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Voltage Clamp on Helix Pomatia Neuronal Membrane; Current Measurement over a Limited Area of the Soma Surface

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Zusammenfassung. Es wurde eine Methode entwickelt, die es gestattet, während eines „Voltage-Clamp" Experiments nur diejenigen Ströme zu messen, die über ein begrenztes Stück der Somamembran fließen.

Axonströme, die beim üblichen „Voltage-Clamp" die Ergebnisse verfälschen, werden aus der Messung ausgeschlossen. Um dies zu erreichen, wird zusätzlich zur intracellulären Clampelektrode eine flammenpolierte Pipette auf das Zellsoma aufgesetzt. Das Innere der Pipette wird mittels eines einfachen weiteren Clampmechanismus auf dem Potential des Außenmilieus gehalten, die dazu erforderlichen Clampströme werden gemessen.

In einer alternativen Anwendung verbleibt das Zellinnere und dadurch auch das Axon mittels des intracellulären Clamps im Ruhezustand, während dem Pipetteninneren ein negativer Spannungssprung aufgeprägt wird. Über dem beobachteten Membranstück herrschen dann dieselben Spannungsverhältnisse wie oben. Diese letztere Methode ist immer dann von Vorteil, wenn bei größeren Zellen oder infolge sehr großer erforderlicher Stromdichten in der intracellulären Stromelektrode kein homogener Clamp über die ganze Zelle erreicht werden kann.

Schlüsselwörter: „Voltage-Clamp" über begrenzte Membranstücke — Schneckenganglienzellen — Membranen.

Summary. A method was developed, to measure during a voltage clamp the current which flows through a limited area ($10^4\ \mu^2$) of the soma membrane. In addition to an intracellular voltage clamp electrode, a fire-polished glass micropipette is placed on the soma surface (with slight suction applied), and only the clamp current through the pipette is measured. This measurement excludes uncontrolled currents from the axon. Alternatively the membrane "spot" (area under the pipette) can be voltage clamped while the rest of the cell is kept in the resting state. The latter method is an advantage with bigger cells where homogeneous clamp over the whole surface cannot be achieved.

Key-Words: Voltage Clamp on limited Membrane Patches — Snail Neurons — Membranes.

During a voltage clamp on the soma of snail neurons the interior of the cell body can be considered an equipotential, since the electrolyte resistance of the soma interior and the maximum current flows observed cause potential variations smaller than 2—3 mV (see also HELLERSTEIN, 1968). However, more distant parts of the axon are increasingly separated

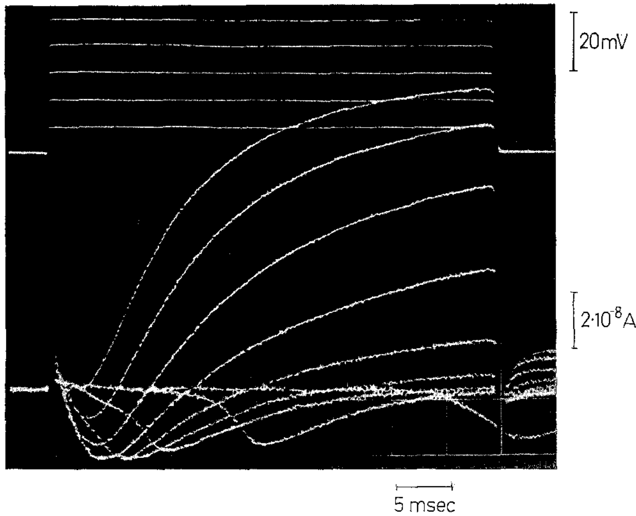

Fig. 1. Overall intracellular voltage clamp. Inward currents downward going; superimposed traces for 10, 20, 30, 40, 50, 60, 70, 80, 90 mV depolarizing steps. Upper voltage traces not on screen

from the soma by the longitudinal electrolyte resistance, and therefore are not necessarily equipotential with the cell soma. Depolarizations applied to the soma will spread electrotonically down the axon and may elicit a spike in the spike initiation region. This spike will propagate towards the soma and contribute appreciably to the clamp current although attenuated by clamping action. These contributions are typically seen in Fig. 1, where they cause transient inward currents. The transients here occur at the end of the voltage clamp pulse at very low depolarizations (10 mV). They occur earlier and originate probably at varying axonal sites during higher clamp voltages. They finally merge with the inward clamp current of the soma membrane (at 30—40 mV). It was our objective to exclude these uncontrolled axon currents, and to obtain voltage clamp data on a limited patch of soma membrane. To achieve this, we placed a fire-polished micropipette filled with the extracellular fluid medium on the cell surface (with some suction)[1], to measure the current flow through the pipette during a voltage clamp with the pipette interior kept at the same potential as the solution outside (Fig. 2). FRANK and TAUC (1963) used a similar method without suction. We

[1] The suction was between 2 and 10 mm Hg. We found it to be helpful during the approach of the pipette. In most cases it resulted in an appreciable increase of leakage resistance (R_2 in Fig. 3). Once this was obtained, suction was found no longer to be necessary.

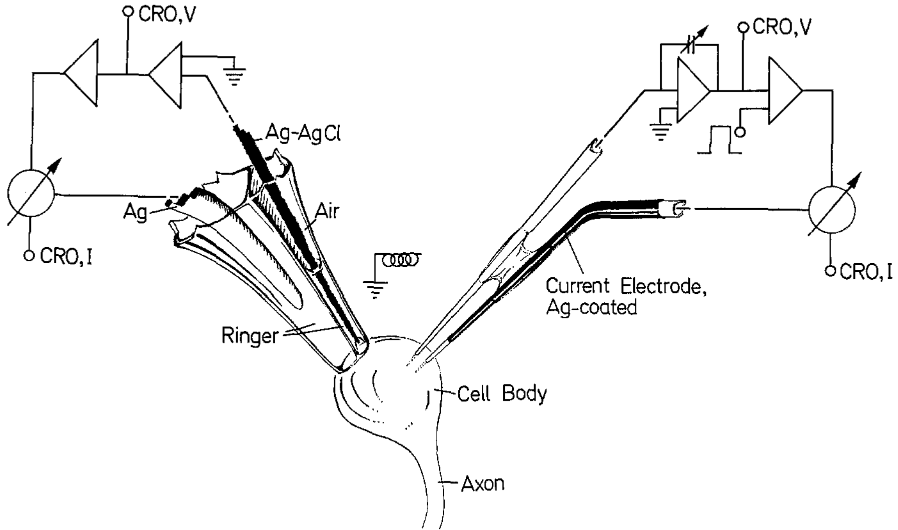

Fig. 2. Schematic diagram of the electronic system, and geometry of the pipette. Current is measured with high input impedance operational amplifiers

examined the reliability of this type of current measurement and modified it to get physically well defined results.

It was assumed by FRANK and TAUC that the currents being measured in the pipette were exactly equal to those that flowed transverse to the patch of membrane underlying the opening of it. We checked this hypothesis by measuring the resistance between the interior of the pipette and the indifferent electrode in the preparation chamber, while moving the tip towards the cell surface. One would expect this resistance to increase considerably as soon as the membrane covers the pipette tip. We found initially that the resistance increase was only by a factor of 1.2. This would mean that the pipette tip was separated from the soma membrane by a distance comparable to the tip diameter (100—150 μ) in these experiments, although it seemed with visual observation that the tip was sitting directly on the spherical unipolar cell body (the connective tissue capsule had been removed). Application of more suction to the pipette did not change the results.

Anatomically, these cells are packed within glial cells and connective tissue. Therefore we cut deeper at the side of cell clusters until some cells seemed to protrude. By this procedure many cells were damaged but those remaining intact showed a different behaviour with pipette measurements. When approaching an intact protruding cell, we observed an increase of resistance by factors up to 30. In this state action potentials of up to 90 mV could be measured with an intracellular electrode for

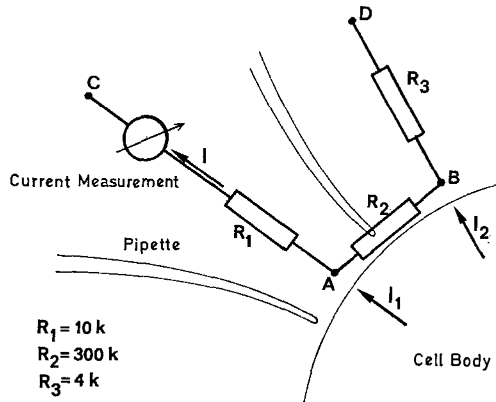

Fig. 3. Equivalent circuit of a simple pipette in contact with a spherical cell body. Point A represents an equipotential inside at the tip. Point B represents a surface enclosing all the rest of the soma. C and D are points far away from the soma. They are connected via the indifferent electrode. A current flow I_1 through the patch of membrane under the pipette opening acts in the circuit as if it was injected into it at point A. Similarly a current I_2 flowing through the rest of the membrane appears as injected at B. R_1 is the volume resistance within the pipette, R_2 is a leakage resistance under the rim of the pipette, and R_3 is the volume resistance between the cell surface and the indifferent electrode. All values are for a pipette without a voltage clamp

hours, indicating that the cells did not suffer from the procedure. The described handicap was probably less in aplysia (Frank and Tauc, 1963) where single neurons protrude readily after opening of the capsule. With this large resistance between the pipette and the surrounding solution, reliable current measurements can be made. Looking at the equivalent circuit (Fig. 3) one can see that a current I_1 passing through the membrane under the pipette will flow through R_1 (10 kΩ) rather than through the series combination of R_2 and R_3 (300 kΩ).

Similarly a current I_2 through the rest of the membrane will flow toward the indifferent electrode.

For measuring I (see Fig. 3), we clamped the potential of the pipette interior (not to be confused with the voltage clamp on the cell itself) to that of the indifferent electrode and recorded this clamp current. In this way the effective value of R_1 is decreased by a factor equivalent to the amplification factor of the feedback loop (50—100). Thus almost complete separation of currents I_1 and I_2 is obtained.

An improvement over this method can be made by clamping the voltage difference across the pipette wall to zero volts (making the voltage across R_2 zero).

Simultaneous measurement of the currents recorded by the intracellular clamp electrode and the current measured through the pipette

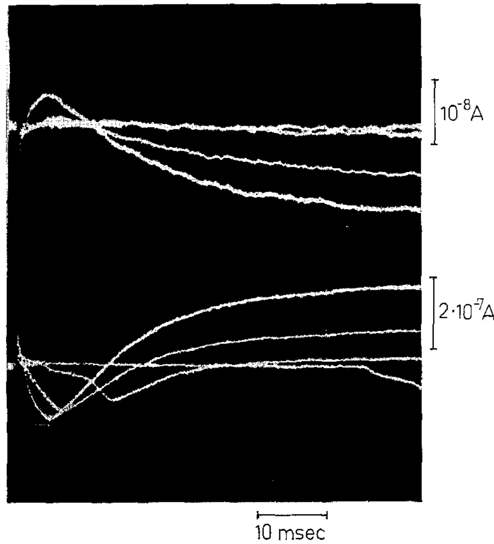

Fig. 4. Simultaneous measurement of the overall intracellular clamp current (lower traces) and the current through the pipette (upper traces, inversed polarity). Superimposed for 30, 40, 50 and 60 mV depolarizing pulses. Note the late inward transients in the lowest traces which are not present in the corresponding upper traces. Active soma current is present only for depolarizing pulses of 50 mV and more

shows a clear suppression of the late inward transient mentioned above (Fig. 4). This proves that this transient current is generated somewhere remote from the soma, probably in the axon, and that the soma patch current can behave quite differently from the overall clamp current. A systematic study of different types of patch currents will appear later (NEHER, in preparation).

An alternative way of performing this experiment is to keep the potential inside the soma constant with the intracellular clamp, and to apply a negative going potential step to the pipette interior. With this the membrane patch under the pipette is depolarized the same way as before, whereas the remainder of the cell stays in the resting condition. Currents entering the cell through the depolarized region leave it via the current electrode of the intracellular clamp where they can be measured. This experiment has the advantage that all axon interference is eliminated by keeping the axon in the resting state. However, current measurement is difficult since the noise current of the whole cell clamp is superimposed on the clamp current of a small part of this cell. Nevertheless, this method will be of advantage in all cases when the relation between volume resistance of the intracellular electrolyte

and the resistance of the membrane does not allow a homogeneous potential over the excitable area. The method is also useful when instantaneous overall clamp currents are too high to be conducted by a microelectrode.

Preparation. The suboesophagael ganglion of Helix pomatia snails was mounted in a lucite chamber and the connective tissue sheath removed under a dissecting microscope. The dorsal surface was cleaned as carefully as possible and clusters of cells exposed as described in the text. Various giant cells with diameters of 150—300 μ were used.

Intracellular Clamp. Two microelectrodes were glued together with a tip separation of 20—30 μ. One of them, the current electrode, was electrolytically coated with silver to within 50 μ of the tip (LUX, 1967). This silver coating was grounded to reduce capacitive coupling. Tip diameters and resistances of the electrodes were 1—2 μ and 5—10 MΩ respectively. The input signal from the other electrode was fed directly (over a distance of ~ 6 cm) to the input pin of a Nexus-FET operational amplifier which was mounted on the micromanipulator. The input amplifier (QFT-2B Philbrick/Nexus) had an input resistance of 10^{11} Ω and an offset current of 20 pA. The gain was adjusted to 10. With subsequent inversion of the signal and further magnification (Tektronix 1A7A and 132) a time constant for the clamp of 20 μsec could be obtained (with amplification factors up to 10,000).

Pipette spot clamp. For measuring the pipette current, the interior of the pipette tip was clamped to the indifferent electrode potential (see Fig. 2). This clamp is not very difficult because of the low resistances involved. Since all time constants are extremely small, one large time constant (100 μsec) is introduced in an amplification stage, to provide dynamic stability of the feedback loop for magnification factors up to 100. This procedure resulted in an overall pipette clamp time constant of about 20 μsec.

The pipette should contain a silver wire as a current electrode, and an Ag-AgCl electrode for measuring the potential at the pipette tip. Both electrodes have to be uncoupled capacitively as well as possible.

We found it easiest to make pipettes from 2 mm tubing asymmetrically divided into two chambers (delivered by W. Krannich KG, Göttingen). This tubing was pulled like normal pipettes, the tip cleaved on a microforge, and a small piece of the center divider removed. The opening (100—150 μ) was then fire-polished. Details of the pipette geometry are seen in Fig. 2. It is significant that the area of close approach of the electrolytes in both chambers is kept very small thereby minimizing capacity.

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