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ORIGIN OF THE LIGHT PEAK: *IN VITRO* STUDY OF *GEKKO GEKKO*

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SUMMARY

1. The light peak is a large, light-evoked increase in standing potential recorded in mammals, birds and reptiles. We have studied the cellular origin of the light peak in an *in vitro* preparation of neural retina-pigment epithelium (r.p.e)–choroid from the lizard, *Gekko gekko*. The tissue was mounted between two separate bathing solutions; the trans-tissue potential was recorded retinal-side positive; micro-electrodes were introduced to measure the trans-epithelial potential (t.e.p.) and to record intracellularly from the r.p.e.

2. A 10 min stimulus of diffuse white light evoked an increase in trans-tissue potential that reached maximum amplitude, the light peak, about 15 min after stimulus onset. Since the light peak is present *in vitro*, it must originate in either the neural retina or the r.p.e.

3. A micro-electrode was positioned in the subretinal space and the trans-retinal potential and t.e.p. were measured simultaneously. A 10 min stimulus produced an increase in t.e.p. equal in magnitude and time course to the trans-tissue light peak; no potential was present across the retina. The light peak is therefore generated solely across the r.p.e.

4. Intracellular r.p.e. recordings were made to determine whether the light peak was generated at the apical or basal membrane or across the paracellular shunt. A 10 min stimulus first caused a hyperpolarization of both membranes with a time course similar to the r.p.e. c-wave followed by a depolarization of both membranes with the time course of the light peak. We conclude that whereas the r.p.e. c-wave results from a hyperpolarization of the apical membrane, the light peak is generated by a depolarization of the basal membrane of the r.p.e.

5. Changes in tissue resistance, R_t, and the ratio of apical to basal membrane resistances, a, were monitored during the light peak by passing current across the tissue and measuring the appropriate current-induced voltages. R_t decreased and a increased with the time course of the light peak. Assuming that the paracellular shunt resistance is constant, we conclude that the light peak is accompanied by an increase in basal membrane conductance.

6. This and the following paper present the first direct demonstration of an interaction between the neural retina and the basal membrane of the r.p.e. The light peak, initiated by absorption of light by photoreceptors, results in a depolarization and conductance increase of the basal membrane.

INTRODUCTION

In darkness, a potential difference (standing potential or corneoretinal potential) can be recorded across the vertebrate eye with the cornea positive to the posterior pole. Illumination produces a fast sequence of changes in the standing potential, the electroretinogram (e.r.g.), which is followed by a series of much slower oscillations in potential, the largest of which is called the light peak (see Täumer, 1976; Krogh, 1979; Marmor & Lurie, 1979). In humans, although continuous DC recording of the light peak is possible (Skoog, 1975; Täumer, Hennig & Wolff, 1976), it is generally recorded indirectly in the electro-oculogram (e.o.g.) (e.g. Täumer, 1976; Arden & Kelsey, 1962). Direct-coupled recording is more feasible in animals and large light peaks can be recorded from many species (Kikawada, 1968; Noell, 1953; Niemeyer, 1980; Steinberg & Niemeyer, 1981; Gouras & Carr, 1965*a*, *b*; Valeton & van Norren, 1981). However, the light peak has only been observed in the vertebrate classes Reptilia, Aves, and Mammalia (Kikawada, 1968), suggesting that it may be a relatively recent development in evolution.

Previous experimental and clinical studies have suggested that the light peak depends on the integrity of both the neural retina and the retinal pigment epithelium (r.p.e.). Noell (1953) demonstrated that the rabbit light peak could be abolished by intravenous injection of sodium iodate, which caused degeneration of the r.p.e., while layers vitreal to the external limiting membrane were preserved. He concluded that the light peak (as well as the c-wave and azide response) is generated by the r.p.e.; this result was later confirmed by Imaizumi, Atsumi, Takahashi & Yoshida (1968). Also, it is known that certain diseases that affect the r.p.e. are accompanied by a reduction in the amplitude of the light peak (see Marmor & Lurie, 1979; Täumer, 1976). Other experimental and clinical studies, however, indicate that the inner retina may also contribute to the generation of the light peak. Gouras & Carr (1965*a*), for example, examined the DC response to light before and after experimental central artery interruption in the rhesus monkey. This procedure destroyed the innermost portion of the retina as far as the inner nuclear layer, and abolished the light peak. They concluded that the light peak depends upon structures at or vitreal to the external plexiform layer. A similar interpretation has been offered recently for the effects of an intravitreal injection of aspartate that eliminates inner retinal function, as indicated by the disappearance of the b-wave, and abolishes the light peak (Madachi-Yamamoto, 1980; Lieberman, 1977; but see Hu & Marmor, 1981).

These investigations into the origin of the light peak are based on treatments that are presumed to destroy selectively structures essential for the generation of a light peak. Since the degree to which a perturbation may affect other retinal elements is not precisely known, these studies are inconclusive. We have, therefore, begun an extensive micro-electrode study of the origin and mechanisms involved in the generation of the light peak. This first paper analyses the light peak in an *in vitro* preparation of retina–r.p.e.–choroid from the lizard, *Gekko gekko*; the second paper (Linsenmeier & Steinberg,1982) analyses the light peak in the intact cat eye. The light peak involves a sequence of steps that are initiated by the capture of light by photoreceptors and produce a potential across the r.p.e. In these papers we show that the light peak is generated by a depolarization of the basal membrane of the r.p.e.

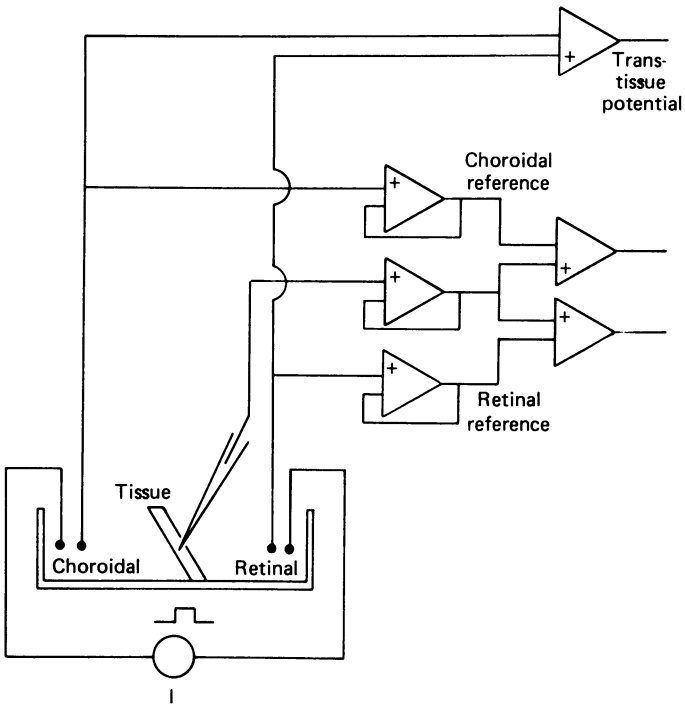

Fig. 1. Diagram of recording system. The trans-tissue potential was recorded differentially, retinal side positive. The micro-electrode was differentially referenced to the retinal and choroidal baths simultaneously. Pulses of constant current could be passed across the tissue.

Although the functional significance is as yet unknown, the light peak is another example of the physiological interaction between the neural retina and the r.p.e.

METHODS

Preparation

Tokay geckos, *Gekko gekko*, were obtained from Charles Sullivan, Inc., kept in glass terrariums, and fed a diet of crickets according to the suggestions of Pawley (1966). Prior to an experiment the animals were refrigerated (10 °C) to facilitate handling, and light-adapted (room lights) to minimize retinal detachment during the dissection. Animals were anaesthetized by packing in ice and then dissected under red light. The eye was enucleated and sectioned behind the lens into anterior and posterior portions. The posterior portion, consisting of neural retina, r.p.e., choroid and sclera was submerged in perfusate and sectioned through the pecten. Each piece was trimmed approximately 5 mm square and dissected free from the sclera. The remaining tissue, consisting of neural retina, r.p.e. and choroid was then mounted in a chamber as previously described for bullfrog (Miller & Steinberg, 1977*a*).

The two sides of the tissue, retinal and choroidal, were continuously superfused at 2 ml./min by a gravity feed system from two large reservoirs. The area of tissue exposed to the perfusate was 0·07 cm^2. The perfusate was a modified Ringer solution having the following composition (mM): NaCl, 82·5; $NaHCO_3$, 27·5; KCl, 2; $MgCl_2$, 1; $CaCl_2$, 18, glucose, 25, bubbled with 95 % O_2/5 % CO_2: pH = 7·4. The solution was maintained at 25 °C to minimize the occurrence of retinal spreading depression (Martins-Ferreira & de Oliveira Castro, 1971; van Harreveld, 1978).

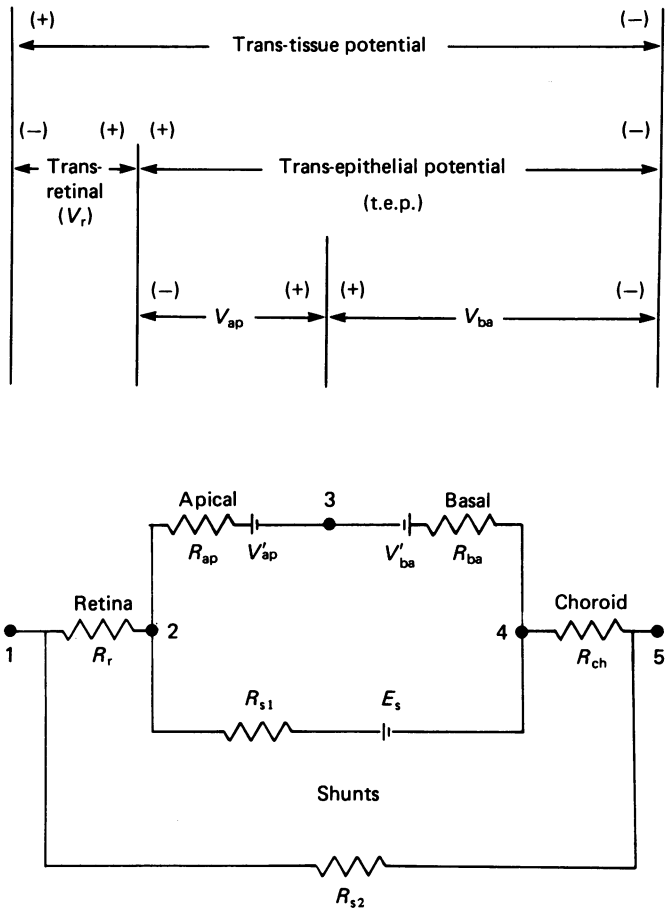

Fig. 2. Equivalent circuit of the retina–r.p.e.–choroid preparation. The resistors represent the resistances of the neural retina, the choroid, the apical and basal r.p.e. membranes, and the two shunt pathways. R_{s1} represents the paracellular shunt of the junctional complexes; R_{s2} represents a shunt pathway at the edges of the tissue. Batteries, V'_{ap} and V'_{ba}, represent voltages generated at the apical and basal membranes, and a diffusion potential, E_s, across the paracellular shunt. Electrode positions are indicated by numbered dots. The potential across the neural retina (V_r) and the t.e.p. were recorded with the micropipette in the subretinal space (position 2) referenced to the retinal bath (position 1) and choroidal bath (position 5), respectively. The choroidal potential was measured with the electrode at position 4. The apical (V_{ap}) and basal (V_{ba}) membrane potentials were recorded intracellularly (position 3).

Recording and stimulation

The methods for recording were similar to those described by Miller & Steinberg (1977*a*). Two pairs of agar–Ringer bridges placed on each side of the tissue were used to record the trans-tissue potential and to pass current across the tissue. Glass micropipettes were used to record intracellularly from r.p.e. cells and extracellularly in the subretinal space. Micropipettes were fabricated from 1·0 mm tubing (Omega Dot, Glass Co. of America) using the electrode puller of Brown & Flaming (1977), filled with 5 mM-K acetate, and bevelled (Brown & Flaming, 1975) to an impedance of 50–100 MΩ. The micropipette was mounted on a hydraulic microdrive (David Kopf), which itself was held by a positioner.

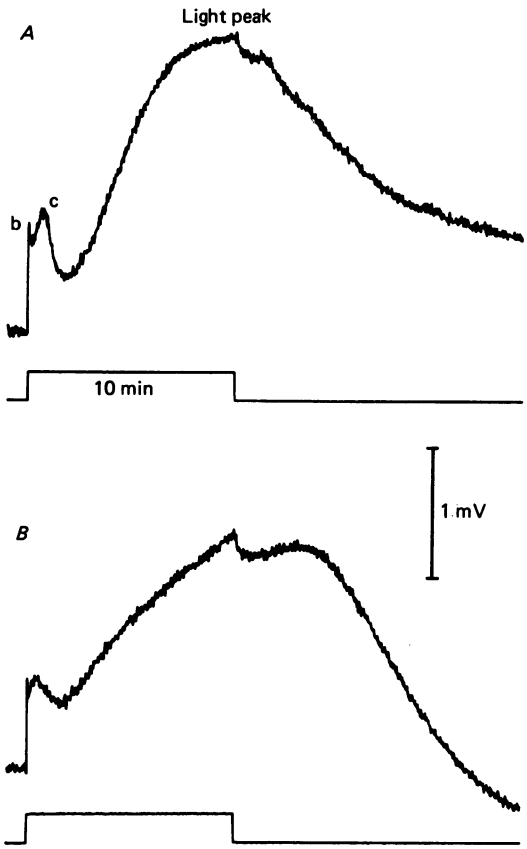

Fig. 3. Trans-tissue recording of light peak. The trans-tissue light peak was recorded differentially, retinal side positive in response to a 10 min stimulus. In this and all subsequent Figures, the stimulus was diffuse white light about 4 log units above b-wave threshold. The c-wave of the e.r.g. is followed by a large increase in potential, the light peak. Two examples are shown. *A*, the c-wave reaches its maximum amplitude about 1 min after stimulus onset, while the light peak reaches its maximum at about 10 min, just before the stimulus terminates; the potential then slowly returns towards the dark potential. *B*, the c-wave peaks faster, but the light peak continues to increase in amplitude after the stimulus terminates; the potential then falls rapidly to a level below the dark potential.

Fig. 1 is a diagram of the electrical recording configuration, while Fig. 2 presents an equivalent circuit of the tissue, emphasizing the r.p.e. The trans-tissue potential was measured differentially (Tektronix 5A22N) between two calomel electrodes that were connected to the retinal and choroidal baths, respectively, by a pair of agar–Ringer bridges. A unity-gain preamplifier (Winston 1090) with an input impedance of 10^{14} Ω was used to measure the potential of the micropipette. In order to reference the micropipette to the retinal and/or choroidal baths, an additional high-impedance preamplifier (Winston 1090) was connected to each bath via the calomel electrodes. The potential between the micro-electrode and the retinal bath was measured differentially (10 times gain), filtered at 50 Hz and further amplified (Tektronix 5A18). The potential between the micro-electrode and the choroidal bath was simultaneously measured in a similar manner. Stable intracellular recordings (drift < 2 mV/hr) could be obtained for over an hour. For measurements of the iR drops across the tissue or across the r.p.e. cell membranes, constant currents were supplied by a square wave generator (Grass S 44) and a stimulus isolation unit (Grass SIU 5). Current pulses (1·0 μA for 1·0 sec) were injected via two silver-chlorided silver wires connected to the retinal and choroidal

baths by the second pair of agar–Ringer bridges. All potentials were displayed on both a storage oscilloscope (Tektronix 5111) and a pen recorder (Brush 220) and stored on magnetic tape (Tandberg 115 or Racal 4 DS). Recordings were subsequently sampled and digitized (DEC PDP 11/03) and plotted on a digital X–Y plotter (Tektronix 4662).

White light from a 100 W tungsten halogen lamp (3300 °K) was used to stimulate the preparation. The lamp filament was focused by aspheric condensing lenses, passed through a water heat filter, and directed at the preparation by a front-surface mirror. All responses in this study were evoked by a stimulus attenuated by 0·1 neutral density, that delivered 92 mW/cm^2 to the preparation. Stimulus durations were determined by an electronically controlled shutter (Vincent Assoc. Inc.).

RESULTS

Each perfused tissue was dark-adapted for 1–2 hr or until the trans-tissue potential and resistance, which tended to increase slowly for several hours, and the e.r.g. b-wave reached relatively stable values (drift <1 mV/hr). For a sample of thirty-three preparations the mean trans-tissue potential was $8{\cdot}4 \pm 3{\cdot}2$ mV (S.D.) (range 3·5–14·1 mV), and the mean tissue resistance, R_t, was $5{\cdot}4 \pm 1{\cdot}4$ kΩ (S.D.) (range 3·7–9·0 kΩ).

The trans-tissue light peak

Fig. 3 shows responses to a 10 min stimulus of relatively high illuminance (see Methods) recorded across the *in vitro* retina–r.p.e.–choroid preparation. The b- and c-waves, which were saturated at this illuminance, were followed by a large increase in trans-tissue potential. The c-wave usually reached its maximum amplitude 20–30 sec after the onset of illumination (range 12–72 sec), after which the potential fell towards and in some cases reached the dark potential. The potential then rose slowly to a peak amplitude, the light peak, in 15–20 min from stimulus onset (range 8–23 min). The stimulus was terminated at 10 min, at which time most responses had plateaued (Fig. 3*A*), and in one or two instances had started to descend during the stimulus. After the stimulus was terminated, the potential returned slowly towards the dark level, in some cases going below the dark level (Fig. 3*B*). The rise to the light peak was generally faster than the return. The potential changes recorded in this study of an *in vitro* retina–r.p.e.–choroid preparation from the gecko were similar, although somewhat slower in time course than the variations in the corneo-retinal standing potential of intact reptiles (Kikawada, 1968; E. R. Griff, unpublished).

In most experiments 2 h of dark adaptation were allowed between successive light peaks. Typically, the initial stimulus did not produce a detectable light peak, the light peak first appearing and then increasing in amplitude over the next few stimuli; it could then be recorded for up to 12 h, one stimulus every 2 h. Amplitudes of these light peaks ranged from 600 to 3500 μV, with a mean of 1423 ± 657 μV (S.D.) (fifty-one responses). In contrast, the c-wave usually decreased in amplitude during the course of an experiment, generally from over 2000 μV to about 600 μV, while the b-wave was relatively constant at a mean amplitude of 523 ± 160 μV (S.D.).

Since a light peak was recorded *in vitro*, we can eliminate several sites and mechanisms for its generation. The light peak is not primarily caused by blood flow, intraocular pressure, or by the pupillary response. Furthermore, the light peak does not depend upon structures in the anterior portion of the eye, or upon neurones outside the eye. Finally, the light peak probably does not originate from a change

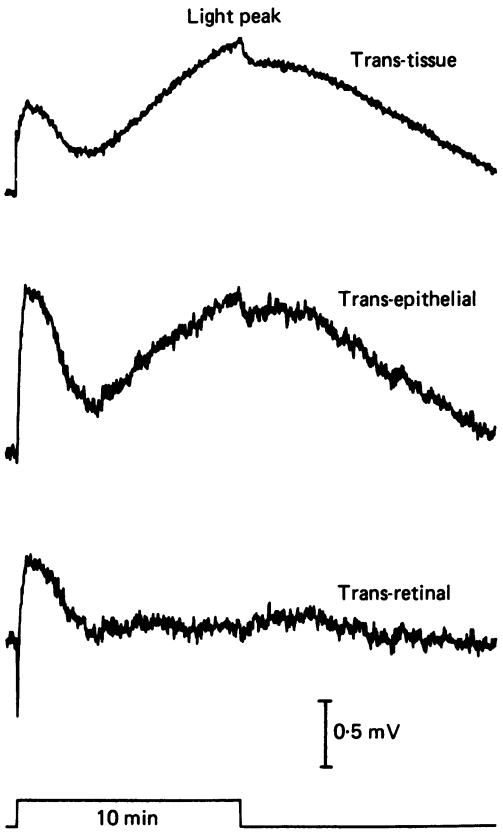

Fig. 4. Extracellular recordings. The micro-electrode was positioned in the subretinal space. The lower trace shows the potential recorded across the neural retina. The middle trace shows the potential across the r.p.e. plus choroid (t.e.p.). The upper trace shows the trans-tissue potential. All responses were recorded simultaneously in response to a 10 min stimulus.

in the composition of the vitreous or choroidal fluid since the response was studied during continuous superfusion of the retinal and choroidal surfaces.

The trans-epithelial light peak

We recorded extracellularly with a micro-electrode to determine whether the light peak is generated in the neural retina or across the r.p.e. The micro-electrode was positioned in the subretinal space by first impaling an r.p.e. cell and then withdrawing the electrode 10–20 μm. By recording the potential between the micro-electrode and a reference in the retinal bath, we measured the potential across the neural retina (trans-retinal potential); with a reference in the choroidal bath, we measured the potential across the r.p.e.–choroid (see Fig. 2). Fig. 4 presents simultaneous recordings of the trans-tissue potential (top), the trans-retinal potential (bottom), and the potential across the r.p.e.–choroid (trans-epithelial, middle). Observe that during the trans-tissue light peak a potential appeared across the r.p.e.–choroid but was not present across the neural retina. The potential that developed across the r.p.e.–choroid had the same polarity and the identical time course as the trans-tissue light peak.

We recorded the potential across the choroid by advancing the micro-electrode through the r.p.e. and referencing it to the choroidal bath. During the light peak either no change in potential, or a small inverted potential, presumably an iR drop across the choroidal resistance, was observed. The increase in potential across the r.p.e.–choroid during the trans-tissue light peak thus primarily reflects an increase in the trans-epithelial potential (t.e.p.). It follows, therefore, that the light peak recorded across the tissue represents an increase in the t.e.p. By contrast, the c-wave could be recorded across both the neural retina and the r.p.e, a finding consistent with the hypothesis that the trans-tissue or corneal c-wave can have two components, one in the neural retina and one in the r.p.e. (Faber, 1969; Rodieck, 1972). As expected, an inverted b-wave was recorded across the neural retina, and a small potential with the time course of the b-wave, presumably an iR drop, was recorded across the r.p.e.–choroid (Oakley, 1977).

R.p.e. intracellular recording

From Fig. 4 we saw that the light peak recorded extracellularly with a micro-electrode originates from an increase in the t.e.p. At the cellular level the t.e.p. is the difference between the apical and basal membrane potentials of the pigment epithelium in the dark, the apical membrane being more hyperpolarized than the basal (Miller & Steinberg, 1977*a*). The light peak, an increase in t.e.p. (see Fig. 2 for polarity of recording electrodes), could be caused by (1) a hyperpolarization of the r.p.e. apical membrane, (2) a depolarization of the basal membrane or (3) both a hyperpolarization of the apical membrane and a depolarization of the basal membrane. Hence, we recorded intracellularly from r.p.e. cells to measure changes in the membrane potentials during the light peak. Interpretation of these potentials, however, is complicated by paracellular shunting.

Theoretical considerations. The retinal pigment epithelium consists of a single layer of cells each of which is connected to the adjacent cells by a junctional complex (Frömter & Diamond, 1972; Hudspeth & Yee, 1973), These complexes form a barrier of finite resistance separating the apical and basal membranes of the r.p.e. cells. In an electrical equivalent circuit of the r.p.e. (see Fig. 2) the junctional or paracellular resistance is designated R_{s1}. In parallel with R_{s1} is a shunt around the edge of the tissue, R_{s2}, caused by incomplete sealing of the tissue in the chip (Miller & Steinberg, 1977*a*, *b*). These shunts allow current generated across one r.p.e. membrane to flow across the other membrane also.

In the steady state the shunt current, i, is given by:

$$i = \frac{V'_{ap} - V'_{ba} - E_s}{R_{ap} + R_{ba} + R_s} \tag{1}$$

where V'_{ap} and V'_{ba} are the potentials that would be measured across the apical and basal membrane, respectively, if the total shunt resistance, R_s (see below) were infinite; E_s is the diffusion potential across the paracellular shunt (see below). Since the shunt resistance is finite, the measured apical and basal membrane potentials, V_{ap} and V_{ba}, respectively, are given by:

$$V_{ap} = V'_{ap} - iR_{ap} \tag{2}$$

$$V_{ba} = V'_{ba} + iR_{ba}. \tag{3}$$

If a change in potential were generated across the apical membrane alone (a change in V'_{ap}; V'_{ba}, E_s, and membrane resistances constant), then i would change (eqn. (1)). This change in i will also change the current flowing across the basal membrane, causing a change in basal membrane potential (eqn. (3)).

During the c-wave, for example, the apical membrane hyperpolarizes due to a decrease in the potassium concentration of the sub-retinal space (Oakley & Green, 1976). Since the apical membrane is permeable to potassium (Miller & Steinberg, 1977*a*), an increase in the equilibrium potential for potassium causes an increase in the apical membrane potential; the c-wave at the r.p.e. thus represents a potential generated (a battery) on the apical membrane. Because of shunting, current flows through the shunt resistances and across the basal membrane, also hyperpolarizing it; the hyperpolarization of the basal membrane is less than that of the apical membrane. In general, a battery on one cell membrane of the r.p.e. changes the potential of both membranes in the same direction. The potential change of the membrane having the battery is, of course, larger than the potential change across the other membrane (Miller & Steinberg, 1977*a*; Oakley, Miller & Steinberg, 1978).

Changes in the trans-epithelial potential can also be produced by diffusion potentials across the paracellular shunt, a shunt battery, E_s (Frömter & Diamond, 1972). If, for example, there were a change in the concentration of an ionic species in the subretinal space to which the paracellular junction was permeable, then a change in shunt potential would cause current to flow across the r.p.e. cell membranes, flowing in across one membrane and out across the other. Thus a change in shunt potential would also polarize the r.p.e. cell membranes, but in this case the membrane potential changes would be opposite each other.

The size of the shunt current, and also the relative magnitudes of the apical and basal membrane potentials (or changes in these potentials), depend on the values of R_{ap}, R_{ba} and R_s (see eqns. (1)–(3)). Although the individual resistances of the neural retina, the r.p.e. cell membranes, and the shunts were not measured, there are two resistance ratios that could be obtained by passing constant current pulses across the tissue and monitoring the appropriate iR drops. For simplicity, since the light peak is not generated across either the neural retina (Fig. 4) or the choroid, and since the retinal and choroidal resistances, R_r and R_{ch}, are each much less than the resistance across the r.p.e. (S. S. Miller & R. H. Steinberg, personal communication), R_r and R_{ch} will be eliminated from this analysis. The resistance ratios are defined as follows (Miller & Steinberg, 1977*a*). R_t is the equivalent resistance across the r.p.e.:

$$R_t = \frac{(R_{ap}+R_{ba})\,R_s}{R_{ap}+R_{ba}+R_s}, \tag{4}$$

where R_s is the total shunt resistance consisting of the parallel combination of R_{s1} and R_{s2} (Fig. 2). The second ratio is

$$a = \frac{R_{ap}}{R_{ba}}. \tag{5}$$

For a given pulse of current, R_t is determined by the iR drop across the tissue and a from the ratio of apical to basal cell membrane iR drops (Frömter, 1972).

R.p.e. membrane potential measurements. After impaling an r.p.e. cell with a micro-electrode, we recorded an apical membrane potential (mean potential =

78±8 mV (S.D.), nineteen cells) that was always more negative than the basal membrane potential by an amount equal to the t.e.p. Fig. 5 shows simultaneous recordings of the intracellular r.p.e. membrane potentials and the trans-tissue potential during a light peak. In this Figure the apical and basal membrane potentials in the dark have been superimposed to facilitate comparison of the two potentials.

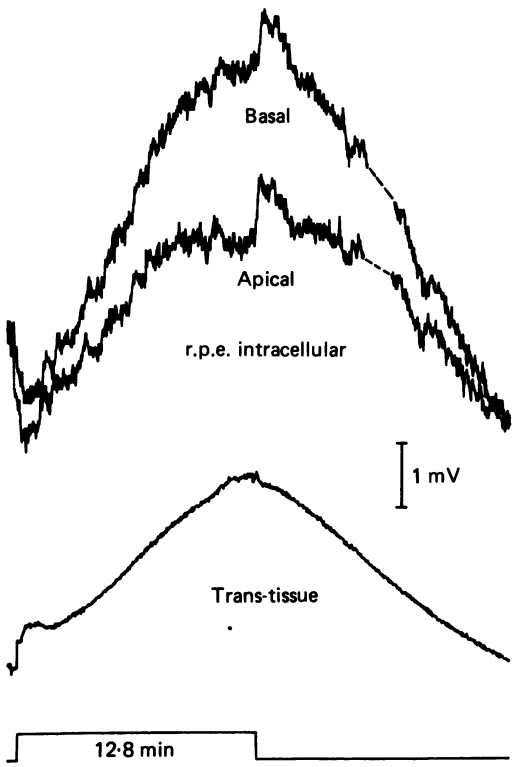

Fig. 5. R.p.e. intracellular recordings. When the micropipette was referenced to the choroidal bath, the potential across the basal membrane plus choroid, V_{ba}, was recorded. With a retinal reference, the potential across the apical membrane plus neural retina, V_{ap}, was recorded. V_{ap} and V_{ba} have been superimposed to facilitate comparison. The trans-tissue potential is also indicated; the three potentials were recorded simultaneously in response to a 12·8 min stimulus.

In response to light there was first a hyperpolarization of the apical membrane with a time course similar to the rise of the trans-tissue c-wave; the basal membrane hyperpolarized by a smaller amount. During the fall of the trans-tissue c-wave the apical membrane repolarized and the basal membrane followed. We then recorded a large depolarization of the basal membrane that had almost the same time course as the trans-tissue light peak, and the apical membrane depolarized to a lesser degree. At the termination of the stimulus there was a small trans-tissue off c-wave (Noell, 1953; Faber, 1969; Steinberg, Oakley & Niemeyer, 1980), accompanied by an apical membrane depolarization and a smaller basal depolarization. Following the off c-wave, both membranes repolarized to the dark level. At every time during the response the

apical and basal membranes were polarizing in the same direction. By subtracting the digitized records of the apical potential from the basal potential, we verified that the amplitude of the trans-tissue light peak was the algebraic difference between the basal and apical membrane potentials. Similar results were obtained from every r.p.e. cell (sixteen cells). The light peak was always accompanied by a depolarization of

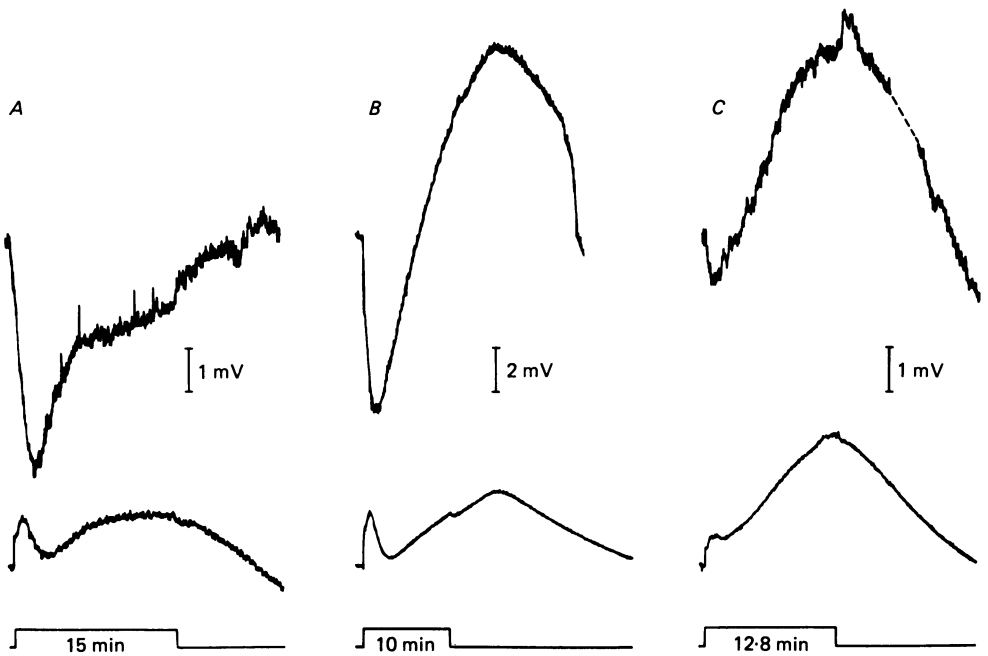

Fig. 6. Relationship between basal depolarization and dark potential. The trans-tissue (bottom) and basal membrane potentials (top) from three preparations are compared. *A*, the c-wave hyperpolarization dominates the intracellular response so that the basal membrane is hyperpolarized relative to the dark potential during the light peak. *B*, a large c-wave hyperpolarization is followed by a depolarization several millivolts above the dark potential. *C*, the c-wave hyperpolarization is relatively small, allowing an estimate of the magnitude of the basal depolarization (same response as Fig. 5, basal and trans-tissue). Stimulus durations in *A*, *B* and C were 15, 10 and 12·8 min, respectively.

the r.p.e. basal membrane and a smaller depolarization of the apical membrane.

Estimation of the magnitude of the basal membrane depolarization depended on whether the measurement was taken from the dark potential or in relation to the recovery from the c-wave hyperpolarization. Fig. 6 shows three examples of the basal membrane depolarization during a light peak; the relationship between the membrane depolarization and the dark potential can differ greatly. In Fig. 6*A*, for example, the c-wave hyperpolarization dominated the intracellular recording so that during the light peak the basal membrane actually was hyperpolarized relative to the dark potential. However, a trans-tissue light peak was present because the basal membrane depolarized relative to the apical membrane. The relationship of the light peak depolarization to the dark potential, however, did not depend solely upon the size of the preceding hyperpolarization. As shown in Fig. 6*B*, following a large c-wave

hyperpolarization the basal membrane depolarized several millivolts relative to the dark potential. Another difficulty in assessing the magnitude of the basal membrane depolarization is that its latency relative to the recovery from the c-wave hyperpolarization is unknown. When the c-wave hyperpolarization was small, as in Fig. 6*C*, depolarizations of 4–6 mV relative to the dark potential were observed.

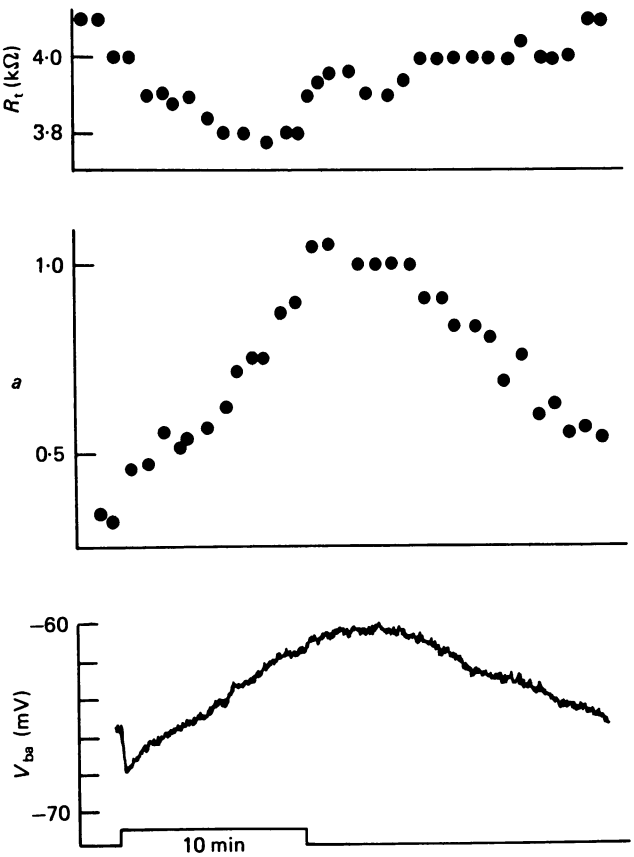

Fig. 7. Resistance measurement. An r.p.e. cell was impaled and 1 μA pulses of current were passed across the tissue. R_t was calculated from the iR drops across the tissue, while a is the ratio of the iR drops across the apical and basal cell membranes. In the upper graph the calculated values of R_t are plotted *versus* time. In the middle graph the ratio a is plotted *versus* time. The lower graph is a record of basal membrane potential *versus* time and shows the stimulus duration for all three graphs.

Resistance measurements

If the basal membrane depolarization during the light peak were associated with a change in conductance of the basal membrane, then this should be reflected in a change in the trans-tissue resistance, R_t, and in the ratio of apical to basal membrane resistances, a. R_t and a were monitored during the light peak by passing 1·0 μA, 1·0 sec square current pulses across the tissue and recording the current-induced voltage changes (iR drops) across the tissue and separately across each r.p.e. cell membrane. The iR drop across the tissue is proportional to R_t, while the ratio of the iR drops of

the cell membranes, apical to basal, is equal to a. The mean value of a in the dark for seventeen r.p.e. cells was $0{\cdot}8 \pm 0{\cdot}4$ (S.D.). Fig. 7 shows the changes in R_t and a that occurred during the light peak; the simultaneously recorded potential of the basal membrane is also shown. The values for a and R_t in the dark were 0·35 and 4·1 kΩ, respectively. During the light peak, a increased to 1·05 and R_t decreased to 3·8 kΩ. For twelve cells, regardless of the values of a and R_t in the dark, a increased and R_t decreased with approximately the same time course as the light peak. When there was a large depolarization of the basal membrane relative to the dark potential, a 2–3-fold increase in a was observed (six cells).

DISCUSSION

In simultaneous recordings of the potential across the neural retina and across the r.p.e., the light peak was observed only across the r.p.e. as an increase in the trans-epithelial potential (Fig. 4). The amplitude and the time course of the trans-epithelial light peak are identical to those of the trans-tissue light peak. We conclude, therefore, that the light peak is generated across the r.p.e. The same conclusion was recently reported by Valeton & van Norren (1982). based on intraretinal recordings in the rhesus monkey. Although the potential change associated with the light peak occurs across the r.p.e., the light that triggers this response is not absorbed by the r.p.e. Spectral sensitivity measurements of the light peak in humans and monkeys have shown a mixed rod–cone action spectrum (Gouras & Carr, 1965*b*; Täumer, Rohde & Pernice, 1976). Thus the light peak represents a series of events, initiated by the photoreceptors, that subsequently cause a potential change across the r.p.e.

Our results support the conclusions of Noell (1953) and Imaizumi *et al.* (1968) that the light peak is generated by the r.p.e. In their results, however, the elimination of the light peak also could have resulted from an effect of iodate on the photoreceptor outer segments, although the light peak was reduced before the a- and b-waves of the e.r.g. were affected. The light peak was also abolished by treatments that lesioned the inner retina (Gouras & Carr, 1965*a*; Madachi-Yamamoto, 1980; Lieberman, 1977). The effects of intravitreal injection of aspartate and central retinal artery occlusion on the r.p.e. and on photoreceptor function are not known, but could explain the elimination of the light peak. Although our results do not rule out a role for the inner retina in some step leading to the generation of the light peak across the r.p.e., results in the following paper suggest that the light peak represents an interaction primarily between the photoreceptors and the r.p.e. (see Linsenmeier & Steinberg, 1982).

The light peak is an r.p.e. basal membrane depolarization

There are three sites at the r.p.e. where the light peak could originate: the r.p.e. apical membrane, the r.p.e. basal membrane, or the junctional complexes between r.p.e. cells. For the apical membrane to generate an increase in t.e.p., it would have to hyperpolarize with the time course of the light peak. If the site of origin were the basal membrane, then that membrane must depolarize. If a change in the battery at the junctional complex, E_s, is responsible for the light peak, then current flow across the r.p.e. membranes would hyperpolarize the apical membrane *and* depolarize the basal membrane.

Fig. 5 shows that during the light peak both the apical and basal membranes *depolarize*. The basal membrane depolarization is larger than the apical depolarization, implying that the depolarization is generated by a battery at the basal membrane (Miller & Steinberg, 1977*a*; Oakley *et al.* 1978). The apical membrane depolarizes due to the flow of shunt current. We cannot rule out the presence of a small change in E_s that also contributes to the light peak, depolarizing the basal membrane and hyperpolarizing the apical membrane. However, since we record a depolarization of *both* apical and basal membranes, the light peak must be considered to originate primarily from a depolarizing basal membrane battery.

The depolarizations of the r.p.e. membranes associated with the light peak are preceded by a hyperpolarization associated with the c-wave. The hyperpolarization in turn reflects the decrease in $[K^+]$ in the sub-retinal space (Oakley, Steinberg, Miller & Nilsson, 1977; Oakley, 1977). Maintained illumination produces a transient decrease in $[K^+]$ followed by a re-accumulation to a steady-state level that is still decreased relative to the dark $[K^+]$ (Steinberg *et al.* 1980). In the frog, an animal that lacks a light peak, the changes in $[K^+]$ produced by maintained illumination cause a transient hyperpolarization of the apical membrane that decays to a plateau hyperpolarization; a smaller hyperpolarization occurs at the basal membrane due to shunting (Oakley & Steinberg, 1982). In the gecko preparation we assume that maintained illumination causes similar decreases in $[K^+]$; these changes in $[K^+]$ should cause a transient followed by a maintained hyperpolarization of the apical membrane. The basal depolarization associated with the light peak will thus be superimposed on the plateau hyperpolarization shunted from the apical to the basal membrane (see Fig. 6). The relative magnitudes of the basal depolarization and the shunted hyperpolarization determine the basal potential during the light peak.

The existence of a paracellular shunt also complicates the relationship between the trans-tissue light peak and the basal membrane depolarization. If the shunt resistance were infinite, so that no current flowed through the shunt, the amplitude of the basal depolarization would equal the trans-tissue light peak (eqns. (1)–(3)). During the light peak, however, current does flow across the shunt and depolarizes the apical membrane. In the responses shown in Fig. 6*B* and *C*, approximately equal trans-tissue light peaks were produced by basal membrane depolarizations of different magnitudes at least in part because the amount of shunting during the light peak must have differed for the two preparations.

Resistance measurements

The trans-tissue resistance, R_t, decreases during the light peak (Fig. 7). Thus, at least one of the resistances contributing to R_t must decrease. We assume that the retinal and choroidal resistances, which are small compared to the resistance across the r.p.e., do not contribute significantly to the decrease in R_t during the light peak. The decrease in R_t reflects, therefore, decreases in either R_{ap}, R_{ba} or R_s. The ratio a of R_{ap} to R_{ba} increases during the light peak, implying that either R_{ap} increases, R_{ba} decreases or that both occur. We can explain both the change in R_t and the change in a solely by a decrease in R_{ba}. A change only in R_s would not cause a change in a. We cannot exclude the possibility that R_{ap} increases *and* that R_s decreases. Since the light peak is generated by a depolarization of the basal membrane, however, it

is tempting to favour an interpretation involving solely a decrease in resistance of the basal membrane. We conclude that the light peak is generated by a depolarization of the basal membrane, accompanied by a decrease in basal membrane resistance.

Preliminary results suggest that passing a step of current (5 min duration) across the tissue to depolarize the basal membrane causes a time-varying decrease in basal membrane resistance. This voltage-dependent, time-varying decrease in resistance was produced by a basal membrane depolarization of the same amplitude as occurs during the light peak. At least part of the decrease in basal membrane resistance during the light peak therefore, is probably a consequence rather than the cause of the basal membrane depolarization.

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REFERENCES

ARDEN, G. B. & KELSEY, J. H. (1962). Changes produced by light in the standing potential of the human eye. *J. Physiol.* **161**, 189–204.

BROWN, K. T. & FLAMING, D. G. (1975). Instrumentation and technique for bevelling fine micropipette electrodes. *Brain Res.* **86**, 172–180.

BROWN, K. T. & FLAMING, D. G. (1977). New microelectrode techniques for intracellular work in small cells. *Neuroscience* **2**, 813–827.

FABER, D. S. (1969). Analysis of the slow transretinal potentials in response to light. Ph.D. Thesis, SUNY, Buffalo.

FRÖMTER, E. (1972). The route of passive ion movement through the epithelium of *Necturus* gallbladder. *J. Membrane Biol.* **8**, 259–301.

FRÖMTER, E. & DIAMOND, J. (1972). Route of passive ion permeation in epithelia. *Nature, New Biol.* **235**, 9–13.

GOURAS, P. & CARR, R. E. (1965*a*). Light-induced DC responses of monkey retina before and after central retinal artery interruption. *Invest. Ophthal.* **4**, 310–317.

GOURAS, P. & CARR, R. E. (1965*b*). Cone activity in the light-induced DC response of monkey retina. *Invest. Ophthal.* **4**, 318–321.

HU, K. & MARMOR, M. (1981). Correlation between rabbit electroretinogram and standing potential. *Invest. Ophthal. vis. Sci.* (Suppl.), **20**, 238.

HUDSBETH, A. J. & YEE, A. G. (1973). The intercellular junctional complexes of the retinal pigment epithelia. *Invest. Ophthal.* **12**, 354–365.

IMAIZUMI, K., ATSUMI, K., TAKAHASHI, F., & YOSHIDA, G. (1968). In *Clinical Value of Electroretinography*, ISCERG Symp., 1966, pp. 74–82. Basel, New York: Karger.

KIKAWADA, N. (1968). Variations in the corneo-retinal standing potential of the vertebrate eye during light and dark adaptations. *Jap. J. Physiol.* **18**, 687–702.

KROGH, E. (1979). The cornealfundal potential and the electro-oculogram: aspects of normal physiology and variability. *Acta ophthal.* Suppl. **140**.

LIEBERMAN, H. R. (1977). Origin of the ocular light-modulated standing potential in cat. Ph.D. thesis, University of Florida.

LINSENMEIER, R. A. & STEINBERG, R. H. (1982). Origin and sensitivity of the light peak in the intact cat eye. *J. Physiol.* **331**, 653–673.

MADACHI-YAMAMOTO, S. (1980). Abolition of the light rise by aspartate. *Societas Ophthalmol. Jap.* **84**, 607–616.

MARMOR, M. F. & LURIE, M. (1979). Light-induced electrical responses of the retinal pigment epithelium. In *The Retinal Pigment Epithelium*, eds. ZINN, K. M. & MARMOR, M. F., pp. 226–244. Cambridge, MA: Harvard University Press.

MARTINS-FERREIRA, H. & DE OLIVEIRA CASTRO, G. (1971). Spreading depression in isolated chick retina. *Vision Res.* (Suppl.) **3**, 171–184.

MILLER, S. S. & STEINBERG, R. H. (1977*a*). Passive ionic properties of frog retinal pigment epithelium. *J. Membrane Biol.* **36**, 337–372.

MILLER, S. S. & STEINBERG, R. H. (1977*b*). Active transport of ions across frog retinal pigment epithelium. *Expl Eye Res.* **25**, 235–248.

NIEMEYER, G. (1980). Electrooculography in isolated perfused mammalian eyes. *Experimentia* **36**, 699.

NOELL, W. K. (1953). Studies on the electrophysiology and metabolism of the retina. U.S.A.F. School of Aviation Medicine Project 21-1201-0004. Texas: Randolph Field.

OAKLEY, B. (1977). Potassium and the photoreceptor-dependent pigment epithelial hyperpolarization. *J. gen. Physiol.* **70**, 405–425.

OAKLEY, B., II & GREEN, D. G. (1976). Correlation of light-induced changes in retinal extracellular potassium concentration with the c-wave of the electroretinogram. *J. Neurophysiol.* **39**, 1117–1133.

OAKLEY, B., II, MILLER, S. S. & STEINBERG, R. H. (1978). Effect of intracellular potassium upon the frog retinal pigment epithelium. *J. Membrane Biol.* **44**, 281–307.

OAKLEY, B., II, STEINBERG, R. H., MILLER, S. S., & NILSSON, S. E. (1977). The *in vitro* frog pigment epithelial cell hyperpolarization in response to light. *Invest. Ophthal.* **16**, 771–774.

OAKLEY, B., II & STEINBERG, R. H. (1982). Effects of maintained illumination upon $[K+]_o$ in the subretinal space of the frog retina. (In the Press.)

PAWLEY, R. (1966). *Geckos as Pets.* T.F.H. Publications, Inc., Neptune City, NJ, U.S.A.

RODIECK, R. W. (1972). Components of the electroretinogram–a reappraisal. *Vision Res.* **12**, 773–780.

RODIECK, R. W. (1973). *The Vertebrate Retina.*, pp. 525–532. San Francisco: W. H. Freeman.

SKOOG, K. D. (1975). The directly recorded standing potential of the human eye. *Acta ophthal.* **53**, 120–132.

STEINBERG, R. H. & NIEMEYER, G. (1981). Light peak of cat DC ERG: not generated by a change in K^+. *Invest. Ophthal.* **20**, 414–418.

STEINBERG, R. H. OAKLEY, B. & NIEMEYER, G. (1980). Light-evoked changes in $[K^+]_o$ in the retina of the intact cat eye. *J. Neurophysiol.* **44**, 897–921.

TÄUMER, R. (1976). Electro-oculography – its clinical importance, ed. TÄUMER, R. *Biblthca ophthal.* **85**.

TÄUMER, R., HENNIG, J. & WOLFF, L. (1976). Further investigations concerning the fast oscillation of the retinal potential. *Biblthca ophthal.* **85**, 57–67.

TÄUMER, R., RHODE, N. & PERNICE, D. (1976). The slow oscillation of the retinal potential. *Biblthca ophthal.* **85**, 40–56.

VALETON, J. M. & VAN NORREN, D. (1982). Intraretinal recording of slow electrical responses to steady illumination in monkey: retinal and pigment epithelial contributions *Vision Res.* **22**, 393–399.

VAN HARREVELD, A. (1978). Two mechanisms for spreading depression in the chicken retina. *J. Neurobiol.* **9**, 419–431.