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Evaluation of viability of retinal photoreceptor cells by using their endogenous electrical field

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Abstract

The rod photoreceptor cells are electrical dipoles sustained by cell metabolic energy. Polarity of photoreceptor cells is directly connected to the so-called "dark current" which circulate along the living photoreceptors. Since only the living cells in a good functional state display electrical polarity, the orientation of photoreceptors in static electric field reflects their viability as long as it depends on the functionality of molecular mechanisms that maintain the dark current. Studying the rod cells' orientation in static electric field at different times after their isolation is thus an accurate way to evaluate the cell viability/degeneration. Retinal transplant experiments in animals and humans, which are presently in progress, require a quick and reliable viability test of cells/tissue to be transplanted. Checking the orientation pattern of rod photoreceptors in static electric field prior to transplantation is a candidate method for an accurate cell viability test.

Keywords: Cell viability; Photoreceptors polarity; Orientation patterns; Retinal transplant

1. Introduction

We have previously reported that isolated frog retinal rods behave as electrical dipoles, orienting in DC fields [1]. The rod cell polarity is sustained by cell metabolism being associated to the dark current, which flows along the cell and vanishes when retina is pretreated with ouabaine [2]. It was suggested that inspection of the orientation patterns of the rod cells suspension in static electric field can provide a quick check of the cell structural and functional integrity, which is a condition for maintaining intact their polarity (Fig. 1).

The interest for such a test was reawaken by recent records which show that research on retinal degeneration (*rd*) therapy includes manipulation of groups of viable photoreceptor cells in order to transplant pieces of healthy retina to experiment animals and human volunteers affected by *rd*. Using healthy, viable retinal cells for transplant is one of the crucial conditions for the operation success [3,4].

In this paper, polarity of fresh isolated frog photoreceptor rods is checked at different times after their isolation (15 min, 30 min, 1 h, 1.5 h, 2 h).

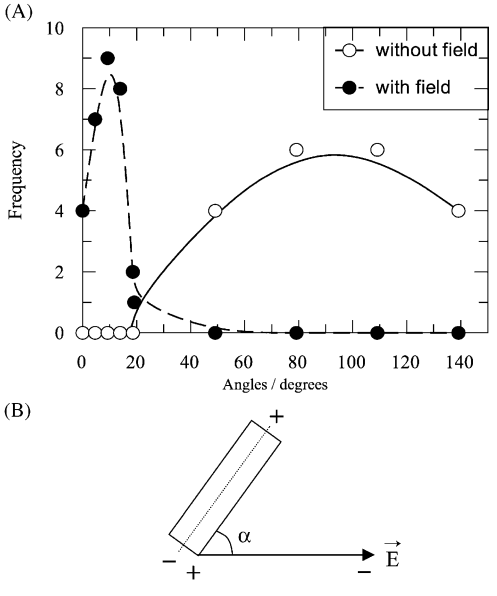

Fig. 1. (A) Angle distribution curves for rod cells in static electric field (●) and in the absence of the field (○). Frequency is the number of rod cells in a given range of angles. (B) The measurement of the angle between the rod cell and the electric field direction. The measurements of angles were made on 103 rods in the absence of DC field and on 97 rods in the presence of the field (from Ref. [1]).

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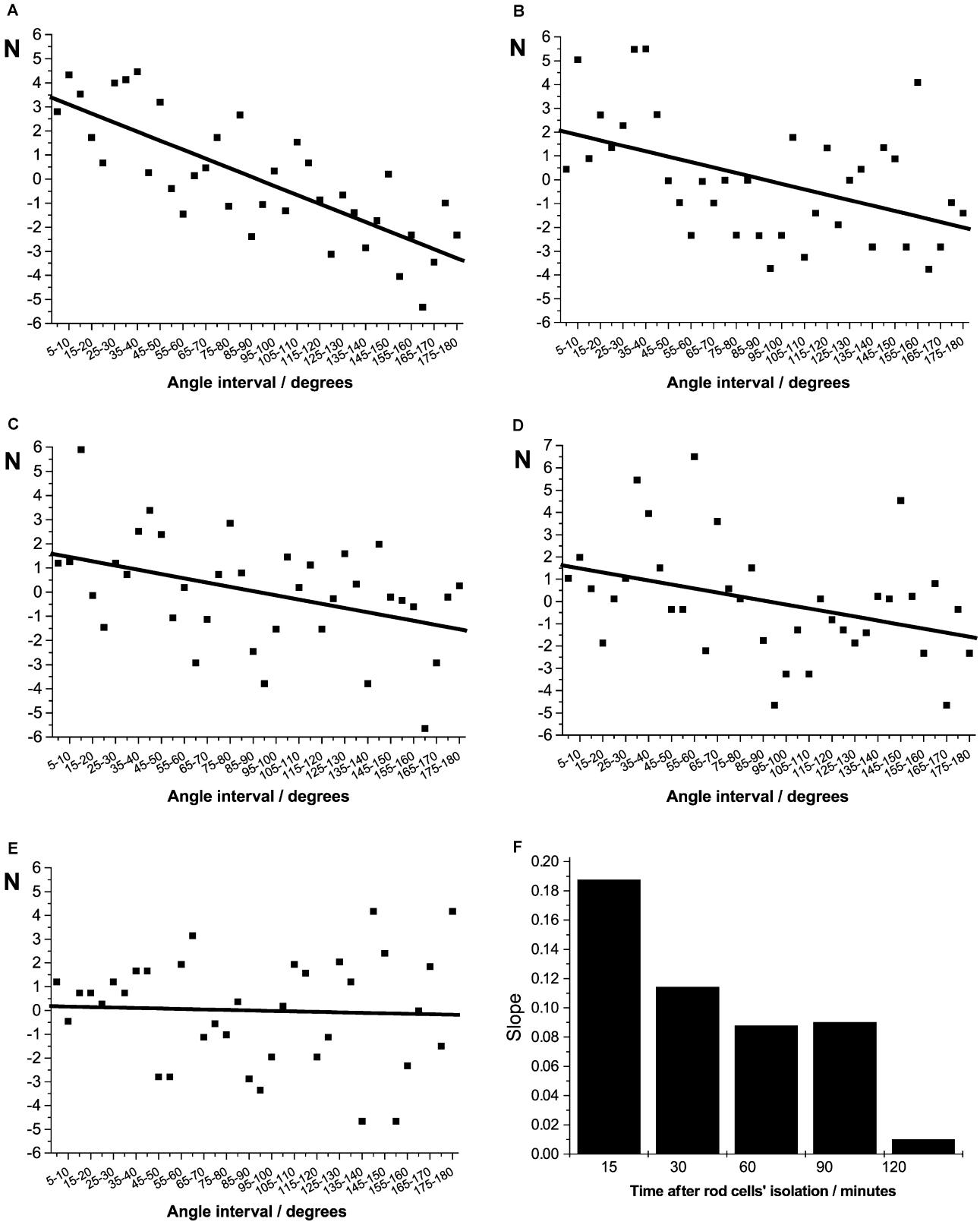

Fig. 2. Orientation degree of rod cells in static electric field at different times after their isolation (linear fit): (A)—15 min, (B)—30 min, (C)—60 min, (D)—90 min, (E)—120 min, (F)—slope of A–E linear fits. $N = n_2 - n_1$. n_2: Number of rod cells in a given angle range after the application of DC field. n_1: Number of rod cells in a given angle range before the application of DC field.

2. Experimental

Retinas were dissected from the eyecup of dark-adapted *Rana ridibunda* frogs. The rod cell suspension was obtained by shaking gently the dissected retinas in 1 ml Ringer solution [2] buffered to pH 7.7–7.8 with NaOH. The electric field was generated by two parallel-platinized platinum electrodes connected to a D.C. power supply.

An amount of 30 μl of suspension fluid was applied on a microscope slide between the two electrodes and then covered with a cover slip. Snapshots were taken before and after the application of the electric field (4 V/cm for 5 min). We used an infrared light microscope and an SL XRS-1 infrared light sensitive video camera connected to a computer with a MiroVideo DRX acquisition board and MGI Video Wave video acquisition software. The procedure was repeated at 15, 30, 60, 90 and 120 min after the rod cell suspension had been prepared.

The angles between the rod cell outer segments and the direction of the external electric field were measured using Image Pro Plus software on the acquired images. Only intact rod cells (that had preserved both outer and inner segment during suspension preparation) were taken into account. By convention, all angles were between 0° and 180° (angles between 180° and 360° were considered as belonging to 180–0° interval). Results were exported in Microsoft Excel for further interpretation. The distribution of the angle orientation was plotted on a chart and a linear fit was performed.

3. Results and discussion

Fig. 2 shows that orientation of rod photoreceptors parallel to the DC field decreases progressively after their isolation. This is illustrated by plotting the number of cells oriented in a certain angle interval vs. angle intervals.

For the sake of accuracy the data were "normalized"; the number of cells oriented in a certain angle interval (N) was computed by subtracting the number found in the absence of electrical field (n_2) from the cell count in the same angle interval in D.C. field (n_1).

We have previously demonstrated [1] that rod photoreceptor polarity is connected to the dark current, being supported by cell metabolic energy. Most probably, it results from asymmetrical distribution of ion pumps along the cell since cells treated by ouabaine are not polar any more [1,2].

Thus, the degree of rod cell orientation indicates the degree of their polarity, which, in turn, reflects the integrity of cell metabolic machinery that drives the dark current.

The decreasing slope of the linear fits in Fig. 2 shows that as the time from the cell isolation increases, less cells belong to the small angles interval (close to "angle **0**", which means parallel orientation to the field direction). Since their electrical polarity decays, they can no more be oriented by the applied DC field.

4. Conclusion

We suggest that the electrical polarity of visual rods can be used for global evaluation of rod cells viability by checking the orientation of cells in weak DC fields. Image processing of cells orientation patterns in DC field, inspected by optical video microscopy, provides the statistical distribution of cells orientation angles which is a relatively quick, sensitive and reliable parameter which reflects the level of cell metabolism/viability.

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