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VOLTAGE CLAMP MEASUREMENT SET-UP FOR INVESTIGATION OF MEMBRANE PARAMETERS

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The voltage clamp measurement method developed by HODGKIN—HUXLEY—KATZ has recently come into widespread use (HODGKIN et al., 1951). According to this method, the potential of the investigated cell membrane is shifted from a defined quiescent value to different values by applying a square-wave, and the potential is held at these shifted values with high accuracy by the use of a feedback amplifier. The potential values are within the normal operating range of the cell; recording of the membrane currents pertaining to different potentials is thus possible with sufficient accuracy. The recorded responses may be used to determine the specific quality and quantity cell parameters, and problems involving the potential generation of the cell may be investigated with sufficient details.

The measurement method is explained by *Fig. 1*, showing the simplified equivalent circuit of the voltage clamp circuit made up of the control amplifier, the cell membrane and the current amplifier. Principal parameters of the method are determined as follows. The difference between amplifier input command voltage E_c and instantaneous membrane voltage V_m will result in amplifier output voltage $A(E_c - V_m) = V_0$. Output voltage V_0 is loaded by

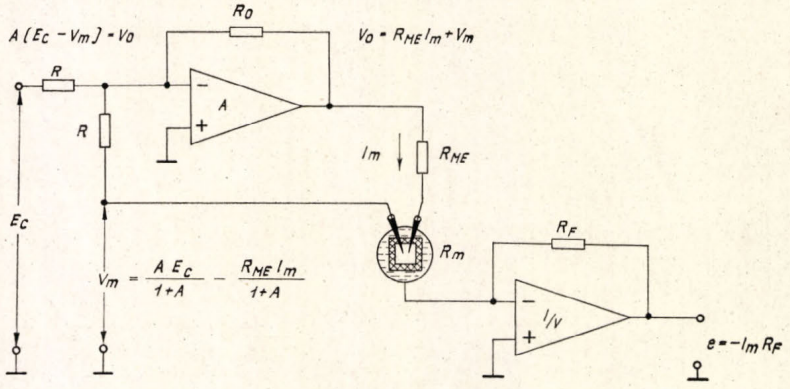

Fig. 1. Equivalent circuit of the voltage clamp measurement set-up

current electrode resistance R_{ME} and membrane resistance R_m. From these, R_m is not constant, and the instantaneous membrane potential V_m is generated across this resistance. From the two equations, V_m may be expressed, and membrane current I_m can be deduced from the I/V converter output voltage.

It is seen from the expressions presented in *Fig. 1* that increasing the gain of the control circuit will decrease the effect of current electrode resistance R_{ME}, and the membrane voltage V_m will approximate voltage $E_cA/\ 1+A$ which is the command voltage E_c. The current measurement accuracy is dependent on the difference between E_c and V_m so the control circuit gain should be chosen as high as possible (NASTUK, 1963; COLE, 1968).

With a given gain, the difference δ between E_c and V_m is determined from the following relation:

$$\delta = E_c - V_m = \frac{E_c + R_{ME} I_m}{1 + A}$$

Thus the gain required from a given difference is given by

$$A = \frac{E_c + R_{ME} I_m}{\delta} - 1$$

Fig. 2 shows the block diagram of the voltage clamp measurement set-up as completed in our Biological Research Institute. A mode switch is provided to choose between two-channel normal recording and voltage-clamp investigation. A chamber for preparation having adjustable temperature is also provided for temperature investigations, in addition to substance investigations.

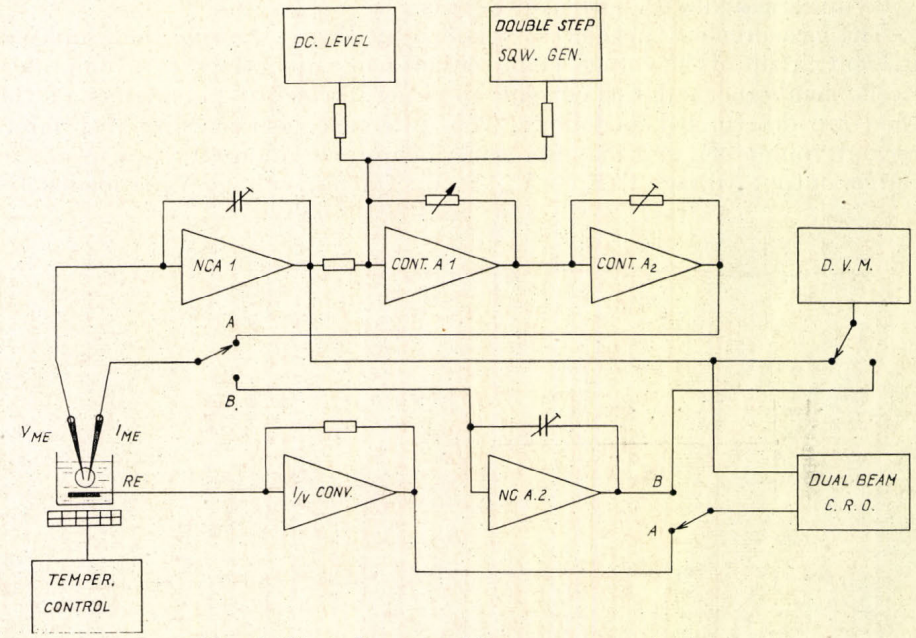

Fig. 2. Block diagram of the measurement set-up

According to the arrangement shown in *Fig. 2*, the change of membrane potential is sensed by a FET input amplifier, the output of which is connected to the input of a two-stage control amplifier. This is also driven by the square-wave generator supplying the command signal and by the adjustable DC signal generating the quiescent membrane potential. The current necessary for fixing the adjusted membrane potential level is generated by the difference of the command signal driving the control amplifier and the instantaneous membrane potential. Thus the necessary membrane current flowing through the current micro-electrode is supplied by the second control amplifier. Membrane current is sensed by a current-voltage converter giving an output voltage proportional to the instantaneous membrane current (CHAMBERLAIN and KERKUT, 1969).

The circuit diagram of the voltage clamp measurement set-up is shown in *Fig. 3*. More important details are explained as follows (TOBEY et al., 1971).

NCA-1. This is a non-inverting type negative capacitance amplifier sensing the membrane potential change through V_{ME}, and having an input impedance of 10^{11} ohms. It has unity gain and a ten times internal gain for neutralizing the input capacitance. The output of this amplifier may be connected to the digital voltmeter thus making possible, without external instrumentation, simple adjustment of zero level, compensation of voltage electrode tip potential and measurement of voltage electrode resistance.

NCA-2. Inverting type negative capacitance amplifier for control of I_{ME} depth of penetration. It has an input impedance of 10^{11} ohms and ten times gain. During measurement, it serves to make possible a switch-over from normal connection to voltage clamp measurement. The NCA-2 amplifier has compensating and control circuits similarly to NCA-1.

CONT. A-1. This is a three-input analog adder with an amplification adjustable from 40 dB to 56 dB. The three inputs are fed by the DC voltage generating the quiescent potential, the instantaneous membrane voltage and the square wave generator command signal. The amplifier output, supplying the algebraic sum of the three signals multiplied by amlifier gain A, is connected to amplifier CONT. A-2.

CONT. A-2. Noninverting type amplifier with a gain adjustable between 20 and 26 dB. The amplifier output supplies the current, flowing through the current electrode, necessary for clamping the cell membrane voltage.

The two-stage control amplifier solution was justified by the requirement for following rapid potential changes. With the gain required, this was the only means to realize the required bandwidth.

I/V CONV. Operational amplifier with current amplification circuit. The current of the high impedance signal source, flowing through the feedback resistance, generates an output voltage $e_0 = -I_m R_F$. Thus the error of current measurement is given by the accuracy and stability of resistance R_F. In the current-voltage converter for membrane current sensing, a high stability $R_F = 1$ megohm $\pm 1\%$ resistor has been applied.

Double step SQW gen. The second generator is triggered by the square wave of the first generator. Time durations, amplitudes and polarities of the two square waves are independently adjustable. Maximum pulse duration is 40 seconds and maximum amplitude is ± 200 mV, which data meet all test requirements. The square wave generator is single-shot operated by a push-button, but an external trigger signal may also be used.

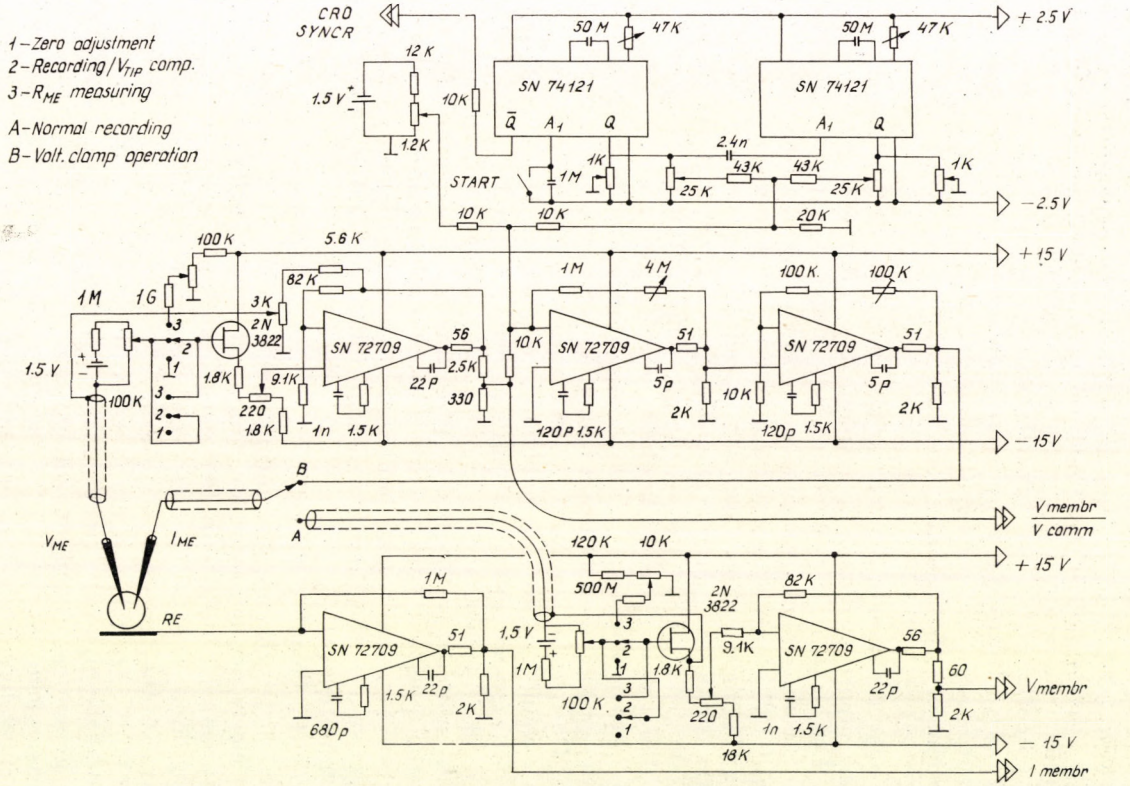

Fig. 3. Circuit diagram of the measurement set-up.
1 — Zero adjustment, 2 — Recording/V_{tip} comp, 3 — R_{ME} measurement, A — normal recording, B — voltage clamp operation

DC LEVEL. The membrane quiescent potential within the cell operating range is set by the DC level at the summing amplifier input. DC adjustment range is zero to +200 mV.

MODE SWITCH (A, B). This serves for simplifying and speeding up the test procedure. According to the test sequence, switch position *A* is used for two channel recording. In this mode, the zero level adjustments of amplifiers NCA-1 and NCA-2, the tip-compensation of V_{ME} and I_{ME} and their resistance measurements by the digital voltmeter are performed. Following these procedures, the suitable penetration depth of the two electrodes can be checked on a double-beam scope. In switch position *B* the circuit is suitable for voltage clamp measurements. In this arrangement, the quiescent potential of the cell can be checked by the digital voltmeter. According to the measurement, one scope channel serves for membrane potential indication and the other channel for membrane current indication.

The highest ion current which may be compensated by the voltage clamp circuit of *Fig. 3* can be calculated by the expressions given in *Fig. 1.* With a 5 megohm current electrode, the highest current which can be compensated is $\pm$ 3 μA which is suitable for our purposes.

The accuracy of membrane current measurement may be determined from Fig. 1. Our tests showed that a membrane potential change of E_c + 70 mV caused a membrane current change of $I_m = 800$ nA. Current electrode resistance was $R_{ME} = 5$ megohm and amplification was $A = 10^4$. Thus accuracy is calculated as follows:

$$\delta = \frac{E_c + R_{ME} I_m}{1 + A} = \frac{70 \,.\, 10^{-3} + (5 \,.\, 10^6 \,.\, 8 \,.\, 10^2 \,.\, 10^{-9})}{10^4} = 0.407 \text{ mV}$$

$\delta = 0.407$ mV voltage error corresponds to 4.65 nA current error, so the accuracy of current measurement is 581% if a linear relation between command voltage and current is assumed. The actual δ-error will be worse because of nonlinear membrane current sub-ranges, but will still meet the requirements.

A photograph of the voltage clamp measurement set-up together with the chamber for preparation is shown in *Fig. 4.* The circuits are housed in subracks corresponding to the block diagram groupings. The photograph shows in the right column the amplifier NCA-1, the I/V converter and control amplifier (in a single subrack), the NCA-2 amplifier and the $\pm$ 15 V power supply feeding all circuits. The center part shows the digital voltmeter and the mode switch, and the unit supplying the temperature control of the chamber for preparation is shown at the left side.

Summary

According to the voltage clamp method, measurement is performed by the aid of the two intracellular glass microelectrodes applied to the nerve cell. One of the electrodes is used to record the changing voltage of the cell membrane. This voltage and a defined command step voltage are applied to a summing network, and the current corresponding to the sum is applied to the other (current) electrode. Membrane voltage is thus constant with good

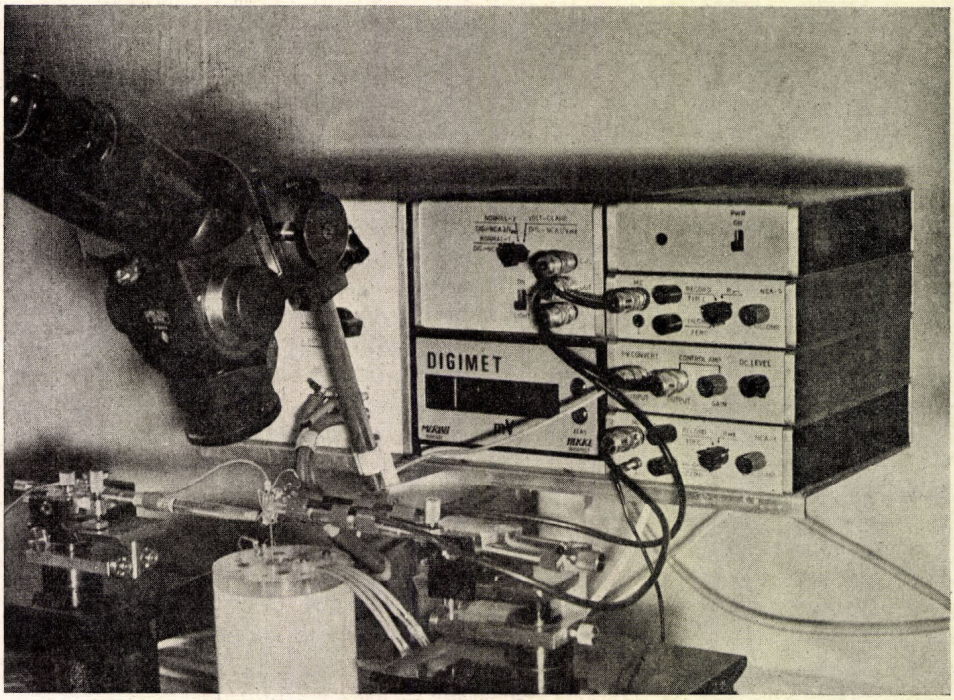

Fig. 4. Photograph of the measurement set-up

approximation, and the ion currents flowing during the measurement may be recorded by a suitable current-voltage converter.

In the paper, the basic measurement principle, the design criteria of the amplifier parameters, the data of the double step square wave generator and the detailed circuit description used for actual measurements are presented.

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VOLTAGE CLAMP MÉRÉSI ÖSSZEÁLLÍTÁS MEMBRÁN PARAMÉTEREK VIZSGÁLATÁHOZ

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Összefoglalás

A voltage clamp módszernek megfelelően a mérés az idegsejtben intracellulárisan bevitt két üvegmikroelektróda segítségével történik. Az egyik elektróda a sejtmembrán változó feszültségét regisztrálja. A membrán feszültség egy meghatározott idejű konstans feszültséggel együtt összegző áramkörre kerül és az eredménynek megfelelő áram folyik át a második, úgynevezett áramelektródán. A membrán feszültsége a mérés alatt jó közelítéssel állandó, így a mérés alatt folyó ionáramok megfelelő áram-feszültség konverter segítségével regisztrálhatók.

A dolgozat ismerteti a mérés alapelvét, az erősítők paramétereit meghatározó szempontokat, a konstans feszültséget szolgáltató négyszöggenerátor adatait és a konkrét méréshez használt összeállítás részletes áramköri megoldásait.