Polarized Light Responses from Octopus Single Retinular Cells

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(*With 1 Text-figure*)

In cephalopods, octopus and squid, it is well known that their retinae consist of rod-shaped retinular cells and supporting cells. The outer segment of each retinular cell has close packed microtubules of about 50 nm in diameter (Moody et al., 1960; Young, 1960; Zonana, 1961). The tubule structure is quite similar to that of retinae of arthropod compound eyes in which the retinular cells were known to be capable of analysis of the plane of polarized light. The ability of analysis of plane of polarized light has also been, therefore, expected in the cephalopod retinae (Moody et al., 1960). The behavioural observations by conditioned training of octopus have shown that mobility of octopus was preferentially activated with the polarized light of either vertical or horizontal plane of polarization, and sometimes of $\pm 45°$ (Moody et al., 1961; Moody, 1962; Jander, 1963).

An electrophysiological approach has also been tried in octopus retina. Tasaki and Karita (1966) reported that electroretinogram (ERG) was modulated in amplitude clearly when an isolated retina was illuminated intermittently by test polarized light, of which plane of polarization was rotated, after the retina had been adapted by polarized light of fixed plane of polarization. The size of ERG was minimal when the planes of test polarized light coincided with that of adapting polarized light, whose plane of polarization was placed either vertically or horizontally with respect to normal orientation of the eye, and maximal when they were at right angles. When the plane of adapting polarized light was placed at 45°, the ERG became smaller as a whole but no waxing and waning occurred. From these results as well as the morphological evidences of the two sets of photoreceptors, each of which is found to have microtubules arranged perpendicularly along vertical or horizontal axis of the retina, they concluded that

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each set of retinular cells functions as analyzer of electric vector of polarized light. The present authors tried a direct electrophysiological examination of ability of polarized light discrimination of octopus by recording intracellularly from single retinular cells.

Materials and Method

The animals used were *Octopus vulgaris*, and occasionally *O. ochellatus*, being kept in aquarium tank of laboratory. Before every experiments, they had been adapted in dark while kept in aerated sea water. After half an hour they were operated under deep red light, to which octopus retina is insensitive. The dissected eye was opened at an equater to remove the another half, and was cut with a pair of scissors into 2 or 4 pieces, depending on size of the eye. One of the pieces was then transfered on to a piece of filter paper, black coloured and moistened with vitreous humor of eye, to be attached with an indifferent Ag-AgCl electrode. Then it was mounted on the electromagnetic jolter which gives linear acceleration to the retina. The jolting method for penetrations of micropipette by which the recording of single cone responses was first succeeded in the carp retina (Tomita, 1965) was found to be also indispensable in studying octopus retina. Natural *in situ* orientation of the eye was carefully retained so that horizontal axis of retina, which always corresponds to the orientation of slit pupil of the eye, was usually placed parallel to the frontal edge of experimental stage. Other pieces of the retina were kept in dark, and refrigerated at about 5°C, for more trials. A micropipette for recording, having tip diameter of less than 0.1 μm and 100–200 MΩ in tip resistance and filled with 2M-KCl, was advanced into the retina with the aid of micromanipulator in step of several micrometers, while the jolter was drived by large current pulses which was synchronizing at the lasting of each sweep of an oscilloscope beam at time interval of about 2 seconds. White light of xenon lamp was used for stimulation of retina. The polarized light was obtained by mounting a polarizing filter just behind the final lens of the optical arrangements. Intensity of light measured by a solar diode was 3.9×10^5 quanta/$\mu m^2 \cdot$sec at the retinal level, without both of neutral denstiy filter and polarizing filter. In the optical system, even it included some reflecting prisms, no significant fluctuation of output current of solar diode was detected when the polaroid was rotated, there is no conceivable interference by the 'Fresnel's effect'.

Results and Discussions

In active retina, the ERG, recorded as a negative slow potential change induced by light flash, appears on surface of retina. The response are 3 to 5 mV to a spot of polarized light of 200 μm in diameter. This potential reverses its polarity at proximal layer of the retina. During a single excursion of micropipette across the retina under jolting operation, usually at depth range from 200 to 250 μm apart from inner limiting membrane, several chances were obtained for recording of negative resting membrane potentials, of about 30 to 40 mV indicating successive penetrations of adjacent single photoreceptors. Whenever such chances are encountered jolting was stopped. A sharp depolarization of membrane potential, was induced if the single test flash of non-polarized white light was applied to the retina providing the penetration was successful. The depolarization of the

potential thus recorded disappeared abruptly by displacement of the light spot, even the distance from the site of micropipette tip to the border of light spot was no more than a few micrometers. On the contrary, the ERG which was recorded on the same retinal site showed gradual reduction in amplitude by passing of light

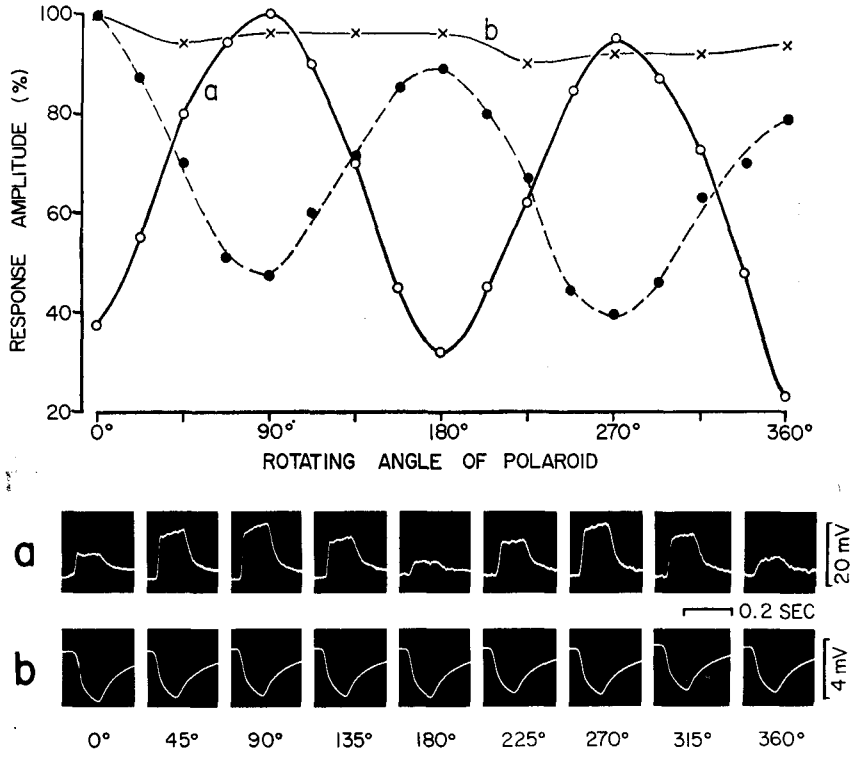

Fig. 1. The relation of the rotating angle of polaroid filter to the response amplitude of single octopus retinular cells (a) and to the ERG as a control (b). The angle on abscissa refers to the horizontal plane of the physiological position of the eye *in situ*.

spot. The effect of changing of spot size of light, the area effect, on the depolarizing responses were markedly less than that of the ERG, the field potential. These results were the same as that observed in the single cone responses of carp retina, except for a hyperpolarization of the receptor potential in the cone reported by Tomita (1965). From the depth of the recorded sites and from the characteristics of response types, it is reasonable to conclude that depolarizing potential in octopus retina is single receptor potential recorded from retinular cell. The earlier maximal phase of depolarizing response of retinular cell

was almost close to 0 mV level but no record of the overshoot could be so far obtained.

In successful records, remarkable cyclic fluctuations in amplitude of single cellular response could be observed when the plane of polarized light flash was rotated slowly by the step of several degrees. After confirmation of this, the film record was made until deterioration of response occured. The duration and interval of polarized light stimulus were fixed at 100 msec and 2 sec respectively. In every cells, intensity of stimulus was limited in the range where the response does not saturate in size, by chosing an appropriate neutral density filter from a series mounted on a revolving disc. About fifty units of receptor potentials have been analyzed.

Two typical examples of single cellular responses are shown by curves (a) (solid and dotted lines) in the upper diagram of figure 1. The recorded wave shapes are also shown in frame record (a) in the same figure. The angles of the polaroid of 0°, 180° and 360° correspond where the plane of polarized light is parallel with the horizontal axis of the retina. Both curves show clear cyclic modulation in amplitude, with a manner of approximate sinusoidal wave, with a constant period of 180°, following rotation of polaroid by the step of 22.5° each. The range of fluctuating amplitude extended more than one half of the peak of response in the most stable units and this was equivalent to 1.0 to 1.5 difference in log unit. The flat curve (b) and frame record (b) are controls, the surface ERG which was recorded from the site where the intracellular penetration had been accomplished. As expected from the previous study of Tasaki and Karita (1966), the rotation of polaroid has shown little effect on the ERG size under no adapting polarized light. The most characteristic fact in intracellular responses may be the shifting in phase of fluctuations between different groups of retinular cells. One type of retinular cell showed maximal receptor potential when the plane of polarized light was parallel to vertical axis of retina as shown by the solid line and frame record (a) in the lower part of the figure. While, the response curve of another showed peak when the plane of polarized light was just agreed with the horizontal axis as shown by the broken line. The phases of oscillation of all the responses recorded approximately overlapped either with the former, or with the latter. These results had been expected to be highly probable from the structural evidence of two sets of retinular cells which arrange in mosaic fashion. It may be also expected that the two types should be approximately fifty-fifty in all recorded population of retinular cells. In the present experiment, 22 and 26 each were observed to be units of the two types, having the maximal response at 0° and 90° of the plane of polaroid respectively. This result agrees well with the mor phological knowledges of octopus retina as mentioned above. Resting membrane potentials of supporting cells which pack the proximal layer of retina in which the cell bodies of receptors were placed (Yamamoto et al., 1965) were found to be extraordinarily deeper than those of retinular cells, usually of well over 100 mV. It depolarized by light and its wave shape was similar with that of retinular cells, but the size was very small, only about 5–10 mV. No significant amplitude

modulation of response could be detected during the rotation of polaroid.

From the present direct examinations and the Tasaki's results (1966), it is confirmed that analyzers of plane of polarized light in the retina are each of the two sets of retinular cells which is similar to that of the compound eyes of arthropods. As is already known in the microtubules of rhabdomeres in the arthropods (Langer et al., 1966), it is conceivable that the function of polarized light perceptions should be attributable to the nature of dichroic absorption of light by photopigment, of which molecules may be arranged unidirectionally, as demonstrated in rhodopsin of frog rods (Denton, 1959) and in that of cephalopod rhabdomeres (Moody, 1964).

In octopus, maximal contrast of sensory outputs between the two sets of receptors may be most effective in discrimination of the plane of e-vector, and furthermore, even in no contrast at $+45°$ or $-45°$ of e-vector, they can discriminate dynamic orientations. Therefore, there is no doubt in that true discrimination of plane of polarized light is owing to neural integrations of sensory informations from both sets, which may occur in the plexiform layer of retina and in the brain. The next step should be in determination of the relation between microtubulous orientation in the distal segment of receptors and plane of e-vector of polarized light to which the receptor is most sensitive.

Summary

Discrimination of the plane of polarized light was examined directly by intracellular recording from single retinular cells of the *Octopus*.

1. The resting membrane potentials of 30 to 40 mV, were obtained at depth from 200 to 250 μm from inner limiting membrane of the isolated retina. The sharp depolarization was induced by a flash of stimulus white light to the retina.

2. Remarkable cyclic fluctuation in amplitude of the single retinular response could be observed when the plane of the polarized light was rotated. One group of the retinular cells showed peak receptor potential when the plane of e-vector was parallel to vertical axis of the retina in normal orientation *in situ*, and another showed peak potential when the plane of e-vector was parallel to the horizontal axis of the retina.

3. The probability of encounter of each group was approximately 50% in all the penetrations of micropipettes. Thus, it is confirmed that the two sets of retinular cells each of which arranges in mosaic fashion are analizers of plane of polarized light in the octopus retina.

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