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# The polar behavior of frog photoreceptors

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**Abstract**

It was observed that the outer segments of the frog visual rods orient along the direction of an externally applied static electric field. The orientation ability of the rod outer segments seems to be fuelled by the cell energy. The dipolar moment per rod was determined using a model which considers rod outer segments as rigid dipoles interacting with the electric field in a viscous medium. The mean dipolar charge of ROS was determined as being $(2.10 \pm 0.17) \cdot 10^{-14}$ C.

*Keywords:* Rod outer segment; Photoreceptor cell; Electric field; Orientation

## 1. Introduction

In spite of the rich information accumulated concerning the photochemistry and photobiochemistry of retinal receptors [1–3], little is known about the functional significance of the morphological design of retina and retinal receptor cells. Due to the early work of Di Francia [4] and of Enoch [5,6] we know that retinal receptors act as light funnels and that their axes align along the direction of incident light in order to catch maximum of light energy [7–9]. Their outer segment axes misalign whenever receptors are kept in darkness for extended periods [10,11]. More recently, the optimal design criteria of photoreceptor rods were discussed with respect to maximal photon absorption and noise control [12,13].

How is the rod 'sensing' the amount of light caught? If sensitive to the direction of the externally applied electric or magnetic field, the rod outer segments (ROS) might be suspected to act as tiny antennas which rod cells are able to orient in order to catch the maximum of the incident light. It was previously shown by Chalazonitis et al. [14] and Hong et al. [15] that frog ROS are oriented by an externally applied magnetic field. This was explained by magnetic anisotropy of the retinal rods [15]. Here we investigate the behavior of the frog ROS in weak static electric field (200–1100 V $m^{-1}$).

## 2. Materials and methods

### 2.1. Preparation of ROS suspensions

Frogs (*Rana ridibunda*) were dark-adapted for 24 h and their retinae were dissected from the eye cup and pigment epithelium in aerated Ringer solution which contained (in mM): NaCl, 111; KCl, 2.5; $CaCl_2$, 1; $MgCl_2$, 1.6; Hepes, 3; EDTA, 0.01; glucose, 1; buffered to pH = 7.7–7.8 with NaOH [16]. The ROS were then isolated by a gentle brushing of retinae into a 45% saccharose solution. The outer segments were not subjected to subsequent purification and washing procedures in order to make the studied processes relevant to the functionally intact photoreceptors. A significant fraction of the rods retained their ellipsoids in the isolation procedure; the ellipsoid, a 15 $\mu$m region lying just under the outer segment and connected to it by a ciliate neck, contains a dense mass of mitochondria that supplies the ATP needed to maintain the dark current and for other metabolic processes of the cell. Cells that have ellipsoids maintain normal dark currents and light response kinetics for well over an hour [17].

All operations were performed in dim red light.

### 2.2. Observation of ROS behavior in the electric field

A drop of ROS suspension was placed on a microscope slide between two platinized platinum electrodes and a

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cover slip was applied on two spacers in order to establish a distance between the two planes of about 0.1 mm. Preparations were observed during application of constant electric fields of 1 min duration; the polarity of the electrodes was reversed every 1 min in order to limit chemical effects of a possible electrolysis [18]. The maximum intensity of the applied electric field was 1100 V $m^{-1}$, which led to a maximum current intensity of 9.9 $\mu$A. This was found to be just below the electrolysis threshold.

The ROS tips and bases could be easily identified for those ROS which kept an observable fragment of the ellipsoid. The polarity of the ROS tips could be checked by placing small fragments of retina under the cover slip and watching the direction of the rods bending in the electric field.

A video microscope was used for measurements of the rotation velocity (Labphot-2 Nikon microscope, DXC-107P Sony Color CCD Video Camera, Panasonic TC-1470Y Color Video Monitor).

### 2.3. KCN incubation experiments

Freshly extracted pair retinae belonging to the same animal were incubated for 5 min, each retina in 1 ml aerated Ringer solution, with 10 mM KCN being added to one of the solutions. Small strips of retina as well as ROS suspensions from both retinae were then observed under microscope, in the electric field. In alternative experiments, small strips or ROS suspensions from the same retina were observed before and after incubation with KCN.

### 2.4. Computation of the ROS dipolar momentum

For evaluating the dipolar momentum of the ROS, the healthy detached rod outer segments have been considered as rigid dipoles and their dipolar moment has been determined using an electromechanical model which disregards the complex phenomena taking place at charged interfaces.

The mechanical momentum of rod rotation ($M_i$) in the electric field is supposed to result from contributions of the electric ($M_e$) and frictional ($M_f$) moments.

$$M_i = M_e - M_f$$

If the rod is assumed to be an homogeneous cylinder of length $l$, diameter $d$, specific mass $\rho$ and dipolar momen-

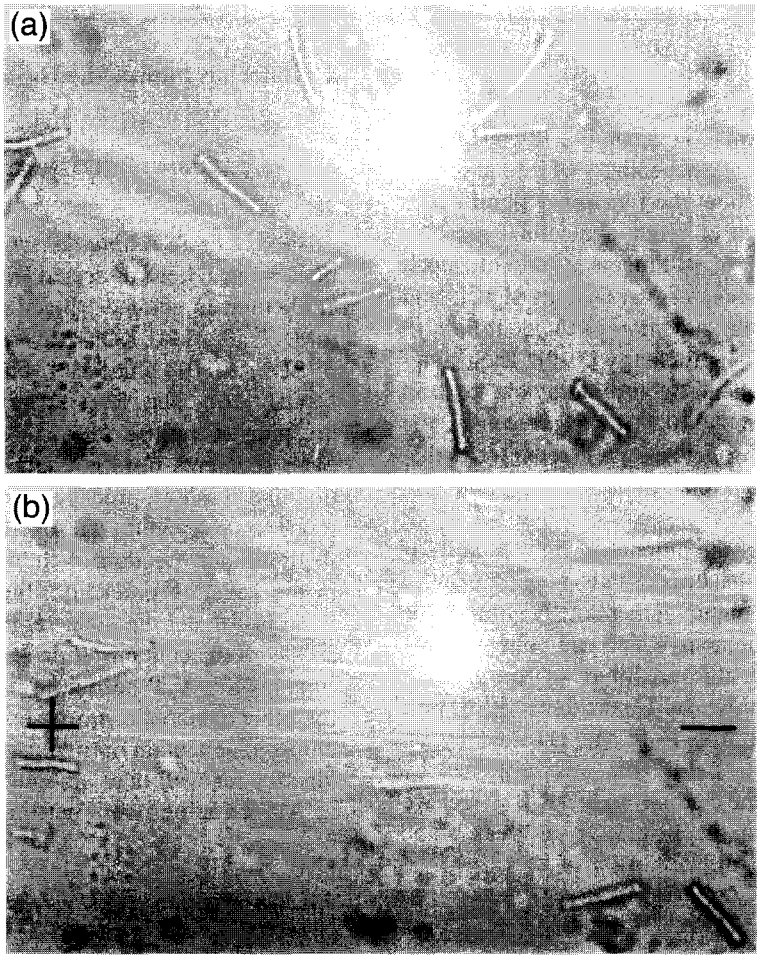


Fig. 1. A ROS suspension: (a) before and (b) 5 min after the application of the electric field (700 V $m^{-1}$). The positions of the oriented cells are slightly changed due to thermal motion.

tum $p$, suspended in a solution with a dielectric constant $K$, then

$$M_i = \frac{\pi d^2 l^3}{48}\ddot{\theta} \text{ and } M_e = \frac{pE_0}{K} \cdot \sin\theta$$

The further computations have been made for small angles which allow the approximation $\sin\theta \cong \theta$ and $M_f = \xi\dot{\theta}$ (where

$$\xi = \frac{16\pi\eta(l/2)^2}{-3 + 6\ln(2l/d)}$$

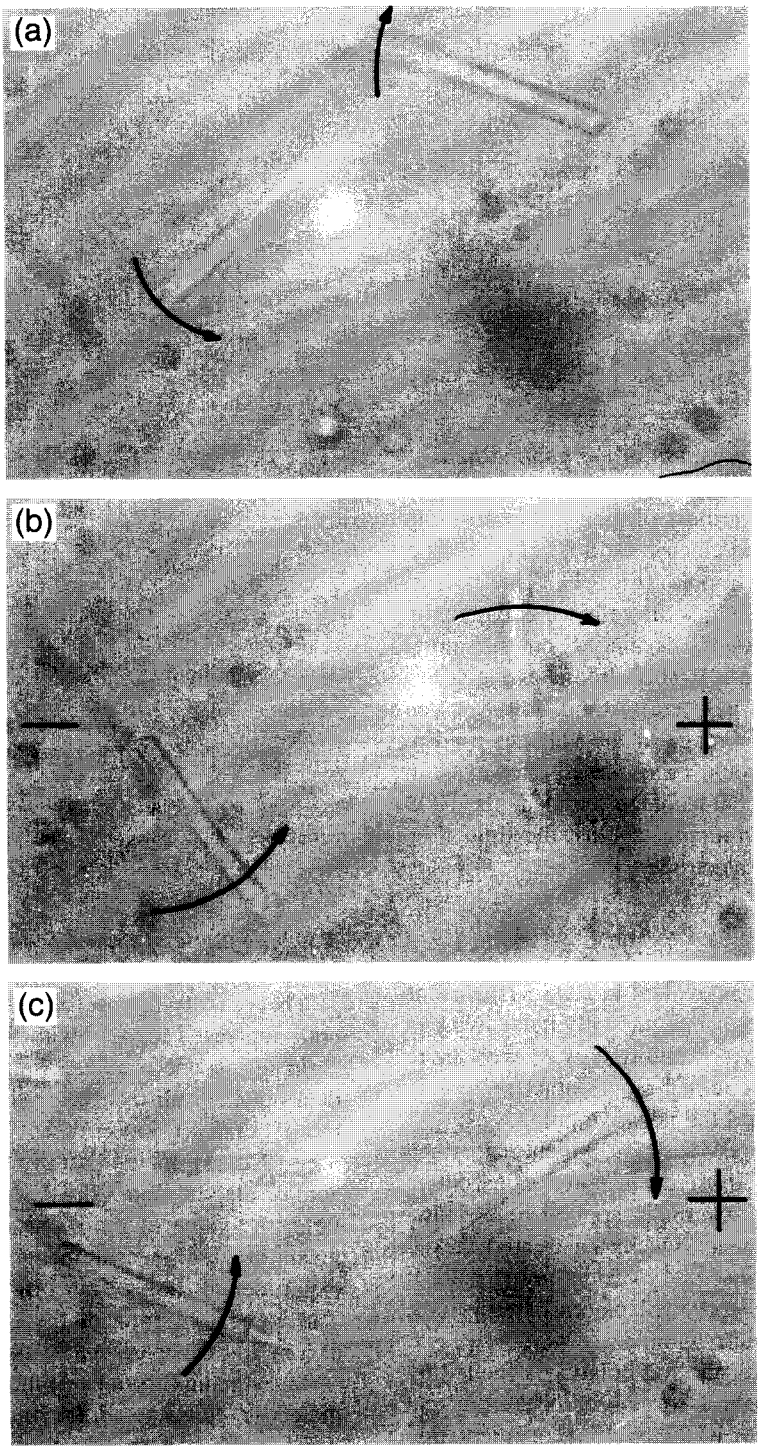


Fig. 2. Three consecutive positions of two ROS, influenced by the electric field. The arrows show the directions of rod rotation. The tips of both ROS are orienting towards the negative pole.

is the rotational frictional coefficient, according to Hong et al. [15]). Thus, the equation describing the rod rotation becomes:

$$\frac{\pi d^2 l^3}{48}\ddot{\theta} + \xi\dot{\theta} - \frac{pE_0}{K}\theta = 0$$

The coefficient of $\ddot{\theta}$ being very small ($8.68 \cdot 10^{-24}$ kg $m^2$) and the observed rod rotation appearing to be uniform ($\ddot{\theta} \cong 0$), the first term of the equation may be neglected, obtaining:

$$\dot{\theta} = \frac{p\theta}{\xi K}E_0$$

This shows a linear dependence of $\dot{\theta}$ versus $E_0$ with a slope

$$b = \frac{p\theta}{\xi K} \quad (1)$$

## 3. Results

### 3.1. ROS sensitivity to the direction of the applied field

It was observed that, regardless of their initial position, ROS start to orient when an electric field is applied, their tips 'seeking' the negative pole (Fig. 1). Thus, when observing the microscope field, some cells were apparently rotating in opposite directions, depending on the initial orientation of their tips (Fig. 2). This eliminates the suspicion that rotation might be induced by fluid flow between the electrodes. At the lower range of the voltages used in our experiments no flow could be observed. However, in some cases reverse flow could not be avoided: the ROS tips could be observed 'swimming' against the fluid flow 'in search' of the negative electrode.

### 3.2. Cell energy dependence of the ROS polarity

The orientation ability was evident in ROS suspensions from freshly decapitated frogs and gradually faded away in 1.5–2 h of microscopic observation. It was even more evident for small pieces of retina, since intact rods were seen to bend strongly toward the negative pole.

Fig. 3 shows a micrograph of a ROS, which maintained its connection with the ellipsoid, in three consecutive positions controlled by the externally applied field. However, many rod outer segments which, apparently, lacked their ellipsoids, were also observed to orient in the field. It is possible that they still kept small pieces of ellipsoids, invisible to microscopic observation.

In order to check whether the rod polarity is fuelled by cell energy, the ROS behavior was observed before and after incubation with 10 mM KCN. No trace of orientation movement could be observed in the presence of this inhibitor.

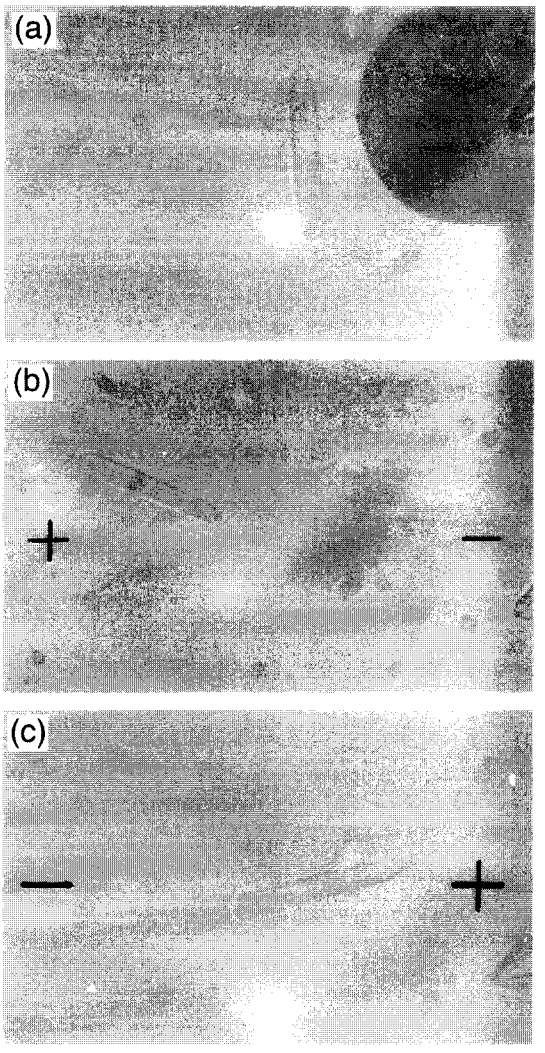


Fig. 3. The orientation of a ROS by the electric field; the cell position (a) before field application; (b) 1 min after field application (700 V $m^{-1}$); (c) 2 min after changing the polarity of the field.

### 3.3. Magnitude of the ROS dipolar momentum

The velocity of rod rotation in the electric field apparently increased linearly with the field intensity.

In Fig. 4 one can see, as an example, the experimental data obtained on measuring the time required for rotation by 10° of a ROS ($l = 60 \cdot 10^{-6}$ m, $d = 6 \cdot 10^{-6}$ m, $\xi = 3.2 \cdot 10^{-17}$ kg $m^2$ $s^{-1}$) at different values of the externally applied electric field ($E_0$). For each intensity of the electric field, the value of the rotation velocity is expressed as a mean of five measurements. The straight line fits the experimental values. The correlation coefficient is $r = 0.99$. The computed slope of the graph is $b = 2.2 \cdot 10^{-4}$ $V^{-1}$ $s^{-1}$ m and the intercept is, as expected, negligible.

Introducing the value of the slope in Eq. 1 and using the dielectric constant of the medium $K = 30$, one obtains $p = 1.2 \cdot 10^{-18}$ C m, which corresponds to a dipolar charge:

$$q = p/l = 1.2 \cdot 0^{-18}\,\text{C m}/60 \cdot 10^{-6}\,\text{m} = 2 \cdot 10^{-14}\,\text{C}$$

Table 1

| Rod No. | 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|
| $\xi$ (kg m$^2$ s$^{-1}$) | $3.2 \cdot 10^{-17}$ | | $2.8 \cdot 10^{-17}$ | | $1.9 \cdot 10^{-17}$ | |
| $l$ (m) | $40 \cdot 10^{-6}$ | | $38 \cdot 10^{-6}$ | | $30 \cdot 10^{-6}$ | |
| $d$ (m) | $6 \cdot 10^{-6}$ | | $6 \cdot 10^{-6}$ | | $8 \cdot 10^{-6}$ | |
| $E_0$(V m$^{-1}$) | $t$ (s) | $\dot{\theta}$ (s$^{-1}$) | $t$ (s) | $\dot{\theta}$ (s$^{-1}$) | $t$ (s) | $\dot{\theta}$ (s$^{-1}$) |
| 400 | $2.00 \pm 0.08$ | 0.0873 | $2.60 \pm 0.060$ | 0.0671 | $1.60 \pm 0.20$ | 0.1091 |
| 500 | $1.7 \pm 0.10$ | 0.1027 | $1.90 \pm 0.40$ | 0.0919 | – | – |
| 600 | $1.20 \pm 0.00$ | 0.1454 | $1.80 \pm 0.20$ | 0.0970 | $1.30 \pm 0.04$ | 0.1343 |
| 700 | $1.10 \pm 0.00$ | 0.1587 | $1.50 \pm 0.20$ | 0.1164 | – | – |
| 800 | $1.00 \pm 0.00$ | 0.1745 | $1.20 \pm 0.20$ | 0.1454 | $1.00 \pm 0.09$ | 0.1745 |
| 900 | $0.90 \pm 0.07$ | 0.1939 | $1.10 \pm 0.05$ | 0.1587 | – | – |
| 1000 | $0.80 \pm 0.02$ | 0.2182 | $0.90 \pm 0.06$ | 0.1939 | $0.76 \pm 0.06$ | 0.2296 |
| 1100 | $0.70 \pm 0.05$ | 0.2493 | $0.87 \pm 0.10$ | 0.2006 | – | – |
| $b$ (mV$^{-1}$ s$^{-1}$) | 0.00022 | | 0.000197 | | 0.0002 | |
| $r$ | 0.99 | | 0.99 | | 0.99 | |
| $p$ (C m) | $3.03 \cdot 10^{-18}$ | | $2.37 \cdot 10^{-18}$ | | $1.64 \cdot 10^{-18}$ | |
| $p/l$ (C) | $7.575 \cdot 10^{-14}$ | | $6.237 \cdot 10^{-14}$ | | $5.467 \cdot 10^{-14}$ | |

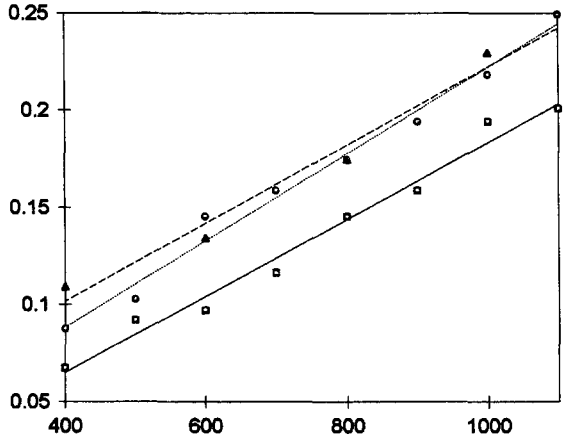


Fig. 4. Plot of rotation velocity ($\dot{\theta}$) of a ROS ($l = 60 \cdot 10^{-6}$ m, $d = 6 \cdot 10^{-6}$ m) vs. the intensity of the applied electric field ($E_0$).

Similar results were obtained from 53 different ROS. The value of $p$ depends on the length of each individual ROS, the quantity $q = p/l$ being, however, very reproducible for all ROS tested: $(2.10 \pm 0.17) \cdot 10^{-14}$ C. See Table 1 for a summary of results.

## 4. Discussion

The alignment of elongated particles in AC and DC fields was observed and explained many years ago [19–22]. More recently, Eynard and Teissié [23] reported the alignment of *Escherichia coli* in an AC field.

Our experiments show that the ROS polarity we observe is not a simple electrically induced phenomenon, since:

1. Only freshly prepared and metabolically active ROS are orienting.
2. The cells which are orienting at the beginning, are getting 'tired' after 1–1.5 h, losing gradually their sensitivity to the applied field.
3. The field sensitivity disappears under the action of inhibitors such as KCN.
4. The field sensitivity of ROS disappears during the winter torpidity (metabolic sleep) of the animals. In our country this lasts approximately from October to February [24].

There are morphological, electrophysiological as well as spectrophotomeric (and biochemical) studies which reveal the structural and functional asymmetry of the ROS along its axis. It is known that new discs are continuously synthesized at the outer segment's base while the old ones are pushed to the rod tip where they degenerate and are phagocytized by pigment epithelium (Young [25]). It was also shown that, at the two ends of the outer segment, the regeneration ability of rhodopsin is different (Makino et al. [26,27]). The pattern of the dark current may be also regarded as the cause/consequence of morphological and functional asymmetry of ROS. Baylor et al. [28] and later Schnapf [29] reported a longitudinal gradient of rod sensitivity as well as the different photoresponse kinetics at tip and base of the toad ROS. Trying to explain the variation of light response with longitudinal position in the outer segment, Baylor suggests the existence of a gradient of a diffusible metabolite which originates in mitochondria in the inner segment and which plays a role in controlling the concentration of the internal transmitter (known today to be cGMP), while Schnapf recalls data which show that the dark steady state concentration of sodium should be higher at the tip than at the base (Sillman et al. [30]; Hagins and Yoshikami [31]; Baylor et al. [28]; Yau et al. [32]). Schnapf estimates that the intracellular concentration of sodium would be about 28 mM higher at the tip than at the base (for an outer segment 50 $\mu$m in length, 6 $\mu$m in diameter,

a dark current of 30 pA flowing through 1/100 of the total cross-sectional area of the rod). Using this model and considering the specific resistance of the rod cytoplasm to be 1 $\Omega$ m (Baylor and Nunn [33]), one can calculate the intracellular resistance to be $2.15 \cdot 10^{8}$ $\Omega$. A current of $30 \cdot 10^{-12}$ A flowing along this resistance would produce a voltage difference of $\Delta V = 6.5 \cdot 10^{-3}$ V, which may be considered as a difference in membrane potential between the tip and the base regions of the ROS, with the tip having a membrane potential such that the interior is less negative with respect to the exterior than at the base. Assuming that this voltage difference occurs across an area of membrane equal to one quarter of the total ROS surface area ($s = 2.8 \cdot 10^{-10}$ m$^2$, for $l = 60 \cdot 10^{-6}$ m and $d = 6 \cdot 10^{-6}$ m) which has a specific membrane capacitance $C = 1.0 \cdot 10^{-2}$ F m$^{-2}$ (Hagins et al. [34]), one may calculate a charge difference of

$$q = \Delta V \, C \, s = 1.8 \cdot 10^{-14} \, C$$

which is in a good agreement with the experimentally found value.

It must, however, be kept in mind that any of the asymmetries mentioned above may generate a charge asymmetry which would determine the behavior of the ROS as an electric dipole.

At present it is difficult to assign the observed dipolar behavior to a certain structural or functional characteristic of the rod. Further refinement of the described experiments, revealing the possible difference in sensitivity to the applied field between bleached and dark-adapted rods, is needed. This would probably help to discriminate between the candidate origins of the rod cell polarity as well as to reveal the functional role (if any) of this property.

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