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THE ORIGIN OF THE EARLY RECEPTOR POTENTIAL OF THE RETINA

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SUMMARY

1. The early receptor potential of the isolated retina of the frog has the following properties:

(*a*) It is unaffected by reversing the direction of incidence of the light, and by soaking the retina in isotonic solutions of salts containing neither sodium nor chloride.

(*b*) Soaking the retina in solutions containing high concentrations of salts or glycerol greatly alters its time course.

(*c*) Soaking the retina in solutions containing formaldehyde or *N*-ethyl maleimide slightly shortens its time course but does not otherwise much affect it.

2. We argue that the early receptor potential depends on displacements of charge within the monolayers of visual pigment as they undergo a sequence of chemical changes after absorbing light. The time course of the observed response must differ from that of the movement of charge because of filtering by the resistance-capacity network in the receptors. Changes in the resistive components of this filter will partly or wholly account for the effects of salts and glycerol on the early receptor potential, but cannot explain those of temperature. It seems that at least two successive movements of charge must occur, the second of them strongly influenced by temperature. Neither movement requires the presence of free amino or thiol groups.

3. The above hypothesis requires that all or most of the monolayers of visual pigment in a receptor be orientated the same way; they cannot face alternately in opposite directions.

INTRODUCTION

Brown & Murakami (1964) found that when a monkey's eye was stimulated with an extremely bright brief flash of light, an electrical response with no detectable latency could be recorded by means of an intraretinal micro-electrode. Cone (1964) showed that this 'early receptor

potential' could be recorded with large electrodes outside the retina. In the present paper we examine some of its properties in the isolated retina of the frog, and draw conclusions about its origin.

METHODS

Retinas were dissected from excised eyes of frogs as described by Hamasaki (1963). They were immersed for minutes or hours in various solutions. Then each retina was mounted on a solid dome pierced from base to apex by a capillary channel which was filled with the solution in which the retina had been immersed, and a cotton wick soaked with the same solution was placed in contact with the surface of the retina that was not touching the dome. A preamplifier and cathode ray oscilloscope recorded the difference of electrical potential between the wick and the solution in the capillary channel of the dome. The metal surfaces which made contact with the electrolyte were of platinum and well shielded from the light.

The stimulus was provided by a Mazda FA 5 flash tube through which a capacitor of 50 μF charged to 1000 V was discharged. The light was concentrated on the retina by a system of lenses and mirrors. Very strong flashes were used; a single flash sufficed to bleach a substantial fraction of the rhodopsin in a retina exposed to it, as could be seen by inspection. Usually we recorded only one response from each retina.

RESULTS

Reversal of the direction of incidence of the light. We usually mounted the retina with the rods and cones against the dome and illuminated it from the vitreal surface (Figs. 1*A*, 2–5). Figure 1*B* shows the response of a retina mounted with the vitreal surface against the dome and illuminated from the choroidal surface. It can be seen that the record is simply inverted, with the possible exception of the first few microseconds, where all our records are marred by a small artifact (Fig. 1*E*). Thus no effect of direction of incidence of the light upon the response is demonstrated.

Replacement of sodium chloride by foreign salts. The early receptor potential could be obtained with normal amplitude and time course from retinas that had been immersed for 2 hr in 250 m-osmolar solutions of potassium chloride, potassium nitrite, calcium chloride, ammonium oxalate, magnesium sulphate or the trishydroxymethylaminomethane salt of ethylenediaminetetra-acetate. As would be expected from the observations of Furukawa & Hanawa (1955) and Hamasaki (1963), the electroretinogram as ordinarily understood (i.e. excluding the early receptor potential) is totally abolished by immersion for 10 min in any of these solutions.

Variations in ionic concentration. Figure 2*A* shows the responses of a retina that had been immersed for 1 hr in quarter-strength Ringer's solution. The principal effect of dilution that can be seen is that the responses are slightly expanded along the time axis.

The opposite effect, a shortening of the time course, can be produced by increasing the concentrations of ions. Immersion for from $\frac{1}{2}$ hr to 2 hr in

a M/2 solution of sodium chloride, potassium chloride or potassium nitrite, or in a M/3 solution of ammonium oxalate, causes a contraction of the first part of the response (as far as the vitreous-negative peak, but not including the final return to the base line) by a factor of between 1·5 and 5 as compared with Ringer's solution. The potassium salts are more effective than sodium chloride or ammonium oxalate (see Fig. 2*B* and *C*). Figure 2*D* shows that M/2 magnesium sulphate does not behave in the same way. A likely explanation is that magnesium and sulphate ions do not penetrate to the site where other ions act.

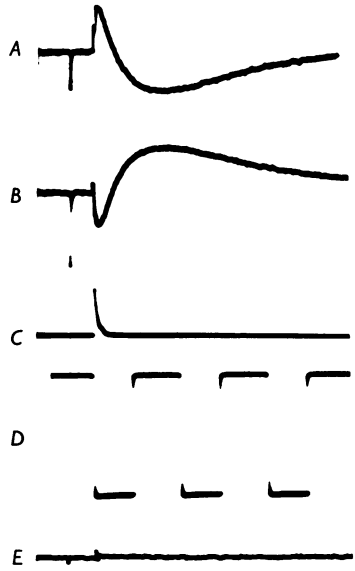

Fig. 1. Early receptor potentials recorded at 24° C. *A*: Receptoral side down (i.e. in contact with the dome). The receptoral side becomes first negative and then positive in relation to the vitreal side. *B*: Vitreal side down. The polarity of the response is inverted in relation to the electrodes, i.e. unchanged in relation to the retina. *C*: Photocell record of the flash. *D*: 1000 c/s and 1 mV. *E*: The artifacts obtained with no retina in the apparatus. One precedes the flash and is unpredictably variable in size and timing; the other coincides with the early part of the flash and is fairly constant.

As can be seen in Fig. 2*E* and *F*, a great expansion on the time axis can be produced by addition of glycerol to the solution in which the retina has been soaked.

Empirically, if those with magnesium sulphate are excluded, the changes illustrated in Fig. 2 are correlated with the electrical conductivities of the solutions used (see Table 1).

Temperature. The effect of temperature on the early receptor potential has been examined by Pak & Cone (1964) and Pak & Ebrey (1965). Our

findings for the frog (Fig. 3) are in general agreement with theirs for the rat, though we have not covered as wide a range of temperature. Our new and striking finding is shown in Fig. 3*E*: the prolongation of the vitreous-positive phase associated with the suppression of the vitreous-negative phase on cooling to 2° C (Fig. 3*A–D*) can be counteracted by the addition of a salt, in this instance M/2 potassium nitrite, so that the total length of the vitreous-positive phase is returned to its value at 20 °C.

TABLE 1. Conductivity of solutions at 24°

Solution	Conductivity (m-Mho/cm)
Ringer's solution (115 m-mole/l. NaCl, 2·5 m-mole/l. KCl, 1·8 m-mole/l. $CaCl_2$, 2·15 m-mole/l. Na_2HPO_4, 0·85 m-mole/l. NaH_2PO_4)	11
M/2 potassium chloride	52
M/2 potassium nitrite	49
M/2 sodium chloride	43
M/2 magnesium sulphate	26
M/3 ammonium oxalate	45
Equal volumes of glycerol and Ringer's solution	1·4
1·3 mole/l. formaldehyde, 140 m-mole/l. sodium chloride	12

Hydrogen-ion concentration, formaldehyde, a thiol-blocking agent, and an aldehyde-blocking agent. Responses recorded after 30 min soaking in 120 mM phosphate buffers between pH 8·5 and 2·8 are shown in Fig. 4*A* and *D*. Hydrogen-ion concentration has a rather small effect on the response.

Figure 5*A* and *B* show the effects of 30 min soaking in 0·33 and 1·30 M formaldehyde. We were surprised to see any response at all in such an environment; but as can be seen, the responses are not much reduced in amplitude. They are shortened in time scale and at the higher concentration the early vitreous-positive phase is greatly reduced in comparison with the late vitreous-negative phase. Whether it is completely abolished cannot be judged with certainty because of the unavoidable stimulus-artifact.

The thiol-blocking agent *N*-ethyl maleimide had no effect on the early receptor potential except to shorten its time course (Fig. 5*C*).

Retinas that had been soaked for 1½ hr in a 125 mM solution of hydroxylamine hydrochloride brought to pH 6·5 by addition of sodium hydroxide gave responses of normal shape and amplitude, but these responses decreased more than normally in amplitude when several flashes were given in sequence; after five flashes the retinas were permanently unresponsive and colourless.

DISCUSSION

Does the 'early receptor potential' come from receptors? The general belief that it does rests on its extremely short latency (Brown & Murakami, 1964 and all later writers), its persistence in mammalian eyes that have been so long excised that their nerve cells are likely to be dead (Cone, 1964), the agreement of its action spectrum with that of rhodopsin (Cone, 1964), the

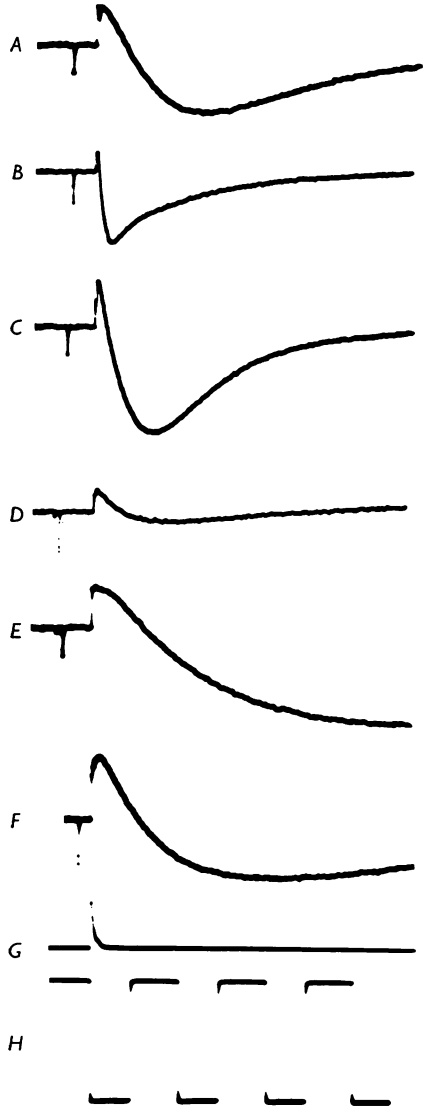

Fig. 2

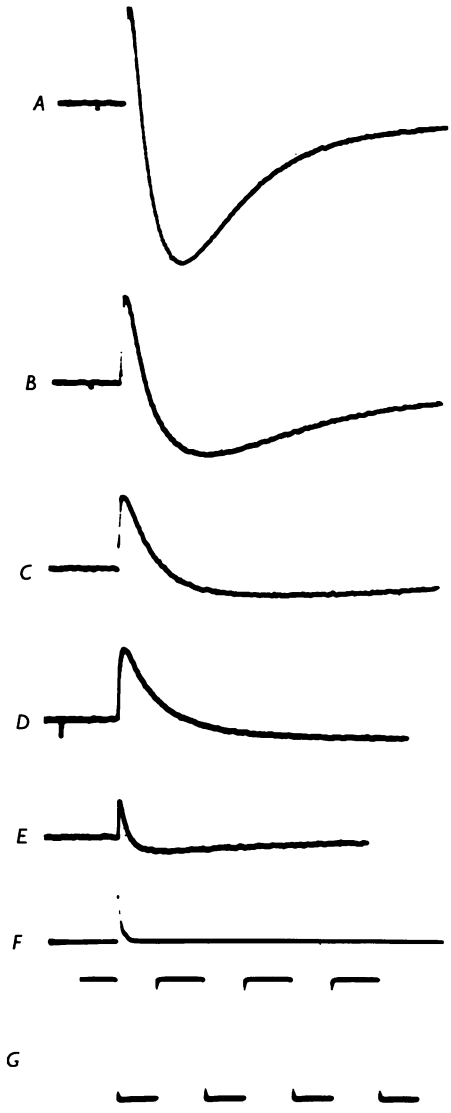

Fig. 3

Fig. 2. Early receptor potentials recorded at 24° C after prolonged immersion of the retina in various solutions. *A*: After immersion for 60 min in a mixture of 1 part Ringer's solution and 3 parts distilled water. *B*: After immersion for 30 min in M/2 potassium nitrite. *C*: 30 min in M/3 ammonium oxalate. *D*: 30 min in M/2 magnesium sulphate. *E*: 50 min in a mixture of 1 part Ringer's solution and 1 part glycerol. *F*: Another retina soaked for 55 min in a mixture of 1 part Ringer's solution and 1 part glycerol, recorded with a time base twice as slow as that used for all other records. *G*: photocell record of the flash. *H*: 1000 c/s and 1 mV.

Fig. 3. Early receptor potentials recorded at different temperatures. *A*: 27° C. *B*: 19° C. *C*: 9° C. *D*: 2° C. *E*: 2° C after immersion for 10 min in M/2 potassium nitrite. *F*: Photocell record of the flash. *G*: 1000 c/s and 1 mV.

direct proportionality of its amplitude to the energy of the stimulus (Cone, 1964), and the persistence of its vitreous-positive peak at temperatures as low as −35° C (Pak & Ebrey, 1965). Though no one of these grounds is conclusive, together they make a very strong case. The case is further strengthened by our finding that the early receptor potential persists in grossly abnormal chemical environments.

Can the early receptor potential be due to downhill movement of ions across a membrane? When we began the experiments of this paper, we thought it likely that the early receptor potential would resemble a post-synaptic

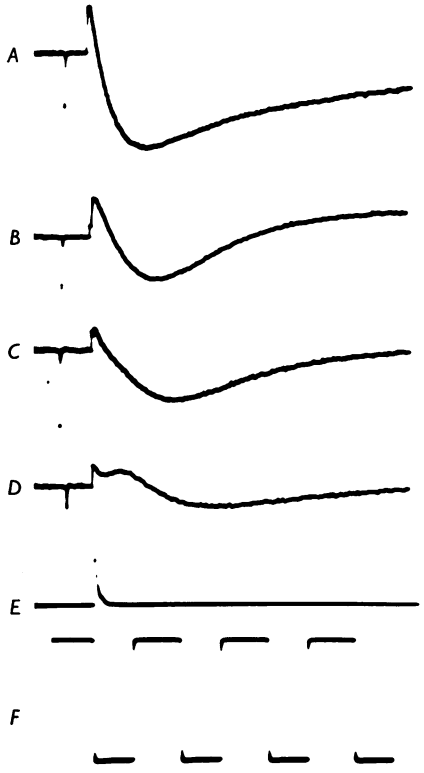

Fig. 4

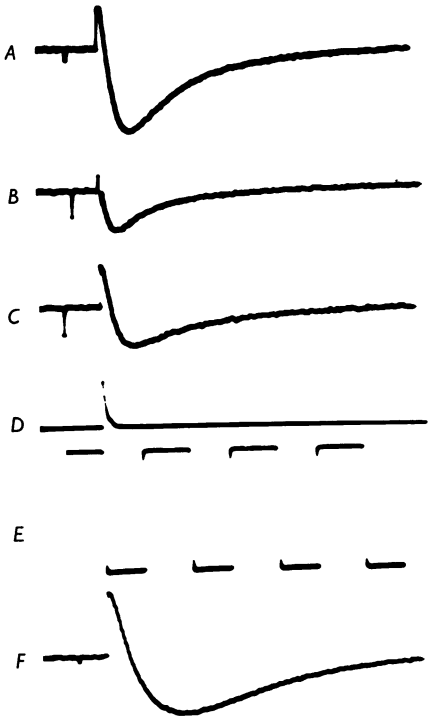

Fig. 5

Fig. 4. Early receptor potentials recorded after immersion of the retina for 40 min in 120 mM phosphate buffers at 24° C. *A*: pH 8·25. *B*: pH 5·5. C: pH 3·7. *D*: pH 2·8. *E*: Photocell record of the flash. *F*: 1000 c/s and 1 mV.

Fig. 5. The effects of formaldehyde and *N*-ethyl maleimide at 24° C. *A*: After immersion of the retina for 35 min in a solution containing 330 m-mole/l. formaldehyde and 150 m-mole/l. sodium chloride, pH 5·2. *B*: 25 min in a solution containing 1·3 mole/l. formaldehyde and 140 m-mole/l. sodium chloride, pH 4·8. *C*: 45 min in a solution containing 20 m-mole/l. *N*-ethyl maleimide and 154 m-mole/l. sodium chloride, pH 6·7. *D*: Photocell record of the flash. *E*: 1000 c/s and 1 mV. *F*: Control response (a retina that had been immersed only in Ringer's solution).

potential or the regenerative component of a nerve impulse; that is, it would be due to a movement of ions from a region where they are at high electrochemical potential to one where they are at low electrochemical potential. The movement would be initiated by a specific or unspecific change in the permeability of the membrane separating the two regions; this might be the surface membrane of the receptors or possibly some membrane within them.

The hypothesis of generation by downhill movement of ions was made very unlikely when we found that the early receptor potential was unaffected by replacing the sodium chloride of the bathing fluid by foreign salts. In the face of this observation the hypothesis can be rescued only if the active membrane is screened from the applied solutions by a barrier impermeable to all the salts that we tested, or if the downhill movement is the outflow of an ion different from any that we tested. The first of these escapes is almost excluded by the fact that M/2 potassium chloride greatly shortens the response but M/2 magnesium sulphate slightly lengthens it. Conductivity and osmotic pressure, the two properties by which these two solutions might plausibly be supposed to act at a distance, are nearly enough equalized, so one or both of the solutes must reach the site where the response is generated. The second escape could be excluded if every ion that could conceivably carry the current were tested and found to have no specific effect on the early receptor potential. But we are disinclined to take this search further than we have done in view of the persistence of the response in the presence of high concentrations of formaldehyde. It is very hard to believe that any biological membrane could retain its normal permeabilities in such an extreme chemical environment.

Our hypothesis of the origin of the early receptor potential. Figure 6 represents part of the outer segment of a rod or cone in longitudinal section. We assume that each of the 'sacs' or 'disks' visible in electron micrographs of receptors contains one, two, or perhaps more monomolecular layers of visual pigment, and that in association with each such layer there is a continuous layer of high electrical resistance that contains the carotenoid parts of the visual pigment molecules. Each of these dielectric layers is represented by a white band in the figure, and each intervening conducting layer by a black band. Within each dielectric layer we assume that there is a sublayer, shown by the interrupted line, that is electrically charged. The origin of the early receptor potential, on our hypothesis, is movement of this charge. Let the thickness of each dielectric layer be d, its dielectric constant be k, its area be A, the resistance across it be R, and the number of such layers be n. Then if in each dielectric layer a sublayer with charge q per unit area moves instantaneously through a distance x, a potential $4\pi nqx/k$ will be developed instantaneously across

the whole system, and will decay exponentially with time constant $AkR/4\pi d$ if the transverse resistance of the conducting layers is negligible. If not negligible, this transverse resistance will cause the decay curve to lie below the given exponential in the early stages of decay and above it in the late stages.

The probable need to postulate two successive movements of charge. If the output given by the system of Fig. 6 with a single rapid movement of charge as input were fed into a simple high-pass resistance-capacity filter of time constant short compared with $AkR/4\pi d$, it would yield a diphasic response roughly resembling the observed early receptor potential, but with zero time-integral. Linear filters can be designed that convert a step

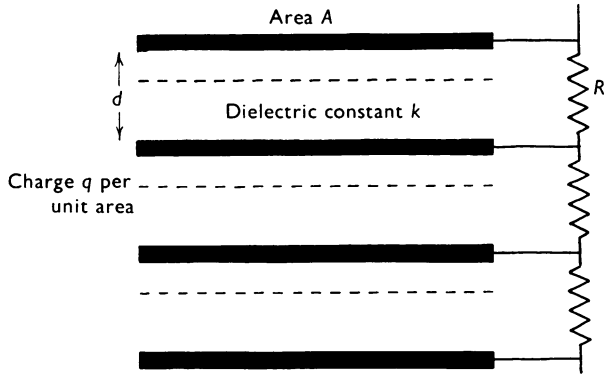

Fig. 6. A hypothesis of the origin of the early receptor potential. For explanation, see text.

input into a diphasic response with non-zero time-integral, and in particular having, as the early receptor potential usually has, the second phase much larger than the first; but we have been unsuccessful in our attempts to design a filter that will do this and at the same time be plausibly reconcilable with the structure of rods and cones as seen with the electron microscope. Even if a system of this kind could be reconciled with the structure, it could not easily explain the specific depression of the late phase of the response by cooling and of the early phase by formaldehyde.

The simplest hypothesis by which these difficulties can be overcome is that there are two successive movements of charge, the first extremely fast and perhaps corresponding to the transition between the ground state and the excited state of the molecules of visual pigment, and the second opposite in direction, greater in amplitude (at least at 27° C), proceeding exponentially with a time constant of the order of 1 msec at 27° C, and corresponding to one of the many changes that can be detected spectroscopically in visual pigments after they have absorbed light. There seems

to be nothing chemically unlikely in this hypothesis. More than two of the changes in visual pigments that follow the absorption of light may be associated with movements of charge, but we can suppose that the rest are too slow to contribute to the early receptor potential.

The effects of ions and glycerol. On our hypothesis the observed responses must be greatly affected by the conductivities in the various conducting spaces of the retina, and it is clear that, roughly speaking, increasing the conductivities should tend to make the observed responses faster and decreasing the conductivities to make them slower. If appropriate *ad hoc* assumptions are made about the size and shape of these spaces and the permeabilities and thicknesses of the membranes separating them, all the effects of ions and glycerol can be explained without need to postulate that these agents influence the speed or magnitude of the primary movements of charge.

The effects of temperature. Cooling depresses the vitreous-negative phase of the response. It has been supposed by Pak & Ebrey (1965) that it also decreases the speed of the process responsible for the vitreous-positive phase, but this is not a necessary conclusion. If on our hypothesis we assume that the linear electrical filter is unaffected by temperature between 2 and 27° C, that the first movement of charge is always instantaneous, and that cooling acts on the second movement of charge either by slowing it or by decreasing its amplitude, all our observations can be well explained. On this view the response observed at 2° C is practically the response of the electrical filter to a step input, the second movement of charge being either too slow or too small in amplitude to be effective. The action of potassium nitrite is then just what would be expected, since it should decrease the resistances in the electrical filter. On the opposing view that the return to the base line of the response in Fig. 3*D* is determined by the speed of a chemical process, it is difficult to explain the action of potassium nitrite.

At temperatures below 0° C, Pak & Ebrey found that the response became even slower. If our interpretation of Fig. 3*D* is correct, the effects of these very low temperatures may be largely due to changes in the distribution of conductivities from the formation of ice crystals.

Formaldehyde and N-ethyl maleimide. The actions of these substances make it fairly clear that no part of the response involves or requires pre-existing free amino or thiol groups.

Orientation of the monolayers. From the appearance of electron micrographs it would be natural to guess that the two sides of each of the 'sacs' or 'disks' of the outer segment of a rod or cone contain layers of visual pigment facing in opposite directions. Our hypothesis of the origin of the early receptor potential is incompatible with this guess; if we are right, all

or most of the layers of visual pigment in a receptor must face in the same direction. It seems unlikely that this can be an accident arising from the way in which outer segments develop, and we suggest that it is probably important for their functioning.

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