15. ULUMBEKOV, E. G., Arkhiv anatomii gistol. i embriol. 12: 89, 1964
16. ULUMBEKOV, E. G., Yubileinaya nauchnaya sessiya Kazan. med. inst. (Jubilee Sci. Sess. Kazan Med. Inst.) 1964
17. LOEWENSTEIN, W. R. and ALTAMERANO-ORREGO, R., J. Physiol. 141: 4, 805, 1958
18. LEIDLER, K. (1958) quoted by PASYNSKII, A. G., Biofizi. khim. (Biophysical Chemistry). Moscow, 1963

Biophysics 11 740-748 (1966)

INVESTIGATION OF THE MECHANISM OF ACTION OF CURRENT ON THE CELLS OF THE *L* TYPE OF THE RETINA*

YU. A. TRIFONOV and I. A. UTINA

Institute of Problems of Information Transmission, U.S.S.R. Academy of Sciences, Moscow

(*Received* 2 *February* 1966)

IN ALL nerve cells it is now known the electrical response to stimulation is associated with change in the resistance of the cell membrane. However, the information on cells of the *L* type, which sources of the *S* potential and are apparently horizontal cells [1], suggests that their electrical reaction is not accompanied by change in the membrane resistance. In fish, it has been shown that in these cells a correlation between the resting potential and the degree of the reaction is absent [2]; in addition, shift in the membrane potential by current passed through an intracellular electrode does not lead to change in the value of the reaction of these cells [3–5]. Below are given new findings confirming the absence of an effect of an alteration in the membrane potential on the value of the reaction of cells of the *L* type of the tortoise (section 1 in the "results"). However, these findings still cannot be taken as evidence that the membrane resistance of these cells does not change during the reaction.

The geometry of the cells may be such that the changes in the resistance in remote parts of the dendrites will have little effect on the input resistance of the cell which we measure by passing current through a microelectrode introduced into the cell body [6]. This, for example, may happen if the longitudinal resistance of the dendrites is sufficiently high (long and thin dendrites). At the same time, for certain ratios of the intracellular and extracellular resistances it may be that passage of current through the whole tissue will elicit changes in the resistance of the membrane in individual parts of the dendrites. We shall illustrate this by a scheme (Fig. 1). In a highly simplified equivalent circuit of the distribution of resistances it is shown that a current passing through the retina in the radial direction (between the sclera and vitreous humour) must flow into the horizontal cells. If the resistance R_3 changes during the reaction to light this must lead to change in the current passing within the cell which in turn

* Biofizika 11: No. 4, 646–652, 1966.

will cause a variation in the potential at resistance R_1. This variation is summated with the initial reaction of the cell to light. Depending on the direction of the current and the sign of change of the resistance, increase or reduction in the response will be obtained.

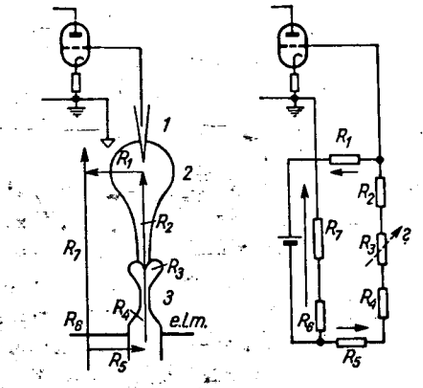

FIG. 1. Diagram depicting in simplified form how current passed through retina from sclera to vitreous humour must flow into a horizontal cell. For simplicity we show only one process of the horizontal cell. Continuous arrows show direction of current. Right—equivalent electric circuit: *1*—microelectrode; *2*—horizontal cell; *3*—receptor; R_1—resistance of cell membrane in somatic part of horizontal cell; R_2—longitudinal resistance of process of horizontal cell; R_3—resistance of post-synaptic membrane of horizontal cell and pre-synaptic membrane of cell of receptor; R_4—longitudinal resistance of process of receptor; R_5—resistance of membrane of inner member of receptor; R_6—resistance of external limiting membrane e.l.m.); R_7—resistance of extracellular medium; E—voltage produced by external current in corresponding layers of retina. To simplify the diagram we do not show the currents crossing the membrane of the processes of the horizontal cell and the process of the receptor outside the synaptic region. The capacitance of cell membranes is not shown since in this instance it is a question of direct current.

In fact, unlike the current passed through the intracellular electrode, a current passed through the whole retina changes the value of the reaction of cells of the *L* type: a current coming from the vitreous humour to the sclera reduces the reaction while a current of opposite direction usually increases it [5]. Such changes may, in principle, occur if the resistance of the post-synaptic membrane increased during the reaction to light (R_3 in the diagram). A current acts outwardly in similar fashion, for example, on the value of the evoked response of the cerebellum of the cat [7]. For the cerebellum it has been shown that this effect is well explained by change in the resistance of the tissues during the evoked response.

This paper describes experiments aimed at establishing whether it is possible to explain the change in the response of the cells of the *L* type under the influence of current by change in the resistance of their membrane in the region of the synapses with the photoreceptors (section 2 in "Results").

From the available electrophysiological evidence it may be thought that the current influencing the response of the cells of the *L* type acts on the receptors (probably

on their presynaptic endings) [5]. This assumption may be checked by the method of indicator cytochemistry: if the photoreceptors are excited by current they must accumulate ribonucleic acid (RNA). The results of the cytochemical experiments are presented in section *3* in "Results".

METHODS

The work was carried out on the eye of the pond tortoise (*Emys orbicularis*). The intracellular reactions of the cells of the *L* type were recorded. The recording was made with liquid microelectrodes with a resistance of 50–200 MΩ. The recording macroelectrode was placed on the surface of the retina. The technique of recording has been described in detail previously [8]. The microelectrode was inserted into the retina close to the recording macroelectrode in order as far as possible to reduce the interference appearing on passage of current through the eye cup. Alternating currents of different frequency and strength were passed with two electrodes one of which was located on the sclera, the other on the surface of the retina. As source of alternating current we used a audio frequency generator assembled by Engineer K. V. Golubstov. The frequency could be changed from 3 to 500 c/s.

In the experiment with passage of direct current through the intracellular microelectrode we used sealed double microelectrodes with low resistance coupling (less than 20 KΩ). One of the electrodes was used for recording, the other for passage of current. The current microelectrodes had a resistance of about 50 MΩ. To stabilize the current in these experiments a resistance of 1000 MΩ was connected in series with the current microelectrode. A second current electrode was located on the sclera. The arrangement of the source of direct current enabled us to change the strength and direction of the current.

The histochemical part of the work was carried out by means of quantitative ultraviolet cytophotometry. The technique of this part of the work has been previously described [9].

RESULTS AND DISCUSSION

1. Experiments with passage of direct current through a microelectrode inserted into a cell of the L type of the tortoise. This section describes the experiments in which the response of cells of the *L* type was recorded for different levels of the membrane potential. The membrane potential was shifted by passing direct current through the microelectrode inserted into the cell.

Similar experiments were carried out in the previous work [5] and from them it was concluded that change in the membrane potential by 30–40 mV towards both hyper- and depolarization does not influence the reaction of the cells of the *L* type. A defect of these experiments was that the same electrode was used both for passage of current and for recording the reaction so complicating evaluation of the actual value of the shift in the membrane potential on passage of current. In this work these experiments were reproduced with double microelectrodes. Two microelectrodes were so sealed that their tips were at the same level separated by 3–4 μ. One of the microelectrodes was used for recording the reaction, the other for passage of current. Thanks to the comparatively large distance between the tips of the microelectrodes the impedance coupling in Ringer solution did not exceed 20 KΩ. On insertion of the double microelectrode into the retina the impedance coupling rose in some layers of the retina to 100 KΩ. as a result of the fact that the specific resistance of the layers of the retina is higher than Ringer solution [10].

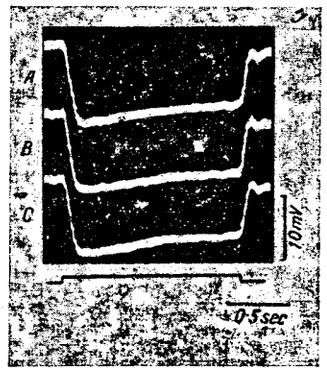

FIG. 2. Response of horizontal cell of retina of tortoise for different levels of membrane potential. Two connected microelectrodes are inserted into the cell, one being used to pass current producing shift in the membrane potential, the other for recording the reaction. *A*—Response with no current; *B*—response with current hyperpolarizing cell membrane by ~30 mV; *C*—response for current depolarizing cell membrane by ~30 mV. Strength of current in both cases 3×10^{-8}A. Input resistance of cell ~1MΩ.

The criterion that both microelectrodes had been introduced into the same cell was the sharp rise in the resistance coupling—through the resistance of the cell membrane. Through the current microelectrode which could be connected to the input of the cathode follower we recorded approximately the same response as through the recording one.

The shift in the potential produced by current was measured directly on the screen of an oscilloscope. Knowing the strength of current in the circuit and the shift in potential it was possible to calculate which part of the resistance was determined by the cell membrane. The resistance of the cell membrane thus measured (to be more exact, the input resistance of the cell) was 0·8–2 MΩ i.e. roughly ten times less than the figures in the previous study [5]. Two reasons for such a difference in values may be assumed. Firstly, the double electrode must damage the cell membrane more than a single one. Secondly, it is natural to assume that a double electrode could by introduced only into comparatively large cells with a low input resistance. Experiments with passage of current through the current microelectrode when both microelectrodes were within the cell confirmed that the change in the membrane potential by a value of about 30 mV towards hyper- and depolarization does not influence the reaction of cells of the *L* type (Fig. 2). If the reaction of these cells were accompanied by change in the input resist-

ance greater than 3–4 per cent, we would already notice the effect of the shift in membrane potential on the reaction. The accuracy of the measurement was limited by the instability of recording in work with double microelectrodes.

2. *Effect of alternating current passed through the eye cup.* The microelectrode was inserted into a cell of the *L* type as could be judged from the appearance of a reaction of up to 10 mV and greater on stimulation with light. Then through the eye cup we passed alternating currents of different frequency and strength. In so doing we recorded a fluctuation in potential by passage of current both through the extracellular medium and through the cell (see diagram Fig. 1). If on stimulation with light the resistance of the cell membrane in the post-synaptic region changes (R_3 changes in the diagram) the amplitude of variations in potential on passage of alternating current must change in the light as compared with the dark.

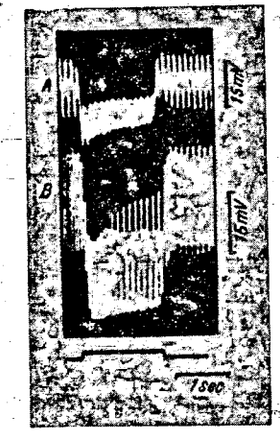

FIG. 3. Recording of variations in potential by microelectrode inserted into a horizontal cell of the tortoise retina on passing through eye cup of alternating current of low frequency in the dark and light. *A* and *B*—two different cells; *A*—frequency of alternating current c/s, peak strength of current about 50 μA; *B*—frequency of 16 c/s, strength of current about 100 μA.

However, with such an experimental arrangement measurements of the resistances are complicated by the fact that the current produces in cells of the *L* type a response which differs in the light and in the dark [5]. The response of the cells to a current superimposed on sinusoidal variations in potential may be a change of their amplitude. Therefore, it is difficult to judge how far the changes in amplitude of variations in potential are due to change in resistance.

Figure 3 presents two records made at frequencies of 8 and 16 c/s and Fig. 3*B* shows that alternating current produces a response of the cell since we observed alternation of the amplitude of variations in potential which cannot be attributed merely to change in the passive-electrical properties of the cell on stimulation with light. In Fig. 3*A*, the upward deviation was produced by a current which, being switched on constantly, usually increases the response of the cells of the *L* type to light. The

downward deflection was produced by current reducing response of the cell. Correspondingly, the upper margin of the record describes a somewhat increased response of the cell to light and the lower a somewhat reduced one. Simply speaking, at low frequencies of alternating current (8 c/s and lower) the effect of direct current on the reaction of the cells of the *L* type to light is reproduced.

Thus, in order to establish whether the membrane resistance of cells of the *L* type changes on stimulation with light it was necessary to select the parameters of alternating current such that it would not produce a response of these cells. In principle, it is possible to use subthreshold currents. However, a special check showed that even the lowest current at which the measurements could still be made gave a reaction of the cells. Another method is to choose a sufficiently high frequency of alternating current at which it would not produce a reaction thanks to its inertia [5]. Of course, the higher the frequency the more will the current be shunted by the capacitance of the cell membranes; however, this effect may be compensated if with increase in frequency the strength of current is also increased. If we assume that the specific capacitance of the membrane of horizontal cells is roughly the same as in the motor neuron, then it may be considered that the shunting of current through the capacitance of the cell membrane will be compensated if the strength of current is increased proportionately to the increase in frequency.

One such experiment is presented in Fig. 4. In order to facilitate the measurements of the amplitude of the sunusoidal variations on passage of alternating current the record was made through an intermediate capacitor. In Fig. 4*A* and *B*, the same reaction is recorded with the intermediate capacitor (Fig. 4*B*) and without it (Fig. 4*A*). Thanks to the capacitor the reaction to light was recorded in the form of two peaks: to switching on the light, lower and to switching off, upper. At a frequency of

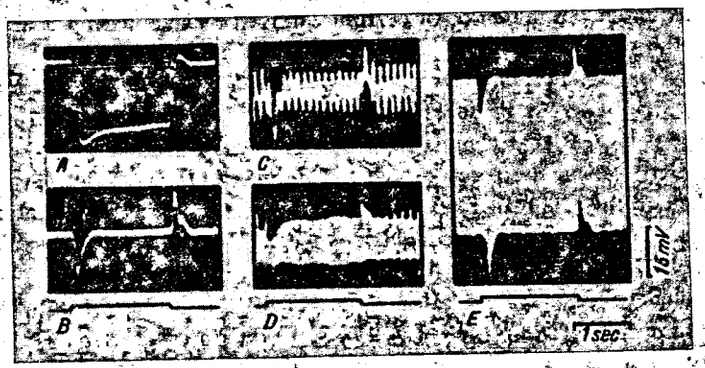

FIG. 4. Recording of variations in potential from horizontal cell of tortoise retina on passing alternating current of different frequencies through eye cup. *A*—response of cell to light (no current); *B*—same response recorded through intermediate capacitor; *C*, *D* and *E*, passage through eye cup of alternating current of different frequencies and strength. Recording made through an intermediate capacitor. *C*—frequency 8 c/s, strength 20 μA; *D*—frequency 16 c/s, strength 20 μA; *E*—frequency 88 c/s, strength 390 μA.

It is interesting to compare these findings with the results of psychophysical experiments showing that the electrical sensitivity of the human eye increases in light as compared with dark [13].

SUMMARY

(1) The response to light of the cells of the *L* type (probably horizontal cells) does not change on shift on the membrane potential by 30 mV, by current passed through an intracellular electrode, both towards hyperpolarization and depolarization. Bearing in mind the accuracy of measurement it may be concluded that the input resistance of these cells if it changes, does so by not more than 3–4 per cent.

(2) No changes in the resistance of the membrane of these cells in response to light could be detected in the experiments with passage through the entire retina of alternating currents of quite high frequency. Intracellularly, the recorded sinusoidal variations in potential in this case were the same in the dark and light. Therefore, the changes in the re ponse of the horizontal cells observed on passage through the whole retina of direct current cannot be ascribed to changes in the resistance of post-synaptic membranes of these cells in response to light.

(3) Experiments on the frog showed that prolonged electrical stimulation of the retina produces roughly the same accumulation of RNA in the horizontal cells and in the photoreceptors. This suggests that the current acts on the receptor cells probably on their presynaptic endings. In conditions of constant illumination accumulation of RNA in the horizontal and receptor cells in response to electrical stimulation was appreciably greater than in the dark. This correla es with well-known high electrical sensitivity of the human eye to light as compared with dark.

The authors wish to thank A. L. Byzov for constant assistance in the work and preparation of the manuscript and V. V. Maksimov for valuable discussion of the results.

REFERENCES

1. McNICOL, E. I. and SVAETICHIN, G., Amer. J. Ophthalm. 46: 26, 1958
2. GOURAS, P., J. Physiol. 152: 487, 1960
3. TASAKI, K., Arch. Ital. Biol. 98: 81, 1960
4. WATANABE, K., TOSAKA, T. and YOKOTA, T., Jap. J. Physiol. 10: 132, 1960
5. TRIFONOV, Yu. A. and BYZOV, A. L., Biofizika 10: 673, 1965
6. ARSHAVSKII, Yu. I., BERKINBLIT, M. B., KOVALEV, S. A., SMOLYANINOV, V. V. and CHAILAKHYAN, L. M., Dokl. Akad. Nauk SSSR 163: 994, 1965
7. ARSHAVSKII, Yu. I. and CHAILAKHYAN, L. M., Biofizika 11: 143, 1966
8. BYZOV, A. L. and KHANICH, R., Fiziol. zh. SSSR 51: 250, 1965
9. UTINA, I. A., NECHAYEVA, N. V. and BRODSKII, V. Ya., Biofizika 5: 749, 1960
10. BYZOV, A. L., Biofizika 3: 658, 1958
11. UTINA, I. A., Dokl. Akad. Nauk SSSR 157: 1216, 1964
12. UTINA, I. A. and BYZOV, A. L., Biofizika 10: 1088, 1965
13. BOGOSLOVSKII, A. I., Probl. fiziol. optiki 2: 136, 1944

alternating current of 8 c/s (Fig. 4C) as in Fig. 3A, illumination changed the amplitude of the variations in potential and these changes were noticeable already for a current of 20 μA (for alternating current the peak value is quoted, i.e. in this case the instantaneous values of the strength of current change from +20 μA to −20 μA). When we passed current with a frequency greater than 80 c/s through the eye cup the amplitude of the sinusoidal variation in potential to light did not change as compared with the dark even if the strength of current was several times greater than in the first case.

In Fig. 4E, is given a record with alternating current with a frequency of 88 c/s and strength of 390 μA. Since the band occupied by the sinusoidal variations was too wide, its upper and lower margins were photographed separately and then brought together in one Figure. The reaction to light was recorded in the form of a brief shift in the whole band downwards on switching on the light and upwards on switching off the light. The amplitude of the sinusoidal variations determined from the width of the band remained unchanged in the light and in the dark.

Thus, at low frequencies of alternating current (8 c/s and lower) the amplitude of variations in potential recorded on passage of alternating current changes in response to stimulation with light but at higher frequencies (80 c/s and higher) it does not change. If in the first case the changes in amplitude of the variations were determined by the change in the resistance of the cell membranes, then similar changes in amplitude ought to be observed also at high frequencies since the strength of current was increased more than the frequency. Since this did not occur, it may be concluded that the changes in amplitude of variations are not determined by change in the resistances.

Thus, in the experiments with passage of current through the whole retina it was not possible to perceive change in the resistance in any part of the membrane of cells of the *L* type. This confirms the previous conclusion [5] that change in the reaction of these cells under the influence of current cannot be explained by change in the resistance of the cell membrane.

3. *Changes in the concentration of RNA in the cells of the retina under the influence of current.* Since current passed through the retina influences the response of cells of the *L* type it is clear that it must act either on these cells themselves or on the link preceding them, i.e. on the receptor cells. Previous electrophysiological findings suggest that the current acts rather on the receptor cells (to more precise their pre-synaptic parts [5]). The validity of this conclusions may also be checked in the histochemical experiment.

On prolonged rhythmic stimulation with light RNA accumulates both in the photoreceptors and in the horizontal cells [11, 12]. Since the pulses of current produce a reaction in the cells of the *L* type (i.e. apparently in the horizontal cells) it may be expected that on prolonged rhytmic stimulation by current RNA will accumulate in the horizontal cells. Whether RNA will accumulate in the photo receptors in these conditions will depend on whether they are excited by current.

Experiments were carried out on intact frogs rendered motionless by diplacin. Before the experiment all the frogs including the controls were kept in the dark in identical conditions for 20 hr. During the experiment for one hour we passed through the eye pulses of current of 0·1 sec duration at a frequency of 5 per sec, strength of current 400–500 μA. The cathode was on the cornea, the anode on the skin in the immediade proximity of the eye, i.e. the direction of current was such that it produced a reaction of the horizontal cells. Then, by the technique described determined the concentration of RNA in the nuclei of the horizontal cells and photoreceptors.

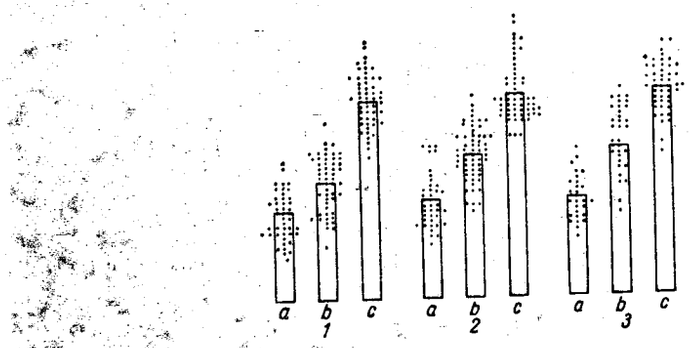

FIG. 5. Accumulation of RNA in horizontal and receptor cells of frog retina on prolonged stimulation of eye with short current pulses. *1*—rods; *2*—cones; *3*—horizontal cells. Height of columns—mean concentration of RNA in nuclei of cells, dots—results of measurements in individual cells; *a*—concentration of RNA in control frogs; *b*—concentration of RNA after electrical stimulation of eye, in the dark for 1 hr; *c*—same conditions of constant illumination of eye (about 100 lx on cornea).

In the experiment we used six frogs: two controls (without treatment with current in the dark), two with application of current in the dark; two with application of current with constant light. The light was passed through a thermal filter (cuvette with solution of $CuSO_4$), the intensity of light on the cornea was about 100 lx.

The results are presented in the graph in Fig. 5 which shows that both in the rods and cones the current produced considerable accumulation of RNA, in particular, in conditions of constant illumination. Consequently, current undoubtedly excites the receptors. In the horizontal cells accumulation of RNA was roughly the same as in the photoreceptors. Therefore, the reaction of the horizontal cell to current can naturally be explained as a consequence of excitation of the photoreceptors.

It should be noted that both in the photoreceptors and the horizontal cells accumulation of RNA in response to pulses of current was considerbly greater in constant light than in the dark. Constant light as such does not produce accumulation of RNA in the horizontal cells [11] and consequently, it increases the effectiveness of the action of pulses of current on the accumulation of RNA in the horizontal cells. Apparently, this is connected with the fact that in some cells of the *L* type light increases the response to current [5] and in the frog this cell variant is encountered more often than are cells in which light reduces the response to current.