controller's front panel (Fig. 1) are divided into five functional groups containing, from left to right, the functions of: current monitoring, series-resistance compensation, capacitance compensation, command signal processing, and the power switch.

1. Current monitor group

PIPETTE CURRENT meter. This is designed to provide a rough indication of the pipette current. Each division corresponds to approximately 1 volt at the CURRENT MONITOR outputs, so that full scale is ± 3V.

GAIN switch. This control sets the scaling of the CURRENT MONITOR

output signals. It also automatically selects one of the two current-measuring resistors in the Probe. In the low range, selected by GAIN settings between 0.5 and 20 mV/pA, the current measuring resistor is 500 MΩ. In this range the relatively low value of the resistor means that the background noise is larger, but the full 100 kHz bandwidth is available; and currents up to about 25 nA can be recorded. This range is used mainly for whole-cell recordings, and for this purpose the special features of the 100 pF transient cancellation range, series resistance (R_s) compensation and the current-clamp modes are made available. On the other hand, the high range, which is selected by GAIN settings from 50 to 1000 mV/pA, is intended for single-channel recording. It has a very low noise level, but this is obtained at the expense of a maximum current limit of 250 pA. The maximum available bandwidth is about 60 kHz, and the special features mentioned above do not function in this range.

CLIPPING indicator. This LED lights whenever an amplifier saturates in the current monitor pathway. The indicator is mainly useful in voltage-clamp experiments where capacitive artifacts will be subtracted in a computer; the subtraction can work well as long as no saturation occurs, and this indicator serves as simple monitor of this condition. It is particulary useful since it will indicate clipping by internal amplifiers even in cases where, because of filtering, the output voltage is not saturated.

CC ZERO trimmer. This trimmer adjustment is used to zero the leakage current in the Probe input which otherwise might cause errors in membrane potentials measured in the Current Clamp mode. The leakage current is typically less than 1 pA, and can be trimmed to zero by this screwdriver adjustment. The adjustment procedure is described in Chapter IX.

FILTER 1 switch. This switch allows a 10kHz, 3-pole Bessel filter to be inserted into the current monitor pathway. Under most conditions a 10 kHz bandwidth is more than ample, and the filtering reduces the high-frequency noise substantially. The resulting signal is provided at the left-hand CURRENT MONITOR output.

FILTER 2 switch. This switch allows the right-hand CURRENT MONITOR output to receive either the same signal as the left-hand one, or this signal after filtering through an additional 3 kHz, 3-pole Bessel filter.

CURRENT MONITOR outputs. The output signals are filtered as described above. Positive voltages correspond to currents flowing out of the pipette. Typically, the left-hand output is fed to a data recorder (e.g. an FM tape recorder or a PCM device / video recorder combination) to record the signal at wide bandwidth, while the additionally-filtered signal from the right-hand output is applied to an oscilloscope for monitoring the progress of the experiment.

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

MODE switch. The various operating modes are described in detail in Chapter 3. Briefly, the VC (voltage-clamp) mode is the usual mode in which the pipette current is recorded while the pipette potential is controlled by command signals. In the CC (current-clamp) modes the pipette potential is measured while the current is held at zero (CC mode) or at a commanded value (CC+COMM). The SEARCH mode is like the VC mode except that a feedback loop acts slowly to keep the current signal from drifting off-scale while a seal is being established. In

the TEST mode the signal at the STIM IN connector is converted into a current (scaling: 100 pA per volt) and injected as a test signal into the probe input.

RESET button. Pressing this button speeds up the feedback in the SEARCH mode, providing a rapid zeroing of the current signal.

2. Series resistance (R_s) compensation group

The R_s compensation circuitry corrects for errors in the membrane potential that arise in whole-cell recordings due to the access resistance between the pipette electrode and the cell interior. See Chapter III for a detailed description of the principles and use of these controls.

RS COMP indicator. This light is on whenever R_s compensation is in use.

FAST/SLOW switch. This switch selects the fast R_s compensation, which requires more critical adjustment of the controls but provides the maximum voltage-clamp speed, or the slow compensation.

%-COMP control. This control turns R_s compensation on and selects the amount of compensation, up to 90% of total.

3. Capacitance compensation group

The capacitance compensation circuitry is used to cancel the large artifactual currents that flow when the patch potential is suddenly changed, for example as is done in experiments on voltage-activated channels. The "C-FAST" circuitry is used to cancel the rapidly-decaying currents that charge the pipette and other stray capacitances, while the "C-SLOW" circuitry is mainly used in

whole-cell recordings to cancel the slower transients arising from the charging of the cell capacitance. The use of these controls is discussed in Chapter VII.

SLOW RANGE switch. Selects the scaling of the transient-cancellation signal controlled by the C-SLOW and G-SERIES potentiometers. In the 10 pF and 100 pF ranges, C-SLOW can be set to cancel capacitance transients due to the membrane capacitance in whole-cell recordings, up to these values. In the C_p range, cancellation is provided for smaller and slower transients arising from pipette charging currents in patch recordings.

C-SLOW control. When it is set (along with G-SERIES) to cancel the capacitance transient in a whole-cell recording, the knob reading gives the cell membrane capacitance; full scale is 10 or 100 pF depending on the SLOW RANGE setting. In the C_p mode, full scale corresponds to a capacitance of 1 pF.

G-SERIES control. Adjusts the conductance in series with the capacitance (C-SLOW) of a model network that gives rise to the transient cancellation signal. The control is calibrated, with full scale corresponding to 1 μS (1 MΩ of series resistance). Lower settings of the control correspond to higher series resistances. For example, one turn (0.1 μS) coresponds to 10 MΩ. The setting of this control is used by the R_s compensation circuitry as the measure of the series resistance as well. In the C_p range, full scale corresponds to 0.01 μS.

C-FAST control. This adjusts the size of the rapid transients that cancel the charging current of the pipette and other stray capacitances. Full scale is 10 pF. With nothing connected to the Probe input, cancellation is typically obtained at a setting of 1-2 pF

due to the residual input capacitance of the current-measuring amplifier.

τ-FAST control. This control adjusts the time constant of the cancelling transient to best match that of the charging currents to be cancelled.

4. Command signal processing

These controls allow a steady voltage (set by V-HOLD) to be added to an external stimulus (scaled by STIM SCALING) to provide the command signal. In the VC mode, this command signal determines the pipette voltage, while in the CC+COMM mode, the command determines the current. The calibrations are given for the VC mode, but they can be readily converted to the corresponding currents in the CC+COMM mode by the simple scaling of 1 pA/mV.

STIM INPUT. Pulses from an external stimulus source are applied here. The input impedance is approximately 8.5 kΩ.

T_r switch. The stimulus signal is passed through a 2-pole filter to round off stepwise changes in voltage. This avoids nonlinearities (from slew-limiting amplifiers) in the command-processing circuitry and also reduces the amplitude of the current transients from rapid charging of the pipette. Two degrees of filtering, specified as the risetimes (time from 10% to 90% of the amplitude of a step change) are available. 2 μs is the minimum required to avoid nonlinearities in the internal circuitry, while the 20 μs is preferable for all but the fastest measurements to make the capacitive transients smaller.

STIM SCALING. The stimulus signal is scaled by a factor selected by this switch. Positive and negative factors are provided to allow the

user to apply pulses of either polarity to the membrane under study. The largest factor is 0.1; the attenuation by 10 is desirable to reduce external noise contributions to the command voltage. The small factors of 0.01 and 0.001 are useful in applying test pulses for measuring the pipette resistance before sealing the pipette onto a cell.

[EPC-5 users note: the definition of STIM SCALING is different in the EPC-7, with the switch setting representing the true scaling factor between STIM IN and the pipette potential.]

V-HOLD. This control adjusts the constant voltage that is added to the stimulus signal. The range is -200 to +200 mV, with a setting of 5.00 on the knob corresponding to 0 mV.

V_{COMM}/V_{RMS} display. This LCD display normally indicates in millivolts the average command potential, i.e. the sum of V-HOLD and the scaled stimulus. In the current clamp modes it indicates the pipette potental, not the commanded current.

NOISE TEST switch. When this switch is pressed either upward or downward from its center position, the V_{COMM}/V_{RMS} display shows the RMS voltage (i.e. the standard deviation) of the CURRENT MONITOR signal in the frequency band of 300 Hz to 3 kHz. In the NOISE TEST mode the decimal point on the display disappears, and a single unit on the display corresponds to 0.1 mV RMS. This facility is useful for checking the noise level of single-channel current recordings. At the typical GAIN setting of 100 mV/pA, the display will register in units of fA; under good conditions, readings in the range of 160-200 fA can be obtained in actual patch recordings.

V-MONITOR output. This output signal provides a monitor of the command potential. It is scaled up by a factor of 10 relative to the

potential applied to the pipette. The output impedance is 50Ω.

V_P/V_{COMM} switch. The MONITOR output can sample either the simple command potential V_{COMM} or the command after it has been modified by the R_s compensation circuitry (the 'true' pipette potential or V_P). With R_s compensation off, these signals are identical.

V_P-OFFSET control. As the final step in the command processing a variable offset voltage is added to compensate for electrode offset potentials. The range of this control is ±50 mV, which is adequate for most stable electrodes.

5. Power switch and chassis connector

POWER switch. It is not a good idea to turn off the power switch during a patch recording but, surprisingly, membrane patches will sometimes survive this treatment!

CHASSIS connector. The chassis is connected to the ground line of the power cord, as is typical of most instruments. The SIGNAL GROUND is kept separate from the chassis to avoid ground loops, but is connected to it through a 10Ω resistor.

II. Operating modes

The EPC-7 is fundamentally an instrument for measuring small electrical currents. It uses a current-to-voltage (I-V) converter circuit (Fig. 2A) to convert the currents to an analog voltage which is then made available at the CURRENT MONITOR outputs for display or recording. At the same time that pipette currents are being recorded the potential must be specified, and the various operating modes of the EPC-7 correspond mainly to different ways of controlling that potential.

A. Voltage-Clamp mode

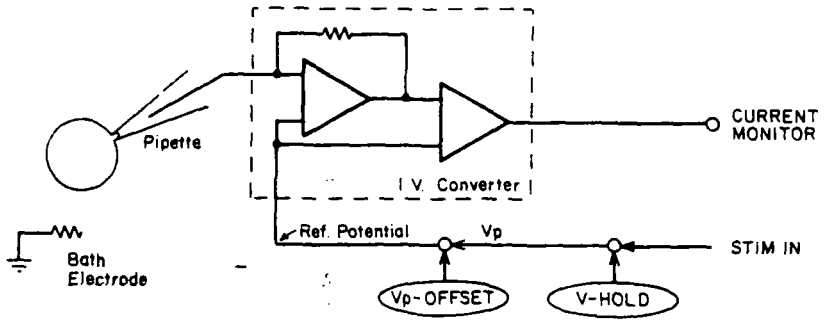
This is the basic patch-clamp mode, and is implemented by the circuitry shown in Fig. 2A. The pipette potential is derived from the signal applied to the STIM INput, with a variable offset added from the V-HOLD control. The sum of these two sources is what is displayed and monitored as the V_{COMM} signal. (Unless R_s compensation is in use, this signal is identical to the V_p signal.) Before being applied to the pipette a further, variable offset is added from the V_p-OFFSET control to allow the user to cancel electrode offsets.

B. SEARCH mode

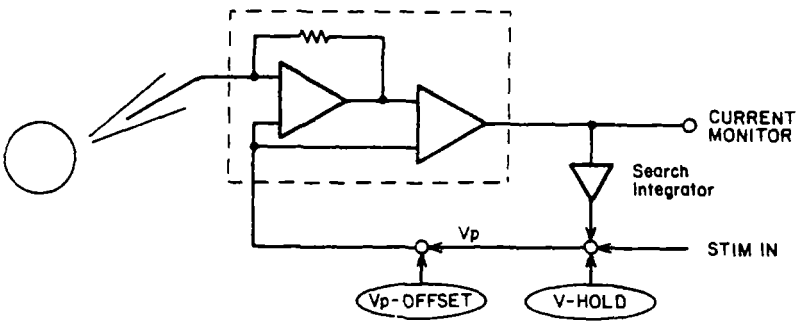
The SEARCH mode is essentially the same as the Voltage-Clamp mode, except that V_{COMM} also receives an input from the search integrator, which is an amplifier that provides negative feedback between the current monitor and pipette potential signals (Fig. 2B). It acts to slowly adjust the pipette potential to make the average pipette current zero. This mode of operation is most useful when one is first approaching a cell to make a seal, as the feedback will compensate for

MAIN OPERATING MODES OF THE EPC-7

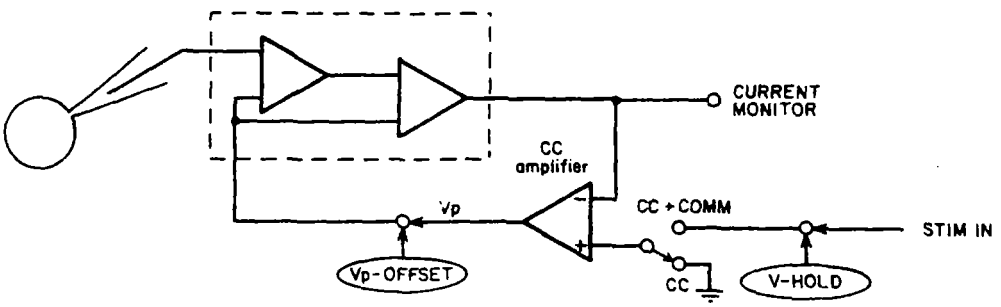
A. Voltage Clamp Mode



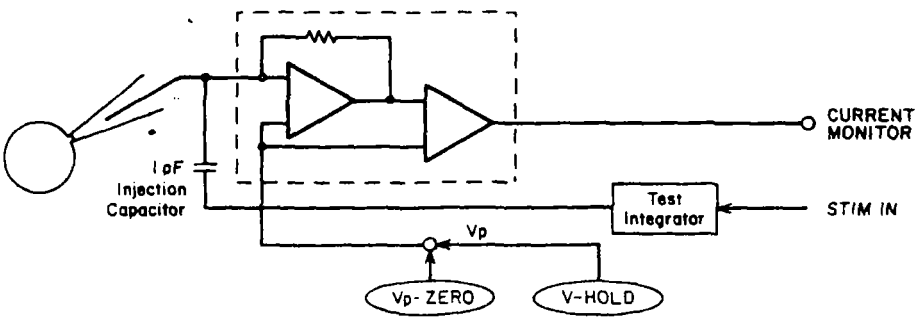
B. Search Mode



C. Current Clamp Modes



D. Test Mode



small electrode potential drifts that otherwise would drive the current monitor signal off-scale. The speed of the feedback is inversely proportional to the resistance between the pipette electrode and the bath, with a time constant of about 1 s at 10 MΩ. When a gigohm seal is established the feedback becomes very slow, so to avoid drifts in the pipette potential you should be sure to switch to the VC mode before starting a recording.

C. Current Clamp modes

The current clamp modes are similar in principle to the search mode, in that feedback is employed between the current monitor signal and the pipette potential (Fig. 2C). In the CC mode the feedback acts rapidly (with a time constant of about 30 μs) to keep the current at zero by varying V_p appropriately; in this way a high-impedance voltage follower is created, with the output voltage available at the V_p or V_{COMM} output (in the CC modes these signals are the same). Note that, unlike the SEARCH mode, the CC modes do not apply the STIM IN and V-HOLD signals directly in determining V_p. In the CC mode these signals are ignored, while in the CC+COMM mode they are used to set the pipette <u>current</u> through the action of the feedback amplifier.

You can use the CC mode to measure the resting potential or spontaneous action potentials in a whole-cell recording, and the membrane potential will be shown on the V_{COMM} display. In the CC+COMM mode, a commanded current can be injected while the pipette potential is measured. The commanded current is determined by the sum of voltages from the STIM IN signal and the V-HOLD control. The scaling is such that any combination of commands that would give rise to 1 mV in the VC mode instead give rise to 1 pA in the CC+COMM mode. [This relationship of 1 pA/mV holds regardless of the GAIN setting, unlike

the EPC-5.] For example, if a 1 V pulse applied to the STIM IN connector and if STIM SCALING is set at 0.1, a 100 pA current pulse will result. The polarity is the usual one, in which positive stimuli result in currents flowing out of the pipette. See Chapter VII for more details on current-clamp recording.

The CURRENT MONITOR output can be used to monitor the currents being passed, with the GAIN control determining the sensitivity. Note: if GAIN settings in the high range are used, the current-clamp operation will be normal but the scaling of the CURRENT MONITOR signal will be in error. Also, we should note that the current-clamp amplifier is only capable of varying V_p over a range of about $\pm$ 150 mV, which means that the voltage-follower function works only over this potential range. Outside this range the pipette current will not be zero, though no damage will occur to the probe unless much larger voltages (several volts) are applied from a low-impedance source to the Probe input. Finally, EPC-5 users should note that in the EPC-7 the current injection scaling is independent of the GAIN setting, which is a change from the EPC-5. See Chapter VII for more details on current-clamp recording.

D. TEST mode

This mode (Fig. 2D) is very much like the VC mode except that the STIM IN signal is not used to determine the pipette potential (only the V-HOLD control is used). Instead the STIM IN signal is used to inject small test currents into the I-V converter input. The scaling of the current that is injected is fixed at 100 pA per volt applied at the STIM IN connector. This scaling is independent of the STIM SCALING setting and is calibrated at the factory to provide a quantitative test of the current monitor circuitry. The signal is not DC coupled,

but square waves of >20 Hz, pulses >30 ms in duration, and sine waves at >1 Hz are reproduced faithfully. For accurate frequency response and to avoid degrading the sensitivity of the I-V converter the test currents are injected through a capacitor (rather than a resistor) into the Probe's input terminal. The STIM IN signal is integrated by an auto-zeroing integrator before being applied to the capacitor. The integrator may require several seconds of recovery time when a signal with a substantial DC component is applied to the STIM INput.

The TEST mode is particularly useful for measuring the step response of the entire recording system, which is a necessary step in high-resolution channel analysis, for example as described by Colquhoun and Sigworth (1983). The current injection circuitry has a useful bandwidth greater than 100 kHz in the high range of GAIN settings and can be used for quantitative measurement of the step response. In the low range however the response is significantly distorted above about 50 kHz, so that critical measurements of the current monitor response should be made with an external signal source. [For example, a high-quality triangle wave generator can be used, whose output is coupled capacitively through a wire placed near the pipette holder.] The distortion in the internal TEST circuitry arises from a small amount of feed-through of the STIM IN signal directly into the current injection capacitors. For best results in either the high or low range, the feed-through can be reduced by setting STIM SCALING to 0 and T_r to 20 μs.

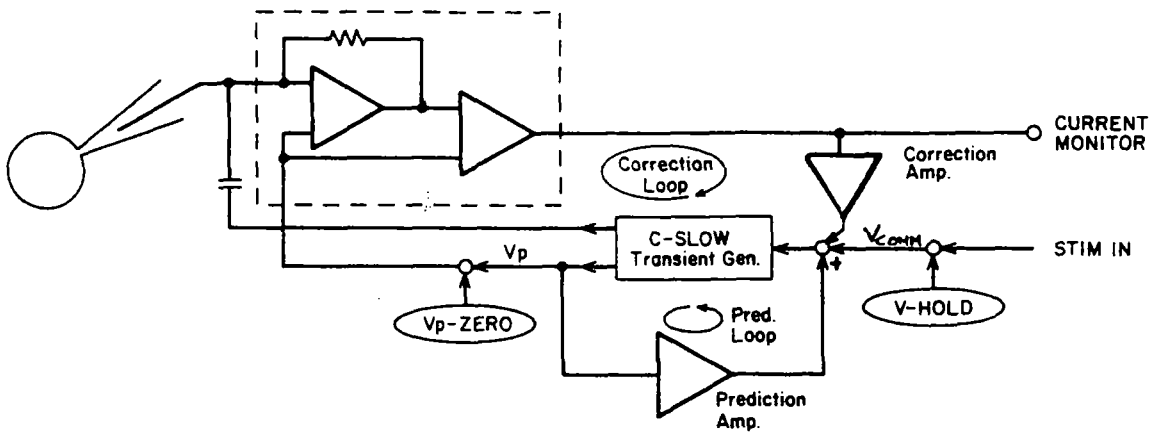
III. Series resistance compensation

In whole-cell voltage-clamp recording the membrane potential of the cell is controlled by the potential applied to the pipette electrode. This control of potential is not complete, but depends on the size of the access resistance between the pipette and the cell interior, and on the size of the currents that must flow through this resistance. This access resistance is called the series resistance (R_s) because it constitutes a resistance in series with the pipette electrode. Part of the series resistance arises from the pipette itself, but normally the majority arises from the residual resistance of the broken patch membrane which provides the electrical access to the cell interior. In practice we find that the series resistance usually cannot be reduced below a value about three times the resistance of the pipette alone.

Series resistance has two detrimental effects in practical recording situations. First, it slows the charging of the cell membrane capacitance because it impedes the flow of the capacitive charging currents when a voltage step is applied to the pipette electrode. The time constant of charging is given by $\tau_u = R_s C_m$, where C_m is the membrane capacitance. For typical values of R_s = 5 MΩ and C_m = 20 pF, the time constant is 100 μs. This time constant is excessively long for studying rapid, voltage-activated currents such as Na currents in nerve, especially since several time constants are required for the membrane potential to settle at its new value after a step change. The second detrimental effect of series resistance is that it yields
errors in membrane potential when large membrane currents flow. In the case of R_s = 5 MΩ, a current of 2 nA will give rise to a voltage error of 10 mV, which is a fairly large error; for studying

III. Series resistance compensation

SERIES-RESISTANCE COMPENSATION



voltage-activated currents, errors need to be kept to ~2 mV at most.

Electronic compensation for series resistance in voltage-clamp systems has been in common use since the days of Hodgkin and Huxley. The principle of the compensation in the case of a patch clamp is that a fraction of the current monitor signal is scaled and added to the command potential (Correction pathway, Fig. 3). When a large current flows in the pipette, the pipette potential is altered in such a way to compensate for the potential drop in the series resistance. This arrangement constitutes positive feedback, and can become unstable when overcompensation occurs.

The EPC-7 incorporates additional circuitry to allow capacitance-transient cancellation to occur while R_s compensation is in use. It is shown as the Prediction pathway in Fig. 3, and it accelerates the charging of the membrane capacitance by imposing large, transient voltages on the pipette when step changes are

commanded. These voltages would occur due to the action of the Correction pathway alone as the large capacitive charging currents elicit pipette voltage changes; howeve, when these currents are cancelled by the transient cancellation, their effect must be predicted by the cancellation circuitry: hence the Prediction pathway.

Together the two parts of the EPC-7 R_s compensation circuitry cancel the effects of a fraction α of the series resistance. This means that the charging of the membrane capacitance is accelerated, with a time constant under compensation of

$$\tau_c = (1-\alpha)\tau_u,$$

where τ_u is the uncompensated time constant. Similarly, the voltage errors due to membrane currents are also reduced by the factor $(1-\alpha)$. The fractional compensation α is determined by the setting of the %-COMP control on the front panel. For proper compensation, however, the circuitry needs to have an estimate of the total series resistance (for the correction pathway), and both the series resistance and membrane capacitance must be known for capacitance transient cancellation (C-SLOW) circuitry. In the EPC-7 the estimation of series resistance has been combined with the transient cancellation, in that the G-SERIES control has a dual effect. It is calibrated in terms of the reciprocal of the series resistance, and its setting affects both the kinetics of the transient cancellation and the scaling of the correction feedback signal. This means that in practice the estimation of the series resistance consists of adjusting the C-SLOW and G-SERIES controls to cancel the transient currents due to the cell membrane capacitance. Once this has been done, the relative amount of R_s compensation can then be selected with the %-COMP control.

III. Series resistance compensation

Theoretically it is desirable to compensate as much of the series resistance as possible. In practice however a degree of compensation above 90% can involve considerable technical problems, and in some recording situations a value below 90% is preferable. To illustrate one technical problem, consider the case when a 100 mV potential change is commanded, and 90% compensation is in use. This degree of compensation means that the cell membrane capacitance will be charged 10 times faster than normally. The rapid charging is accomplished in the compensation circuitry by forcing the pipette potential to (very transiently) reach a potential of 1V. The resulting large current causes the membrane capacitance to charge quickly to its final value of 100 mV. In general, when a voltage step of size ΔV is commanded, the pipette potential actually receives an initial transient of size $\Delta V/(1-\alpha)$ due to the compensation effect. The technical problem comes from the fact that the maximum pipette potential excursion in the EPC-7 is about ± 1.4 V, implying that 90% compensation can be used for steps only up to about 140 mV in amplitude. Overload of amplifiers (obvious in practical use due to the loss of proper transient cancellation) will occur if larger pulses are applied, unless the %-COMP setting is reduced.

The degree of R_s compensation is also limited by stability considerations. Stable R_s compensation requires that the C-FAST control is properly set to cancel the fast capacitance transients; when the series resistance is high, say above 10 MΩ, misadjustment of C-FAST can easily cause oscillation. In cases where R_s is this size or larger (i.e. G-SERIES is less than 0.1 μS) it is often advisable to use the SLOW setting of the R_s switch, which in slowing down the speed of the compensation feedback makes it less susceptible to high-frequency oscillations. In cases where R_s is relatively small,

on the other hand, it is sometimes not possible to use full 90% compensation because of the limited speed of the compensation feedback, even in the FAST setting of the switch. This problem arises when the time constant τ_u is smaller than about 100 μs, and comes from the fact that compensated membrane time constant τ_c cannot be made smaller than a value that depends on the speed of the R_s compensation feedback. If you turn up the %-COMP control to try to obtain a smaller τ_c, you will observe ringing or oscillation in the current monitor signal. The minimum value for τ_c is given by

$$\tau_{c(min)} = \sqrt{\tau_u \tau_f},$$

where τ_f, the effective time constant of the feedback loop, is about 2 μs for the FAST setting, and 6 μs for the slow setting. The corresponding maximum α values are given by

$$\alpha_{max} = 1 - \sqrt{\tau_f/\tau_u} .$$

The table below gives maximum α values (i.e. %-COMP settings) and the resulting τ_c values, in the FAST setting for some values of the uncompensated time constant τ_u.

τ_u (μs)	α	τ_c (μs)
90	.85	13
50	.80	10
30	.75	8
22	.70	7
13	.60	5
8	.50	4

At the SLOW setting, full 90% compensation may be used only for time constants τ_u greater than about 500 μs. In practice you can estimate τ_u from the ratio of the settings on the C-SLOW and G-SERIES knobs. For example, if C-SLOW is set to 10 pF (1.00 on the knob in the 100 pF range) and G-SERIES is at 0.1 μS (likewise 1.00 on the knob) the time

constant is (10pF)/(0.1μS) = 100μs.

The use of the R_s compensation circuitry can be summarized as follows. When you set the capacitance transient-cancellation controls (C-SLOW, G-SERIES, C-FAST, τ-FAST) to minimize the size of the transients when voltage pulses are applied, you have also properly set them for series-resistance compensation. Then to turn on the compensation, you turn up the %-COMP control to the desired value. Any misadjustment of the transient-cancellation controls will be apparent and can be touched up, since uncancelled transients are magnified by the R_s compensation. The procedure will be described in more detail in Chapter VII.

IV. Setting up the EPC-7

A. Power

A switch on the rear panel of the controller selects between 110 and 220 volt operation. **Make sure that the switch is in the proper position**, and that the correct fuse is installed, before applying power. Irreversible damage to the circuitry may otherwise occur.

B. Static electricity

The input circuitry of the probe can be damaged by static electricity. To avoid this, please observe the following rules:

1. Avoid touching the input terminal unnecessarily.
2. When it is necessary to touch the input (e.g. while inserting a pipette into the holder), ground yourself first by touching a grounded metal surface.

C. Initial checkout

The TEST mode allows one to easily check the basic current measuring circuitry of the EPC-7. Connect the Probe cable to the Controller (this should be done with the power off); then set the MODE switch to TEST, connect a function generator or stimulator to STIM IN, and connect the right-hand CURRENT MONITOR output to an oscilloscope. With the GAIN set to 10 mV/pA, any signal applied to the STIM IN connector should be reproduced with the same amplitude at the CURRENT MONITOR output. An applied signal amplitude of about 1 volt is appropriate. Please note the following:

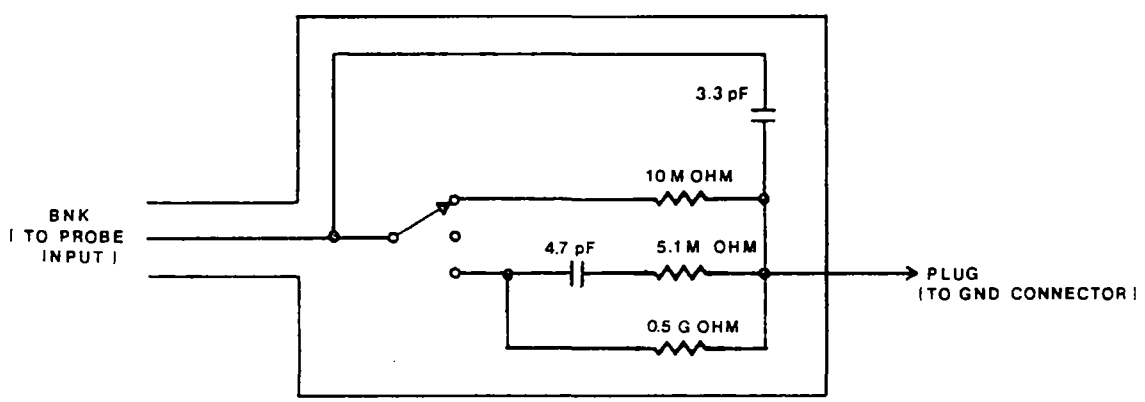
1. The probe input usually needs to be shielded from low-frequency noise. A simple way to do this is to cover the

input connector with a cap made from aluminium foil.

2. The TEST mode current-injection system is intrinsically AC-coupled, which means that long (>30 ms) pulses may be distorted in shape. Also, large low-frequency inputs can cause the current-injection circuitry to saturate.

The action of the internal filters on the background noise level and the temporal response can be observed by switching in FILTER 1 and FILTER 2. An improved signal-to-noise ratio should be apparent when the GAIN is increased to 50 or greater (which selects the 50 GΩ measuring resistor). If the signal source is a sine-wave generator, the frequency response of the patch clamp can be verified directly.

Further tests can be performed with the MC-7 Model Circuit, which is diagrammed below.



MC-7 MODEL CIRCUIT

The model circuit connects to the probe input via a double-female BNC

adapter and the plug goes to the GND connector.

In the 10 MΩ setting, the model circuit simulates a pipette that is open to the bath solution. In the SEARCH mode the action of the auto-null integrator can be verified and small-amplitude stimulus pulses should elicit appropriate current responses.

The center switch position leaves only a capacitance of about 3 pF connected. This simulates a gigaseal, and the C-FAST controls can be used to cancel the residual capacitive spikes resulting from stimulus pulses. (Note: Because of poor dielectric properties in the internal switch, the model circuit introduces excess random noise above the level that can be obtained with a gigaseal.)

In the 0.5 GΩ position a "model cell" is switched in. In the VC mode this can be used to verify the C-SLOW controls and the action of series resistance compensation with C-SLOW enabled (SLOW RANGE set to 100) and disabled (SLOW RANGE set OFF). In the CC+COMM mode, the current clamp action can be demonstrated. Note that this model cell has a long "membrane" time constant (about 25 ms).

V. The patch-clamp setup

A. Mounting the Probe

For low-noise recording the pipette holder must be attached directly to the EPC-7 probe. (Although the Probe amplifier can tolerate the additional capacitance of a short connecting cable without instability or oscillations, we find that the dielectric and electrostrictive properties of coaxial cables introduce excessive noise.) In typical setups, the Probe is therefore mounted directly onto a 3-axis micromanipulator. The EPC-7 probes are supplied with a plastic mounting plate for mounting on a flat surface. Holes can be drilled through the protruding surfaces for attachment to a matching plate or other surface.

Because of the extreme sensitivity of the EPC-7, special care must be taken in grounding all surfaces that will be near the probe input to minimize line-frequency interference. Even one millivolt of AC on a nearby surface, as can easily arise from a ground loop, can result in significant 50 or 60 Hz noise. A high-quality ground is available at the GND terminal of the probe, which is internally connected through the probe's cable directly to the SIGNAL GND in the controller. The GND terminal on the Probe is best used for the bath electrode and perhaps for grounding nearby objects such as the microscope. It is a good idea to run a separate ground wire from the SIGNAL GND jack on the controller to ground large objects such as the table, Faraday cage, etc. It is best to have this wire run parallel to the Probe's cable to avoid magnetic pickup and ground loop effects.

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. In a well-grounded setup the microscope can provide most of the shielding.

1. Make sure there is electrical continuity among the various parts of the microscope, especially between the microscope frame and the stage and condenser, which are usually the large 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.

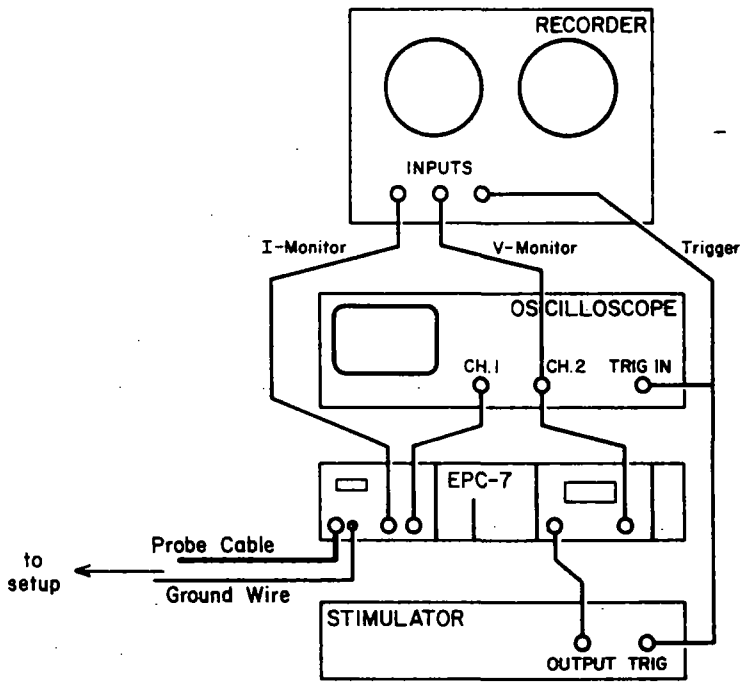
C. External shielding.

Especially when an unshielded pipette holder is used (see section E below) some electrostatic shielding of the experimental setup is necessary to avoid line-frequency pickup from lights and power lines in the room. Most experimenters use a table-top Faraday cage with a closable front, and lead all of the cables (e.g. from the microscope lamp, Probe, cooling system, ground lines) through a hole in the cage to an equipment rack mounted outside. If the pipette holder is somewhat exposed, or if the Faraday cage has an open front, a small grounded screen placed near the pipette holder may help.

D. Connections to other instruments.

Fig. 5 shows the connections in a typical recording setup. Voltage pulses for testing the electrode resistance

CONNECTIONS FOR A TYPICAL RECORDING SETUP



and for stimuli are provided by a stimulator. [Actually, if voltage pulses are not required for stimulating the membranes under study, a full-blown stimulator is not required, and a very simple square-wave or pulse generator is sufficient for monitoring the pipette resistance. On the other hand, the stimulus source for applying pulses in single-channel recordings should have a low noise level--see Chapter VIII.] The monitor signals from the EPC-7 are applied to an oscilloscope for observation during the experiment, and to a data recorder, which is typically either an FM tape recorder or a PCM adapter-video recorder system. Both the stimulator and data recorder

functions can also be fulfilled by a computer with suitable analog input and output interfaces.

Filtering beyond that provided in the EPC-7 is sometimes needed in observing channel activity during an experiment. For this reason many users insert a variable filter (typically a 4 or 8-pole Bessel filter) between the CURRENT MONITOR output and the oscilloscope. Also, it is often convenient to observe the voltage and current monitor signals at the recorder's outputs, rather than directly from the patch clamp. In most data recorders the input signals are passed through to the outputs, and observing the signals there can increase the experimenter's confidence that the data are being recorded correctly.

E. Pipette holder and electrode.

A shielded version of the pipette holder is available; this holder and a properly grounded microscope can provide sufficient shielding from 50-60 Hz interference even without the use of a Faraday cage. The shielded holder however introduces much more random noise than the unshielded one. This random noise arises from the nonideal dielectric properties of the plastic in the holder and from thermal voltage fluctuations in films of aqueous solutions, and the metal shield allows more of this noise to be coupled capacitively into the amplifier input. For single-channel recordings the unshielded holder is strongly recommended. The difference in background noise level between the two holder types is roughly a factor of two.

The unshielded holder is made from a plastic having low dielectric loss. If you make your own holder, you should give some thought to the choice of materials. The insulating parts of the

holder should be of a low-loss material, and should have a hydrophobic surface to prevent the formation of conducting water films. Teflon^{R} fulfills these criteria the best of any material we have tried. You can test the noise level of a holder by mounting it (with the electrode wire installed, but dry) on the Probe input, and measuring the noise using the NOISE TEST facility. The Probe should be in a shielded enclosure, so that no line-frequency pickup is visible on an oscilloscope connected to the CURRENT MONITOR output at 3 kHz bandwidth. A good holder increases the rms noise only by about 25%, e.g. from 95 to 120 fA. Noise sources are discussed in more detail in Chapter VIII.

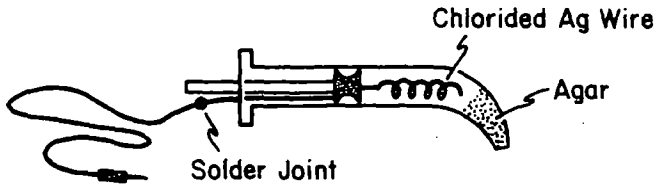
The pipette electrode is simply a thin silver wire that is soldered onto the pin that plugs into the probe's BNC connector. The chloride coating on the wire is scratched in exchanging pipettes, but we find that this does not degrade the stability very much; the wire does need to be re-chlorided occasionally, perhaps once per month. A wire for the standard electrode holder should be about 4.5 cm long; after it is chlorided an O-ring is slipped onto it and it is inserted into the holder. Chloriding can be done by passing current (e.g. 1 mA) between the wire and another silver or platinum wire in a Cl-containing solution (e.g. 100 mM KCl, or physiological saline). Current is passed in the direction to attract Cl^- ions to the electrode wire, and results in the formation of a gray coating.

F. Bath electrode.

The main requirements for a bath electrode are that it have a stable electrode potential and that it does not disturb the composition of the bathing solution. A bare, chlorided silver wire makes a good bath electrode except that the Ag^+ ions are tolerated only by some types of

cells, such as muscle cells. A good alternative is an electrode incorporating an agar salt bridge, such as illustrated in Fig. 6.

A SIMPLE BATH ELECTRODE



The body of the electrode is a 1 ml plastic syringe body that has been heated and pulled to form a small, bent tip. The electrode proper is a chlorided Ag wire that is first inserted with the plunger into the fluid-filled body; then hot agar is sucked into the tip by withdrawing the plunger partially. The filling solution (which might as well be the solution that is used to make the agar) can be a typical bath solution or something similar, such as 150 mM NaCl. More concentrated salt solutions are not necessary, and they can leak out, changing the composition of the bath solution.

4% Agar w/v

VI. Patch Pipette Lore

Procedures for fabricating pipettes are presented in some detail in the paper by Hamill *et al.* and in the chapter by Corey and Stevens. We will summarize the procedure and present some tips that we have found helpful. The main steps in pipette fabrication are to form a smooth tip on the pipette (to allow seals to be formed without damaging the cell membrane) and to coat the pipette with a suitable insulating coating to reduce the background noise.

A. Glass capillaries.

Pipettes can be made from many different types of glass. Our impression is that different types of glass work better on different cell types. Glass capillaries are available from soft (soda glass, flint glass, ~30% Na_2O) or hard glasses (borosilicate, aluminosilicate). Soft glass pipettes have a lower melting point (800°C *vs.* 1200°C), are easily polished, and can be pulled to have a resistance of 1-2 megohms. They are best for whole-cell recording where series resistance rather than noise is the limiting criterion. Hard glass pipettes often have a narrow shank after pulling and consequently a higher resistance. Hard glasses tend to have have better noise properties, however: the important parameter here is the dielectric loss parameter, which describes the AC conductivity of the glass. [Although the DC conductivity of most glasses is very low, soft glasses especially have a conductivity around 1 kHz that is sufficiently high to become the major source of thermal noise in a patch clamp recording.] Borosilicate and, especially, aluminosilicate glasses (Rae and Levis, 1984) have low dielectric loss and are desirable for the lowest-noise recordings. They do not necessarily form the best seals, and this might be due to evaporation of metal

onto the glass surface during the high-temperature pulling and polishing steps.

Some sources of glass pipettes

Soft glass	OD, mm
Blu-Tip® hematocrit tubing	1.3
Cee-Bee® capillaries, Bardran Lab. Supplies, Denmark	1.3
Drummond Microcaps®, Drummond Scientific, Bloomall, PA	1.4

Hard glass	OD, mm
Boralec® 100 λ Rochester Scientific	1.7
Pyrex®, Jencons Scientific Catalog #H 10/15	1.8
Corning® Sealing Glass #7052	1.6
Aluminum Glass GCASS 150-4 A-M Systems, Everett, Wa.	1.5

B. Pulling.

Pipettes are pulled in two stages: the first to thin the glass to 200-400 μm at the narrowest point over a 7-10 mm region, and the second to pull the two halves apart, leaving clean, symmetrical breaks. Both halves can be used. The length of the first pull and the heat of the second pull are the main determinants of the tip diameter of the final pipette.

A number of commercial pullers can be used to make pipettes. For reproducibility, however, a regulated current supply to the heater coil is best. A mechanical stop to set the length of the first pull is also important for reproducibility.

C. Coating.

The capacitance between the pipette interior and the bath, and also the noise from dielectric loss in the glass, can be reduced by coating the pipette with an insulating agent such as Sylgard^{R} (Sylgard 184 silicone elastomer kit, Dow Corning Corp., Midland, MI 48640) or Q-dope (G-C Electronics, Rockford, IL 61101). Sylgard^{R} is much superior in noise reduction. It is pre-cured by mixing the resin and catalyst oil and allowing to sit at room temperature for several hours (or in an oven at 50°C for 20 min) until it begins to thicken. It can then be stored at -10°C for many weeks until use. The Sylgard is applied around the lower few mm of the electrode to within ≈10-20μm of the tip and then rapidly cured by a hot-air jet or coil. Coating should be done before the final heat-polishing of the pipette, so that the heat can evaporate or burn off any residue left from the coating process.

D. Heat polishing.

Heat polishing is used to smooth the edges of the pipette tip and remove any contaminants left on the tip from coating. It is done in a microforge or similar setup in which the pipette tip can be observed at a magnification of 400-800x. The heat source is typically a platinum or platinum-iridium wire; to avoid metal evaporation onto the pipette, the filament is coated with glass at the point where the pipette will approach it. To produce a steep temperature gradient near the filament (which helps make the pipette tip sharply

convergent) an airstream can be directed at the filament. The amount of current to pass through the filament must be determined empirically for each type of glass, but a good place to start is with the filament barely glowing. Typical practice is to turn on the filament current and move the filament toward the pipette (which, being stationary, should remain in focus). Since the opening in the pipette tip is typically at the limit of resolution of viewing, you might not see the change in shape at the tip, but instead only a darkening of the tip. You can tell whether you have melted the tip closed, and also get an idea of the tip diameter, by blowing air bubbles in methanol with air pressure supplied by a small syringe.

E. Use of Pipettes.

Pipettes should be used within 2-3 hours after fabrication even if stored in a covered container; small dust particles from the air stick readily to the glass and can prevent sealing. Pipettes can be filled by sucking up a small amount of solution through the tip and then back filling. It is very important to filter the filling solutions (e.g. using a 0.2 μm syringe filter). The pipette should only be partially filled, just far enough to make reasonable contact with the electrode wire (The pipette holder is not filled with solution, but is left dry). Overfilling the pipette has disastrous consequences for background noise because the solution can spill into the holder, wetting its internal surfaces with films that introduce thermal noise. Bubbles left in the pipette from filling can be removed by tapping the side of the pipette.

For low-noise recording, the electrode holder should be cleaned before each experiment with a methanol flush followed by drying with a nitrogen jet. Before you insert a pipette into the holder, it is a

good idea to touch a hand to a metal surface of the setup to discharge any static electricity that you may have picked up. Be sure to tighten the holder firmly enough that the pipette does not move (on a scale of 1 μm) when you give suction. Then, when you change pipettes during an experiment, check the noise level of the empty holder using the NOISE TEST function; if it increases, solution has probably spilled inside the holder, which should be cleaned again and dried thoroughly.

VII. 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.

A. Forming a seal

1. *Initial setup.* The object is to apply voltage pulses to the pipette and observe the CURRENT MONITOR signal on an oscilloscope to monitor the pipette resistance. The approach to the cell membrane and the formation of a gigaseal cause the resistance to increase, reducing the currents. A convenient pulse amplitude is 1 mV, which can be obtained by applying 100 mV pulses, say 10 ms in duration at a rate of maybe 5 Hz, to the STIM IN connection and setting the STIM SCALING to 0.01. The 1 mV pulses will cause 1 nA to flow in a 1 MΩ pipette, or 0.5 nA in a 2 MΩ pipette, and so on. For observation of the current pulses it is convenient to pick a GAIN setting and oscilloscope sensitivity so that the current through the open pipette results in deflection of 2-3 divisions on the screen. For example, if you are recording with 1 MΩ pipettes (a good size for whole-cell work) you might want to set the GAIN to 1 mV/pA and the oscilloscope to 0.5 V/division, yielding a scaling of 0.5 nA per division on the scope. Set the MODE to SEARCH, and V-HOLD to give a reading of zero on the V_{COMM} display. Set FILTER 1 to 10 kHz and FILTER 2 to 3 kHz, %-COMP to *OFF*, *SLOW-RANGE* to *OFF*, T_r to 20 μs, *MONITOR* to V_{COMM}. Before the pipette is inserted into the bath, the current trace should be flat except for very small capacitive pulses due to the stray capacitance of the pipette and holder.

2. *Entering the bath.* The surface of the solution is relatively

"dirty", even if (as we strongly recommend) you aspirate some solution from the surface to suck off dust and contaminants. For this reason it is important to apply a small amount of positive pressure to the pipette before you move its tip into the bath, and also to avoid going through the air-water interface more than once before forming a seal. When you do move the pipette tip into the bath the current trace may go off scale; it will be brought back to zero in a few seconds by the SEARCH circuitry, and the feedback can be accelerated by pushing the RESET button. [Note: the speed of the SEARCH feedback depends inversely on the pipette resistance. It is nominally 1 second at 1 MΩ; for very low-resistance pipettes the feedback may act sufficiently quickly during the voltage pulse to distort the shape of the current pulse. This distortion is of no consequence in patch recording, however.] From the size of the current response to the test pulses, the pipette resistance can be calculated. If there should be 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.

There is invariably a small offset potential between the pipette and bath electrodes. The V_p-ZERO control is designed to provide a bucking potential to cancel this offset. With the pipette in the bath, turn this knob to bring the displayed V_{COMM} to zero. (If a high-resistance pipette is in use, pushing the RESET button will speed the response to changes in the V_p-ZERO setting.)

3. *Forming a gigaseal.* When the pipette is pushed against a cell, the current pulses will become slightly smaller to reflect the increasing seal resistance; when the positive pressure is released, the resistance usually increases further. Some cell types require

more "push" from the pipette than others, but an increase in resistance of 1.5 (i.e. a reduction in the current pulses by this factor) is typical. Application of gentle suction should increase the resistance further, and result (sometimes gradually, over maybe 30 sec; sometimes suddenly) in the formation of a gigaseal, which is characterized by the current trace becoming essentially flat again. To verify gigaseal formation, increase the GAIN to perhaps 50 mV/pA; the trace should still appear essentially flat except for capacitive spikes at the times of the start and end of the voltage pulse. You can use the C-FAST and τ-FAST controls to minimize the size of these spikes; this will be essential if you will be giving voltage pulses in your experiment, but it is a good practice anyway because it will reduce any current noise resulting from noise appearing at the STIM INput. Turn the C-FAST control first to reduce the size of the pulses, and then the τ-FAST control to reduce the residual biphasic component.

B. Cell-attached recording.

1. *VC mode.* Switch the MODE to VC (voltage clamp). The pipette voltage will now become the level originally set by the V-HOLD potentiometer. (It is a good idea to start out with it set to zero, as we specified above; an alternative is to start with it set to the holding potential you desire, e.g. -70 mV.) A note of caution: because the SEARCH and VC modes are functionally very similar, it is easy to forget to switch to VC mode. Please be sure to do this, however, since in SEARCH mode the pipette potential will drift slowly with time. This drift shows up on the V_{COMM} display.

2. *Transient cancellation.* If no voltage jumps are required, turn the stimulus off to avoid introducing artifacts. If voltage jumps are

to be applied, switch the GAIN and FILTER controls to the values you will be using, but leave the STIM SCALING at a low value (e.g. 0.01) to make a coarse adjustment of the C-FAST and τ-FAST controls before switching to the full-sized stimulus. This will allow you to see the transients that otherwise might be off-scale on the oscilloscope screen. Then switch STIM SCALING to the final value you will be using and repeat the adjustments. Usually there is a slower (100 μs to 1 ms time constant) transient component present that cannot be cancelled with the C-FAST controls. To reduce its size, Set SLOW RANGE to C_p and adjust C-SLOW and G-SERIES for best cancellation. It is often easiest to adjust G series first by zeroing the initial value, and then adjusting C-slow to flatten the remainder of the component. The slow component typically arises from dielectric relaxation in the pipette glass, or from aqueous films on the surface of uncoated pipettes. The component usually does not have a single-exponential decay, so perfect cancellation cannot be expected. The use of glasses and coating techniques that reduce the size of the slow component also generally result in lower noise levels in the recording.

3. *Recording.* Be sure to use GAIN settings of 50 mV/pA or above for lower noise. Keep the FILTER 1 switch set at 10 kHz unless you actually will need the full 60 kHz bandwidth for some reason; otherwise you might drive the CURRENT MONITOR output or your recorder's input amplifiers into saturation with the very large amount of high-frequency noise. Should you use the full bandwidth, you should avoid GAIN settings above 100 mV/pA for the same reason.

If you are applying voltage pulses to the patch membrane, you probably will want to use a computer to subtract control traces from the traces containing the channels of interest to remove the capacitive transients. Nevertheless, it is important to try to cancel

the capacitive transients as well as you can to avoid saturating any amplifiers or the recording medium or A to D converter. It is a good idea to set the C-FAST and τ-FAST controls while you observe the CURRENT MONITOR signal without any filtering beyond the internal 10 kHz filter. Then during the recording, watch to see if the CLIPPING light blinks. When it does, it means that internal amplifiers in the EPC-7 are about to saturate, and/or that the CURRENT MONITOR output voltage is going above 13 V peak, on the peaks of the transients, and you should readjust the transient cancellation controls. Otherwise it is likely that the recording will be nonlinear, so that subtraction will not work correctly.

The fast transient cancellation is not sufficient to cancel all of the capacitive transients in a patch recording. This is partly because the pipette capacitance is distributed along the length of the pipette; therefore each element of capacitance has a different amount of resistance in series with it, so that a single value of τ-FAST will not provide perfect cancellation. The time course of the transients also reflects dielectric relaxation in the plastic of the pipette holder and in the pipette glass. These relaxations are not simple exponentials, but occur on a time scale of about 1 ms. If you use pipette glass with low dielectric loss (e.g. aluminosilicate glass) or if you are careful to coat the pipette with a thick coating and near to the tip, the relaxations will be smaller. You can cancel part of these slow relaxations by using the C-SLOW controls, with the SLOW RANGE switch set to C_p. The C_p setting selects a small amount of transient cancellation suitable for the pipette glass relaxations, with G-SERIES setting the initial amplitude of the cancellation current, and C-SLOW setting the total area of the exponential transient.

Note that 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 Positive pulses applied to the 10X STIM INPUT will result in hyperpolarizations when the STIM SCALING is set to a positive value. The table below shows the polarities for all four of the common recording configurations.

Recording configuration	+ Stimulus results in	Outward membrane current results in
Cell-attached patch	hyperpolarization	- at CURRENT MONITOR
Inside-out patch	hyperpolarization	- at CURRENT MONITOR
Outside-out patch	depolarization	+ at CURRENT MONITOR
Whole-cell recording	depolarization	+ at CURRENT MONITOR

C. Whole-cell recording.

1. *Breaking the patch membrane*. After a gigaseal 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 cell's input resistance, a shift in the current level. Additional suction sometimes lowers the access resistance, causing the capacitive transients to become larger in amplitude but shorter. As was discussed in Chapter III, low values of the access (series) resistance are desirable, and when R_s compensation is in use, it is important that the resistance be stable as well. A high level of Ca^{2+} buffering

capacity in the pipette solution (e.g. with 10 mM EGTA) helps prevent spontaneous increases in the access resistance due to partial resealing of the patch membrane.

2. *Transient cancellation.* If the fast capacitance cancellation was adjusted as described above before breaking the patch, then all of the additional capacitance transient will be due to the cell capacitance. Cancelling this transient using the C-SLOW and G-SERIES controls will then give estimates of the membrane capacitance and the series conductance (reciprocal of series resistance). For adjusting these controls it is a good idea to observe the transients at high time resolution, perhaps with 10 kHz filtering. This will allow you to observe the effect of the G-SERIES control, which sets the initial amplitude of the transient, as opposed to the C-SLOW control, which sets the total area. Select the appropriate SLOW RANGE (10 pF for small cells up to about 10 μm diameter, 100 pF for larger cells) and start with C-SLOW set at a nonzero value. If the transient is not too rapid, you will be able to see the initial value of the transient change as you adjust G-SERIES; bring it to zero, leaving no initial step in the transient (see Fig. 7, below), and then adjust C-SLOW to reduce the overall size of the transient. After an iteration or two it should be possible to reduce the transient to only a few percent of its original amplitude. However, if the cell has an unfavorable shape (for example a long cylindrical cell or one with long processes), the cell capacitance transient will not be a single exponential, and the cancellation will not be as complete.

3. *Series-resistance compensation.* As was discussed in Chapter III, series-resistance (R_s) compensation is important when the membrane capacitance is large or when the ionic currents are large enough to introduce voltage errors. To use R_s compensation you first adjust the

transient-cancellation controls (including C-FAST and τ-FAST if necessary) to provide the best cancellation. Then you turn up the %-COMPensation control to provide the desired degree of compensation. When compensation is in use the uncancelled transients become larger in amplitude, so it is best to touch-up the settings of C-FAST, τ-FAST, C-SLOW and G-SERIES with R_s compensation turned on. Note: the G-SERIES control determines (along with the %-COMP control) the amount of positive feedback being applied for compensation. It should be adjusted with some care, since too low a setting causes overcompensation (the EPC-7 will think that R_s is larger than it is) which can cause oscillation and possible damage to the cell under observation.

How you should set the R_s compensation controls depends on the approximate value of the uncompensated membrane-charging time constant τ_u, which you can calculate as the ratio of the C-SLOW and G-SERIES settings. [For example, suppose C-SLOW is 20 pF and G-SERIES is 0.1 μS; τ_u is then (20pF)/(0.1μS) = 200 μs.] If τ_u is smaller than about 500 μs, you should use the FAST setting of the R_s-compensation switch to provide the necessary rapid compensation; otherwise, the SLOW setting will provide compensation that is less prone to oscillate from misadjustment of the controls. How much compensation you can apply is also determined by τ_u. If τ_u is larger than about 100 μs, you can use any degree up to the maximum of 90% compensation without serious overshoot or ringing in the voltage-clamp response. For smaller values of τ_u the %-COMP. setting should be kept below the point where ringing appears in the current trace, or below the values given in Chapter III.

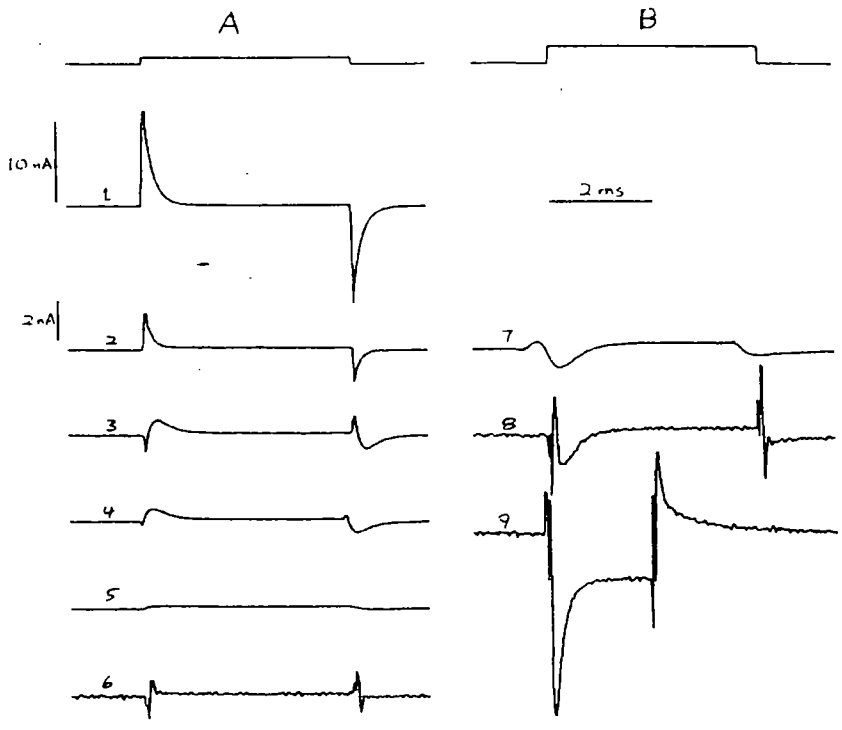
4. *Voltage monitor, filtering, polarities.* The V_P/V_{COMM} switch allows you to monitor the command voltage (V_{COMM}; this is normally

what one observes) or the command after it has been modified by the R_s-compensation circuitry (V'P). The latter is helpful mainly to advanced users who wish to see how much correction is being made by the R_s circuitry to get an estimate of the size of possible errors. In the absence of R_s-compensation this switch has no effect but is best left in the V_p position.

As in the case of patch recording, there is rarely need to use the FULL bandwidth setting of the FILTER 1 switch in whole-cell recording. This is because typical membrane charging time constants (even after R_s-compensation) are considerably longer than 16 μs, which is the time constant corresponding to a 10 kHz bandwidth. Thus the current monitor signal is expected to contain no useful information beyond this bandwidth.

In whole-cell recording the voltage and current monitor signals follow the usual convention, with outward currents being positive. This is because the pipette has electrical access to the cell interior.

5. *An example of whole-cell recording.* Figure 7 shows traces from a whole-cell voltage-clamp experiment on a relatively large N1E-115 Neuroblastoma cell, having a membrane capacitance of 70 pF. The pipette solution contained Cs^+, which blocked most of the cell's K currents, so that mainly Na currents were recorded. In part *A* of the figure the various current traces were elicited by 20 mV depolarizing pulses of 4 ms duration (indicated by the top trace) as the slow transient cancellation and R_s controls were adjusted. The pulses were selected to be too small to elicit active membrane currents, so that the membrane capacitive and leak currents could be observed. *Trace 1*



CLAMP OF NEUROBLASTOMA CELL

shows the uncompensated capacitive currents at a low vertical scaling. The membrane charging time constant* quite slow, about 200 μs. In *trace 2* the SLOW RANGE switch has been set to 100 pF, and the currents have been partly compensated, but both G-SERIES and C-SLOW are set too low. In *trace 3* G-SERIES has been turned up too high, causing an initial overshoot. *Trace 4* shows the correct initial adjustment of G-SERIES, where no initial "jump" in the trace is visible. Proper adjustment of C-SLOW, as shown in *Trace 5*, then leaves only a small pulse of current with rounded rising and falling edges. This current is the residual that flows through the membrane leakage conductance as

* = is

the cell gradually (over 200 μs) charges and discharges to the pipette potential. Finally, *trace 6* shows the result of turning up the %-COMP control of the R_s compensation circuitry (the speed has been set to FAST): the trace becomes noisier, and the transient artifacts become larger in amplitude but shorter in time. At this stage a slight readjustment of G-SERIES and C-SLOW would reduce the size of the artifacts.

Part *B* of Figure 7 shows current traces elicited by larger depolarizations that elicited Na currents. *Trace 7* shows a distorted Na current recorded under the same conditions as Trace 5, that is with proper transient cancellation but no R_s compensation. The current was elicited with a 50 mV depolarizing pulse. *Trace 8* shows the change in this recording when R_s compensation was turned on. *Trace 9* was obtained later in the experiment with a larger (70 mV) depolarization and after more careful adjustment of G-SERIES, C-SLOW and C-FAST. The residual capacitive artifacts in these traces can be eliminated by subtracting suitably scaled control records.

D. Current-Clamp recording.

To switch from Voltage Clamp to Current Clamp recording, just turn the MODE switch to CC. This provides a recording of the cell membrane potential, which can be monitored at the V-MONITOR output and appears on the V_{COMM} display.

When you switch to the CC mode the following things happen inside the EPC-7 Controller: it forces the current monitor circuitry into the the low gain range (regardless of the GAIN setting); it sets the 10 kHz Bessel characteristic for FILTER 1; it disables R_s compensation; and it disables both fast and slow capacitance

cancellation. For the user these changes are of little consequence, and are mainly designed to make CC recording simple and reliable. There are two fine points to consider, however. (1) The GAIN switch will not show the correct scaling for the CURRENT MONITOR output unless you have set it somewhere in the low range. [The CURRENT MONITOR output is of academic interest anyway in the CC mode, but in the CC+COMM mode provides a nice way to monitor the injected current.] (2) As we said, the capacitance cancellation is disabled. This was intended to make the current-clamp circuitry unconditionally stable so that the user would not have to worry about oscillation due to excessive negative capacitance. The disadvantage is that the Probe input presents an input capacitance of ~1 pF, which, along with pipette and holder capacitance, results in a total of about 3 pF presented to the pipette. Recordings with very high resistance pipettes therefore will suffer in their time resolution, but pipettes of ~10 MΩ or lower resistance will yield time constants below 30 μs, which is the intrinsic speed of the current-clamp amplifier itself. Further, when you inject current using the CC+COMM mode the current will cause a small voltage to appear across the access resistance (i.e. R_s), since no R_s-compensation is in effect. Since patch pipettes give such low resistances compared to conventional microelectrodes, this voltage is so small (it is 1 mV for a 10 MΩ access resistace and 100 pA of current) that in designing the EPC-7 we assumed that simplicity of use would be more important than providing the functions of a "bridge" circuit or capacitance neutralization.

While you are in the CC mode you can do whatever you want to the setting of the V-HOLD control or to the STIMulus INput since these are functionally disconnected. For example, you can change the V-HOLD control to its mid-scale setting (5.00) so that when you switch to

CC+COMM no holding current will be passed. Once you have switched to CC+COMM you can then use V-HOLD to set a holding current (±200 mV full scale) and you can apply stimulus pulses via STIM IN. The rule for the scaling of current stimuli in the CC+COMM mode is easy to remember:

Any combination of applied stimulus and STIM SCALING settings that would provide 1 mV of stimulus in the VC mode actually provides 1 pA of stimulus in the CC+COMM mode.

VIII. Techniques for low-noise recording

A. Patch recording technique

The EPC-7 amplifier has a particularly low background noise level. The noise level is in fact low enough that in many experimental situations it can be neglected, in view of other background noise sources that make larger contributions to the total. As we consider these other sources, first let us make it clear that in this section we are concerned with *random* noise which is fundamentally due to the thermal motion of electrons and ions; we assume that any user who is interested in low-noise recording has shielded and grounded his setup sufficiently well to take care of any *synchronous* noise due to line-frequency pickup, computer power supplies, TV cameras, etc. Synchronous noise can be readily identified as stationary features on an oscilloscope trace when the oscilloscope is triggered by the appropriate signal source, for example line-frequency triggering. Grounding and shielding is discussed in Chapter V.

The NOISE TEST feature of the EPC-7 makes it easy for the user to identify important noise sources. When the NOISE TEST switch is pressed either upward or downward, the LCD display shows the rms noise voltage present in the CURRENT MONITOR signal in the frequency band of 300 Hz to 3 kHz. Each unit on the display corresponds to 0.1 mV rms; for noise measurements the standard setting of the GAIN control is 100 mV/pA, which yields a scale of 1 fA rms per unit on the display. The 300 Hz lower limit of the frequency band is designed to reject most (though not all) 50-60 Hz noise to make the measurement of random noise simpler. With the Probe placed in a shielded enclosure and with nothing connected to the input, the NOISE TEST reading is usually 90-100 fA. If you get a reading higher than this, first check that

the G-SERIES control is not set at its minimum value, but at a reading of about 1.00 or higher (one turn clockwise or more). Even with the slow transient cancellation turned off, the circuitry can inject a small amount of noise into the input. Secondly, try varying the C-FAST control. If you have a noisy stimulus source connected to STIM IN, the induced current noise will vary with C-FAST, with a minimum occurring with the control set for 1-2 pF. If such a variation occurs, you might consider getting a quieter stimulator.

Starting from the intrinsic noise reading of 90-100 fA one observes increments in the noise level when the holder and pipette are installed and when an actual recording is made. By analyzing these increments you can see where there is the most room for improvement in your technique. Under the best conditions (i.e. with a clean holder, an aluminosilicate pipette, etc.) we have observed the noise reading increase to about 120 fA when the holder and pipette are present, and 160 fA when the pipette tip is in the bath, sealed on a cell. These are rms current values, which means that they are equal to the standard deviation of the fluctuating current. Since the noise sources in the patch-clamp amplifier, pipette holder, pipette and patch membrane are statistically independent, their contributions to the total noise do not add linearly; instead their variances (the squares of the standard deviations) add. This means that the rms reading on the EPC-7's display represents the square root of the sum of the squares of the rms currents from each source. Taking this into account one can calculate the relative contributions from the amplifier, pipette holder, and the combination of pipette immersion and patch noise. The table below shows the relative contributions calculated in this way for the "optimum" situation just described.

Noise source	Contribution to variance	Effective rms current (fA)
Amplifier	35 %	95
Holder	21 %	73
Pipette and patch	44 %	106

The contributions to the variance from the three sources are seen to be comparable in size, and improvements in the amplifier noise level will not help very much unless corresponding improvements are made in the other noise sources. As it is, rms noise values as low as those quoted here are obtained only with considerable care. Some of the important considerations are outlined below.

1. *Pipette holder.* As we mentioned in Chapter V, the unshielded (all-plastic) holder is greatly superior to the shielded one for low background noise. For low noise the holder must be made from a low-loss, hydrophobic plastic; TFE is one of the best, and plexiglas one of the worst materials. [For our purposes, low-loss materials are those that show little dielectric relaxation in the frequency range of a few kHz. Dielectric relaxation involves the reorientation of dipoles within the material; since any dipoles will be in thermal motion, thermal reorientations in this frequency range will result in current fluctuations coupled capacitively into the pipette.]

It is very important that the pipette holder be kept clean and dry. Noise can be coupled into the pipette from the thermal motion of ions in films of aqueous solution, especially on the inside of the pipette. A good practice for low-noise work is to connect a valve to the pipette-suction line, and arrange for dry air or N_2 to flow into the suction line during the time while you change pipettes. This will dry out any such aqueous films and keep the noise level low.

2. *Pipettes*. Films of aqueous solutions and dielectric relaxation are also serious problems with pipette glass. Coating with Sylgard^{R} helps because it is hydrophobic and because it has good dielectric properties. Also, its thickness helps to reduce the capacitance between the pipette interior and the bath. This is mainly important because it reduces the coupling of the glass's dielectric noise into the pipette interior. Clearly, making thicker coatings (especially in the tip region) and coating closer to the tip will reduce the pipette noise. The best glass type we know of is aluminosilicate; this glass requires fairly high temperatures in pulling, and does not necessarily give the best gigaseals; but its dielectric relaxation appears to be about a order of magnitude smaller than soft glass.

Some improvement is probably to be gained by taking steps to prevent formation of aqueous films on the back end of the pipette. It is a good idea to wipe the outside of the pipette to remove any spilled solution, fingerprints, etc. before inserting it into the holder. It might also help to treat the inside of the pipette to prevent the formation of a film, for example by shooting some dimethyldichlorosilane vapor (Caution: nasty stuff!) into the back of the pipette before or after filling it.

3. *Recording practice*. Lower noise is obtained by immersing the pipette a shorter distance into the bath: this reduces the coupling of noise currents arising in the pipette glass. A significant amount of noise seems to arise in the sealed membrane itself and is probably lower in higher-resistance seals. This noise is generally more than one would calculate from the resistance of the gigaseal.

4. *Filtering.* The usual goal of low-noise recording is better time resolution: if the noise level is lower, you can use a wider filter bandwidth to observe single-channel events of a given amplitude. Judicious use of filtering can improve the time resolution of your analysis. For example, if you are using the 50%-threshold-crossing analysis technique to analyze channel open and closed times (see for example Colquhoun and Sigworth, 1983), the best filter bandwidth is - the one that makes the rms background noise about 1/10 of the channel amplitude. Since one rarely wants to go through the process of choosing the optimum bandwidth during an experiment, the best procedure is to record the data at a wide bandwidth and perform any necessary filtering (analog or digital) later, during analysis of the data.

B. Noise in whole-cell recording

In typical voltage-clamp, whole-cell recordings the predominant noise source arises from the combination of the access resistance R_s and the cell membrane capacitance C. Above 1 kHz or so the current variance σ^2 from this source increases with this resistance and capacitance as

$$\sigma^2 \propto R_s C^2$$

so that it is clearly desirable to keep R_s as small as possible, and even more important to select small cells, if you are interested in low noise. See the chapter by Marty and Neher (1983) for a more complete description of this and other fine points of whole-cell recording.

IX. Internal adjustments

The internal adjustments of the EPC-7 can be divided into three groups. The first is the single CC-zero trimmer on the front panel. It is used to trim the probe input current in special cases where a small bias current (>1 pA) might affect the measurement of membrane potentials. The second group consists of ten trimmers located at the left rear of the main circuit board in the controller (see the location diagram on the next page). These trimmers set the gain, offset, and frequency response trims for the current monitor pathway, and are used to match the characteristics of the 500 MΩ and 50 GΩ current-measuring resistors. Adjustment of these trimmers is best left to the factory or authorized repair facilities; however, since the resistor characteristics may change with time, advanced users may want to touch up these trimmers, and for this reason they will be described here.

The remaining trimmers (some 22 of them) constitute the third group and should not need adjustment in normal use. They trim amplifier offsets, calibrate various functions and match the controller to the current-injection capacitors in the probe. Adjustment should be necessary only when components are replaced or when probes are exchanged. Since special fixtures are required, calibration is best performed at the factory.

A. CC-ZERO trimmer.

If the probe input is left open, any bias current will cause the pipette potential to drift when the MODE is switched from VC to CC. An 0.1 pA error causes a roughly 0.1 V/sec drift, since the input capacitance is about 1 pF. The V-MONITOR output gives the pipette

potential scaled up by 10, so this output would slew at 1 V/sec. Adjust the trimmer to give the slowest drift.

B. Current monitor adjustments.

Separate sets of trimmers are provided for adjusting the offset, gain and transient response of the current monitor circuitry in the low and high gain ranges. These trimmers can be reached by removing the rear panel of the Controller. The offsets can be adjusted simply by leaving the probe input open and adjusting for zero output at a CURRENT MONITOR connector in either the low rage (Set the GAIN to 20) or in the high range (set GAIN to 100). The gain and transient response of the current monitor circuitry can be checked using the TEST mode as mentioned in Chapter IV. The low-range gain can be set to provide the same size signal at the CURRENT MONITOR output (with GAIN set to 10) as that applied to the STIM INput. For lowest noise, leave the probe input open, but shielded if necessary. Likewise the high-range gain can be set for an amplification of 5.00 with the GAIN switch set to 50. The transient response trimmers are adjusted for the best response to a square wave input. About 50 Hz is a convenient frequency for initial adjustment; the flatness of the response should be checked carefully at FULL bandwidth in the low range to ensure that R_s compensation will work correctly.

Please note that calibrating the gain using the TEST mode depends on the accuracy of the oscilloscope and of the TEST circuitry, whose scaling is specified within 2%. At the factory, the gain is calibrated to within 1% by the method of injecting currents through a 10 MΩ resistor from a precision millivolt-signal source and measuring the gain at 100 Hz with a lock-in amplifier.

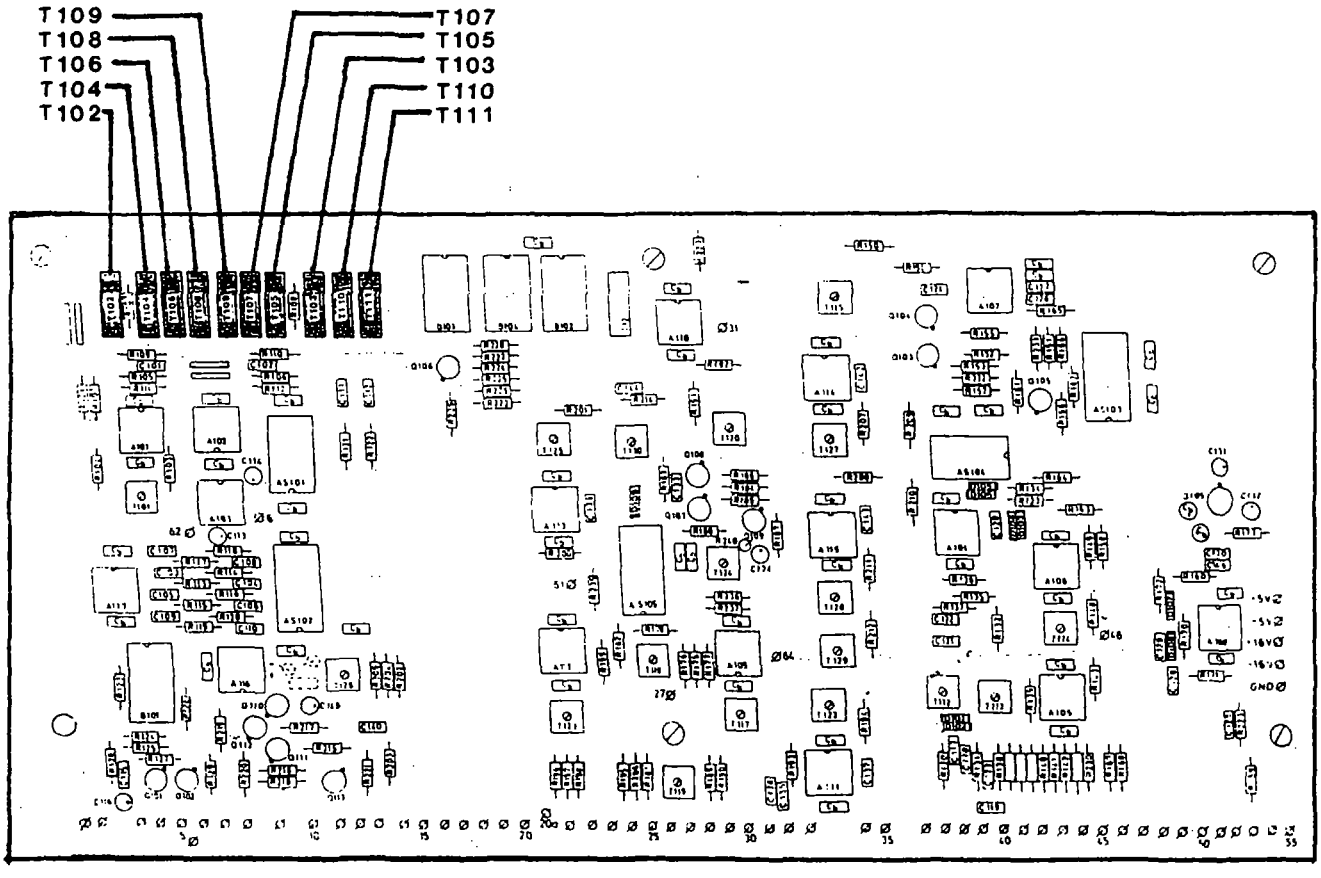


FIG. 8 EPC-7 TRIMMER LOCATION

IX. Internal adjustments
Current monitor adjustments

The trimmer locations are shown in Fig. 8. The functions of the trimmmers are:

T102 High range gain
T104 High range offset
T106 High range aux. τ
T108 High range aux. amplitude
T109 Low range aux. amplitude
T107 Low range aux. τ
T105 Low range offset
T103 Low range gain
T110 High range frequency comp.
T111 Low range frequency comp.

The frequency comp. trimmers are the main adjustments for compensating the transient response. Misadjustments result in under- or overshoots in the square-wave response having time constants of about 1 ms in the high range or about 100 μs in the low range. The aux. amplitude and aux. τ trimmers adjust an additional exponential component that can be used to correct for biphasic deviations in the square wave response. The amplitudes are zero when the wipers of T108 and T109 are to the rear (screws turned fully clockwise). Note: The aux. amplitude and aux τ trimmers interact strongly. It is wise to avoid making large changes in their settings.

X. Circuit Description

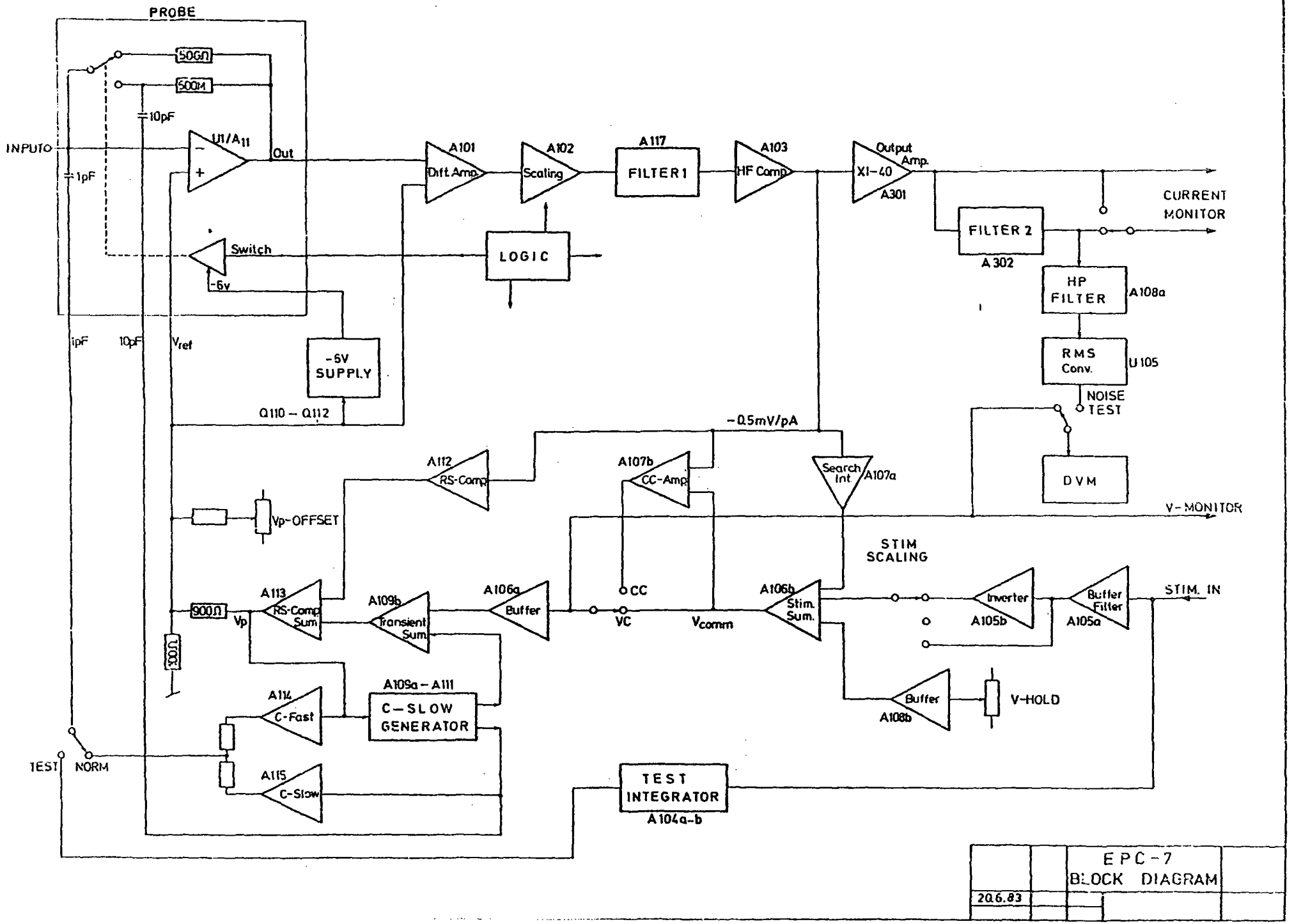
The EPC-7 circuitry (see Fig. 9) consists of two main signal pathways, one for pipette current measurement and the other for setting the pipette potential. These pathways and other functions are described in this section.

A. Current monitor pathway

The basic current measurement is performed in the probe by an operational amplifier connected as a current-to-voltage converter. It forces the input voltage to follow the reference voltage V_{ref}, and the resulting current flows through a measuring resistor of either 500 MΩ (low range) or 50 GΩ (high range). The resistors are selected by logic circuitry controlled by the GAIN switch. All critical input circuit components, including the resistors, input transistors, and switching circuitry are contained in a hybrid integrated circuit. The hybrid also contains two current-injection capacitors which are used for transient cancellation and test functions.

The output from the probe is applied to a unity gain differential amplifier (A101) which subtracts the reference voltage V_{ref} to yield a voltage proportional to input current with a nominal scaling of 0.5 or 50 mV/pA. In the next stage (A102) the gain and offset of the signal is trimmed, and a small transient response correction can be applied (the main correction is made later, in A103).

FILTER 1 is a two-pole, low-pass active filter stage. Normally it yields an overall 100 kHz or 10 kHz Bessel response for the recording system, as selected by the FILTER 1 switch. (A third pole for the 10 kHz response is provided in the output amplifier.) However, when R_s compensation is in use, its response is changed to an


PROBE
50GΩ
500M
10pF
INPUT0
1pF
U1/A11
Out
A101
Dift.Amp.
A102
Scaling
A117
FILTER1
A103
HF Comp.
Output Amp.
X1-40
A301
CURRENT MONITOR
FILTER 2
A 302
HP FILTER
A108a
RMS Conv.
U105
NOISE TEST
DVM
Switch
-6v
LOGIC
-6V SUPPLY
Q110 – Q112
Vref
-0.5mV/pA
A112
RS-Comp
A107b
CC-Amp
Search Int
A107a
V-MONITOR
Vp-OFFSET
STIM SCALING
A113
RS-Comp Sum
900Ω
Vp
A109b
Transient Sum
A106a
Buffer
CC
VC
Vcomm
A106b
Stim. Sum.
Inverter
A105b
Buffer Filter
A105a
STIM. IN
100Ω
A109a – A111
C-SLOW GENERATOR
A114
C-Fast
A115
C-Slow
Buffer
A108b
V-HOLD
TEST
NORM
TEST INTEGRATOR
A104a-b
EPC-7
BLOCK DIAGRAM
20.6.83

underdamped two-pole characteristic with natural frequencies of 30 kHz or 9 kHz, depending on whether FAST or SLOW compensation is selected.

The primary frequency response correction of the current signal is performed by A103. Trimmers can be adjusted to match the characteristic time constants of this stage to the time constants τ_1 of measuring resistors in the probe. Above the frequencies determined by these time constants (about 100 μs for the 500 MΩ resistor, 1 ms for the 50 GΩ resistor), the gain of this stage increases proportionally with frequency up to limits of 50 kHz (in the high range) and 140 kHz (low range). The correction stage follows the Filter 1 to avoid amplifier saturation on high-frequency signals when filtering is in use.

The signal at the output of A103 has a flat frequency response and a scaling of -0.5 mV/pA in the low gain ranges. This signal is used by the Search, Current Clamp, and R_s compensation circuits. It is also scaled by a factor between 1 and 40 by the output amplifier (A301) to provide the low range gains of 0.5 to 20 mV/pA available at the CURRENT MONITOR output connectors. At the 50 mV/pA GAIN setting the probe resistor and other circuitry is switched to the high range, and the output amplifier's gain returns to 1. As the GAIN setting is further increased the output amplifier's gain is increased to a maximum of 20.

A 3 kHz, 3-pole Bessel filter (FILTER 2) follows the Output Amplifier. This filter has two purposes. First, it provides additional filtering for one of the CURRENT MONITOR outputs. Second, it is part of the band-pass filtering of the signal for the Noise Test function. The remainder of the band-pass filter (a 3-pole, 300 Hz

Butterworth high-pass response) is provided by A108, whose output is applied to the RMS-to-DC converter, U105. When the Noise Test mode is selected, the output of the converter is applied to the LCD display unit, scaled such that 0.1 mV RMS corresponds to one unit on the display.

B. Stimulus pathway

The pipette command potential V_{ref} is formed by summing contributions from the stimulus input, the V-HOLD potentiometer, the search integrator (in SEARCH mode) and R_s compensation (when in use). These signals are processed at 10 times their final scaling, and the resulting V_p signal is divided by ten and an offset from the V_p-OFFSET control is added to form V_{ref}.

The stimulus input signal is first buffered and filtered by A105a. The filter has two identical single time constants of either 0.5 or 7 μs which, with the filtering of V_{ref} itself, yields risetimes (10%-90%) of about 2 and 20 μs depending on the setting of the T_r switch. This filter has two purposes. First, the rounding-off of step inputs avoids slew-limiting and the accompanying nonlinear behavior in the succeeding signal processing stages. Secondly, a rounded stimulus produces smaller-amplitude capacitance currents that are easier to compensate.

After variable scaling, the stimulus is added together with the V-HOLD voltage and the output of the search integrator in A106b. In the VC mode the stimulus is then fed directly to the buffer A106a, the output of which is called V_{COMM} and represents the commanded pipette voltage; V_{COMM} is normally displayed on the LCD display (after being scaled down by ten to match the scaling of V_{ref}). The final two

amplifier stages, A109a and A113, can be considered as two inverting, unity-gain stages. Their output is V_p, which differs from V_{COMM} only when R_s compensation is in use.

C. R_s compensation

Series resistance compensation for voltage clamp is performed in two stages in the EPC-7. The first stage is called *prediction*, in which the pipette potential is automatically changed in a way to rapidly charge the cell capacitance whenever a potential change is commanded. The second stage is *correction*. This is the conventional process in which the current monitor signal is appropriately scaled and added to the voltage command with the polarity of providing positive feedback.

The correction circuitry consists of the scaling amplifiers A112a and b, and the summing amplifier A113. The input is the -0.5 mV/pA current monitor signal. The gain of A112a-b is inversely proportional to the G-SERIES setting and proportional to %-COMP.

The prediction part of the compensation is provided by A109a and the C-SLOW generator. When the C-SLOW and G-SERIES controls are properly adjusted, the C-SLOW generator injects a current into the probe input to match the cell capacitance charging current. For proper R_s compensation a voltage equal to this current, times the series resistance value, must be added to the command voltage. In the EPC-7 a voltage is used from the C-SLOW generator which is proportional to the injected current. This voltage is scaled according to the reciprocal of the G-SERIES setting, and by another, tracking section of the %-COMP potentiometer.

D. Capacitive transient cancellation

The probe has two current-injection capacitors, both of which are contained in the hybrid circuit in the probe. One has a capacitance of 1 pF and is always connected to the input. It is used for both fast and slow transient cancellation, and for the TEST mode. The other 10 pF capacitor is switched in with the 500 MΩ measuring resistor and is therefore available only in the low gain range. It is used in the 100 pF C-SLOW range.

The C-fast circuitry imposes a scaled copy of the V_p signal on the 1 pF capacitor. The signal is filtered with a time constant (τ-FAST) that is variable over the range of about 0.2-1.2 μs to allow matching to the time constant of V_{ref} and short time constants in the pipette capacitance. It is scaled by the C-FAST potentiometer, amplified by A114, and applied to the injection capacitor through a resistive mixing network.

Slow capacitance transients to be cancelled are assumed to arise from a capacitance C_{slow} (typically the cell membrane capacitance) in series with a conductance G_s (i.e. the pipette access resistance). The current I_i induced in this combination by a step in voltage has an exponential relaxation with time constant C_s/G_s. To inject the proper cancelling current through a capacitor into the input, a voltage is required that is the integral of I_i. This injection voltage can be derived from the stimulus by filtering with the same time constant and scaling it proportionally to C_{slow}. The C-slow generator forms such a signal, along with a voltage proportional to the injected current for use by the R_s compensation-prediction operation.

The injection voltage is applied to the 1 pF capacitor through the scaling amplifier A115; the output is summed resistively with the C-FAST signal from A114. When the SLOW RANGE is set to 100 pF, the injection voltage is also applied to the 10 pF injection capacitor, and the time constant of the C-SLOW generator is changed appropriately.

E. Test circuit

In the TEST mode the STIM IN signal is disconnected from the normal stimulus pathway by an analog switch. The signal is applied to the TEST integrator, A104. The integrator time constant is 10 ms, so that a 1 V input signal yields a 100 V/sec output. The output is applied through a second analog switch to the 1 pF injection capacitor in the probe; 100 V/sec yields a current of 100 pA. The test integrator has a second-order feedback loop to prevent DC offsets from driving the integrator into saturation.

F. Search and current clamp

Both the search and current clamp circuits act to vary V_{COMM} to keep the current monitor signal (actually the -0.5 mV/pA signal from A103) at zero or at a commanded level. In the search integrator, the main time constant was chosen to give an overall feedback time constant of about 1 s for a 10 MΩ resistance at the probe input. The RESET button speeds up the search integrator by a factor of 100.

The current clamp amplifier A107b acts to keep the pipette current at zero with a response time constant of about 10 μs. A small offset current (±8 pA) can be added by the CC-ZERO trimmer on the front panel

to adjust the probe input current to zero. In the CC+COMM mode a voltage is added to the input circuit for commanding an injected current level. The output of A107b is attenuated (relative to V_{ref}) by a factor of 60, so that the voltage compliance at the probe input is about $\pm$200 mV. In the CC modes the logic circuitry forces the low gain range and the 10 kHz Bessel characteristic for FILTER 1. It also disables both fast and slow capacitance cancellation.

Information for:

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 capacitance transient cancellation. Current-clamp operation can also be checked using the 10 gigaohm switch setting.

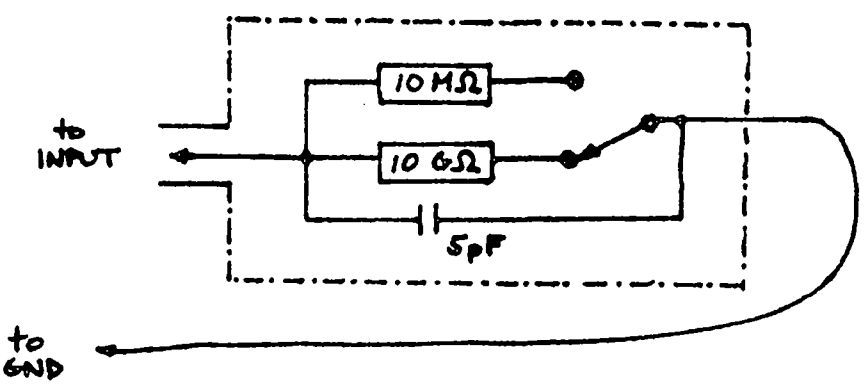


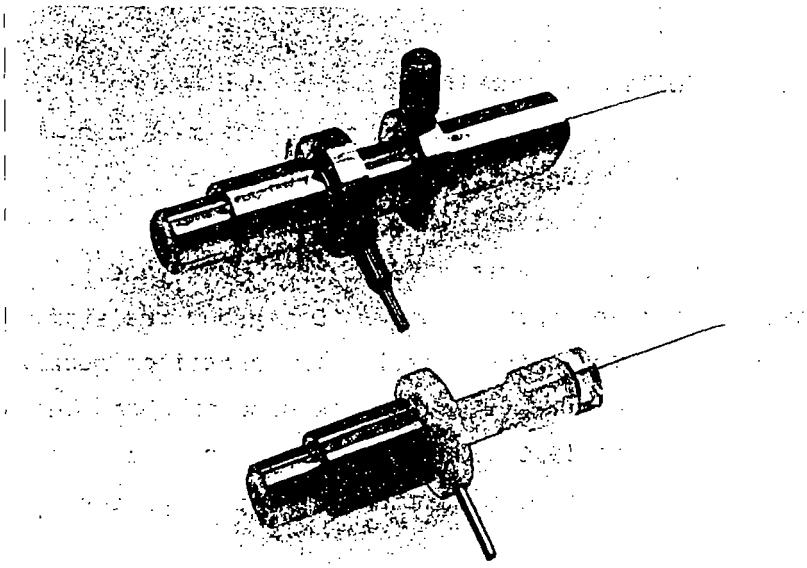
Fig. 3 Diagram of a model circuit. The circuit should be built in a shielded box.

This Model Circuit we deliver as an option to our L/M-EPC-7-System: "MC7"

LIST-MEDICAL
D-6100 DARMSTADT 13
TEL.: (06151) 56000

List-electronic

N E W : Options to the **"L/M-EPC-7"** from LIST-ELECTRONIC, D-6100 Darmstadt West-Germany / PHONE (06151)56000



Accessory to the **"L/M-EPC-7"** patch clamp

Pipette Holders

Two types of pipette holders are available for the **"L/M-EPC-7"** patch clamp which plug directly into the INPUT connector of the probe.

-a shielded version with a sliding aluminum sleeve, providing optimal shielding against line pickup

-a nonshielded version for work inside a Faraday cage. This version optimizes high frequency background noise.

Both types of holders accept ≈ 1.6 o.d pipettes. (Different bores can be supplied upon request.) They provide a post for connnection of 1mm i.d. silicon rubber tubing through which suction is applied to the pipette.

List-electronic

The EPC-7
A second-generation patch clamp

The EPC-7, the successor to the EPC-5 patch clamp, incorporates three major technical advances:

1. Background noise is reduced by nearly half due to the advanced head-stage design. A thin-film integrated circuit incorporates all critical components of the input circuitry, and an improved amplifier design and a 50 GΩ measuring resistance are employed. The resulting noise level is 0.05 pA RMS at 1 kHz, and 0.30 pA RMS at 10 kHz bandwidth.

2. Internal resistance switching. A remote switching circuit in the head stage allows switching from the 50 GΩ resistor (for single-channel recording) to a 500 MΩ resistance for measuring whole-cell currents up to 20 nA.

3. Integrated series-resistance and transient-cancellation functions. Adjustment of the capacitance-transient cancellation controls automatically determines the series resistance. For series-resistance compensation only a "% Compensation" adjustment is required. The transient-cancellation features also function properly while series-resistance compensation is in use.

Other improvements include the following:

4. 100 kHz bandwidth allows the fast kinetics of channels to be studied, and also allows improved series-resistance compensation.

5. Extended cell capacitance compensation -- to 100 pF -- allows step-clamping of cells up to 50 µm diameter.

6. Built-in RMS noise measuring facility for evaluating the background noise level.

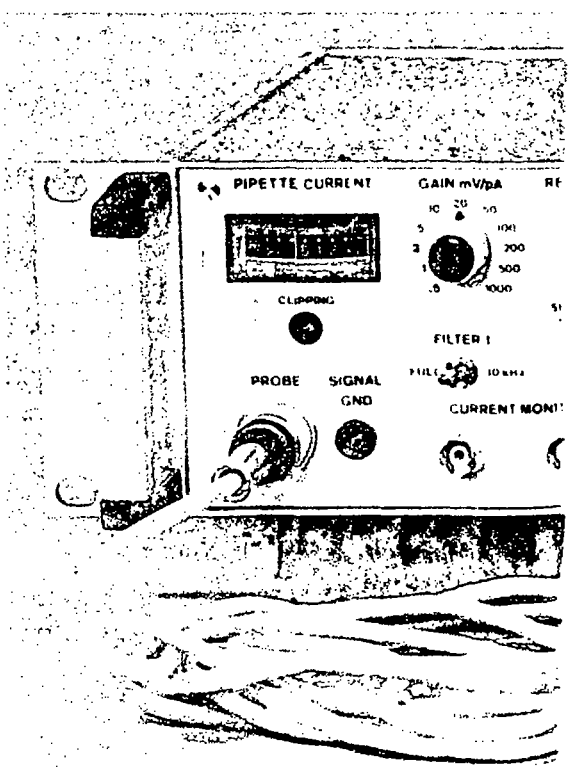
7. Calibration and evaluation of frequency response is simplified by an auto-zero integrator for the response-test signal.

Some notes on the design philosophy of the EPC-7

The tight seal clamp EPC-7 is designed in view of three recent developments in electrophysiology. First, whole-cell recording and clamping is becoming an increasingly important technique for the study of many cell types, such as cultured neurons and cardiac muscle cells. Second, whole-cell recordings and single-channel recordings complement each other. Even when the goal of a study is the analysis of single-channel currents, for example, the ability to gather whole-cell data from the same cells greatly simplifies setting up the proper experimental conditions for patch recording, and saves analysis time. Third, because of improvements in pipette technique, background noise from the recording electronics has remained a limiting factor in the resolution of single-channel recordings. For certain channel currents of low amplitude (e.g. Ca channel currents) improved resolution is needed for an adequate characterization of channel behavior.

Our aim was to design an instrument capable of both single-channel recording and whole-cell recording, and to allow switching between the two modes to be done during an experiment in order that single-channel and whole-cell recordings can be made on the same cell. For single-channel use our main goal was a significantly lower background noise level, ideally so that noise in the electronics could be ignored altogether. By redesigning the headstage amplifier and increasing the current-measuring resistance to 50 GΩ, we were in fact able to reduce the noise level by roughly half. We also wanted to include a noise measurement facility so that the experimenter could check for excess noise sources (e.g. a dirty pipette holder) conveniently in the course of experiments. A built-in, true-RMS voltmeter provides this capability.

Whole-cell recording has been very successful in the past with small cells (10 µm diameter or smaller), but larger cells have presented problems because of their larger currents and membrane capacitance. We wanted to extend the clamp capabilities to cells up to about 50 µm in diameter. We chose an upper limit of about



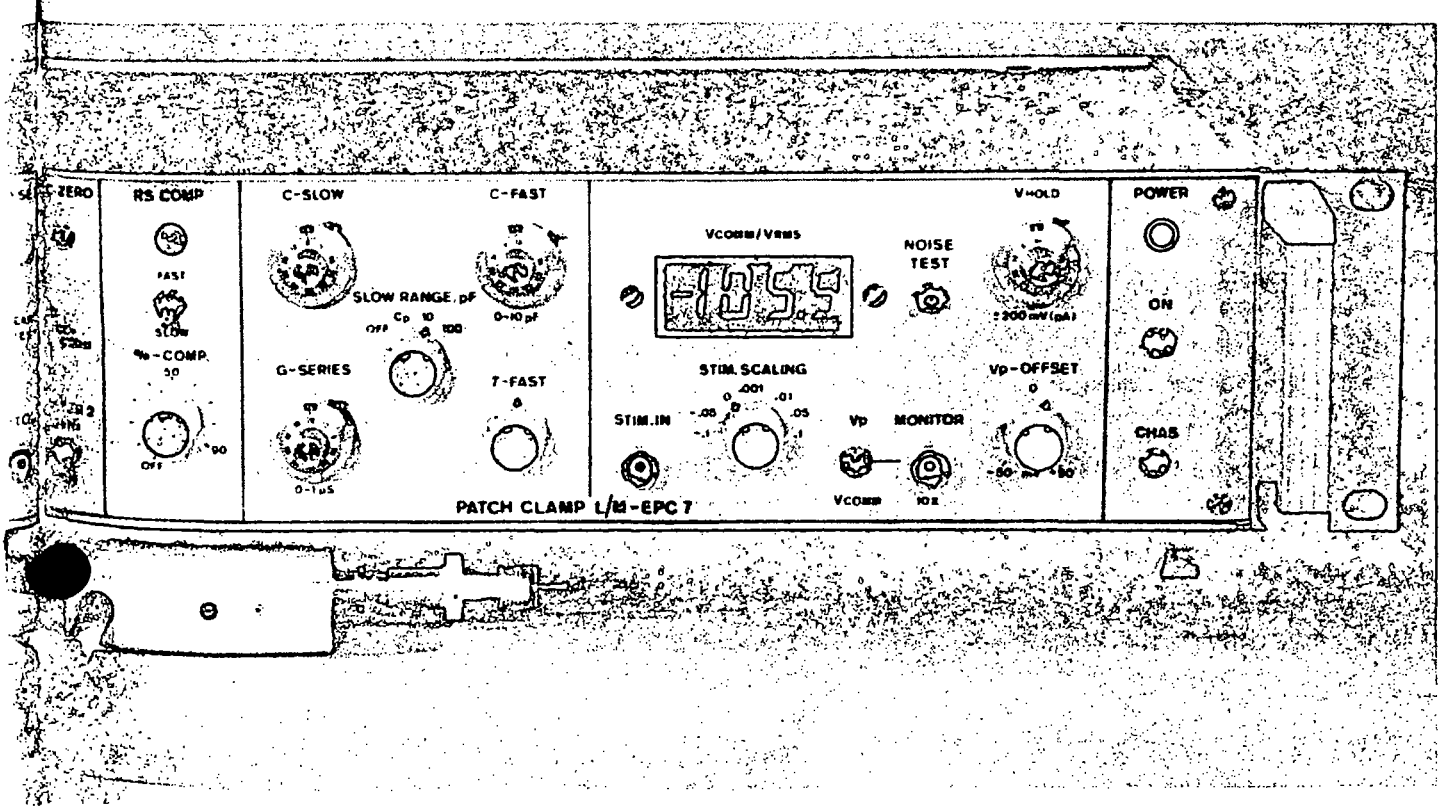
LIST-MEDICAL
D-6100 DARMSTADT 13
TEL.: (06151) 56000

LIST ELECTRONIC
Pfungstaedter Straße 18–20
D-6100 Darmstadt/Eberstadt, West Germany
(0 61 51) 5 60 00

20 nA as the maximum ionic current to be measured, since this is tha largest current that can be clamped , even using series resistance compensation, without incurring a serious voltage error. The choice of a 500 MΩ measuring resistor allowed us to meet this requirement while maintaining a noise level below the intrinsic noise from even small cells.

The transient cancellation for whole-cell recording required special attention for two reasons. First, the capacitance transient from a 100 mV voltage step in a 100 pF cell involves currents in the microampere range. To inject currents of this size without degrading the noise performance required careful design of the transient cancellation circuitry. Second, we wanted the transient cancellation to be operative while series resistance (R_S) compensation was in use. To accomplish this required an intricate feedback network which, however, is remarkably simple to use. It turns out that the settings of the transient cancellation controls are related to the series resistance; our circuit takes advantage of this fact, so that the user has only one additional adjustment to make - the "percent R_S compensation" control - in using the R_S compensation.

We also wanted to include a signal source for calibration and checking of the frequency response of the tight seal clamp. The best way to apply a test signal into the headstage is by injecting current through a small capacitor into the input circuit. Unfortunately, the current injected is the derivative of the voltage applied, so the usual practice is to use a precise triangle wave, which is transformed into a square wave of currents by the capacitor. We developed instead an integrator circuit that allows the experimentator to apply any sort of test signal-pulses, sine wave, or whatever - to the test input, and have a proportional, calibrated current of the same form injected into the headstage.



PRELIMINARY SPECIFICATIONS: EPC-7

(Subject to change without notice)

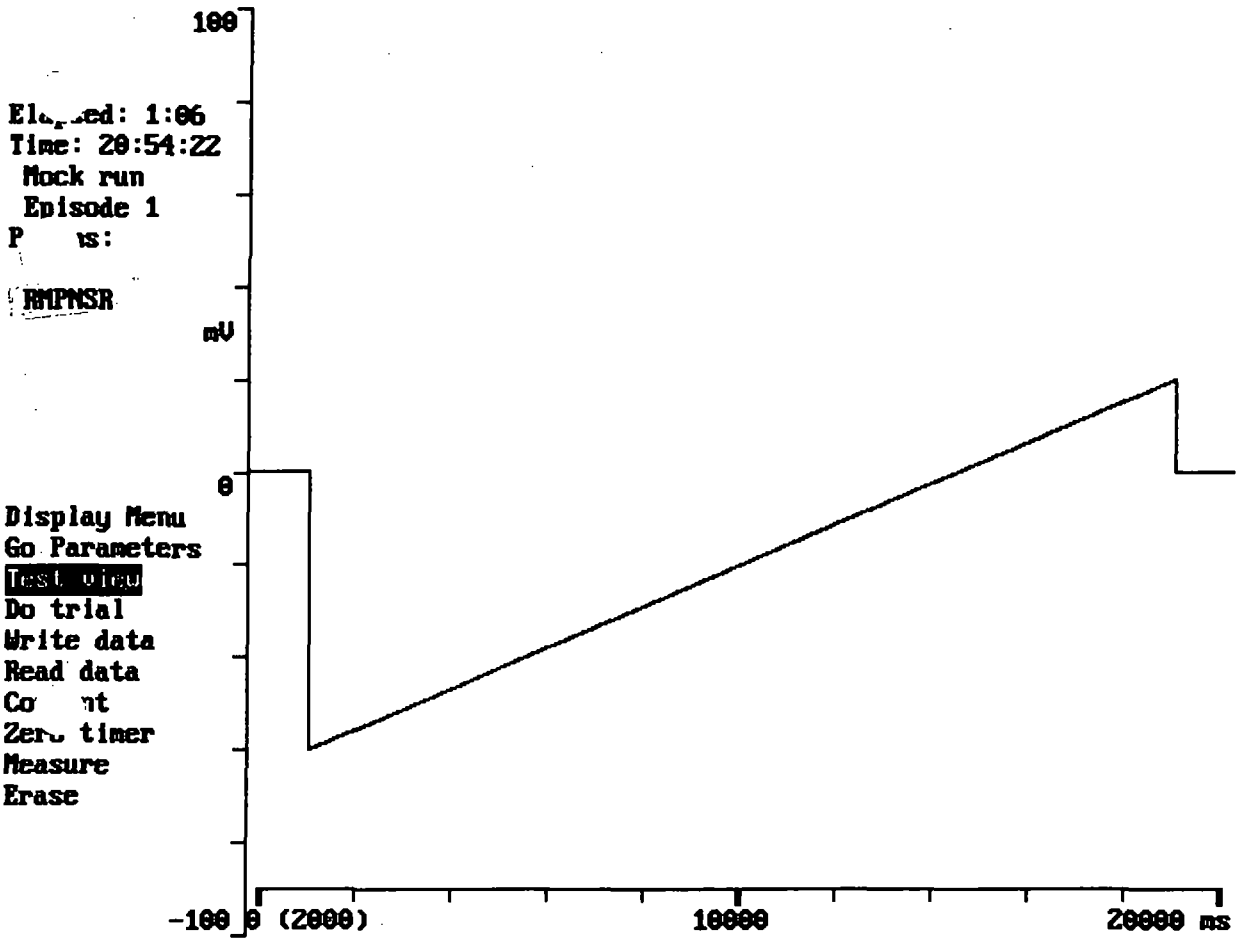
NOISE	
(Equivalent current input noise, measured with either Butterworth or Bessel, 4-pole filter; 50 gigohm range)	
0-1 kHz	0.05 pA, RMS
0-3 kHz	0.1 pA, RMS
0-10 kHz	0.30 pA, RMS
CURRENT MONITOR SIGNAL	
Gain	0.5-1000 mV/pA, switch selected
Measuring resistance	0.5 GΩ (0.5-20 mV/pA GAIN settings) 50 GΩ (50-1000 mV/pA GAIN settings)
Bandwidth	100 kHz
Internal filters	10 kHz and 3 kHz, 3-pole Bessel response
PIPETTE POTENTIAL CONTROL	
Holding potential range	±200 mV
Command input scaling	.1, .05, .01, .001, -.05, -.1, Off
Electrode offset adjustment	±50 mV
Pipette potential display	3 1/2-digit LCD display
Potential monitor output	10x scaling
TRANSIENT CANCELLATION	
Fast time constant	0.5-5 µs
Fast capacitance range	0-10 pF (Calibrated)
Slow capacitance ranges	0.2-10 pF, 2-100 pF (Calibrated)
Series conductance adjustment	0.01-1 µS (Calibrated)
SERIES RESISTANCE COMPENSATION	
Compensation range	1-100 MΩ
R_S determination	Automatically determined from transient cancellation controls
Fractional compensation	0-90% (80% compensation with no overshoot with typical values R=10M, C=20 pF)
CURRENT CLAMP MODE	
Holding current range	±200 pA
Command input scaling	10 to 100 pA/V
HEADSTAGE	
Current-measuring resistances	50 GΩ and 500 MΩ, remotely switched by GAIN control
Largest measurable currents	20 nA (500 MΩ range) 200 pA (50 GΩ range)
Input connector	Standard BNC
Other connections	Ground sense input, Pipette command output
RESPONSE TEST SIGNAL INPUT	Auto-zeroing integrator provides a direct correspondence between input and CURRENT MONITOR output signals.
Frequency response	Flat, 0.5 Hz to 1 MHz
Scaling	100 pA/volt
NOISE MONITOR FACILITY	LCD displays RMS current for checking background noise.
Bandwidth	300 Hz-3 kHz
Range	0-200 mV RMS referred to CURRENT MONITOR SIGNAL
POWER REQUIREMENTS	115 or 220 VAC, 50-60 Hz
DIMENSIONS	
Head stage	approx. 7x4x2 cm; 60 g
Head stage cable	2 m, highly flexible
Controller	9 cm (3.5 inch) high, rack-mountable

PC Tools Deluxe R4.30 Service Impression Rpertoire Label Vol=PRAMFILE

Voie=B:*.*

Nom	Suf	Vol	NbGr	Date	Heure	Attributs
SCR-70S	BPA	1024	2	17/02/92	14:12	Normal,Archive
SCR-70L	BPA	1024	2	30/03/92	20:31	Normal,Archive
SCR-70T	BPA	1024	2	12/03/92	22:05	Normal,Archive
RMP_RISE	BPA	1024	2	17/02/92	13:59	Normal,Archive
RMP_FALL	BPA	1024	2	17/02/92	13:59	Normal,Archive
SCR-100T	BPA	1024	2	17/02/92	14:11	Normal,Archive
SCR-100L	BPA	1024	2	17/02/92	14:09	Normal,Archive
SCR-100	BPA	1024	2	02/04/92	19:51	Normal,Archive
SCR100	BPA	1024	2	17/02/92	14:15	Normal,Archive
SCR-90_1	BPA	1024	2	11/02/92	22:00	Normal,Archive
SCR_90	BPA	1024	2	05/02/92	10:50	Normal,Archive
SCR_90_9	BPA	1024	2	05/02/92	10:49	Normal,Archive
SCR_I(A)	BPA	1024	2	12/03/92	21:47	Normal,Archive
SCRI(A)0	BPA	1024	2	17/02/92	14:17	Normal,Archive
EPCTEST1	BPA	1024	2	22/11/91	10:25	Normal,Archive
RMPNSR	BPA	1024	2	26/03/92	12:58	Normal,Archive
3C90-9S	BPA	1024	2	26/03/92	16:00	Normal,Archive
SCR40	BPA	1024	2	01/04/92	20:43	Normal,Archive

18 fichiers LISTs= 18432 octets. 18 fichiers sous-rp= 18432 octets.
0 fichiers SELECT= 0 octets. Disponibilit volume= 1439232 octets


100
Elapsed: 1:06
Time: 20:54:22
Mock run
Episode 1
RMPNSR
mV
0
Display Menu
Go Parameters
Test view
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (2000)
10000
20000 ms

@

Parameter file RMPNSR

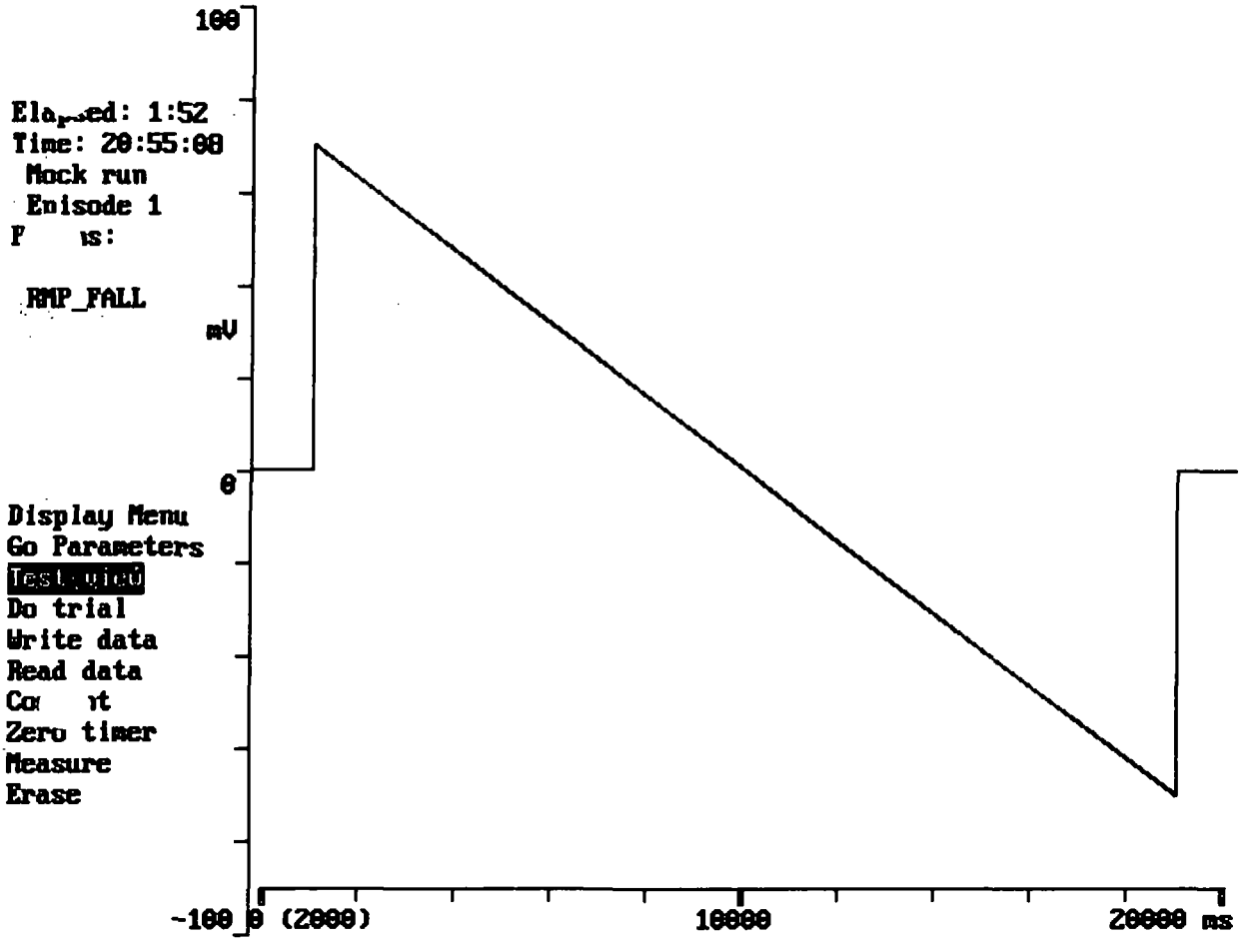
```
      Acquisition
1         Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
1           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
2         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4         Time between start of episodes (s) (0 for maximum rate)
10000       First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)


      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
100         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
-60         Amplitude initial value (mV)
0           Amplitude increment (mV)
1           Duration initial value (samples)
0           Duration increment (samples)
2         C Epoch type: 1 Step, 2 Ramp
20          Amplitude initial value (mV)
0           Amplitude increment (mV)
1800        Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
80          Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
     Display data
5        ADC ch. #15: amplification factor (not 0)
0                     display offset: fraction of full screen
2        ADC ch. #14: amplification factor (not 0)
-0.35                 display offset: fraction of full screen
1        ADC ch. #13: amplification factor (not 0)
0                     display offset: fraction of full screen
1        ADC ch. #12: amplification factor (not 0)
0                     display offset: fraction of full screen

0        Segment number to display (0..4) (0 for all)
1        Skip factor: plot every Nth point (1..4)
0        Display averaged data: 0 After each episode, N After each N runs
0        Autoerase: 0 No, 1 Yes
1        Graph style: 0 Points, 1 Lines
     Units of measure
pA       ADC ch. #15
mV       ADC ch. #14
mV       ADC ch. #13
mV       ADC ch. #12
mV       DAC ch.  #0 (command)
     Peak detection
0        Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0        Optional sample number to measure, negative for negative peaks
0        Channel number to search
0        Number of samples averaged in search (1-20) (0 for default 5)
0        Baseline: 0 Average first 24 samples, 1 Average interval A
0        Display:  0 Screen, 1 File & screen
     Hardware configuration
10       DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1        ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01       ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1          ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1          ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10       ADC range,  V
10       DAC range,  V
3        Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1          Gain multiplier
100000     Filter cutoff frequency
     Times
         Start time for this data file
         Elapsed time at start of this data file
         Date this data file acquired
```

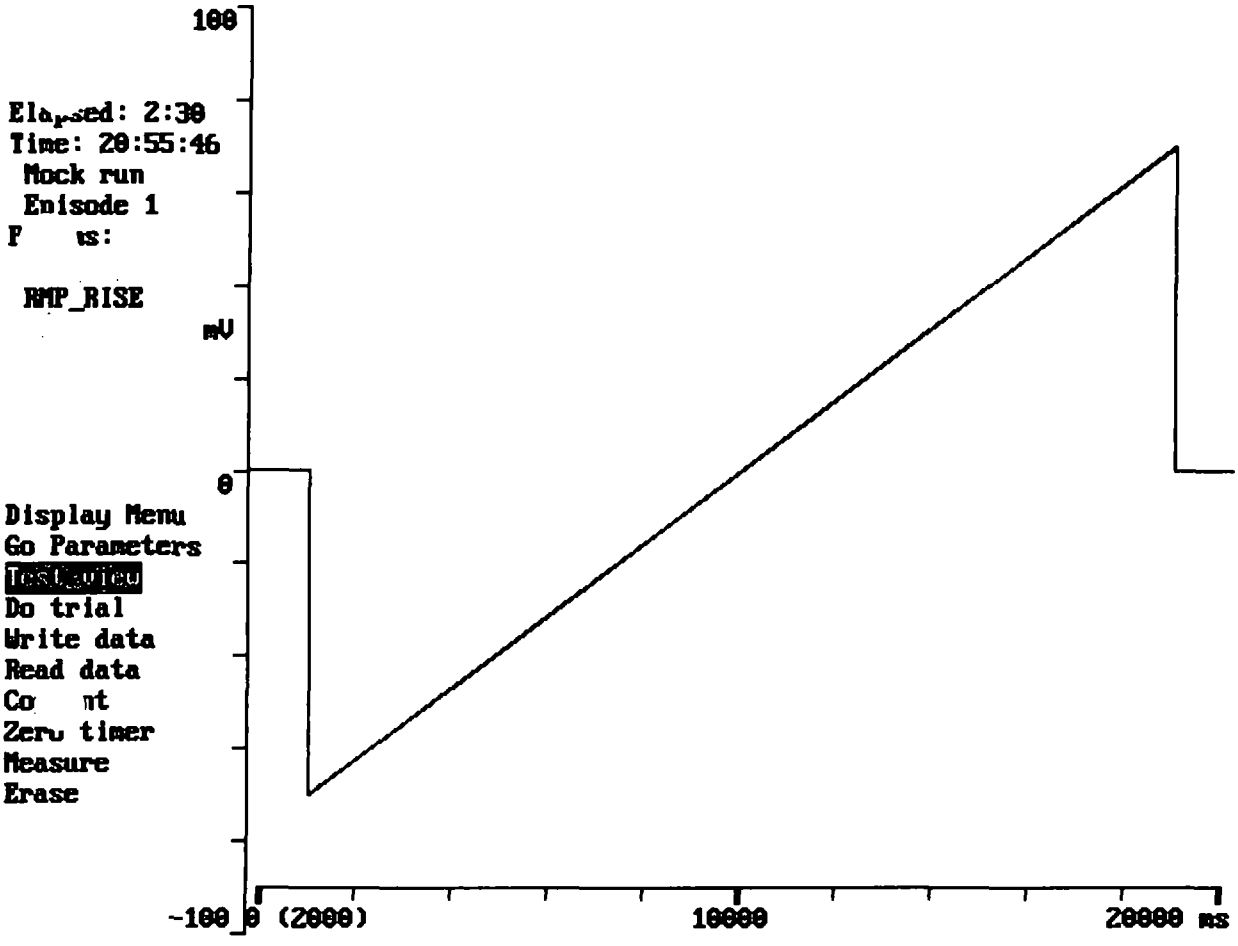

100
Elapsed: 1:52
Time: 20:55:00
Mock run
Episode 1
RMP_FALL
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (2000)
10000
20000 ms

```
     Acquisition
1        Number of trials to perform (-1 for continuous)
1          Number of runs/trial (runs are averaged) (  65536)
1          Number of episodes/run (if no conditioning pulses)
0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1          Starting episode number (normally 1)
4          Number of 512-sample segments/episode (1..4)
2          Number of channels to sample (1..4)
2        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4        Time between start of episodes (s) (0 for maximum rate)
10000      First  clock interval (samples   1-1024) (  8  s)
0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0          Delay between scope trigger and episode start (ms)
     Subtraction
0        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0          ADC channel number
0          Subpulse holding amplitude (mV)
0          Settling time after change of holding amplitude (ms)
0          Time interval between sub-pulses (ms)
     Waveform on analog output channel #0
0        Holding amplitude (mV)
1        A Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
100        Duration initial value (samples)
0          Duration increment (samples)
1        B Epoch type: 1 Step, 2 Ramp
70         Amplitude initial value (mV)
0          Amplitude increment (mV)
1          Duration initial value (samples)
0          Duration increment (samples)
2        C Epoch type: 1 Step, 2 Ramp
-70        Amplitude initial value (mV)
0          Amplitude increment (mV)
1800       Duration initial value (samples)
0          Duration increment (samples)
1        D Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
80         Duration initial value (samples)
0          Duration increment (samples)
0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
     Trigger outputs
0        Duration and polarity of pulse on trigger channel #1 (samples)
0          Sample number at which to start pulse
0        Duration and polarity of pulse on trigger channel #2 (samples)
0          Sample number at which to start pulse
0        First episode at which trigger channels #1 and #2 fire
0        Last  episode at which trigger channels #1 and #2 fire
     Conditioning train
0        Number of pulses in train (0 for none)
0        Pre-conditioning pulse duration (ms)
0        Pre-conditioning pulse amplitude (mV)
0        Conditioning pulse duration (ms)
0        Conditioning pulse amplitude (mV)
0        Post-conditioning train duration (ms)
0        Post-conditioning train amplitude  (mV)
```

```
      Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.35                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen


0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
0         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
      Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
      Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
      Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```

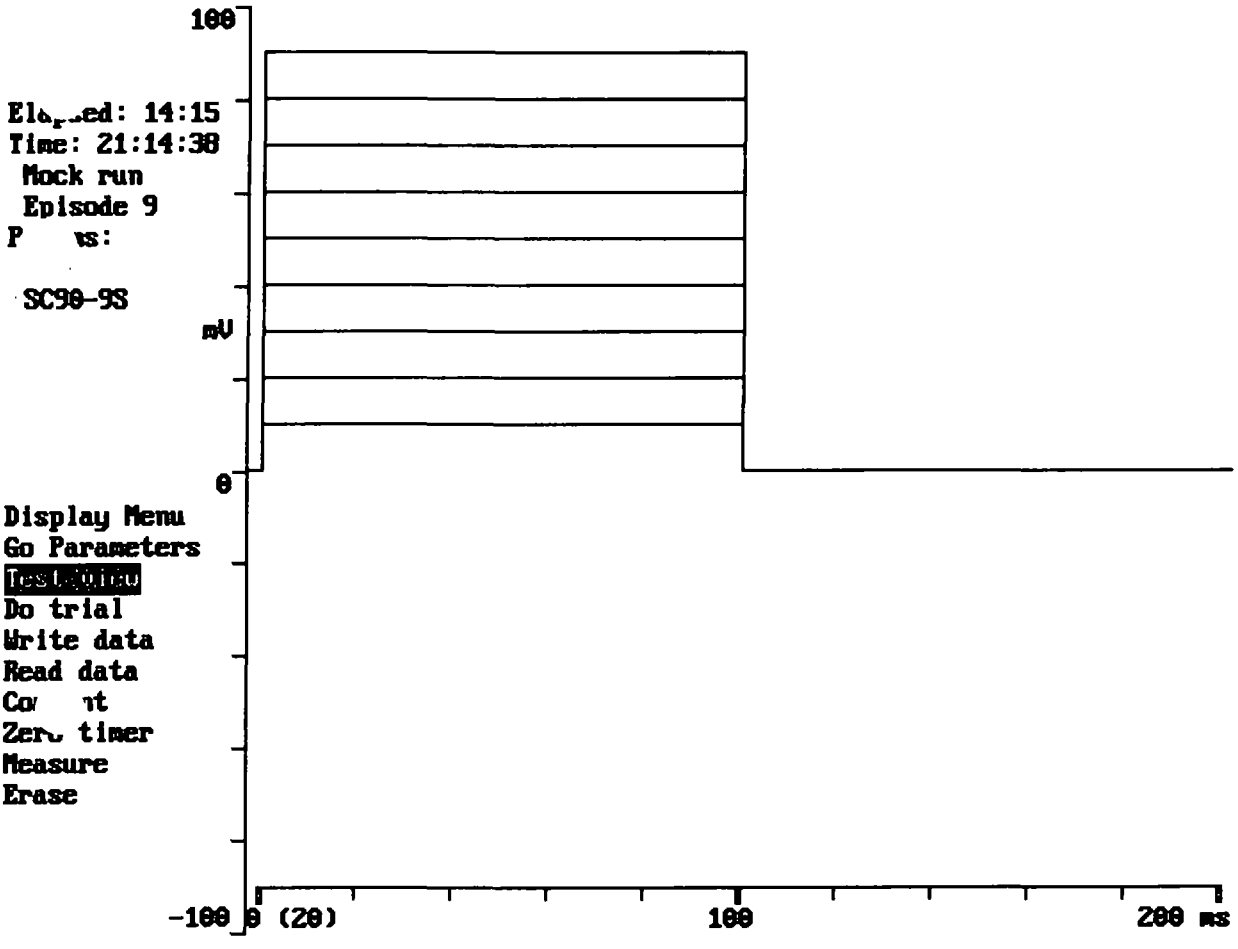

100
Elapsed: 2:30
Time: 20:55:46
Mock run
Episode 1
F us:
RMP_RISE
mV
0
Display Menu
Go Parameters
Test Device
Do trial
Write data
Read data
Co nt
Zero timer
Measure
Erase
-100
0 (2000)
10000
20000 ms

```
      Acquisition
1         Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
1           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
2         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4         Time between start of episodes (s) (0 for maximum rate)
10000       First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
100         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
-70         Amplitude initial value (mV)
0           Amplitude increment (mV)
1           Duration initial value (samples)
0           Duration increment (samples)
2         C Epoch type: 1 Step, 2 Ramp
70          Amplitude initial value (mV)
0           Amplitude increment (mV)
1800        Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
80          Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
      Display data
 5         ADC ch. #15: amplification factor (not 0)
 0                      display offset: fraction of full screen
 2         ADC ch. #14: amplification factor (not 0)
-0.35                   display offset: fraction of full screen
 1         ADC ch. #13: amplification factor (not 0)
 0                      display offset: fraction of full screen
 1         ADC ch. #12: amplification factor (not 0)
 0                      display offset: fraction of full screen

 0         Segment number to display (0..4) (0 for all)
 1         Skip factor: plot every Nth point (1..4)
 0         Display averaged data: 0 After each episode, N After each N runs
 0         Autoerase: 0 No, 1 Yes
 1         Graph style: 0 Points, 1 Lines
      Units of measure
pA         ADC ch. #15
mV         ADC ch. #14
mV         ADC ch. #13
mV         ADC ch. #12
mV         DAC ch.  #0 (command)
      Peak detection
 0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
 0         Optional sample number to measure, negative for negative peaks
 0         Channel number to search
 0         Number of samples averaged in search (1-20) (0 for default 5)
 0         Baseline: 0 Average first 24 samples, 1 Average interval A
 0         Display:  0 Screen, 1 File & screen
      Hardware configuration
10         DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1          ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01         ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1            ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1            ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10         ADC range,  V
10         DAC range,  V
3          Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1            Gain multiplier
100000       Filter cutoff frequency
      Times
           Start time for this data file
           Elapsed time at start of this data file
           Date this data file acquired
```

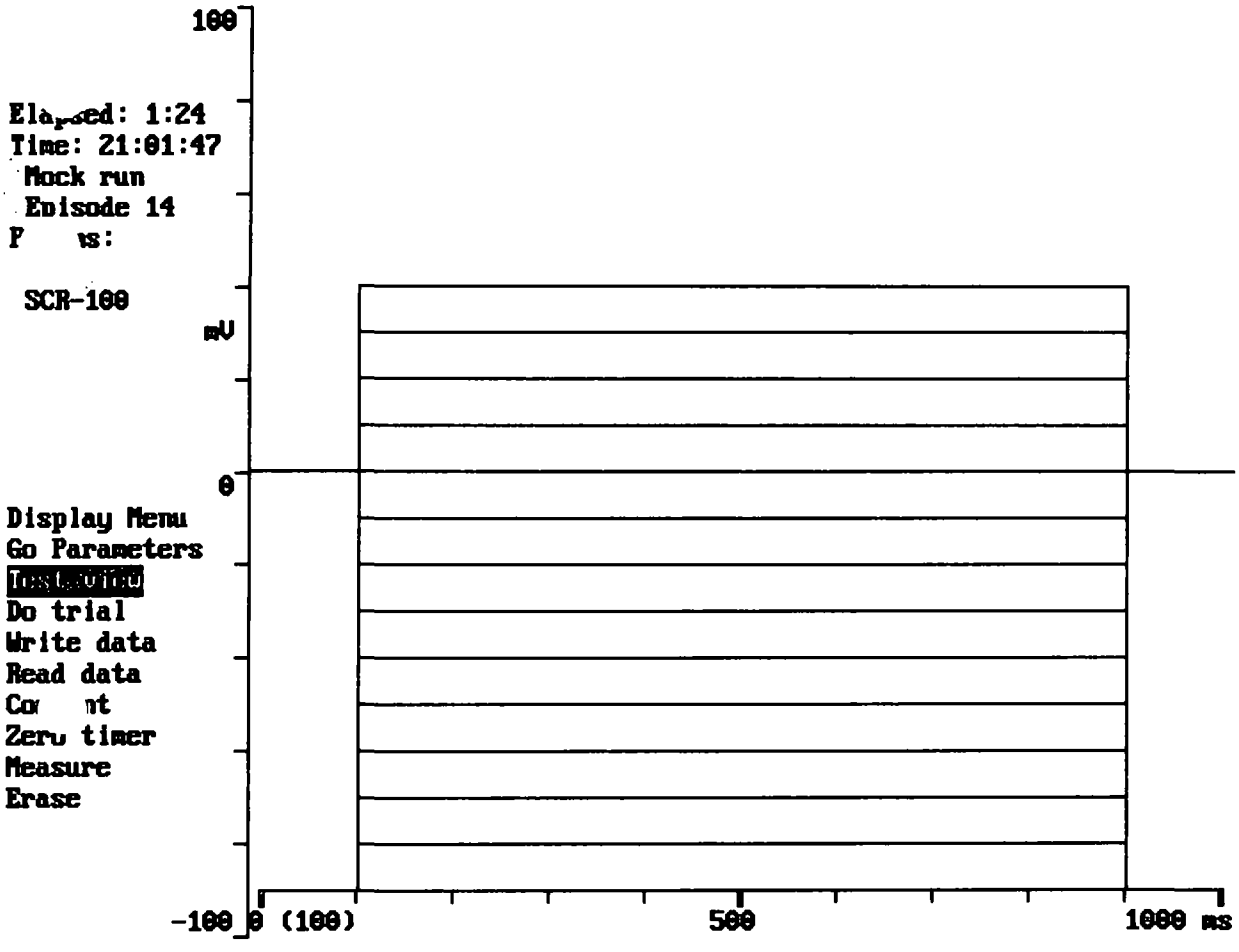

100
Elapsed: 14:15
Time: 21:14:38
Mock run
Episode 9
SC90-9S
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (20)
100
200 ms

```
      Acquisition
12        Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
9           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4         Time between start of episodes (s) (0 for maximum rate)
100         First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
5           Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
10          Amplitude initial value (mV)
10          Amplitude increment (mV)
1000        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
      Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
      Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
      Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
      Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 1:24
Time: 21:01:47
Mock run
Episode 14
SCR-100
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (100)
500
1000 ms

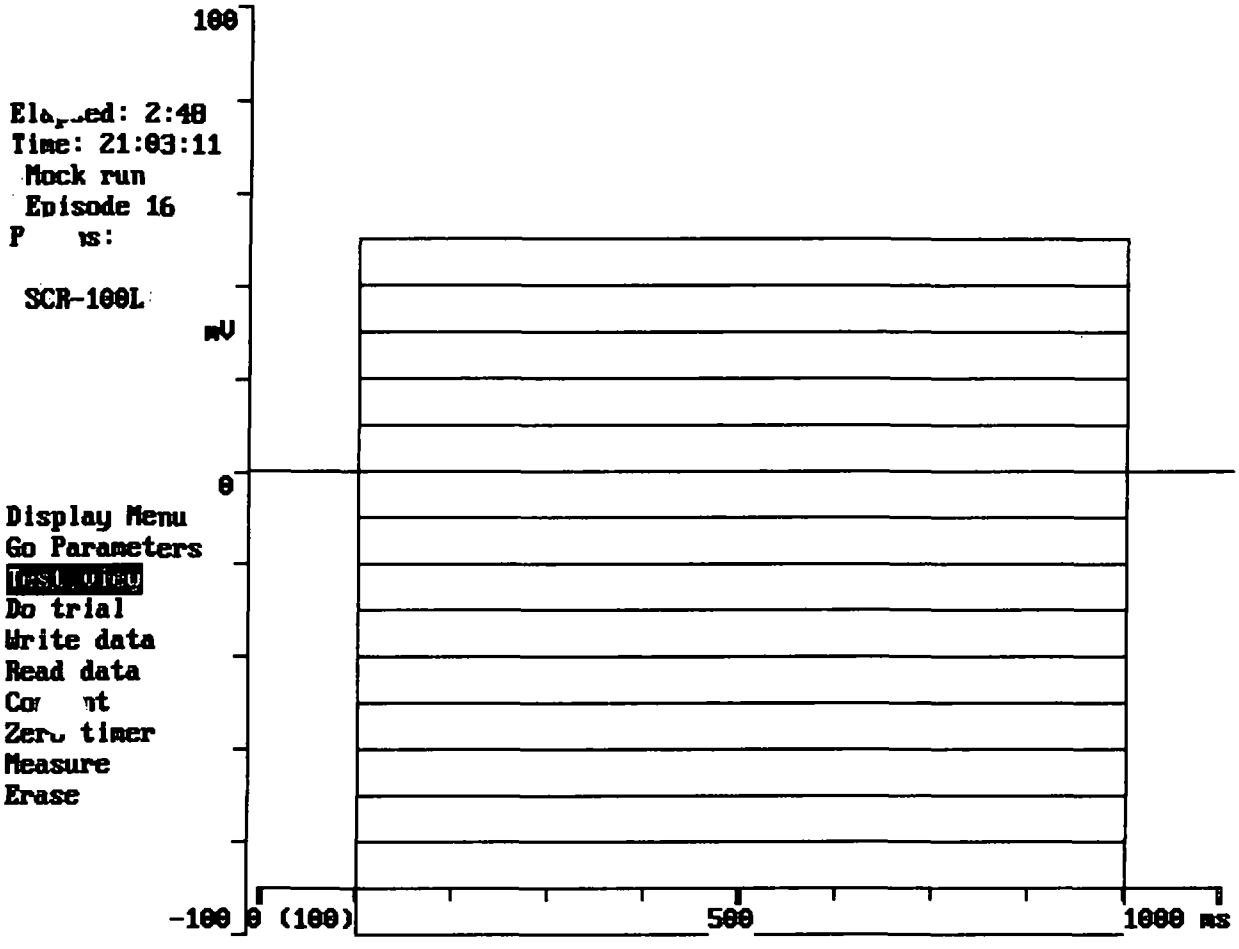
```
      Acquisition
 15       Number of trials to perform (-1 for continuous)
 1          Number of runs/trial (runs are averaged) (  65536)
 14         Number of episodes/run (if no conditioning pulses)
 0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
 1          Starting episode number (normally 1)
 4          Number of 512-sample segments/episode (1..4)
 2          Number of channels to sample (1..4)
 0        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
 10       Time between start of episodes (s) (0 for maximum rate)
 500        First  clock interval (samples   1-1024) (  8  s)
 0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
 0          Delay between scope trigger and episode start (ms)
      Subtraction
 0        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
 0          ADC channel number
 0          Subpulse holding amplitude (mV)
 0          Settling time after change of holding amplitude (ms)
 0          Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
 0        Holding amplitude (mV)
 1        A Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 200        Duration initial value (samples)
 0          Duration increment (samples)
 1        B Epoch type: 1 Step, 2 Ramp
 40         Amplitude initial value (mV)
 -10        Amplitude increment (mV)
 1600       Duration initial value (samples)
 0          Duration increment (samples)
 1        C Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 1        D Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs

 0        Duration and polarity of pulse on trigger channel #1 (samples)
 0          Sample number at which to start pulse
 0        Duration and polarity of pulse on trigger channel #2 (samples)
 0          Sample number at which to start pulse
 0        First episode at which trigger channels #1 and #2 fire
 0        Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
 0        Number of pulses in train (0 for none)
 0        Pre-conditioning pulse duration (ms)
 0        Pre-conditioning pulse amplitude (mV)
 0        Conditioning pulse duration (ms)
 0        Conditioning pulse amplitude (mV)
 0        Post-conditioning train duration (ms)
 0        Post-conditioning train amplitude  (mV)
```

```
      Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
      Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
      Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
      Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 2:48
Time: 21:03:11
Mock run
Episode 16
SCR-100L
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (100)
500
1000 ms

@

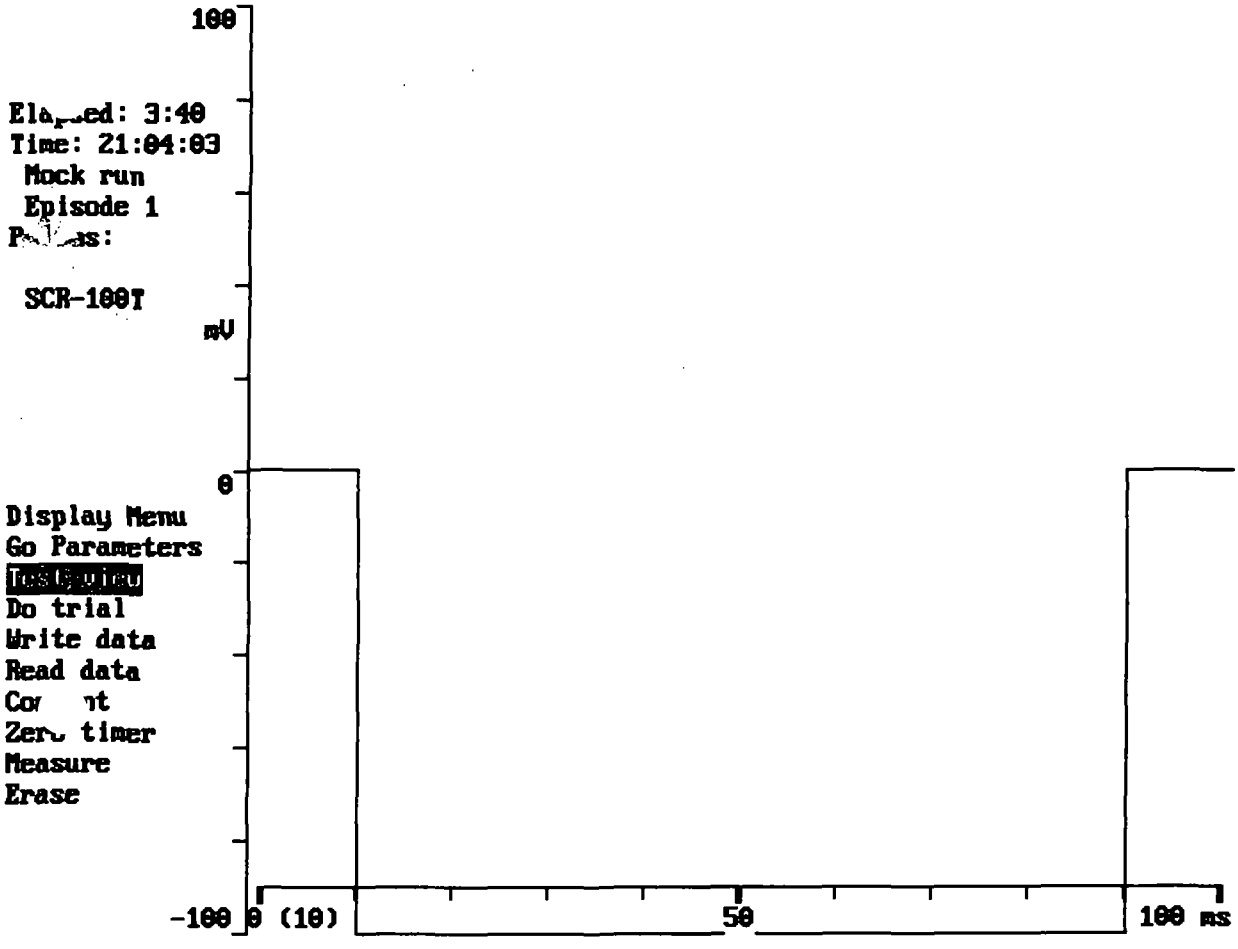
Parameter file SCR-100L

```
      Acquisition
 12       Number of trials to perform (-1 for continuous)
 1          Number of runs/trial (runs are averaged) (  65536)
 16         Number of episodes/run (if no conditioning pulses)
 0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
 1          Starting episode number (normally 1)
 4          Number of 512-sample segments/episode (1..4)
 2          Number of channels to sample (1..4)
 0        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
 15       Time between start of episodes (s) (0 for maximum rate)
 500        First  clock interval (samples   1-1024) (  8  s)
 0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
 0          Delay between scope trigger and episode start (ms)
      Subtraction
 0        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
 0          ADC channel number
 0          Subpulse holding amplitude (mV)
 0          Settling time after change of holding amplitude (ms)
 0          Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
 0        Holding amplitude (mV)
 1        A Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 200        Duration initial value (samples)
 0          Duration increment (samples)
 1        B Epoch type: 1 Step, 2 Ramp
 50         Amplitude initial value (mV)
 -10        Amplitude increment (mV)
 1600       Duration initial value (samples)
 0          Duration increment (samples)
 1        C Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 1        D Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
 0        Duration and polarity of pulse on trigger channel #1 (samples)
 0          Sample number at which to start pulse
 0        Duration and polarity of pulse on trigger channel #2 (samples)
 0          Sample number at which to start pulse
 0        First episode at which trigger channels #1 and #2 fire
 0        Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
 0        Number of pulses in train (0 for none)
 0        Pre-conditioning pulse duration (ms)
 0        Pre-conditioning pulse amplitude (mV)
 0        Conditioning pulse duration (ms)
 0        Conditioning pulse amplitude (mV)
 0        Post-conditioning train duration (ms)
 0        Post-conditioning train amplitude  (mV)
```

```
      Display data
 5        ADC ch. #15: amplification factor (not 0)
 0                     display offset: fraction of full screen
 2        ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
 1        ADC ch. #13: amplification factor (not 0)
 0                     display offset: fraction of full screen
 1        ADC ch. #12: amplification factor (not 0)
 0                     display offset: fraction of full screen

 0        Segment number to display (0..4) (0 for all)
 1        Skip factor: plot every Nth point (1..4)
 0        Display averaged data: 0 After each episode, N After each N runs
 1        Autoerase: 0 No, 1 Yes
 1        Graph style: 0 Points, 1 Lines
      Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
      Peak detection
 0        Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
 0        Optional sample number to measure, negative for negative peaks
 0        Channel number to search
 0        Number of samples averaged in search (1-20) (0 for default 5)
 0        Baseline: 0 Average first 24 samples, 1 Average interval A
 0        Display:  0 Screen, 1 File & screen
      Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 3:40
Time: 21:04:03
Mock run
Episode 1
SCR-100T
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (10)
50
100 ms

@

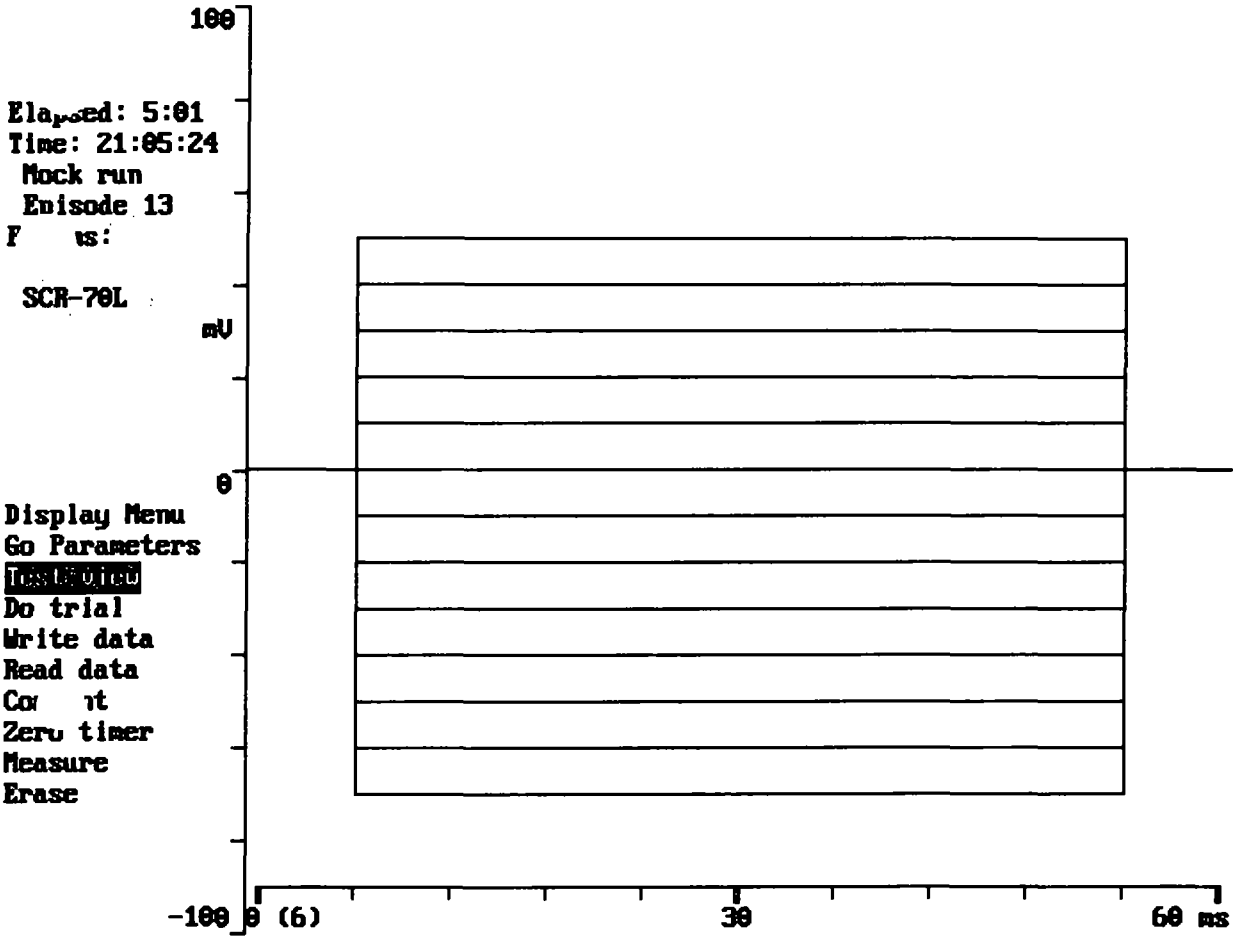
Parameter file SCR-100T

```
     Acquisition
12       Number of trials to perform (-1 for continuous)
1          Number of runs/trial (runs are averaged) (  65536)
1          Number of episodes/run (if no conditioning pulses)
0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1          Starting episode number (normally 1)
4          Number of 512-sample segments/episode (1..4)
2          Number of channels to sample (1..4)
0        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
15       Time between start of episodes (s) (0 for maximum rate)
50         First  clock interval (samples   1-1024) (  8  s)
0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0          Delay between scope trigger and episode start (ms)
     Subtraction
4        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
15         ADC channel number
0          Subpulse holding amplitude (mV)
0          Settling time after change of holding amplitude (ms)
500        Time interval between sub-pulses (ms)
     Waveform on analog output channel #0
0        Holding amplitude (mV)
1        A Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
200        Duration initial value (samples)
0          Duration increment (samples)
1        B Epoch type: 1 Step, 2 Ramp
-100       Amplitude initial value (mV)
0          Amplitude increment (mV)
1600       Duration initial value (samples)
0          Duration increment (samples)
1        C Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
0          Duration initial value (samples)
0          Duration increment (samples)
1        D Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
0          Duration initial value (samples)
0          Duration increment (samples)
0       Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
     Trigger outputs
0        Duration and polarity of pulse on trigger channel #1 (samples)
0          Sample number at which to start pulse
0        Duration and polarity of pulse on trigger channel #2 (samples)
0          Sample number at which to start pulse
0        First episode at which trigger channels #1 and #2 fire
0        Last  episode at which trigger channels #1 and #2 fire
     Conditioning train
0        Number of pulses in train (0 for none)
0        Pre-conditioning pulse duration (ms)
0        Pre-conditioning pulse amplitude (mV)
0        Conditioning pulse duration (ms)
0        Conditioning pulse amplitude (mV)
0        Post-conditioning train duration (ms)
0        Post-conditioning train amplitude  (mV)
```

```
     Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
     Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
     Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
     Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
     Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 5:01
Time: 21:05:24
Mock run
Episode 13
F vs:
SCR-70L
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (6)
30
60 ms

@

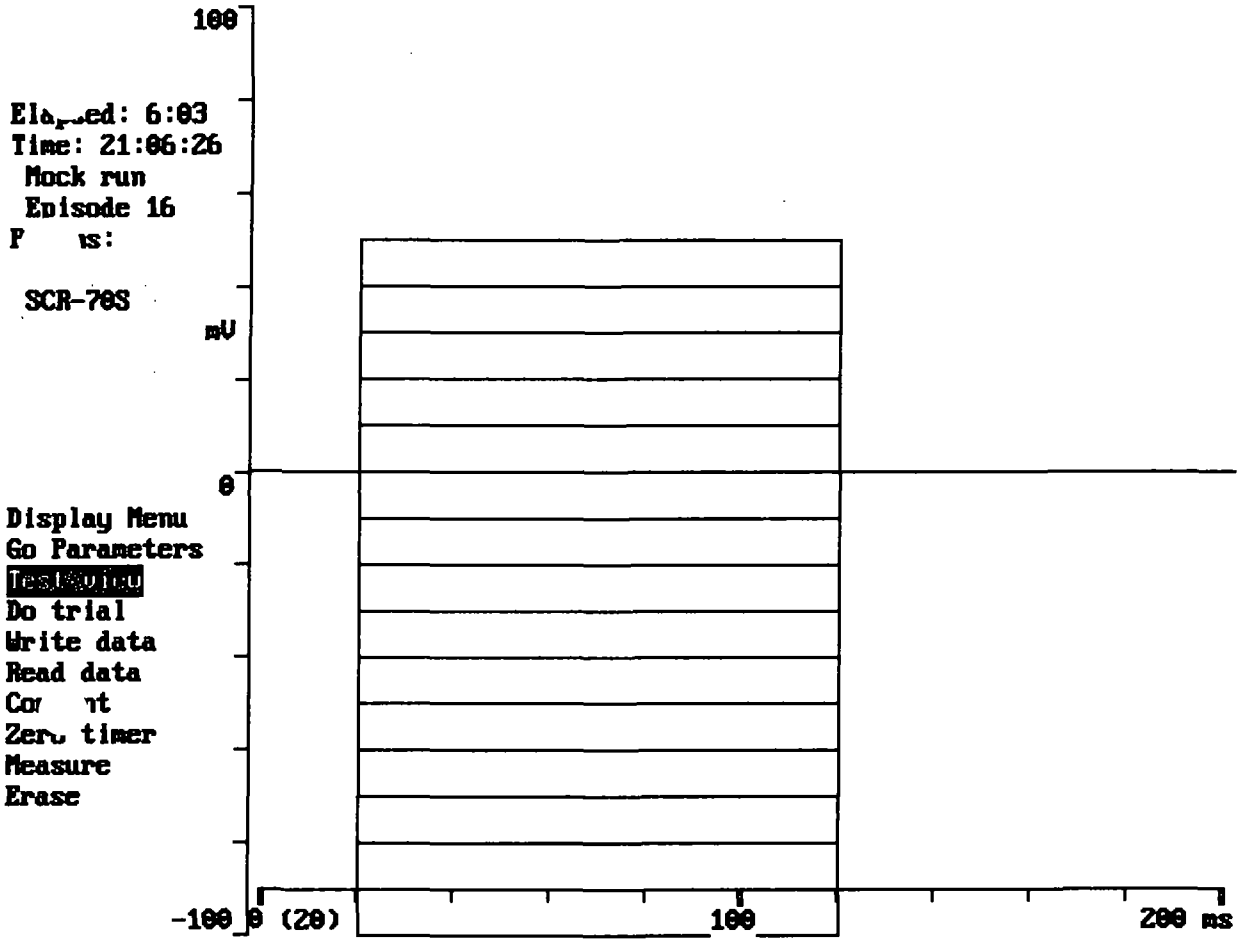
Parameter file SCR-70L

```
     Acquisition
12       Number of trials to perform (-1 for continuous)
1          Number of runs/trial (runs are averaged) (  65536)
13         Number of episodes/run (if no conditioning pulses)
0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1          Starting episode number (normally 1)
4          Number of 512-sample segments/episode (1..4)
2          Number of channels to sample (1..4)
0        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
10       Time between start of episodes (s) (0 for maximum rate)
30         First  clock interval (samples   1-1024) (  8  s)
0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0          Delay between scope trigger and episode start (ms)
     Subtraction
0        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0          ADC channel number
0          Subpulse holding amplitude (mV)
0          Settling time after change of holding amplitude (ms)
0          Time interval between sub-pulses (ms)
     Waveform on analog output channel #0
0        Holding amplitude (mV)
1        A Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
200        Duration initial value (samples)
0          Duration increment (samples)
1        B Epoch type: 1 Step, 2 Ramp
50         Amplitude initial value (mV)
-10        Amplitude increment (mV)
1600       Duration initial value (samples)
0          Duration increment (samples)
1        C Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
0          Duration initial value (samples)
0          Duration increment (samples)
1        D Epoch type: 1 Step, 2 Ramp
0          Amplitude initial value (mV)
0          Amplitude increment (mV)
0          Duration initial value (samples)
0          Duration increment (samples)
0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
     Trigger outputs
0        Duration and polarity of pulse on trigger channel #1 (samples)
0          Sample number at which to start pulse
0        Duration and polarity of pulse on trigger channel #2 (samples)
0          Sample number at which to start pulse
0        First episode at which trigger channels #1 and #2 fire
0        Last  episode at which trigger channels #1 and #2 fire
     Conditioning train
0        Number of pulses in train (0 for none)
0        Pre-conditioning pulse duration (ms)
0        Pre-conditioning pulse amplitude (mV)
0        Conditioning pulse duration (ms)
0        Conditioning pulse amplitude (mV)
0        Post-conditioning train duration (ms)
0        Post-conditioning train amplitude  (mV)
```

```
      Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
      Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
      Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
      Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

 10       ADC range,  V
 10       DAC range,  V
 3        Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
 1          Gain multiplier
 100000     Filter cutoff frequency
       Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 6:03
Time: 21:06:26
Mock run
Episode 16
SCR-70S
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (20)
100
200 ms

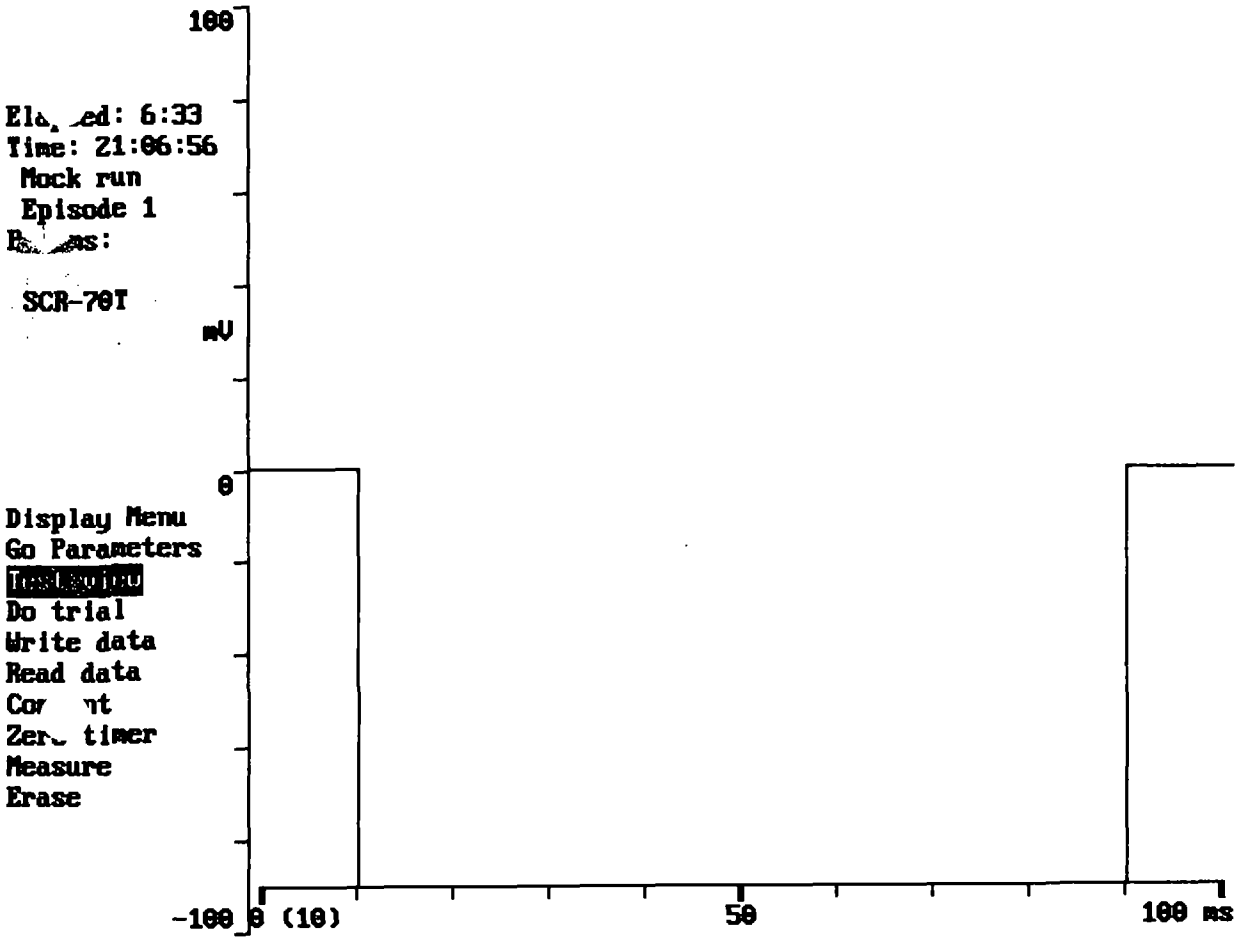
```
      Acquisition
 12       Number of trials to perform (-1 for continuous)
 1          Number of runs/trial (runs are averaged) (  65536)
 16         Number of episodes/run (if no conditioning pulses)
 0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
 1          Starting episode number (normally 1)
 4          Number of 512-sample segments/episode (1..4)
 2          Number of channels to sample (1..4)
 0        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
 10       Time between start of episodes (s) (0 for maximum rate)
 100        First  clock interval (samples   1-1024) (  8  s)
 0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
 0          Delay between scope trigger and episode start (ms)
      Subtraction
 0        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
 0          ADC channel number
 0          Subpulse holding amplitude (mV)
 0          Settling time after change of holding amplitude (ms)
 0          Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
 0        Holding amplitude (mV)
 1        A Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)

 200        Duration initial value (samples)
 0          Duration increment (samples)
 1        B Epoch type: 1 Step, 2 Ramp
 50         Amplitude initial value (mV)
 -10        Amplitude increment (mV)
 1000       Duration initial value (samples)
 0          Duration increment (samples)
 1        C Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 1        D Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
 0        Duration and polarity of pulse on trigger channel #1 (samples)
 0          Sample number at which to start pulse
 0        Duration and polarity of pulse on trigger channel #2 (samples)
 0          Sample number at which to start pulse
 0        First episode at which trigger channels #1 and #2 fire
 0        Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
 0        Number of pulses in train (0 for none)
 0        Pre-conditioning pulse duration (ms)
 0        Pre-conditioning pulse amplitude (mV)
 0        Conditioning pulse duration (ms)
 0        Conditioning pulse amplitude (mV)
 0        Post-conditioning train duration (ms)
 0        Post-conditioning train amplitude  (mV)
```

```
     Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
     Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
     Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
     Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
     Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Ela ed: 6:33
Time: 21:06:56
Mock run
Episode 1
SCR-70T
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (10)
50
100 ms

Parameter file SCR-70T

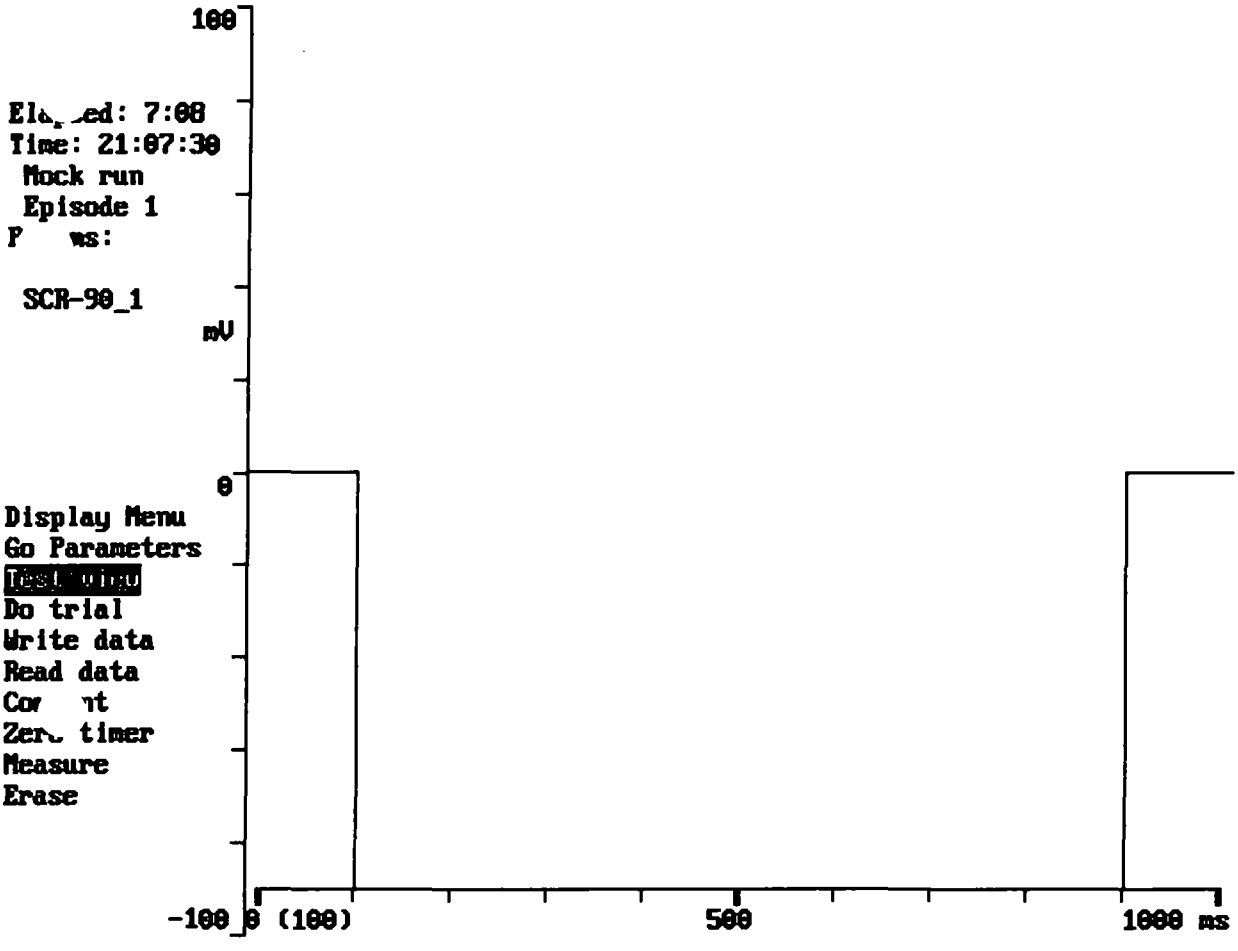
```
      Acquisition
12        Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
1           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4         Time between start of episodes (s) (0 for maximum rate)
50          First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)

200         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
-90         Amplitude initial value (mV)
0           Amplitude increment (mV)
1600        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
     Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
     Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
     Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
     Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
     Times
         Start time for this data file
         Elapsed time at start of this data file
         Date this data file acquired
```


100
Elapsed: 7:08
Time: 21:07:30
Mock run
Episode 1
SCR-90_1
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (100)
500
1000 ms

```
      Acquisition
-1        Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
1           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
10        Time between start of episodes (s) (0 for maximum rate)
500         First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)

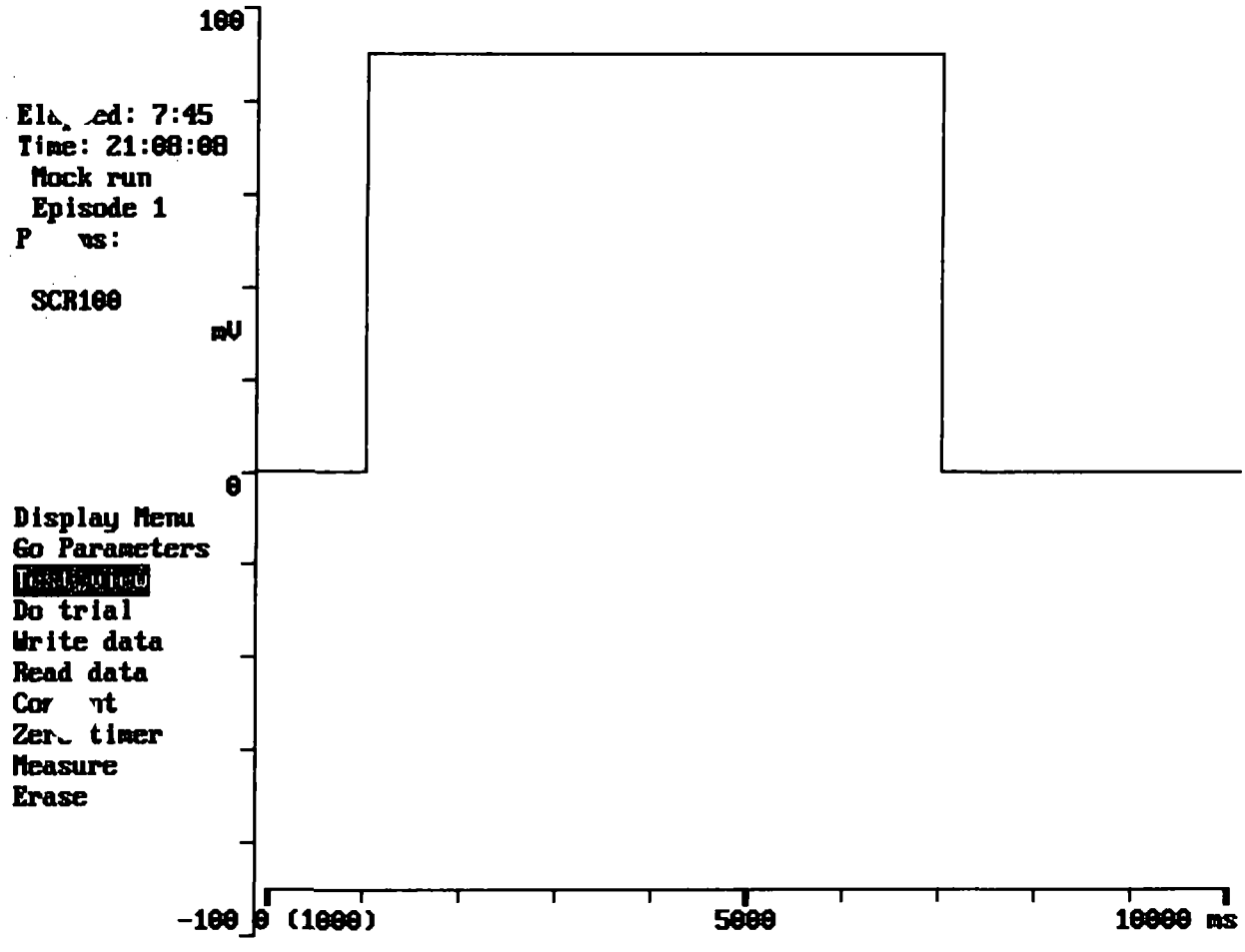
200         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
-90         Amplitude initial value (mV)
0           Amplitude increment (mV)
1600        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs

0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
     Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
     Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
     Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
     Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

 10       ADC range,  V
 10       DAC range,  V
 3        Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
 1          Gain multiplier
 100000     Filter cutoff frequency
     Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Ela ed: 7:45
Time: 21:08:08
Mock run
Episode 1
P vs:
SCR100
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zer timer
Measure
Erase
-100
0 (1000)
5000
10000 ms

@

Parameter file SCR100

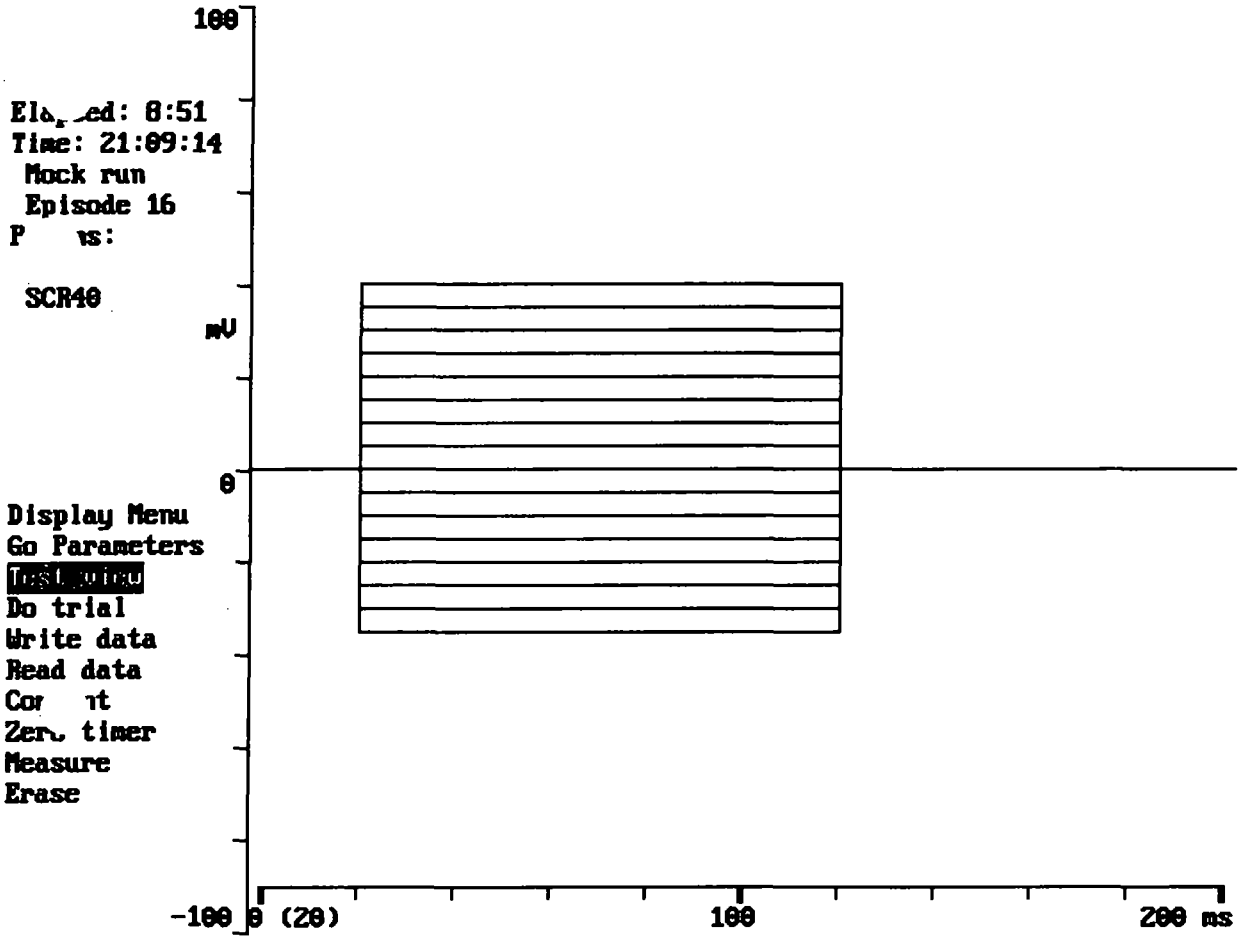
```
      Acquisition
-1        Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
1           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
15        Time between start of episodes (s) (0 for maximum rate)
5000        First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)

200         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
90          Amplitude initial value (mV)
0           Amplitude increment (mV)
1200        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
     Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
     Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
     Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
     Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
     Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 0:51
Time: 21:09:14
Mock run
Episode 16
SCR40
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Zero timer
Measure
Erase
-100
0 (20)
100
200 ms

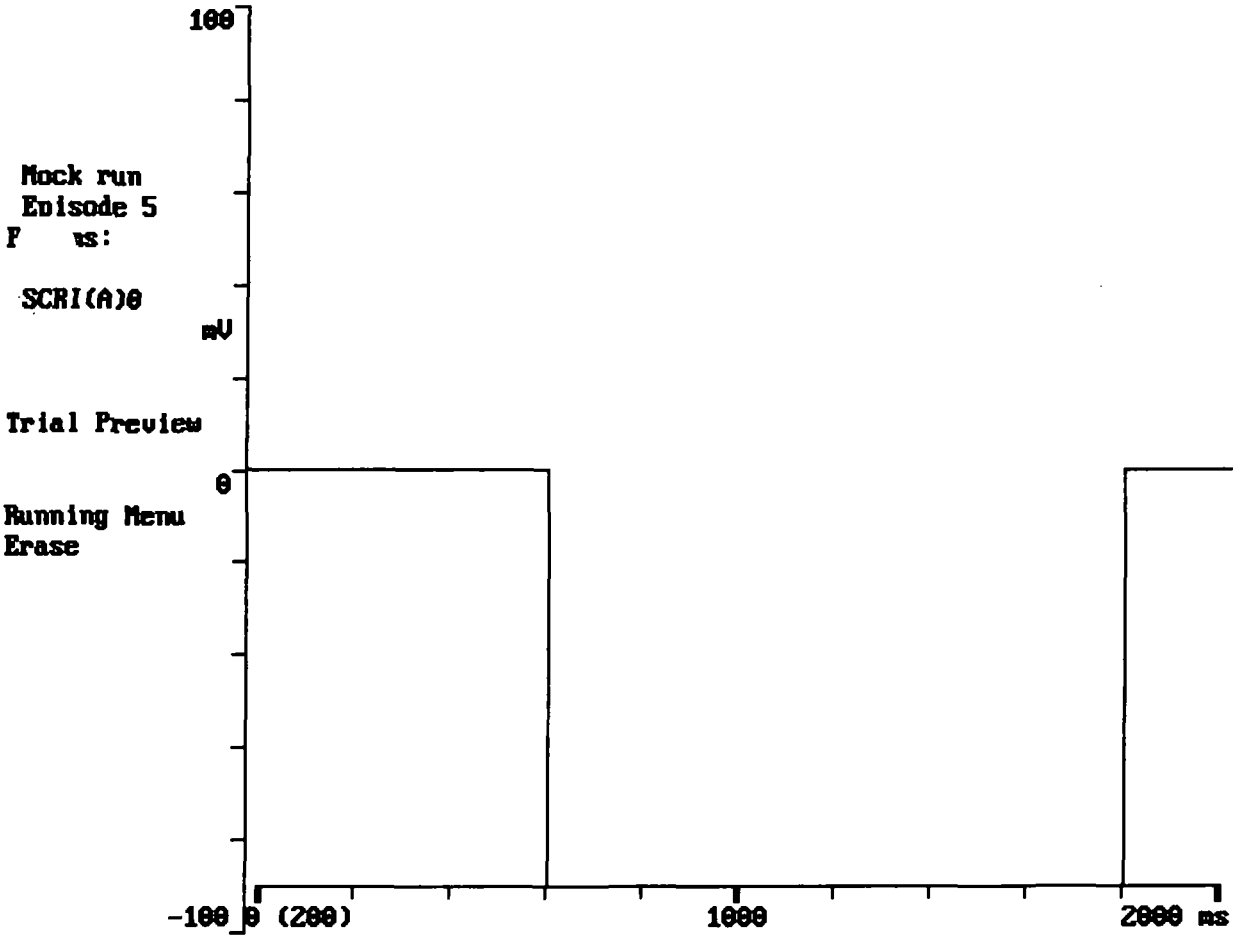
Parameter file SCR40

```
      Acquisition
 12       Number of trials to perform (-1 for continuous)
 1          Number of runs/trial (runs are averaged) (  65536)
 16         Number of episodes/run (if no conditioning pulses)
 0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
 1          Starting episode number (normally 1)
 4          Number of 512-sample segments/episode (1..4)
 2          Number of channels to sample (1..4)
 0        Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
 10       Time between start of episodes (s) (0 for maximum rate)
 100        First  clock interval (samples   1-1024) (  8  s)
 0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
 0          Delay between scope trigger and episode start (ms)
      Subtraction
 0        Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
 0          ADC channel number
 0          Subpulse holding amplitude (mV)
 0          Settling time after change of holding amplitude (ms)
 0          Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
 0        Holding amplitude (mV)
 1        A Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 200        Duration initial value (samples)
 0          Duration increment (samples)
 1        B Epoch type: 1 Step, 2 Ramp
 40         Amplitude initial value (mV)
 -5         Amplitude increment (mV)
 1000       Duration initial value (samples)
 0          Duration increment (samples)
 1        C Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 1        D Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 0       Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
 0        Duration and polarity of pulse on trigger channel #1 (samples)
 0          Sample number at which to start pulse
 0        Duration and polarity of pulse on trigger channel #2 (samples)
 0          Sample number at which to start pulse
 0        First episode at which trigger channels #1 and #2 fire
 0        Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
 0        Number of pulses in train (0 for none)
 0        Pre-conditioning pulse duration (ms)
 0        Pre-conditioning pulse amplitude (mV)
 0        Conditioning pulse duration (ms)
 0        Conditioning pulse amplitude (mV)
 0        Post-conditioning train duration (ms)
 0        Post-conditioning train amplitude  (mV)
```

```
      Display data
 5          ADC ch. #15: amplification factor (not 0)
 0                       display offset: fraction of full screen
 2          ADC ch. #14: amplification factor (not 0)
 -0.25                   display offset: fraction of full screen
 1          ADC ch. #13: amplification factor (not 0)
 0                       display offset: fraction of full screen
 1          ADC ch. #12: amplification factor (not 0)
 0                       display offset: fraction of full screen

0          Segment number to display (0..4) (0 for all)
1          Skip factor: plot every Nth point (1..4)
0          Display averaged data: 0 After each episode, N After each N runs
1          Autoerase: 0 No, 1 Yes
1          Graph style: 0 Points, 1 Lines
     Units of measure
pA         ADC ch. #15
mV         ADC ch. #14
mV         ADC ch. #13
mV         ADC ch. #12
mV         DAC ch.  #0 (command)
     Peak detection
0          Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0          Optional sample number to measure, negative for negative peaks
0          Channel number to search
0          Number of samples averaged in search (1-20) (0 for default 5)
0          Baseline: 0 Average first 24 samples, 1 Average interval A
0          Display:  0 Screen, 1 File & screen
     Hardware configuration
10         DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1          ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01         ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1            ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1            ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10         ADC range,  V
10         DAC range,  V
3          Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1            Gain multiplier
100000       Filter cutoff frequency
     Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


Mock run
Episode 5
F vs:
SCRI(A)0
Trial Preview
Running Menu
Erase
100
mV
0
-100
0 (200)
1000
2000 ms

Parameter file SCRI(A)0

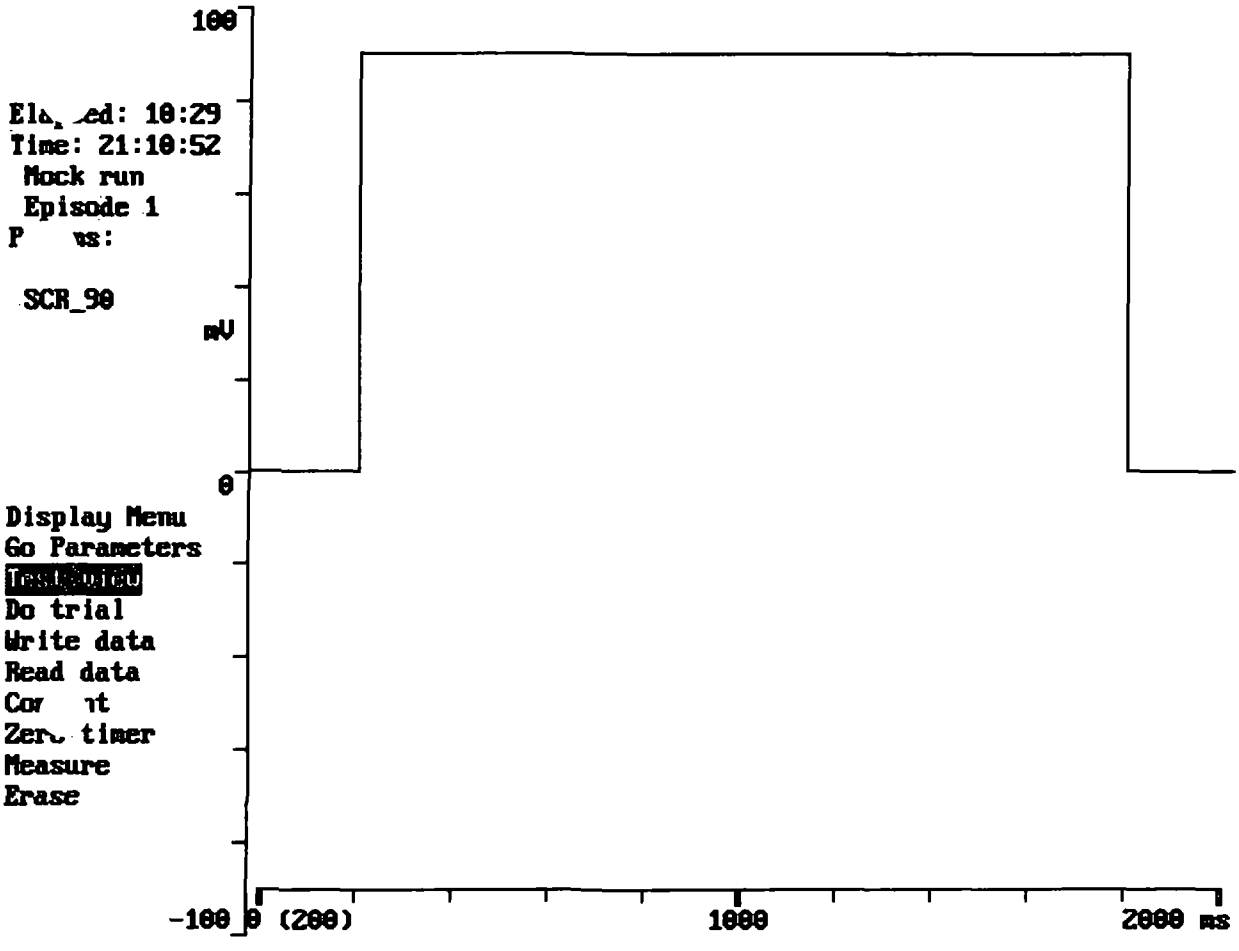
```
      Acquisition
1         Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
13          Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4         Time between start of episodes (s) (0 for maximum rate)
1000        First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)

600         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
-90         Amplitude initial value (mV)
0           Amplitude increment (mV)
1200        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
 0          Duration and polarity of pulse on trigger channel #1 (samples)
 0            Sample number at which to start pulse
 0          Duration and polarity of pulse on trigger channel #2 (samples)
 0            Sample number at which to start pulse
 0          First episode at which trigger channels #1 and #2 fire
 0          Last  episode at which trigger channels #1 and #2 fire
       Conditioning train
 0          Number of pulses in train (0 for none)
 0          Pre-conditioning pulse duration (ms)
 0          Pre-conditioning pulse amplitude (mV)
 0          Conditioning pulse duration (ms)
 0          Conditioning pulse amplitude (mV)
 0          Post-conditioning train duration (ms)
 0          Post-conditioning train amplitude  (mV)
```

```
     Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen

0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
     Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
     Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
     Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
     Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```

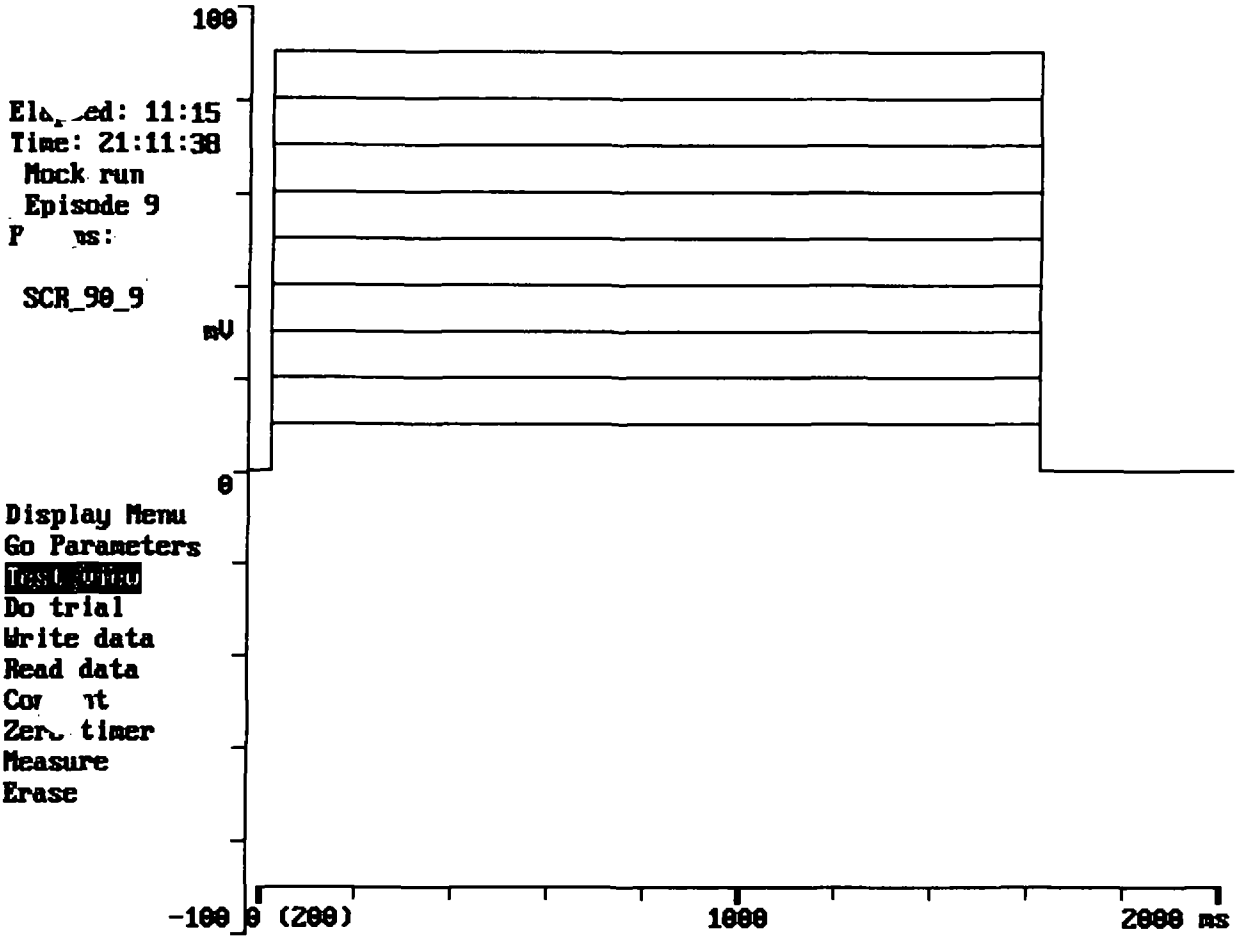

100
Ela ed: 10:29
Time: 21:10:52
Mock run
Episode 1
P vs:
SCR_90
mV
0
Display Menu
Go Parameters
Test Button
Do trial
Write data
Read data
Cor nt
Zero timer
Measure
Erase
-100
0 (200)
1000
2000 ms

```
      Acquisition
-1        Number of trials to perform (-1 for continuous)
1           Number of runs/trial (runs are averaged) (  65536)
1           Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
4         Time between start of episodes (s) (0 for maximum rate)
1000        First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
200         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
90          Amplitude initial value (mV)
0           Amplitude increment (mV)
1600        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0        Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
      Display data
 5          ADC ch. #15: amplification factor (not 0)
 0                       display offset: fraction of full screen
 2          ADC ch. #14: amplification factor (not 0)
-0.25                    display offset: fraction of full screen
 1          ADC ch. #13: amplification factor (not 0)
 0                       display offset: fraction of full screen
 1          ADC ch. #12: amplification factor (not 0)
 0                       display offset: fraction of full screen

 0          Segment number to display (0..4) (0 for all)
 1          Skip factor: plot every Nth point (1..4)
 0          Display averaged data: 0 After each episode, N After each N runs
 1          Autoerase: 0 No, 1 Yes
 1          Graph style: 0 Points, 1 Lines
      Units of measure
pA          ADC ch. #15
mV          ADC ch. #14
mV          ADC ch. #13
mV          ADC ch. #12
mV          DAC ch.  #0 (command)
      Peak detection
 0          Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
 0          Optional sample number to measure, negative for negative peaks
 0          Channel number to search
 0          Number of samples averaged in search (1-20) (0 for default 5)
 0          Baseline: 0 Average first 24 samples, 1 Average interval A
 0          Display:  0 Screen, 1 File & screen
      Hardware configuration
 10         DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
 0.1          ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
 0.01         ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
 1            ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
 1            ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

 10         ADC range,  V
 10         DAC range,  V
 3          Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
 1            Gain multiplier
 100000       Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Elapsed: 11:15
Time: 21:11:38
Mock run
Episode 9
SCR_90_9
mV
0
Display Menu
Go Parameters
Do trial
Write data
Read data
Measure
Erase
-100
0 (200)
1000
2000 ms

@

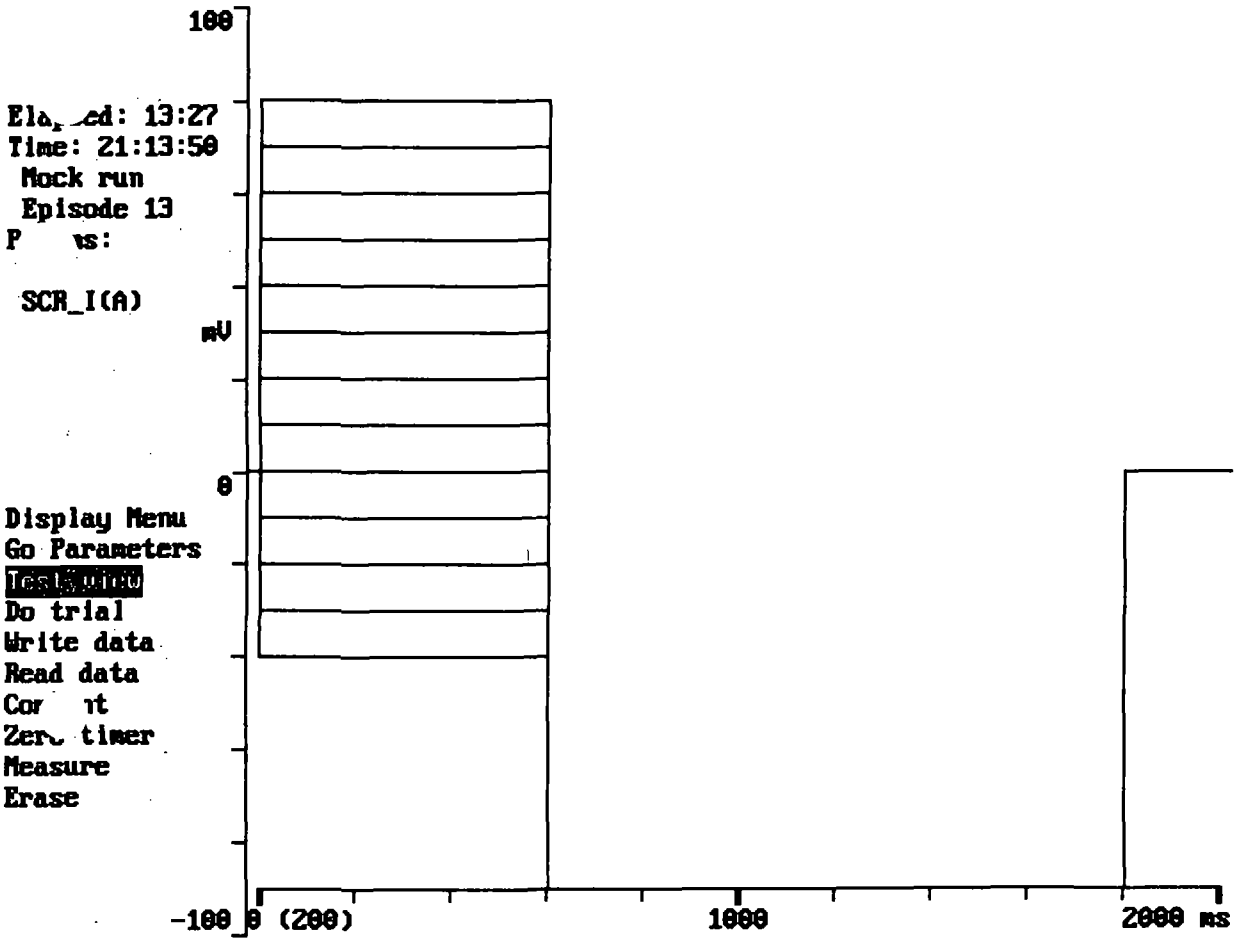
Parameter file SCR_90_9

```
      Acquisition
 12       Number of trials to perform (-1 for continuous)
 1          Number of runs/trial (runs are averaged) (  65536)
 9          Number of episodes/run (if no conditioning pulses)
 0          Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
 1          Starting episode number (normally 1)
 4          Number of 512-sample segments/episode (1..4)
 2          Number of channels to sample (1..4)
 0       Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
 4       Time between start of episodes (s) (0 for maximum rate)
 1000       First  clock interval (samples   1-1024) (  8  s)
 0          Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
 0          Delay between scope trigger and episode start (ms)
      Subtraction
 0       Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
 0          ADC channel number
 0          Subpulse holding amplitude (mV)
 0          Settling time after change of holding amplitude (ms)
 0          Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
 0       Holding amplitude (mV)
 1       A Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 20         Duration initial value (samples)
 0          Duration increment (samples)
 1       B Epoch type: 1 Step, 2 Ramp
 10         Amplitude initial value (mV)
 10         Amplitude increment (mV)
 1600       Duration initial value (samples)
 0          Duration increment (samples)
 1       C Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 1       D Epoch type: 1 Step, 2 Ramp
 0          Amplitude initial value (mV)
 0          Amplitude increment (mV)
 0          Duration initial value (samples)
 0          Duration increment (samples)
 0       Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
 0       Duration and polarity of pulse on trigger channel #1 (samples)
 0          Sample number at which to start pulse
 0       Duration and polarity of pulse on trigger channel #2 (samples)
 0          Sample number at which to start pulse
 0       First episode at which trigger channels #1 and #2 fire
 0       Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
 0       Number of pulses in train (0 for none)
 0       Pre-conditioning pulse duration (ms)
 0       Pre-conditioning pulse amplitude (mV)
 0       Conditioning pulse duration (ms)
 0       Conditioning pulse amplitude (mV)
 0       Post-conditioning train duration (ms)
 0       Post-conditioning train amplitude  (mV)
```

```
      Display data
5           ADC ch. #15: amplification factor (not 0)
0                        display offset: fraction of full screen
2           ADC ch. #14: amplification factor (not 0)
-0.25                    display offset: fraction of full screen
1           ADC ch. #13: amplification factor (not 0)
0                        display offset: fraction of full screen
1           ADC ch. #12: amplification factor (not 0)
0                        display offset: fraction of full screen


0           Segment number to display (0..4) (0 for all)
1           Skip factor: plot every Nth point (1..4)
0           Display averaged data: 0 After each episode, N After each N runs
1           Autoerase: 0 No, 1 Yes
1           Graph style: 0 Points, 1 Lines
      Units of measure
pA          ADC ch. #15
mV          ADC ch. #14
mV          ADC ch. #13
mV          ADC ch. #12
mV          DAC ch.  #0 (command)
      Peak detection
0           Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0           Optional sample number to measure, negative for negative peaks
0           Channel number to search
0           Number of samples averaged in search (1-20) (0 for default 5)
0           Baseline: 0 Average first 24 samples, 1 Average interval A
0           Display:  0 Screen, 1 File & screen
      Hardware configuration
10          DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1           ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01          ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1             ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1             ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)


10          ADC range,  V
10          DAC range,  V
3           Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1             Gain multiplier
100000        Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```


100
Ela ed: 13:27
Time: 21:13:50
Mock run
Episode 13
P vs:
SCR_I(A)
mV
0
Display Menu
Go Parameters
Test
Do trial
Write data
Read data
Cor nt
Zero timer
Measure
Erase
-100
0 (200)
1000
2000 ms

```
      Acquisition
1         Number of trials to perform (-1 for continuous)
10          Number of runs/trial (runs are averaged) (  65536)
13          Number of episodes/run (if no conditioning pulses)
0           Perform interepisode data write: 0 No, 1 Yes(Set future trials >0
1           Starting episode number (normally 1)
4           Number of 512-sample segments/episode (1..4)
2           Number of channels to sample (1..4)
0         Trigger mode: 0 Internal, 1 External, 2 Space bar, 3 Ext-No wavefor
8         Time between start of episodes (s) (0 for maximum rate)
1000        First  clock interval (samples   1-1024) (  8  s)
0           Second clock interval (samples 1025-2048) ( s) (0 to use 1st cloc
0           Delay between scope trigger and episode start (ms)
      Subtraction
0         Number of P/N sub-pulses: 0 None, -N Add, +N Subtract
0           ADC channel number
0           Subpulse holding amplitude (mV)
0           Settling time after change of holding amplitude (ms)
0           Time interval between sub-pulses (ms)
      Waveform on analog output channel #0
0         Holding amplitude (mV)
1         A Epoch type: 1 Step, 2 Ramp
-40         Amplitude initial value (mV)
10          Amplitude increment (mV)
600         Duration initial value (samples)
0           Duration increment (samples)
1         B Epoch type: 1 Step, 2 Ramp
-90         Amplitude initial value (mV)
0           Amplitude increment (mV)
1200        Duration initial value (samples)
0           Duration increment (samples)
1         C Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
1         D Epoch type: 1 Step, 2 Ramp
0           Amplitude initial value (mV)
0           Amplitude increment (mV)
0           Duration initial value (samples)
0           Duration increment (samples)
0         Inter-episode amplitude: 0 Holding, 1 Last epoch amplitude
      Trigger outputs
0         Duration and polarity of pulse on trigger channel #1 (samples)
0           Sample number at which to start pulse
0         Duration and polarity of pulse on trigger channel #2 (samples)
0           Sample number at which to start pulse
0         First episode at which trigger channels #1 and #2 fire
0         Last  episode at which trigger channels #1 and #2 fire
      Conditioning train
0         Number of pulses in train (0 for none)
0         Pre-conditioning pulse duration (ms)
0         Pre-conditioning pulse amplitude (mV)
0         Conditioning pulse duration (ms)
0         Conditioning pulse amplitude (mV)
0         Post-conditioning train duration (ms)
0         Post-conditioning train amplitude  (mV)
```

```
      Display data
5         ADC ch. #15: amplification factor (not 0)
0                      display offset: fraction of full screen
2         ADC ch. #14: amplification factor (not 0)
-0.25                  display offset: fraction of full screen
1         ADC ch. #13: amplification factor (not 0)
0                      display offset: fraction of full screen
1         ADC ch. #12: amplification factor (not 0)
0                      display offset: fraction of full screen


0         Segment number to display (0..4) (0 for all)
1         Skip factor: plot every Nth point (1..4)
0         Display averaged data: 0 After each episode, N After each N runs
1         Autoerase: 0 No, 1 Yes
1         Graph style: 0 Points, 1 Lines
      Units of measure
pA        ADC ch. #15
mV        ADC ch. #14
mV        ADC ch. #13
mV        ADC ch. #12
mV        DAC ch.  #0 (command)
      Peak detection
0         Search mode: 0 None, 1 A, 2 B, 3 C, 4 D, 5 All, 6 Use sample number
0         Optional sample number to measure, negative for negative peaks
0         Channel number to search
0         Number of samples averaged in search (1-20) (0 for default 5)
0         Baseline: 0 Average first 24 samples, 1 Average interval A
0         Display:  0 Screen, 1 File & screen
      Hardware configuration
10        DAC ch. #0: gain from DAC to cell (mV @ cell / V @ DAC)
0.1         ADC ch. #15: gain from cell to ADC (V @ ADC / pA @ cell)
0.01        ADC ch. #14: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #13: gain from cell to ADC (V @ ADC / mV @ cell)
1           ADC ch. #12: gain from cell to ADC (V @ ADC / mV @ cell)

10        ADC range,  V
10        DAC range,  V
3         Autosample Axopatch-1: 0 Manual, 1 Yes, 2 Yes(inverted), 3 Disabled
1           Gain multiplier
100000      Filter cutoff frequency
      Times
          Start time for this data file
          Elapsed time at start of this data file
          Date this data file acquired
```

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