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T19. Patch-clamp

Living cells are covered with a membrane, the structural basis of which is a double layer of lipids that is impermeable to water and practically impermeable to ions. Each cell must exchange various substances and, especially ions, with the external environment. The transport of ions across the membrane plays an important role in the processes of cell excitation and signaling. Ions penetrate into and out of the cell through proteins built into the membrane which called channels. Channels are proteins that function as membrane pores by forming openings through which ions can pass. Membrane channels are selective - they are only permeable to certain substances. Selectivity is determined by the radius of the pores and the distribution of charged functional groups in them. There are channels that selectively allow sodium ions to pass through (sodium channels), as well as potassium, calcium and chloride channels. (See Fig. 1.)

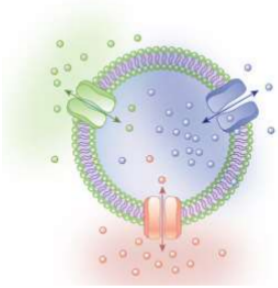


Figure 1. A cell with channels permeable to various substances

Part A. Single channel & Whole cell patch clamp

There are light-gated ion channel - channel rhodopsins. These channels transition from a closed state, in which they are impermeable to ions, to an open state, absorbing photons of a certain wavelength (see Fig. 2).

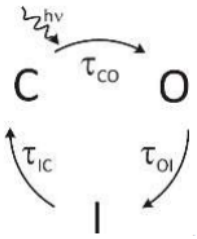


Figure 2. Photocycle of channel rhodopsin, three-state

Next, the protein passes through several intermediate states to return from the open state to the closed state (in the following, we will assume that in intermediate states the channel is also impermeable to any ions). All these states together are called a photocycle (see Fig. 3, which shows a photocycle in which there is only one intermediate state I. C and O - closed and open states, respectively). Transitions between these states occur with some probability. The transition from a closed state to an open state is impossible without the absorption of light. In the presence of light, this transition also occurs with some probability.

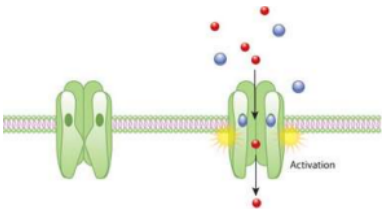


Figure 3. Closed and open states of the channel

The probability of a transition between two states A and B is described by the value τ - the characteristic time of the transition from A to B. This time is defined as the inverse derivative of the transition probability over time: $\tau_{AB} = \left(\frac{dp}{dt}\right)^{-1}$, i.e. the transition probability over time dt is equal to dp.

A1	Consider a protein for which the only two possible states are A and B. Suppose that a direct transition from state A to state B occurs with a known characteristic time τ_{AB}, and the reverse transition does not occur (actually it could be described with $\tau_{BA} \to \infty$). Consider a sample containing N of such proteins. Let them all be in state A at the initial moment. Find the dependence of the number of proteins in each state as a function of time $N_A(t)$ and $N_B(t)$. Express your answers in terms of τ_{AB} and N.

Due to the free passage of charged particles (ions), the channels in the open state effectively increase the electrical conductivity of the cell membrane. To study the electrical properties of the membrane and study the properties of ion channels, there is a method of local potential fixation (Patch-clamp). In this method, a glass pipette is used to make contact with a cell membrane. This contact has a resistance of several gigaohms - this is the so-called gigaohm contact. One electrode is placed in a pipette filled with electrolyte, the second electrode is placed extracellularly, in the external fluid.

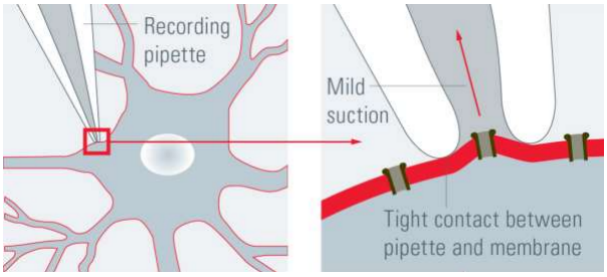


Figure 4. Patch-clamp. Formation of the gigaohm contact.

To measure the conductance of individual channels, the pipette is detached from the rest of the cell with a fragment of the membrane inside. The narrow tip of the pipette leaves such a small portion of the membrane that no more than one channel can be built into it. In the case of photosensitive proteins, the current flowing through a fragment of the membrane is measured: jumps in current when the light is turned on indicate the opening of the light-gated channel. This method is called Single Channel Patch Clamp. (see Figure 5. Obtaining a membrane fragment, the right picture is a selected fragment with one channel).

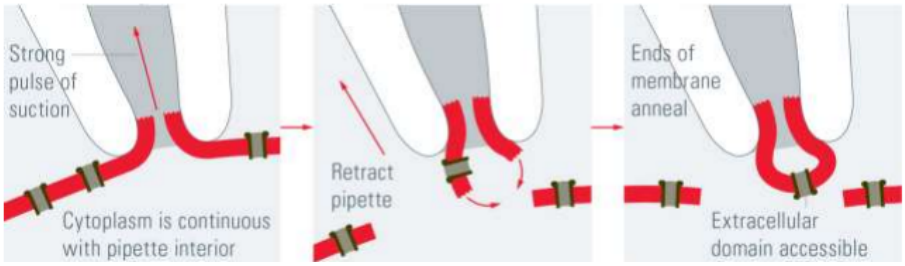
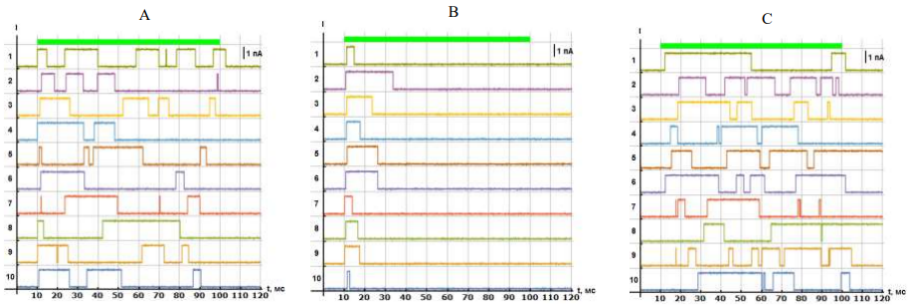


Figure 5. There are the next after Fig.4 steps of obtaining membrane for Single Channel Patch Clamp.

Let's consider measurements using the Single Channel Patch Clamp method for three channels. All three channels have photocycles consisting of three states: C (closed), O (opened) and I (intermediate). The channels have the following characteristic transition times:

1. $\tau_{CO} = 10$ ms, $\tau_{OI} = 10$ ms, $\tau_{IC} = 0{,}1$ ms
2. $\tau_{CO} = 1$ ms, $\tau_{OI} = 10$ ms, $\tau_{IC} = 30$ ms
3. $\tau_{CO} = 1$ ms, $\tau_{OI} = 10$ ms, $\tau_{IC} = 1000$ ms.

The graphs show 10 of current versus time plots for each channel (the green line shows the time when the light is on).



A2	Indicate which ones belong to which channel? The height of the step is the same everywhere and is equal to $I = 1.4$ pA.

When measuring the conductivity of single channels, the current is very small and difficult to detect. In order to avoid this difficulty, the other method is used: a fragment of the membrane is broken through by excess pressure, and the cell remains attached to the pipette. Thus, it turns out that the electrode inside the pipette is separated from the electrode in the external liquid by a complete cell membrane. In this case, the solution inside the cell becomes the same as that which was poured into the pipette. This method is called Whole Cell Patch Clamp (see Figure 5, the left figure shows a Whole Cell contact, see Figure 6, which shows the entire cell) and it allows you to measure the currents caused by the conductivity of all channels located in the cell membrane together.

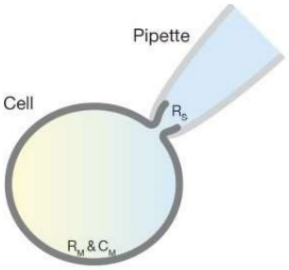


Figure 6. Whole Cell contact

A3	Let's consider 3 cells, the membranes of which have built-in channels described in the previous paragraph. In the membrane of each cell there are many ($\gg 1$) channels, all of the same type. Measurements are made using the Whole Cell Patch Clamp method. The light turns on and in all three cases they wait until the current readings are established, after which the light turns off. Draw qualitatively the current versus time dependence for all three cells. What is the steady current for each of the cells if the voltage applied between the electrodes and the solutions in the pipette and in the external liquid were left unchanged after the experiment in point A2. Express your answers in terms of the number of channels in the cell $N = 1000$, τ_{CO}, τ_{OI} and τ_{IC}.

Part B. Nernst–Planck equation

When the channel is open, ions can pass through it by two factors: an external electric field and diffusion.

B1	Find the total flux J of potassium ions through the selective potassium channel. Express your answer in terms of the diffusion coefficient D, the molar concentration c of potassium outside the cell and its derivative $\frac{dc}{dx}$ along the channel, the mobility μ of the potassium ions, the elementary charge e and the electric field E. *Remark:* mobility is the coefficient of proportionality between the drift velocity v of the particles and the force acting on them: $\mu F = v$.

The connection between the diffusion coefficient D, mobility μ and the temperature T was discovered when Einstein and Smoluchowski independently studied the Brownian motion. Einstein-Smoluchowski equation:

$$D = \mu k T,$$

where k is Boltzmann's constant.

B2	Find at the voltage U_{rev} on the cell membrane (membrane potential) that corresponds to zero flux through the open potassium channel at temperature $T = 20°\text{C}$. Express your answer in terms of T, $c_{\text{out}} = 4$ mmol/l and $c_{\text{in}} = 155$ mmol/l – potassium concentrations outside and inside the cell, and fundamental physical constants. *Remark:* the membrane potential is considered positive if the plus is on the inner surface of the cell membrane.

Part C. Normalization and determination of channel selectivity

In a real electrophysiological experiment, it is necessary to collect statistics, so the dependence of the current on time is measured for different voltages applied to the cell, and the experiment is also repeated for other cells with the same channels in the membrane. But all cells are different sizes and therefore the have different numbers of channels. Hence, it's necessary to normalize the current to a value proportional to the amount of proteins in the cell membrane. In electrophysiology it is common to normalize the current to the electrical capacitance of the cell (because the capacitance is proportional to the membrane area $C \propto S \propto N$).

Other electrical characteristics of the cell (R_M is the electrical resistance of the cell membrane (when all channels are closed), R_S is the so-called «access» resistance to the cell) also affect the signal that is measured during whole-cell patch clamp measurements. An equivalent cell circuit is shown in Figure 7.

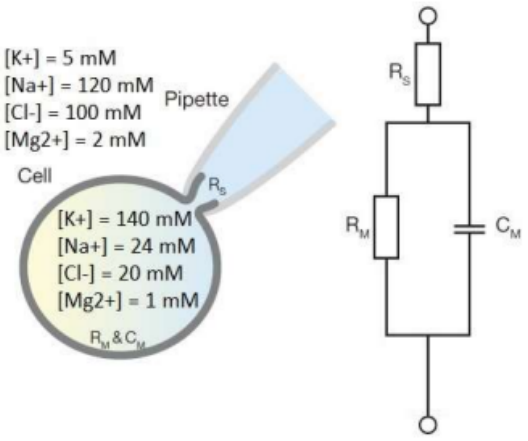
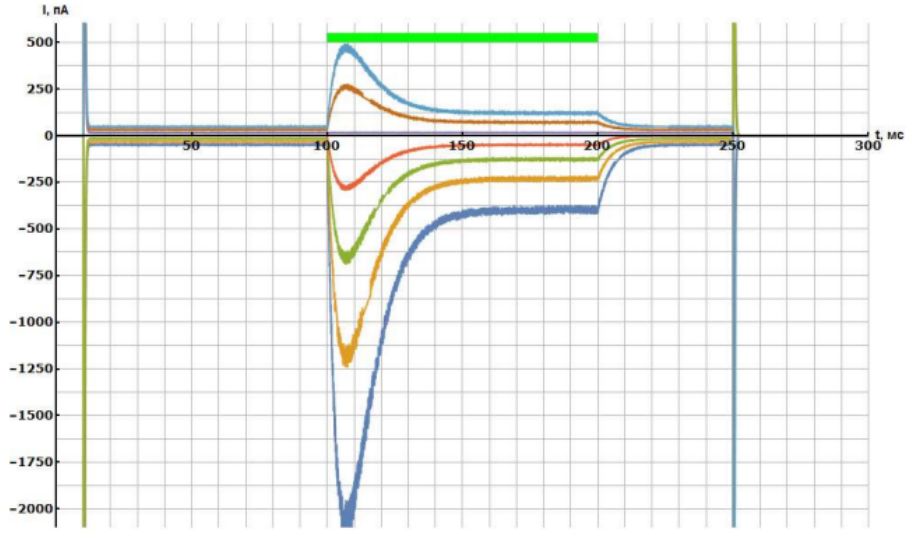
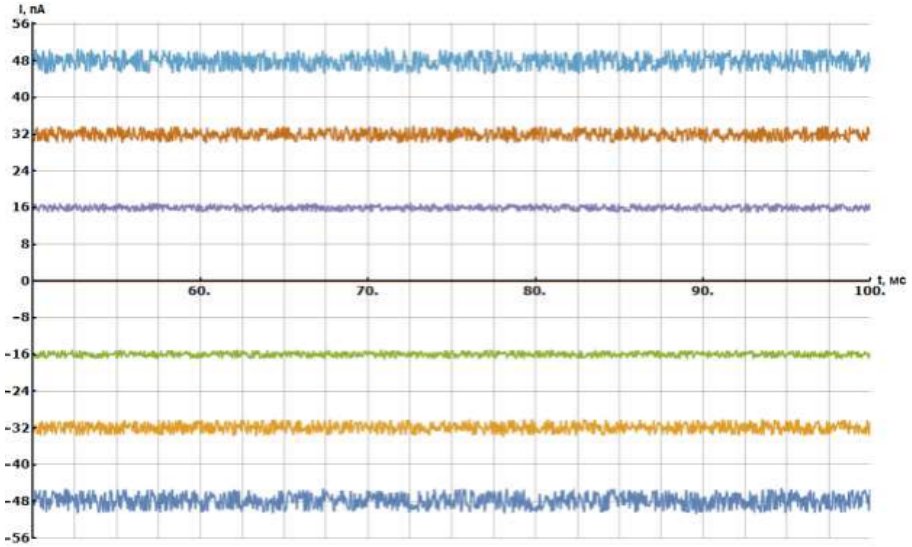
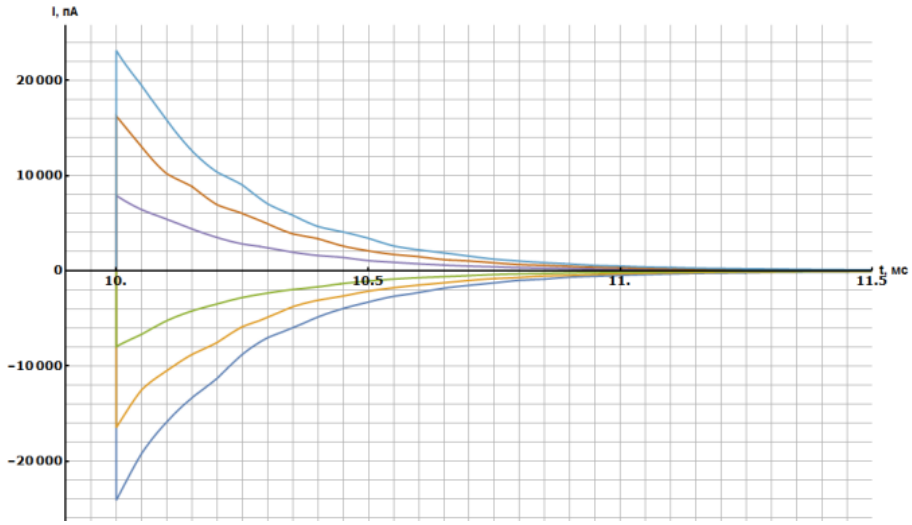


Figure 7. Whole-cell connection and an equivalent circuit of the cell.

C1	From the data shown in the plots, determine the electrical properties of the cell membrane: R_M, R_S, C_M. All three graphs reflect the dependence of the current through the cell membrane on time for the same point (this is all one experiment, different sections are shown in time). Different lines from bottom to top correspond to the voltages applied to the membrane: −120mV, −80mV, −40mV, 0mV, 40mV, 80mV, 120mV. The green line at the top indicates the time the light is on.







C2	Determine which ion freely passes through the channel built into the membrane of this cell? The concentrations of all ions in the pipette and external solutions are shown in Figure 7.