XIX. NEUROPHYSIOLOGY*

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A. MODELLING THE GROUP 2 GANGLION CELL OF THE FROG'S RETINA†

1. Introduction

Since Lettvin, Maturana, McCulloch, and Pitts[1,2] published their measurements of signals in the optic fibers of the frog, considerable effort[3,4] has been given to developing models that could account for the properties they found. Such models are of importance to engineers and neurophysiologists for two reasons. First, models provide clues on which to base advanced and versatile engineering systems. Second, models provide a basis of thought consistent with reported neurophysiological findings. Such a basis could be useful to neurophysiologists in interrelating experimental results.

Lettvin, Maturana, and co-workers have distinguished four major groups of retinal ganglion cell which report to the tectum. These have been designated as follows: Group 1, edge-detector; Group 2, bug-detector; Group 3, dimming-detector; and Group 4, event-detector ganglion cells. Of these, relatively simple explanations can be given[5,6] to the operations of the Group 1, 3, and 4 ganglion cells. The Group 2, or bug-detector ganglion cell, however, is a more intricate and the most exciting cell to model because it is sensitive to small dark convex objects that move centripetally with respect to the responsive retinal field (RRF) of this cell. In essence, it is the most specialized pattern recognition cell of the frog's retina. Gaze and Jacobson[7] suggest that the Group 2 operation may be due to the existence of an excitatory area surrounded by an inhibitory ring, such that large objects will cause inhibition, whereas small objects will be detected by the cell.

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Grusser and co-workers[8] reported that in their experiments no special construction of the receptive field, with respect to inhibitory or excitatory areas, was found. Grusser[9] pointed out that patterns moved outside the RRF can have an inhibitory effect on the response elicited by a small moving object inside the RRF, i.e., the inhibitory effect of the supposed ring appears only if the object moves. Evidence[8] has been given suggesting that these cells are directionally sensitive, although the argument is not definitive.

We present an analytical model that is consistent with the findings of the aforementioned authors. In structuring our model, we follow the anatomy of the Group 2 ganglion cell as understood by Lettvin and his co-workers. They identified[10] the Group 2 ganglion cell as multilevel E-shaped neuron from Ramon y Cajal's drawings. Accordingly, we distribute cell computations in three layers. Those computations are, in general, compatible with commonly accepted neural processes. It has not been necessary to postulate an exclusively inhibitory ring, although the model cell receives information from an area wider than the responsive retinal field. Some cellular properties appear as consequences of the model structure. As a consequence, it is not necessary to make ad hoc hypotheses to explain each of them.

The operation of the model can be summarized as follows. First, a convex function Φ, depending upon the penetration of an object into the responsive retinal field (RRF), is defined. It is only significant when the object moves centripetally. Second, a similar function, Ψ, is defined, which is dependent on the size of the object, being a maximum for one particular size. The coincidence of both is computed by the product $\Phi\Psi$. Third, an inhibitory effect, X, is defined which acts upon the function $\Phi\Psi$. The inhibition is large for bright objects and small for dark objects. As a result, an activity function, Ω, is obtained. The pulse repetition frequency of the cell is assumed to be proportional to Ω.

2. The Model

We assume that, for the purpose of the Group 2 ganglion cell operation, the photoreceptors are connected to two different types of bipolar cells, the outputs of which are pulses of width δt and amplitude r. Each bipolar cell performs a different operation on the retinal image. Let us call $n_I(t)$ and $n_{II}(t)$ the number of bipolar cells (belonging to Types I or II) that fire at time t as a response to a changing image on the retina.

We postulate:

a. $n_I(t)$ is proportional to the total length of the edges in the retinal image which are coincident with a local dimming.

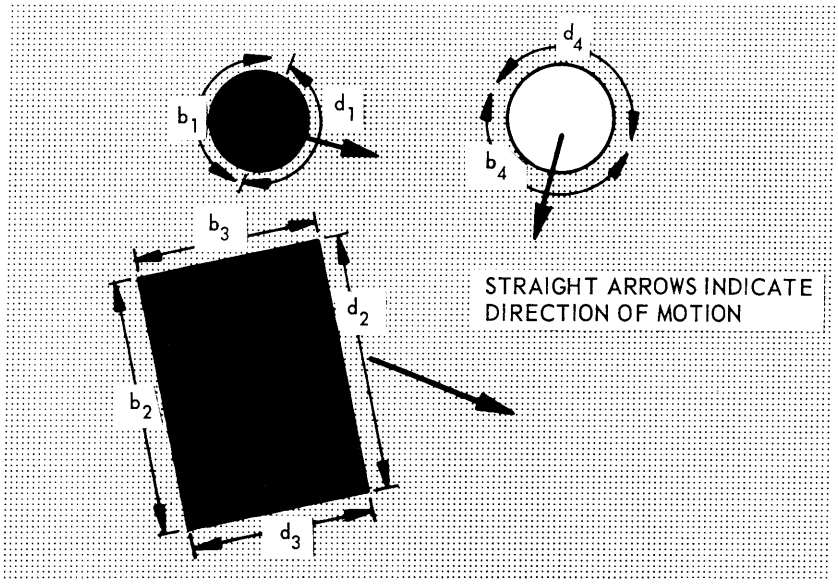
b. $n_{II}(t)$ is proportional to the total length of the edges in the retinal image which are coincident with local brightening.

The author has previously described[11] a model in which photoreceptors and bipolar

cells perform in a manner similar to that postulated here.

Type I bipolar cells we term contrast-dimming detectors, whereas Type II bipolar cells we term contrast-brightening detectors. In both cases, spatiotemporal changes of the illumination on the photoreceptors feeding each bipolar cell are necessary to fire the latter.

Figure XIX-1 illustrates $n_I(t)$ and $n_{II}(t)$ for several bright and dark moving objects.



$$n_I(t) \propto d_1 + d_2 + d_3 + d_4$$

$$n_{II}(t) \propto b_1 + b_2 + b_3 + b_4$$

Fig. XIX-1. Equivalences of $n_I(t)$ and $n_{II}(t)$ for several moving objects.

Consider one Group 2 ganglion cell. It receives signals from Types I and II bipolar cells, and processes these signals in three computation layers (Fig. XIX-2).

In layer 1, pulses emanating from Type I bipolar cells are collected over a circular area equal to the RRF of the ganglion cell. Each pulse originates a signal level that is maintained for a time Δt. (Δt is made equal to the transit time across the RRF of the slowest object to be detected by the cell.) We may regard this as a short-term memory. Let $n_1(t)$ be the number of pulses impinging on layer 1 at time t. $N_1(t)$, the number of existing signals levels at time t, will be equal to the total number of pulses that have reached layer 1 in the previous time interval $(t-\Delta t, t)$. Note that $N_1(t)$ is proportional to the area that has been scanned by dimming, within the RRF, because of a moving object. Furthermore, each existing signal level at time t is affected by divisional inhibition,[13] by the ensemble of incoming pulses at time t. Thus, $N_1(t)$ new signal

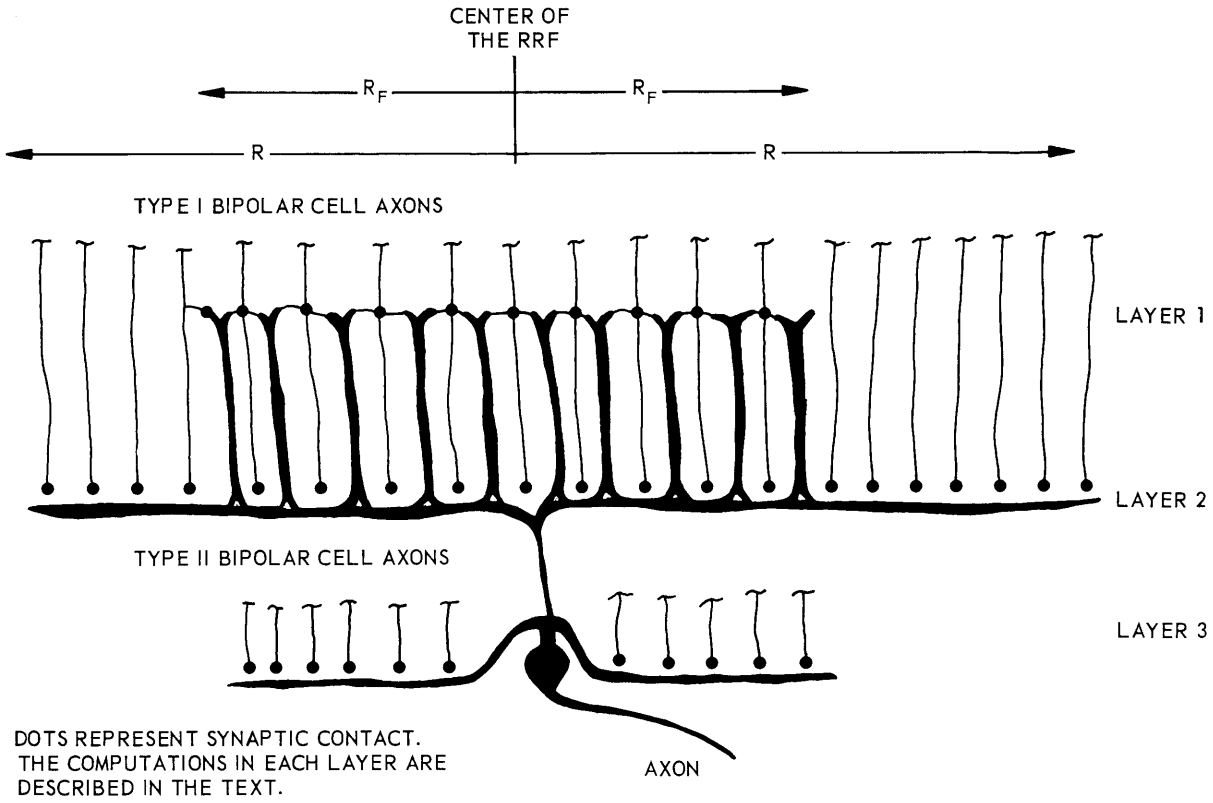


Fig. XIX-2. A section of Group 2 ganglion cell model.

levels are originated, each of them having an identical value, α, defined by

$$\alpha = a/[2+bn_1(t)], \tag{1}$$

where a and b are constants. For $bn_1(t) \gg 1$, Eq. 1 becomes

$$\alpha = K/[n_1(t)], \tag{2}$$

where K = a/b.

In layer 2, three operations are distinguished. First, the $N_1(t)$ signal levels from layer 1, each of them having a value $K/n_1(t)$, interact in a manner such that a signal, $\Phi[N_1(t)/n_1(t)]$, is obtained, which has the convex shape shown in Fig. XIX-3. $\Phi[N_1(t)/n_1(t)]$ is maximum for particular value $[N_1(t)/n_1(t)]_{opt}$, and is zero for $N_1(t) = 0$ and for $[N_1(t)/n_1(t)] \geq [N_1(t)/n_1(t)]_{lim}$. The ratio

$$[N_1(t)/n_1(t)] = \frac{\text{area scanned by contrast-dimming in the RRF}}{\text{length of contrast-dimming in the RRF}}$$

provides a measure of the penetration of a round-shaped dark object moving into the RRF.

By appropriately choosing the value of constants in the function Φ, we can make Φ significant only when the image moves centripetally across the RRF.

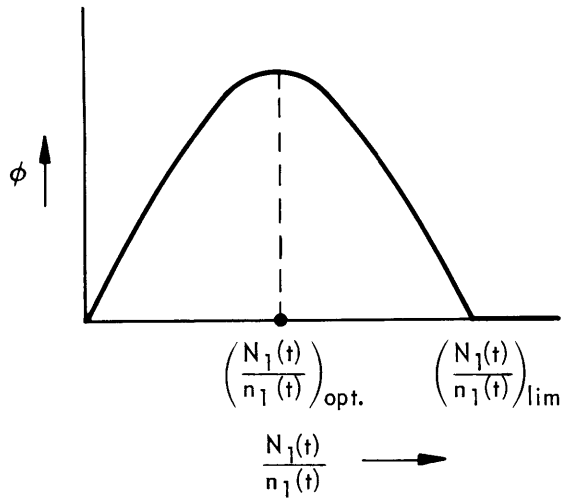


Fig. XIX-3. Shape of the curve $\phi\left(\frac{N_1(t)}{n_1(t)}\right)$.

A particular function, Φ, containing the previously mentioned characteristics can be obtained by assuming that the active-level signals interact by processes commonly accepted in neurophysiology; namely, lateral divisional inhibition, adaptation, and spatial summation. To demonstrate, let us assume that each afferent active line is laterally inhibited by all the others. If the signal level of each line is $K/n_1(t)$, the total inhibition upon each line is

$$\Psi = k[N_1(t)-1] \cdot K/n_1(t), \tag{3}$$

where $N_1(t)$ is the total number of active lines, and k is a constant.

If $N_1(t) \gg 1$, Eq. 3 becomes

$$\Phi = K_1[K_1(t)/n_1(t)], \tag{4}$$

where $K_1 = kK$.

As a result of divisional inhibition, the signal in each active line becomes

$$A_C = [K/n_1(t)]/[1+K_1[N_1(t)/n_1(t)]]. \tag{5}$$

If we assume that each active line is adaptive, i.e., its threshold, θ, increases proportionally to the incoming signal,

$$\theta = A[K/n_1(t)], \tag{6}$$

where A is a constant. This is a form of linear adaptation.

From Eqs. 5 and 6, the net signal in each line is

$$A_C - \theta = \frac{K/n_1(t)}{1 + K_1[N_1(t)/n_1(t)]} - A[K/n_1(t)]. \tag{7}$$

By spatial summation over all the $N_1(t)$ active lines at time t, we have

$$\Phi\left[\frac{N_1(t)}{n_1(t)}\right] = \sum_{\substack{\text{all active}\\ \text{lines}}} A_C - \theta = K\left[\frac{N_1(t)/n_1(t)}{1 + K_1[N_1(t)/n_1(t)]} - A[N_1(t)/n_1(t)]\right]. \qquad (8)$$

Equation 8 is plotted in Fig. XIX-4 for $K_1 = 1/0.22\ R_F$ and $A = 0.25$. R_F is the number of contrast-dimming bipolars contained in one radius of the RRF. K_1 and A have

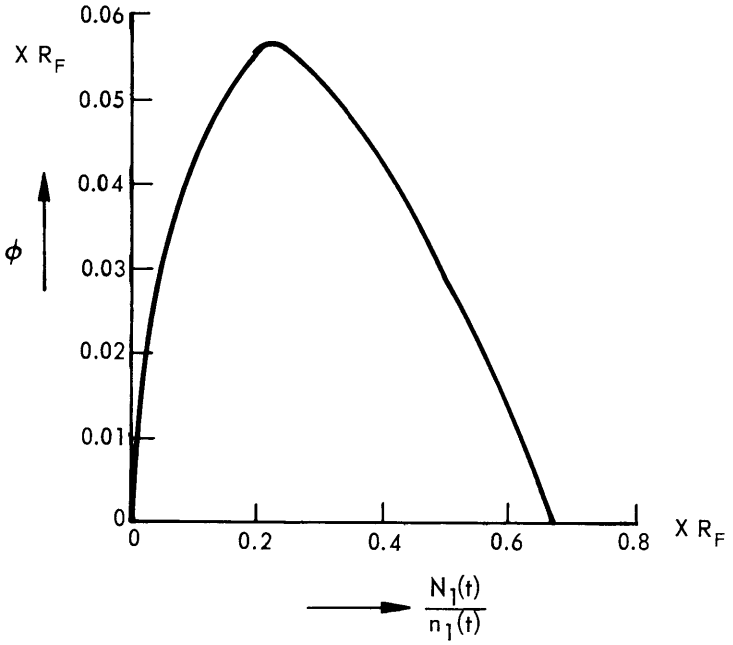


Fig. XIX-4.
Example of function $\phi\left(\frac{N_1(t)}{n_1(t)}\right)$.

been chosen in a manner such that Φ is significant only for a round-shaped dark object when it moves centripetally across the RRF. Again, note that the essential feature of $\Phi[N_1(t)/n_1(t)]$ is its convex shape as shown in Fig. XIX-3. There exists an infinite number of functions with these characteristics. Among them, Eq. 8 is an example, which is compatible with neurophysiological facts. (The author[11] has previously obtained a similarly shaped curve by assuming nonlinear divisional inhibition of the type E/e^I, where E is the excitation and I the inhibition.)

The second operation in layer 2 is performed on afferent pulses from Type I bipolar cells over a circular area of radius R, which is wider than the RRF. Let $n_2(t)$ be the number of incoming pulses collected at time t over this area. $n_2(t)$ is proportional to the total length of contrast-dimming within the circular area of radius R.

The $n_2(t)$ pulses interact in a manner such that a function, $\Psi[n_2(t)]$, is obtained which is similar to Φ. Thus, $\Psi[n_2(t)]$ is maximum for $n_2(t)_{opt}$, and it is zero for $n_2(t) = 0$ and for $n_2(t) \geq n_2(t)_{lim}$. Again, constants in $\Psi[n_2(t)]$ can be computed to adjust $n_2(t)_{opt}$ and $n_2(t)_{lim}$ to the experimental results.

As an example, we assume again that the $n_2(t)$ pulses interact by lateral divisional inhibition and that adaptation and spatial summation exists. Ψ can be expressed as

$$\Psi[n_2(t)] = K'\left[\frac{n_2(t)}{1 + K_1' n_2(t)} - Bn_2(t)\right], \tag{9}$$

where K', K_1', and B are constants.

Experimental results[1,2,9] have shown that when experimenting with dark discs, the ganglion cell output is maximum for a disc of radius $\approx R_F/2$, and is zero for discs of radii larger than R_F. Using these findings, we then compute $K_1' = 3/\pi R_F$ and $B = 0.25$. The radius, R, of wider circular area is estimated to be $R = 3.2\ R_F$ by using the criterion[1,2] that a straight band wider than R_F does not produce a response. The results of Gaze and Jacobson[6] appear then as a consequence of this restriction.

The third layer 2 operation is a multiplication of the functions Φ and Ψ. We do not have enough neurophysiological evidence to support this assumption, although we still tacitly assume its validity. Thus the activity function

$$\Phi[N_1(t)/n_1(t)]\ \Psi[n_2(t)]$$

is generated, and we consider this as the output of layer 2. (An explanation of this hypothesis and the shapes of the curves Φ and Ψ may be given in terms of probability. We shall discuss this point of view in a later report, since it could be applied to the description of any nerve cell.)

In layer 3, the outputs from the Type II bipolar cells are of concern. These afferent pulses are collected over the RRF, and they generate signal levels that are maintained for a time Δt. Let $N_3(t)$ be the number of these levels at time t. $N_3(t)$ is then proportional to the area that has been scanned by contrast-brightening within the RRF in the time interval $(t-\Delta t, t)$.

The $N_3(t)$ signal levels are spatially summed and generate a signal

$$X[N_3(t)] = CN_3(t), \tag{10}$$

where C is a constant. This signal affects, by divisional inhibition, the output from layer 2. Therefore, we have

$$\Omega = \frac{\Phi\Psi}{1 + X}. \tag{11}$$

We assume that the pulse frequency, f, of the ganglion cell is proportional to Ω. Thus,

$$f = f_o\Omega = f_o(\Phi\Psi/1+X), \tag{12}$$

where f_o is a constant.

The value of constant C in Eq. 10 can be chosen to achieve a cell output for bright objects suitably less than that for dark objects.

3. Discussion

The performance of the model can be derived from Eq. 12. For purposes of illustration, we shall assume that Φ and Ψ are given by Eqs. 8 and 9, respectively. Constant C is fixed by the arbitrary condition that the maximum response for a bright disc

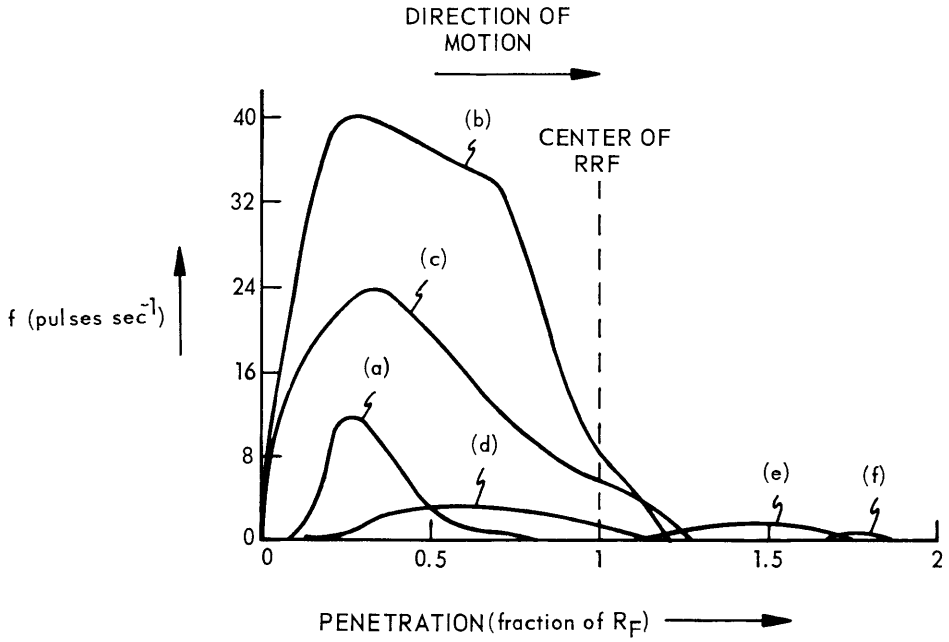


Fig. XIX-5. Output of the model versus penetration of different objects into the RRF.

RRF/8 wide is 1/10 of that which would have resulted without the inhibition produced by X. This condition gives $C = 60\ R_F^{-2}$. The maximum pulse frequency[8] is approximately 40 pulses · sec^{-1}, which fixes $K = K' = 1$ and $f_o = 300/R_F^2$ sec. The pulse frequency of the ganglion-cell model is plotted against the penetration of the leading edge of the object crossing the RRF in Fig. XIX-5. Curves (a), (b), and (c) are for dark discs of radii 0.125 R_F, 0.5 R_F, and 0.75 R_F, respectively. Curves (d), (e), and (f) are for bright discs of radii 0.125 R_F, 0.5 R_F, and 0.75, respectively.

Following are some of the consequences that may be derived from the characteristics of the model.

a. No response occurs to a general change in illumination. (In agreement with Lettvin, Maturana, and co-workers.[1,2,10])

b. A corner may produce a response. (In agreement with Maturana.[2])

c. Several small object images moving simultaneously in the RRF may produce either very small of null responses. This is in accord with Lettvin's observation.[1,2]

d. No evidence of the annulus surrounding the RRF can be detected by fixed dark or light spots with a simultaneous moving testing spot. This is in agreement with Grusser-Cornehls and co-workers.[8]

e. If the spots in the surrounding ring move, however, the response to the testing spot may be either increased or decreased, the amount depending on the size of the spots. This is in agreement with Grusser and co-workers.[9]

The Group 2 ganglion cells respond for approximately one second after an object has entered and stops in the RRF. This response is erased by a corresponding step to darkness.[1,2,10] In the model, however, the response disappears when the object is stopped within the RRF. The persistence of the response might be explained by feedback from the tectum, as suggested by Lettvin.[10] If we assume that tectal feedback acts on the Type I of the bipolar cells, and that the feedback has the same effect as that of dimming, the ganglion cell will provide an output as long as feedback exists. This can be formulated in the following manner. Boolian magnitudes C(t), D(t), F(t), and $B_I(t)$ are defined as follows:

C(t) is 1 if contrast exists, at time t, in the field of a Type I bipolar cell

0 if there is no contrast

D(t) is 1 if dimming occurs, at time t, in the field of a Type I bipolar cell

0 if there is no dimming

F(t) is 1 if there is feedback from tectum, at time t, on a Type I bipolar cell

0 if there is no feedback

$B_I(t)$ is 1 if the Type I bipolar cell fires at time t.

0 if it does not fire.

The condition for Type I bipolar cell firing is then the Boolian expression

$$B_I(t) = C(t) \cdot [D(t)+F(t)]. \tag{14}$$

Feedback from the tectum must be maintained for approximately 1 sec after local dimming has disappeared. This feedback might be provided by the newness cells of the tectum.[10]

R. Moreno-Diaz

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13. "Divisional Inhibition," proposed by Lettvin in Quarterly Progress Report No. 66 (pages 333-335), can be formulated as follows: Let E and I be the excitory and inhibitory signals, respectively, expressed as dimensionless numbers. After inhibition, the resulting signal is $E/(1+I)$; if $I \gg 1$, $E/(1+I) \simeq E/I$.

B. EXPERIMENT DEALING WITH THE DEVELOPMENT OF LOGICAL AND ABSTRACT THOUGHT IN YOUNG CHILDREN

1. A Concept Formation Experiment

The purpose of this research was to study the development of logical and abstract thought in young children. To determine the thought processes used by the child in the solution of such problems.

2. Method

Our method was a modified version of Dr. Jean Piaget's techniques. The child was placed in a typical experimental testing situation. He was presented with familiar

objects such as rubber animals, and questioned as to color, form, size, and number. The child was then led, by the use of clinical techniques, explain and defend his solution. I wanted to know: Did the child understand the question; Was he answering the whole question or only a part of it; Was he answering the question asked or something he thought was being asked; Was he just verbalizing or did he have some degree of insight? Correct and incorrect responses were analyzed.

3. The Subjects

Fifty-one children were tested on three separate occasions. The sample consisted of 36 "average" children from the Newton Public Schools and 15 children from the M.I.T. Day Camp. The age range was from 5 to 11 years. Grades: 1, 2, 3, 5, 6.

4. Results

The thought processes involved were clearly demonstrated in the test asking, "Are there more lions or more animals on the table in front of you?" Seventy-three per cent of our children said that "There were more lions than animals." Only 27% realized that lions were animals as well as being lions. The percentages of lion choice and of animal choice are listed below according to grade.

Grade	Lion	Animal
I	87%	17%
II	57%	43%
III	100%	0%
IV	63%	27%
VI	57%	43%

The children tended to separate lions and animals into distinct groups. There was a juxtapositioning of the two groups. The lions were seen as group A, while the animals were thought to belong to group B. Lions were seen as a different kind of an animal. When answering "Lions" the child felt that his answer was perfectly logical and correct. The child was actually answering only one part of the question, or had changed the meaning of the question into one which he could understand and answer. This appears to follow Piaget's schemata of classification – the application of familiar schemata to a new situation and of application of one different solution at one time.

The children in grades 5 and 6 could realize that a lion was an animal when they were asked. The children in the lower grades remained firm in their conviction that a lion could not be an animal. The younger children, also could not

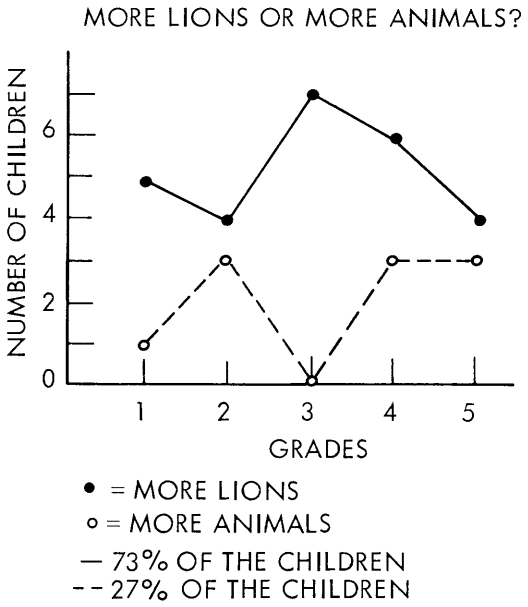

Fig. XIX-6. Results from 36 Newton (Massachusetts) school children.

accept mixed sizes of the same animal as belonging to one group. Color presented no problem.

Figure XIX-6 indicates no real pattern of development of logical thought at a pre-adolescent age. The child just has it or he does not. The children tend to parrot "proper" answers long before they truly understand what they are talking about.

Sylvia G. Rabin

C. STEREOMICROSCOPY WITH ONE OBJECTIVE

It can be very difficult to make out spatial relations in thick histological sections. The obvious answer is to use a stereomicroscope, but most commercial stereomicroscopes do not give useful magnification above approximately 50X, and provide only rudimentary staging and lighting facilities.

Faced with such a problem, we re-invented an old method for obtaining stereo images from a single objective. Our solution takes the form of a simple and inexpensive modification which can be made to most microscopes equipped with a binocular body. It gives a true stereo image, and does not interfere with normal use of the microscope

Because of the wide aperture of microscope objectives, the left- and right-hand parts of the objective "look at" the subject from significantly different angles. Therefore, if the rays from each half of the objective are sent to the corresponding eye, a stereo image

results. (Actually, because the image is inverted the rays from the left half of the objective should go to the right eye, and vice versa, if an orthoscopic image is desired.)

We use Polaroids to sort out the rays from the two halves of the objective. At the objective, we place a disc made from two pieces of Polaroid filter cemented together; one half of the disc is polarized "vertically" (i.e., in the observer's plane of symmetry) and the other half "horizontally." Corresponding Polaroid filters in the oculars sort out the rays.

The split filter can be obtained from the Polaroid Corporation (split field disc 0°-90°). This split filter is placed just below the diaphragm at the top of the objective. This placement works well with objectives up to 40X. With oil immersion objectives, however, there is a vignetting effect – each eye sees only half the field illuminated – and the method is not usable.

The image quality is acceptable for most purposes; there is, however, a noticeable loss of resolution at high powers. Some of the loss may be due to the loss in numerical aperture (N.A.), since each eye sees an image made with only half of the objective. We suspect that some of it is a psychological result of the fact that the "circles" of confusion are no longer round, and are differently shaped for each eye. If viewed through oculars without Polaroids, a nonstereo view is obtained. In this view, there is still some loss of resolution, perhaps owing to the extra optical path length introduced by the filter at a critical point in the system. There is also some loss of contrast, because of scattering at the cemented filter junction, and diffusion in the Polaroid material itself.

Needless to say, the filter at the objective should be of good quality. It also helps to begin with a good image, by using objectives of large N.A. and avoiding high-power eyepieces. The quality of the eyepiece filters is comparatively unimportant.

The axes of the halves of the split filter should be as described – parallel to and normal to the observer's plane of symmetry. It is not sufficient for them merely to be perpendicular to each other, or there will be loss of polarization in the prisms of the binocular body. When they are as described, there is no serious loss of polarization in any style of microscope that we have tried.

One last note is in order. At low powers, say, below 200X, it is possible to obtain a stereo view without any extra equipment at all! All that is necessary is to adjust the oculars so that they are slightly closer together than the observer's interpupillary distance. When this is done, the pupils of the observer's eye each mask off a part of the exit pupil of each ocular. Since the exit pupils are optical images of the objective, this has the same effect as masking the objective itself. This is a trick worth knowing, although it only works at low powers and produces eyestrain if used for prolonged periods.

The use of Polaroids for splitting the objective may be novel; but the fundamental idea is far from new[1,2,4-9] and pupillary masking is mentioned by Ives.[3] For some reason,

binocular microscopes on this principle have almost completely disappeared from the scene. Perhaps it is time for a revival.

D. P. Smith

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D. SPECIAL FUNCTION THEORY

Properties of the polylogarithm function $\mathrm{Li}_\nu(z) = \sum_{n=1}^{\infty} z^n/n^\nu$ have been examined for complex order and argument. The results of this investigation have been submitted for publication to Annali di Matematica. The chief findings are summarized below.

Section 1 dealt with defining relations and integral representations. The principal new result was

$$\frac{d^p \mathrm{Li}_\nu(z)}{dz^p} = \frac{1}{z^p} \sum_{m=1}^{p} S_p^{(m)} \mathrm{Li}_{\nu-m}(z),$$

where the $S_p^{(m)}$ are Stirling numbers of the first kind.

Section 2 presented a generalized proof of the well-known factorization theorem and a derivation of the new expansion

$$\mathrm{Li}_\nu(z^\lambda) = \sum_{m=0}^{\infty} \frac{(\lambda-\lambda_o)^m}{m!} \ln^m z \mathrm{Li}_{\nu-m}\left(z^{\lambda_o}\right).$$

Section 3 discussed various expansions in z, the most novel of which was

$$\mathrm{Li}_\nu(z) \sim -\frac{\gamma^\nu}{\Gamma(\nu+1)} - \frac{\gamma^{\nu-1}}{\Gamma(\nu)} \sum_{m=0}^{M} (1-\nu)_m \gamma^{-m} \cdot \left[\mathrm{Li}_{m+1}(e^{-i\theta}) + (-1)^{m+1} \mathrm{Li}_{m+1}(e^{i\theta}) \right],$$

where $0 < \theta = \arg z < 2\pi$, $\ln |z| = \gamma \gg 1$, and RE $\nu > 0$.

Section 4 discussed various expansions in ν, the most novel of which was

$$\mathrm{Li}_\nu(z) = \sum_{n=1}^{N} \frac{z^n}{n^\nu} + \mathcal{O}\left\{ \frac{|z|^{N+1}}{N^{\nu'}} \frac{\Gamma(\nu')}{|\Gamma(\nu)|} \right\},$$

where $\nu' = \mathrm{RE}\ \nu > 0$ and $0 < \arg z < 2\pi$.

In Section 5 were placed results on the expansion of functions in terms of polylogarithms; the most novel of these was

$$\mathrm{Li}_\nu(z) = z + z \sum_{p=0}^{\infty} a_p(\nu)\, \mathrm{Li}_{\nu+p}(z),$$

where RE $\nu < 0$ and

$$a_p(\nu) = \frac{\Gamma(1-\nu)}{\Gamma(1-\nu-p)\,\Gamma(1+p)}.$$

Section 6 concluded the paper and contained a brief discussion of unsolved problems relating to the polylogarithm.

W. F. Pickard

E. RECEPTOR POTENTIALS IN RETINULAR CELLS IN LIMULUS

The photoreceptor unit of the compound lateral eye of the horseshoe crab, Limulus polyphemus, is the ommatidium. Each ommatidium has two kinds of cells involved in the transduction and transmission of photic information to brain: the retinular cells, numbering 8 to 20, which are the photoreceptor cells, and the (usually) single eccentric cell, which is the first-order neuron in the visual pathway. Light incident on the ommatidium is absorbed by the visual pigment rhodopsin.[1] By some still unknown means this leads to a depolarization of the retinular cells. This depolarization is transmitted to the eccentric cell via an electrotonic synapse.[2] When the eccentric cell is depolarized to a threshold valve, all-or-none action potentials are generated, which propagate along its axon to the optic lobe of the brain.[3,4] Also, some integration of the photic information occurs via lateral inhibition in the plexus just central to the ommatidia.[4]

Thus, while a good deal is known about the means by which photically evoked electrical signals, once produced, are transmitted from cell to cell and conducted to the brain, little is known of the mechanisms by which the absorption of light by rhodopsin leads to a potential change (the receptor potential) across the retinular cell membrane. The nature of this energy transduction from light to electricity remains an important unknown in visual physiology.

In order to gain some insight into the nature of the coupling between the visual pigment and the photoreceptor membrane, the light-evoked potential changes in retinular cells have been investigated with intracellularly placed double-barrel microelectrodes. With such an arrangement, extrinsic current may be passed through the cell membrane via one barrel and the membrane potential measured with the other. The changes in membrane potential produced by light and by the interactions of light and injected current may also be observed.

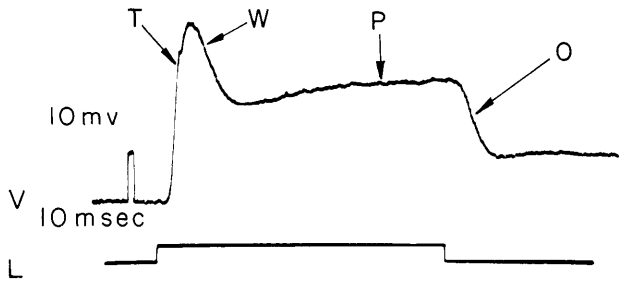

Fig. XIX-7

Receptor potential (upper trace) evoked by long pulse of light (lower trace) of moderate intensity. Pulse on voltage trace is 10 mv and 10 msec. T, W, P and O indicate transient, wave, plateau, and "off" responses, respectively.

The dark-adapted retinular cell has resting membrane potential of 40-50 millivolts, the inside of the cell negative with respect to an extracellularly placed reference electrode. The receptor potential evoked by a long pulse of light is a complicated waveform which may be divided into four components (Fig. XIX-7). Several milliseconds after the onset of the light pulse, the response begins with a depolarizing transient (T in Figs. XIX-7 and XIX-8).

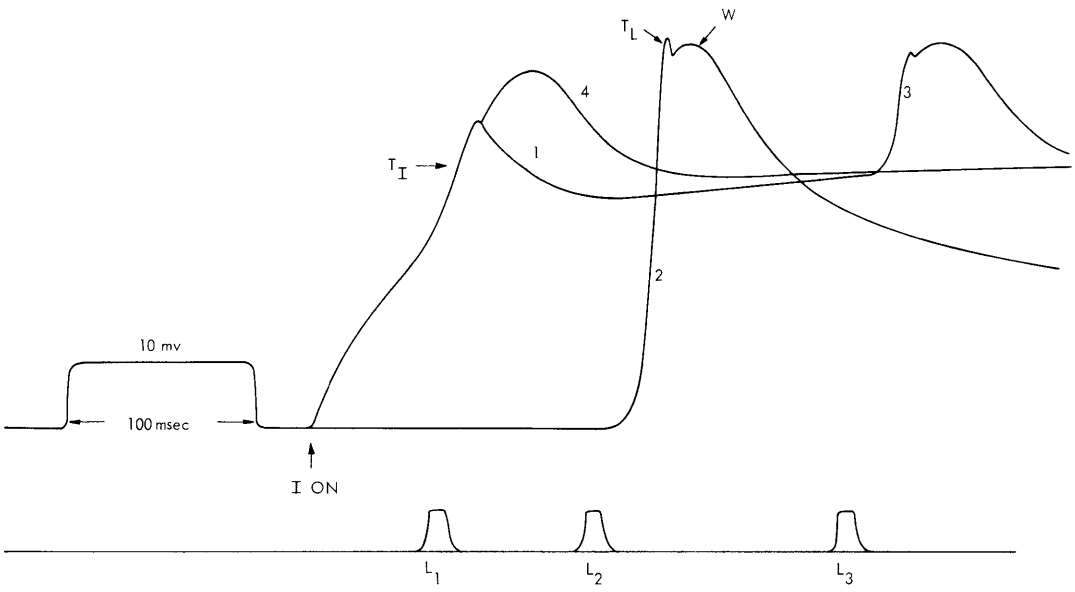

Fig. XIX-8. Occlusion of current and light-evoked transients. Superimposed line drawings from separate single traces. Upper trace is voltage-time recording with intracellular microelectrode from a retinular cell. Lower trace indicates short light pulses. Voltage-time calibration is 10 mv, 100 msec. Trace 1 is response of cell to a step of polarizing current beginning at I ON. Current-evoked transient is T_I. Trace 2 shows transient (T_L) and wave (W) evoked by a short pulse of light (L_2). Trace 3 is interaction of the same light pulse (applied during L_3) and the same current step with a long delay between their onsets. Trace 4 is the same as Trace 3 with a short delay between onset of current and light. Note in 4 the occlusion of T_L.

This is followed by a slow wave, (W), of depolarization. Subsequently, the potential repolarizes to a plateau, P, whose steady-state level, however, is more depolarized than the membrane potential in the dark. Following cessation of the light, the potential returns to its resting level in one of two ways. After long or intense lights, the membrane potential first repolarizes to a potential level greater (more hyperpolarized) than its resting value and then decays to that value. After short or dim lights, the potential shows no hyperpolarizing undershoot, but returns to its resting level (O in Fig. XIX-7). These various components will be examined seriatim.

Extrinsic depolarizing current applied through the microelectrode evokes a transient which has a threshold, T_I (see Fig. XIX-8). Interaction of this current-evoked transient with that generated by light shows that they occlude (see Fig. XIX-8). These data suggest that both transients arise via the same mechanism and are an inherent property of the retinular cell membrane. The transient appears to be regenerative but not propagated. It is not, however, a true all-or-none action potential or spike for several reasons: (i) its peak amplitude is inconstant and varies with the degree of depolarization;

(ii) its duration is an inverse function of its amplitude; (iii) it has a variable refractory period; and (iv) its threshold is a function of the membrane potential (i.e., the greater the resting membrane potential, the larger the membrane potential level of the apparent threshold).

Short light pulses evoke only the transient and wave components (Figs. XIX-7 and XIX-8). Increasing (hyperpolarizing) the membrane potential with current via the microelectrode increases the amplitude of both components (Figs. XIX-9 and XIX-10). Conversely, depolarization decreases both and when the membrane potential is reversed (i.e., the inside made positive) the wave reverses its polarity (i.e., becomes negative-going) (D_1 and D_2, Fig. XIX-9). Since at small values of membrane potential the transient often becomes obscure, its reversal potential is not easy to specify with certainty. It appears (Fig. XIX-9, D_1 and D_2), however, to be at some positive potential value. Time-varying impedance measurements indicate that the wave is associated with a large increase in conductance. Thus the wave appears to be a consequence of a virtual short-circuiting of the membrane impedance.

If, during the plateau phase of the response, a short pulse of current is injected, the steady-state potential evoked is a measure of the slope resistance of the membrane. If, in the dark, the membrane is depolarized with extrinsic current to the same absolute membrane potential reached during the light-evoked plateau and if the same short current pulse is then injected, the identical steady-state potential is recorded. This indicates that a steady-state potential level, whether generated by light or current, is associated with the same membrane conductance and suggests that the plateau response is produced via a light-activated constant-current source.

The plateau response can also be studied by examining the current-voltage characteristics of the retinular cell membrane in the dark and under the condition of continuous illumination (Fig. XIX-10). In the dark (curve D), the membrane shows double rectification. Near resting potential, the resistance is greater to hyperpolarizing than to depolarizing currents. When, however, the membrane potential is reversed (inside positive) the resistance again increases with further depolarization. With intense, continuous light (curve L_2), the increased resistance with hyperpolarization persists, but requires more current to demonstrate. The increased resistance to large depolarizing currents, however, is no longer demonstrable. If from the I-V curve obtained in the light, the $\log \frac{I - I_{s_1}}{I_{s_1}}$ is plotted against V (where I_{s_1} is the saturation current obtained from the "dark" I-V curves and is defined as the asymptotic current measured from resting potential required to produce a graphically projected infinite voltage), a straight line results. If the I-V curve obtained in the light is subtracted from the I-V curve obtained in the dark and, from the resultant curve, the $\log \frac{I - I_{s_2}}{I_{s_2}}$ versus V is plotted

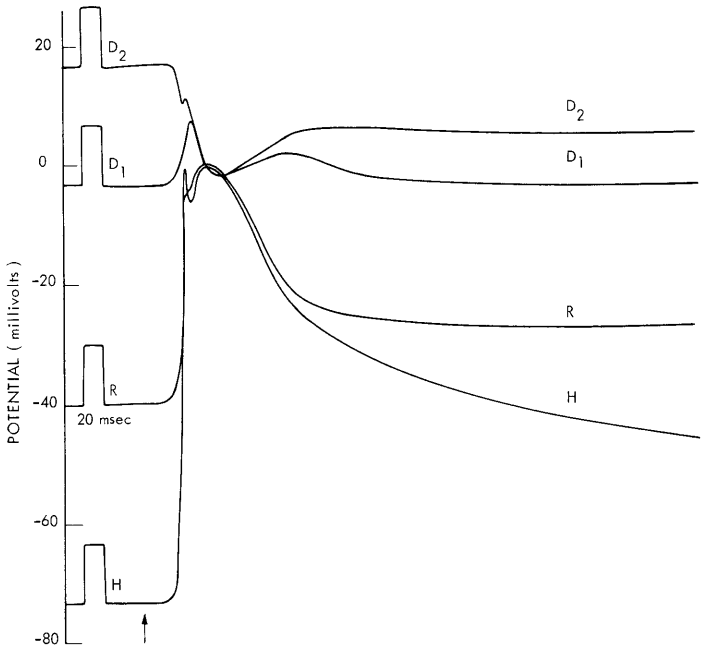

Fig. XIX-9. Effects of changing membrane potential on light-evoked transient and wave. Superimposed line drawings from separate single traces. Calibration is 10 mv and 20 msec. Onset of short light pulse indicated by arrow under Trace H. Potential in millivolts with respect to extracellular reference electrode. Trace R is response to light pulse in the absence of extrinsic current. Trace H is response to identical light pulse after the membrane potential was hyperpolarized with extrinsic current. Traces D_1 and D_2 show response after membrane potential was depolarized to successively higher levels. Note that the peak of the wave reverses near V = 0 while the transient reverses at a positive potential.

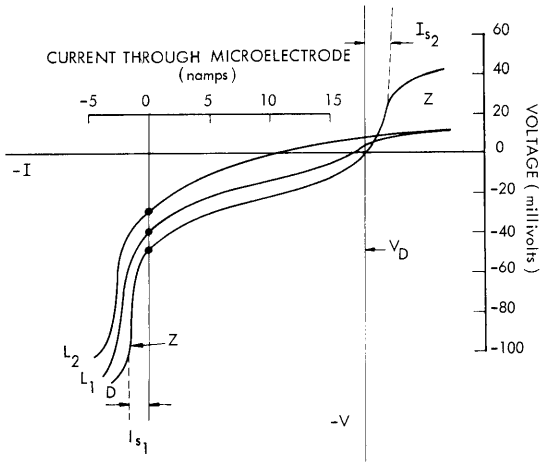

Fig. XIX-10.

Current-voltage characteristics measured with double-barrel microelectrode in retinular cell. Current in namps passed through microelectrode; voltage in millivolts with respect to extracellular reference electrode. V_D is resting potential in the dark. Three curves show characteristic in the dark (D) and at moderate (L_1) and high (L_2) light intensities. Vertical line through closed circles on each curve joins steady-state membrane potentials. I_{s_1} and I_{s_2} are reversed saturation currents for diodes D_1 and D_2 of Fig. XIX-11.

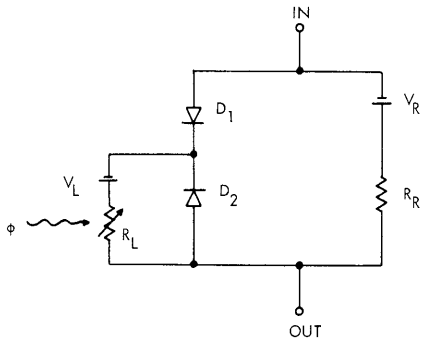

Fig. XIX-11.

Equivalent electrical circuit for retinular cell membrane under steady-state conditions. IN is intracellular; OUT is extracellular. D_1 and D_2 are semiconductor-like diodes. V_R is resting membrane voltage. R_R is a resistance whose value is high with respect to the reverse resistance of diodes D_1 and D_2. R_L is the resistance whose value is greater than the reverse resistance of D_2 in the dark and is reduced by light (ϕ). V_L is a battery whose current output is increased by light.

(with the appropriate adjustments in sign of I and V), another straight line is obtained. (Here, I_{s_2} is also obtained from the "dark" curve and is defined as the asymptotic current, measured from V = 0, required to produce a graphically projected infinite voltage.) These results suggest that the equivalent circuit for the resting retinular cell membrane is two semiconductorlike diodes placed back to back (Fig. XIX-11). At resting potential one diode (D_2) (revealed only by membrane potential reversal in the dark) is partially forward-biased. Moreover, these results suggest that the action of intense light is to short-circuit completely this diode D_2, and that there is a marked increase in conductance of a resistor (R_L) in parallel with D_2. (The conductance of R_L in the dark is much less than D_2; however, its value in intense lights is much greater than D_2.) Also, detectable current is now drawn from V_L, a battery in series with R_L and in parallel with D_2. Furthermore, it would appear that the other diode (D_1, revealed by the increased resistance with hyperpolarization) is not altered by light. This is further suggested by the observation that translation of the "light" I-V curve onto the "dark" I-V curve reveals virtual superposition of the two curves over the range -15 to -100 mv ("dark" values).

If these observations are substantially correct, three conclusions may be allowed. First, the mechanism of the apparent constant-current source is a short-circuiting of one diode (D_2) and the attendant activation of a previously occult battery. The potential changes which result are a consequence of the currents so generated flowing across the other, unaltered diode (D_1). Second, the over-all slope conductance of the membrane changes, but this is mainly due to forward-biasing the passive diode (D_1), which is the major determinant of slope resistance in the physiological range of steady-state membrane potentials, and not to a short-circuiting of the entire membrane, as in the wave response.

The third conclusion is that the Weber-Fechner relationship may be a consequence of the membrane characteristics of the photoreceptor cell. Previous experiments have

shown (i) that the steady-state firing frequency of eccentric cells is a logarithmic function of light intensity[3]; (ii) the firing frequency of eccentric cells is a linear function of their membrane potential[3]; (iii) steady-state membrane potential changes in eccentric and retinular cells are a logarithmic function of light intensity[5]; and (iv) "physiological-range" depolarizations of retinular cells by extrinsic current are linearly transmitted to eccentric cells.[6] We have shown here that light and current can evoke indistinguishable steady-state changes in retinular cells and thus, presumably, light generates a current linearly related to its intensity. This presumption is supported by the previous conclusions and observations, viz., given a logarithmic I-V curve and a logarithmic relationship between light intensity and membrane potential change, light intensity and current must be linearly related over the ranges studied.

Two ancillary observations of the above studies are worthy of note. First, the I-V curves show a hysteresis effect, i.e., the removal of extrinsic current produces an increased conductance of the retinular cell membrane which may last hundreds of milliseconds and is associated with depolarizing oscillations, often of sufficient magnitude to fire the eccentric cell.[2] The second is that large hyperpolarizing and depolarizing currents produce reversible "punch-through" or "breakdown" effects in the two diodes in a manner similar to actual semiconductor diodes (the Z's, in Fig. XIX-10).

The "off" response, produced by the removal of light, has been little studied, even in the experiments reported here. By analyzing the time-varying impedance changes, however, we have found that this phase of the response is associated with an impedance greater than the resting, "dark" impedance. This is the case even in those responses that do not show a hyperpolarizing undershoot.

These various experiments define in an operational way the mechanisms by which light evokes potential changes in Limulus photoreceptors. As in most cells, the recorded potentials are a consequence of a complex electrochemical system involving a membrane and both intracellular and extracellular spaces composed of ionic solutions. It might be expected, therefore, that one might produce alterations, perhaps specific, by manipulation of the ionic composition of the extracellular medium.

Previous experiments have shown that, again as in most cells, the resting potential is essentially a function of the ratio of the concentration of potassium between the inside and outside of the membrane in a manner predicted by the Nernst equation.[6] We have found that alteration of the extracellular concentration of chloride (replacement with sulfate) produces little or no effect on any measured characteristics of retinular cells. Replacement of extracellular sodium with $TrisH^+$ (keeping osmolarity and pH constant), however, completely and reversibly abolishes the responsiveness of retinular cells to light without altering the I-V curves for values near resting potential. Such cells cease to act as photoreceptors. Thus, as far as the receptor potential is concerned, the action of light on photoreceptors appears to be involved with the mechanisms by which the

permeability of the membrane to sodium ions is regulated.

In summary, we have defined the area of interest to be the means by which the chemical alterations in rhodopsin produced by its absorption of light energy are transduced or coupled to the receptor cell membrane. In pursuing this question we have analyzed the mechanisms by which the resultant potential changes are produced. The initial, transient portion of the response appears to be an inherent property of the membrane to any depolarization of sufficient magnitude. The wave seems to involve a virtual but brief short-circuiting of the membrane impedance. The plateau or steady-state response to light appears to involve constant-current type of generator and we have suggested the way in which this unusual and unexpected mechanism operates, viz., the short-circuiting of a diode and the activation of a battery. We have mentioned, in passing, how the receptor membrane characteristic might account for the Weber-Fechner relationship in Limulus photoreceptors. Moreover, we have suggested that the "off" response is an active process involving an increase in over-all membrane impedance. Finally, we have indicated that the alterations in membrane potential by light appear to involve the means by which the absorption of photic energy by rhodopsin leads to an increase in the permeability of the photoreceptor membrane to sodium ions.

A good deal of the credit for any experimental successes and valid interpretations reported here must go to Dr. Fritz Bauman, Institut de Physiologie, École de Médicine, Geneva, Switzerland, and Dr. M. G. F. Fuortes, Ophthalmology Branch, NINDB, NIH, Bethesda, Maryland, who collaborated in various phases of these researches. The author alone, however, assumes responsibility for the present report.

T. G. Smith, Jr.

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F. DIRECT PHOTOELECTRIC EFFECT IN PHOTORECEPTOR CELL MEMBRANES

When an eye is illuminated, the energy in the incident photons is absorbed by a visual pigment (e.g., rhodopsin), which is a constituent of the photoreceptor cells (rods, cones, retinular cells). This process then leads to a change in the membrane potential of the photoreceptor cell, the so-called receptor potential. In the lateral eye of the horseshoe crab, Limulus polyphemus, this potential change appears to be primarily a consequence of an increase in the permeability of the retinular cell membrane to sodium ions (see Sec. XVIII-E).

When the eye is stimulated with a pulse of light of moderate intensity there is a latency of several milliseconds between the onset of the light and the beginning of the receptor potential. This delay may indicate that there are one or more steps intervening between the absorption of light by the visual pigment and the onset of the receptor potential. Considerable interest has been generated, therefore, by the recent discovery the so-called early receptor potential (ERP).[1,2] The ERP, which has been observed only with extracellular recordings in vertebrate eyes, has the following characteristics: (i) its latency of onset is of a few microseconds; (ii) it persists when the cells of the eye are depolarized with potassium ions; (iii) it persists at temperatures as low as -35°C; (iv) it has, in the albino rat, the action spectrum of rhodopsin; and (v) its amplitude is linearly related to the member of rhodopsin molecules bleached.[1-3] For these reasons, the ERP has been interpreted as a direct manifestation of the absorption of light by the visual pigment and perhaps represents a change in the dipole configuration of the pigment molecule.

Efforts to record an ERP in invertebrate eyes have previously been unsuccessful. In an attempt to elicit the response in the lateral eye of the horseshoe crab, a 100-watt mercury-arc lamp and an optical system were employed which would focus several milliwatts of radiant power per square centimeter onto a spot 100 μ in diameter (the diameter of one ommatidium). With a shutter arrangement, light pulses as short as 3 msec could be produced. With such intense lights, considerable care must be taken to shield those components of the recording system (e.g., Ag-AgCl wires) which might generate photoelectric effects. Since the tip of the KCl-filled micropipette, however, must be in the light beam when located inside a retinular cell, it was necessary to investigate the effects of intense lights on microelectrode tips. Lights of the intensity employed during the biological experiments produce a photoconductive effect in KCl microelectrodes. In our recording system (peak-to-peak noise 50 μV, input impedance 10^{12} ohms, grid current less than 10^{-12} amp) this increased conductance is detectable only with currents greater than 10^{-10} amp flowing through the microelectrode. Thus KCl microelectrodes show no detectable photovoltaic effect. Moreover, no effects could be produced by filling the microelectrode with KCl saturated with methylene blue or after plugging the tips of

the microelectrode with tissue fragments from the crab's eye. Thus all observations reported here were recorded in a system with the appropriate light shielding and with grid current of the order of 10^{-12} amp.

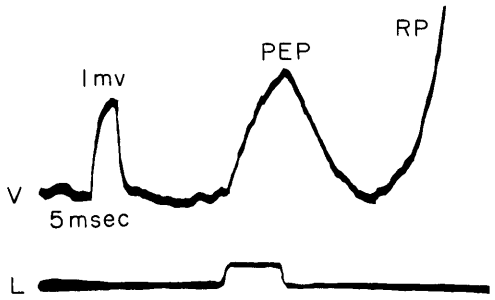

Fig. XIX-12.

Photoelectric potential (PEP) from retinular cell. Upper trace is voltage-time recording via intracellular microelectrode. RP is receptor potential. Calibration pulse is 1 mV and 5 msec. Lower trace shows the application of a brief, intense pulse of light.

When a short pulse of our most intense light stimulates a retinular cell whose transmembrane potential is recorded with an intracellular microelectrode, a depolarizing potential of 0.5-1.2 mV amplitude is evoked (Fig. XIX-12). The latency of the potential is less than a millisecond and the potential lasts for the duration of the light pulse. Since the mechanism underlying this response has yet to be completely elucidated, we shall call it a photoelectric potential (PEP) rather than an ERP, whereby a specific mechanism is implied.

When a micropipette is extracellularly located, but pressed against the retinular cell membrane, the polarity of the PEP and the receptor potential are both reversed in sign (i.e., now negative-going). Withdrawal of the pipette, only a few microns results in simultaneous loss of both the PEP and the receptor potential. This suggests that both a arise from the retinular cell. Like the ERP, the PEP persists when the cell is depolarized with extracellular potassium ions and when the tissue is frozen (to -10°C, the temperature below which recording from a KCl pipette is no longer practicable). Moreover, the amplitude of the PEP is linearly related to light intensity. The PEP shows, however, little evidence of light adaptation. If repetitive light pulses are delivered to a dark-adapted eye, the second and subsequent PEP's are only slightly smaller than the first. This observation does not so readily distinguish the PEP from the ERP as it might first appear. In the first place, the ERP does not completely light-adapt in vertebrate eyes, where the visual pigment is bleached.[4] This unadaptable ERP is presumably due to light-regenerated rhodopsin. Second, most invertebrate rhodopsins do not bleach at physiological temperatures and are readily regenerated by light.[5]

When two separate single-barrel microelectrodes are placed within the same retinular cell, current may be passed through one electrode across the cell membrane and the resultant potential changes observed with the other. The effects of changing membrane potential on the PEP can then be studied without having a photoconductive effect

in the recording electrode. Hyperpolarization of the retinular cell membrane increases the amplitude of the PEP. Conversely, depolarization decreases its amplitude. Moreover, when the membrane potential is forced to values more positive than a level somewhere near zero membrane potential, the polarity of the PEP is reversed. These observations suggest that the PEP arises from some structure electrically in series with the recording system between the inside and outside of the cell. Presumably this is the membrane itself. An alternative mechanism would appear to be excluded, viz. the injection of a positive charge into the intracellular space of the cell from some exclusively intracellularly located compartment or molecule. If such a mechanism were operative, it would not be possible to reverse the sign of the PEP by passing current across the membrane.

Thus the PEP appears to be a response evoked by light from some component of the retinular cell membrane and has some of the characteristics of the ERP. It remains to be shown, however, that the PEP has anything to do with vision. The evidence that we have on this important point is inconclusive. The PEP can be recorded from the eccentric cell of the Limulus eye and from other cells that do not otherwise respond to light. The latter may be the pigment cells known to envelop the ommatidium. In an experiment performed in collaboration with Mr. James Anderson of the Communications Biophysics Group a PEP was recorded from pigmented cells in the cerebral and abdominal ganglion of the sea-slug, Aplysia californica. These experiments would suggest that the PEP is a general property of pigmented cells, which is consistent with the recent observations that ERP-like responses can be recorded from isolated pigment epithelium of the frog and rat eye.[6,7]

Our light source is insufficiently intense to allow a detailed examination of the action spectrum of the PEP with interference filters. Employing broadband and highpass and lowpass interference filters, however, we have found that the response is most sensitive to lights in the visual spectrum and less so to the deep-blue, the near ultraviolet, the far red, and the near infrared. We cannot, at present, specify the wave length of maximum sensitivity. If the PEP were mainly or exclusively due to rhodopsin, a peak in the action spectrum should occur near 520 mμ.[8]

In an effort to define the action spectrum in some detail, we have recently begun a series of experiments employing argon and krypton lasers. Such light sources have sufficient energy at a number of monochromatic lines to evoke the PEP. The results are still not definitive.

While there is insufficient evidence at present to suggest that the PEP, like the ERP, is a direct manifestation of the absorption of light by visual pigments, should that turn out to be the case the experiments reported in this communication bear importantly on the question of the physical location of such pigments in photoreceptors. They indicate that the pigment molecules are so intimately associated with the cell membrane as to be

a component of the electrical circuit between the inside and outside of the cell and may actually be a molecular constituent of that membrane. The location of the photopigment within the photoreceptor membrane has important implications for the possible mechanisms by which its absorption of light leads to the production of the generator potential. Certain kinds of mechanisms would appear to be excluded. For example, the release by an intracellularly located structure of a transmitter-like substance which acts on the inner surface of the receptor membrane would seem not to be involved. Instead some means by which the alteration of the pigment molecule by light can affect those (presumably) nearby membrane components which control the membranes' permeability to sodium ions would appear to be a more probable mechanism.

T. G. Smith, Jr., J. E. Brown

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