# Extracellular K<sup>+</sup> Activity Changes Related to Electroretinogram Components

## II. Rabbit (E-Type) Retinas

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ABSTRACT Electroretinogram (ERG) and extracellular potassium activity  $(K_{\phi})$  measurements were carried out in isolated superfused rabbit eyecup preparations under control conditions and during the application of pharmacological agents that selectively modify the light-responsive retinal network. Light-evoked  $K_{o}^{*}$  changes in the rabbit (E-type) retina resemble those previously described in amphibian (I-type) retinas. Different components of the lightevoked  $K_{\circ}^{+}$  changes can be distinguished on the bases of retinal depth, V vs. log I properties, and their responses to pharmacological agents. We find two separable sources of light-evoked increases in extracellular K<sup>+</sup>: a proximal source and a distal source. The properties of the distal light-evoked K<sup>+</sup><sub>o</sub> increase are consistent with the hypothesis that it initiates a K<sup>+</sup>-mediated current through Müller cells that is detected as the primary voltage of the electroretinographic b-wave. These experiments also support previous studies indicating that both the corneal-positive component of c-wave and the corneal-negative slow PIII potential result from K<sup>+</sup>-mediated influences on, respectively, the retinal pigment epithelium and Müller cells.

## INTRODUCTION

Several distinct light-evoked changes in extracellular  $K^+$  activity ( $K_o^+$ ) have been described, using  $K^+$ -selective microelectrode techniques, in the retinas of a number of vertebrate species. These  $K_o^+$  modulations have been categorized according to waveform, retinal depth of maximum response, sensitivity to ionic manipulations of the extracellular milieu, and sensitivity to pharmacological agents. Some of these light-evoked  $K_o^+$  modulations are either certainly or probably related to the generation of major components of the electroretinogram (ERG). In frog and cat retinas, a light-evoked  $K_o^+$  decrease in the outer (distal) retina has been shown to underlie generation, by the retinal pigment epithelium (RPE), of the corneal-positive component of the c-wave (Noell, 1953; Steinberg et al., 1970; Schmidt and Steinberg, 1971; Steinberg, 1971; Oakley, 1975, 1977;

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J. GEN. PHYSIOL. © The Rockefeller University Press · 0022-1295/85/06/0911/21\$1.00 Volume 85 June 1985 911-931 Oakley and Green, 1976; Steinberg et al., 1980; Vogel, 1980). Light-evoked  $K_{o}^{+}$  changes have also been related to the generation of the b-wave, d-wave, and slow PIII components of the ERG in both amphibians and mammals (Faber, 1969; Miller and Dowling, 1970*a*, *b*; Miller, 1973; Zuckerman, 1973; Witkovsky et al., 1975; Proenza and Freeman, 1975; Karwoski and Proenza, 1977; Dick and Miller, 1978*a*, 1985; Kline et al., 1978; Dick, 1979; Newman, 1979, 1980; Newman and Odette, 1984; Rager, 1979; Lurie and Marmor, 1980; Shimazaki et al., 1984). The present study examines intraretinal light-evoked K<sup>+</sup><sub>o</sub> modulations and electroretinographic components in the E-type retina (Granit, 1935) of the rabbit. The observations of this study suggest that the b-wave is closely associated with a light-evoked K<sup>+</sup><sub>o</sub> increase that is detected near the level of the outer plexiform layer (OPL) and that is probably generated by activity of depolarizing bipolar cells (DPBCs). Preliminary results of this work have been presented elsewhere (Dick and Miller, 1977, 1978*b*).

#### METHODS

#### Preparation, Light Stimulation, and Recording

The superfused rabbit eyecup preparation has been described previously in detail (Dacheux et al., 1973; Dacheux, 1977; Dick, 1979). However, we reiterate that, in this preparation, the rabbit eye is enucleated, followed by a dissecting away of the cornea, iris, and lens. The remaining eyecup is everted over a Teflon dome containing an Ag/AgCl electrode. A superfusion chamber is subsequently secured over the everted eyecup. The Ag/AgCl electrode serves as the ground reference electrode for the ERG and intracellular recordings. ERGs in this study represent potential changes recorded between the ground reference, located behind (sclerad to) the eye, and a second Ag/AgCl electrode immersed in Ringer's solution superfusing the vitreal surface of the retina. Intraretinal ERGs represent potential changes recorded between the reference electrode behind the eye and a Ringer's-filled micropipette advanced into the retina from the vitreal side. The control perfusion medium used in these experiments was a bicarbonate-buffered, plasmated Ringer's solution composed of (mM): 120 NaCl, 5 KCl, 25 NaHCO<sub>3</sub>, 0.8 NaHPO<sub>4</sub>, 0.1 NaH<sub>2</sub>PO<sub>4</sub>, 1 MgSO<sub>4</sub>, 2 CaCl<sub>2</sub>, 10 glucose, and 1% dialyzed horse serum. The pH of the perfusate was maintained between 7.4 and 7.5 by constant aeration of the perfusate with 95% O<sub>2</sub> and 5% CO<sub>2</sub>. Experimental solutions containing ethanol (200 proof, U.S. Industrial Chemicals Co., Cincinnati, OH) and  $\gamma$ -aminobutyric acid (Sigma Chemical Co., St. Louis, MO) were made by addition of these agents to a perfusate of control composition.

Details of the light stimulation techniques used in this study were outlined in the preceding paper (Dick and Miller, 1985).

Details of the recording techniques used in this study were also outlined in the preceding paper (Dick and Miller, 1985). In the present study, all recordings with K<sup>+</sup>-selective microelectrodes were carried out using cross-capacitance neutralization. Illustrations were photographically reproduced from the data displayed on a storage oscilloscope (5102, Tektronix, Inc., Beaverton, OR) or penwriter (model 5, Grass Instrument Co., Quincy, MA, or model 260, Gould-Brush, Cleveland, OH).

#### Horseradish Peroxidase (HRP) Staining

The tip of a microelectrode for intracellular recording was filled with 10% HRP (grade I, Boehringer-Mannheim Biochemicals, Indianapolis, IN) in 0.5 M K acetate (pH 7.6, Tris buffer). After penetration of a cell, HRP was injected for up to 10 min with a 1.0-nA

(peak to peak), 3-Hz current. The light response of the cell was monitored every 30 s. Retinas were fixed in 1.5% gluteraldehyde/1.5% paraformaldehyde for 12 min. Retinas were then washed at least 16 h in rabbit Ringer's or phosphate buffer. HRP was reacted using benzidine dihydrochloride as the chromagen and the retina was subsequently dehydrated through an ethanol series and cleared in xylene. Retinas were mounted in JB-4 embedding medium (Polysciences, Inc., Warrington, PA) and 20- $\mu$ m sections were cut for microscopic inspection.

## Microelectrodes

The details of the techniques used in this study to fabricate double-barreled K<sup>+</sup>-selective microelectrodes were outlined in the preceding paper (Dick and Miller, 1985). Acetylcholine (ACh)-selective microelectrodes were fabricated in the same fashion as doublebarreled K<sup>+</sup>-selective microelectrodes except that a liquid ACh exchanger resin was used



FIGURE 1. Graphic representation of the potentiometric responses of an AChselective and a K<sup>+</sup>-selective microelectrode to an ACh concentration of  $10^{-3}-10^{-7}$ M in Ringer's solution. Over this test range, the K<sup>+</sup>-selective electrode shows an ACh selectivity ~0.4 times that of the ACh-selective electrode.

in place of the K<sup>+</sup> exchanger (Baum, 1970). The solute for the ACh exchanger was synthesized and kindly provided for our use by Dr. George Baum. This ACh salt was dissolved in 1,3-dimethyl-2-nitrobenzene (Eastman Kodak Co., Rochester, NY) to produce a liquid exchanger resin selective for ACh over K<sup>+</sup> and other cations. K<sup>+</sup>- and ACh-selective microelectrodes were calibrated as described in the preceding paper (Dick and Miller, 1985) with one exception. In the present study, the response slope (potential change developed by a K<sup>+</sup>-selective electrode per decade change in external K<sup>+</sup>) of the electrodes over the range of ~4–10 meq/liter of K<sup>+</sup> was used to calculate the magnitude of K<sup>+</sup><sub>o</sub> changes from K<sup>+</sup> electrode responses. Fig. 1 illustrates ACh calibration curves for both K<sup>+</sup>-selective and ACh-selective microelectrodes. Although, as Fig. 1 illustrates, the response slope of the ACh-selective microelectrode shows a pronounced deviation from a Nernstian slope with external ACh concentrations below 10<sup>-5</sup> M, a reproducible potention on the present of the ACh concentrations as low as 10<sup>-7</sup> M.

#### RESULTS

## Depth Profiles

Light-evoked  $K_o^+$  modulations in the rabbit retina are generally similar to those seen in other vertebrates including amphibia (Oakley, 1975, 1977; Oakley and Green, 1976; Karwoski and Proenza, 1977, 1978, 1980; Olsen and Miller, 1977; Dick and Miller, 1978*a*, 1985; Dick, 1979), skate (Kline et al., 1978), and cat (Steinberg et al., 1980). Fig. 2*A* presents simultaneously measured intraretinal



FIGURE 2. (A) Simultaneous recordings of intraretinal ERGs and K<sup>+</sup>-selective microelectrode responses taken at various retinal depths, expressed as percent retinal depth, in a superfused rabbit eyecup. At 98% retinal depth, the K<sup>+</sup> electrode response shows a light-evoked K<sup>+</sup><sub>o</sub> decrease and a return to baseline at light offset. At 82% retinal depth, the K<sup>+</sup> decrease goes through a maximum. At 60% retinal depth, a light-evoked K<sup>+</sup><sub>o</sub> increase is evident in the K<sup>+</sup> electrode response. The maximum light-evoked K<sup>+</sup><sub>o</sub> increase was detected at 36% retinal depth in preparation. A c-wave is not seen in this ERG (see text). The horizontal bar beneath each column defines the duration of the diffuse light stimulus. Irradiance:  $1.0 \times 10^{-7}$  W/cm<sup>2</sup>. (B) Cross-section from a rabbit retina. Abbreviations: OS: outer segments; IS: inner segments; ELM: external limiting membrane; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; ILM: inner limiting membrane. Calibration bar, 50 µm.

ERGs and light-evoked  $K_o^+$  modulations collected at different retinal depths during a microelectrode withdrawal in a rabbit eyecup. With this preparation, the c-wave is typically lost or very much reduced within ~15 min, and the ERG recorded after the loss of the c-wave is essentially identical to that seen in the proximal intraretinal ERG records of Fig. 2A, a positive b-wave followed by a negative slow PIII, which returns to baseline at light offset. This is essentially the same form of the rabbit ERG as seen under conditions of cathodal polarization

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across the eye (Noell, 1953; Faber, 1969). The loss of the c-wave is probably due to surgical trauma, resulting in retinal separation from the pigment epithelium or a change in the resistive properties of the pigment epithelium.

In Figs. 2 and 3, the R membrane was used to denote 100% retinal depth and its position was located through the I-R drop characteristics of the intraretinal ERG recording channel as described in the preceding study (Dick and Miller, 1985). At 98% retinal depth, the intraretinal ERG consists of a negative b-wave followed by a large and positive slow PIII. The K<sup>+</sup><sub>o</sub> response at this level consists of a slow K<sub>o</sub><sup>+</sup> decrease at light onset followed by a gradual return to baseline at light offset. The  $K_{0}^{+}$  decrease response reached a maximum near 82% retinal depth. At 60% retinal depth, the K<sup>+</sup> electrode response shows a transient  $K_{o}^{+}$ increase after the initial development of the  $K_{o}^{+}$  decrease. In the proximal retina, a large K<sub>o</sub><sup>+</sup> increase is seen at light onset (light-evoked) and a comparatively smaller  $K_{o}^{+}$  increase is seen at light offset (dark-evoked). In this experiment, the maximum light-evoked  $K_{o}^{+}$  increase was detected at 36% retinal depth. The magnitudes of light-evoked  $K_{o}^{4}$  modulations became rapidly attenuated at retinal depths proximal to the peak  $K_{o}^{+}$  increase. The pattern of light- and dark-evoked  $K_0^+$  changes illustrated in Fig. 2A is very typical of the intraretinal  $K_0^+$  modulations recorded in an additional 18 depth profile studies. In 11 of these 19 experiments, a light-evoked  $K_{0}^{+}$  increase was detected at, or distal to, 60% retinal depth. A light-evoked K<sub>o</sub><sup>+</sup> increase was detected at, or distal to, 50% retinal depth in all cases.

In Fig. 2*B*, a cross-section of rabbit retina is presented in order to help associate the anatomical layers of the retina, with intraretinal ERGs and K<sup>+</sup> electrode responses mapped according to percent retinal depth. The percent retinal depth at which discrete layers are found varies from central to peripheral retina, and with respect to the distance from the visual streak. The section in Fig. 2*B* was taken from a region near the visual streak and, in this example, the outer plexiform layer (OPL) occurs at ~51-55% retinal depth. In different sections of rabbit retina, the percent retinal depth at which the OPL occurred was found to vary from 60 to 40% retinal depth. Based upon the percent retinal depths at which the OPL occurs and our measurements of K<sub>0</sub><sup>+</sup> increases, we infer that lightevoked K<sub>0</sub><sup>+</sup> increases are detected from near the vitreous to retinal levels at least as distal as the OPL.

#### Does the K<sup>+</sup>-selective Electrode Sense ACh Release?

ACh appears to be released by a subclass of amacrine cells in rabbit retina (Masland and Livingstone, 1976; Masland and Ames, 1976; Massey and Neal, 1979; Massey and Redburn, 1982). The Corning K<sup>+</sup> ion exchanger used in this study is poorly selective for K<sup>+</sup> over ACh (a K<sup>+</sup>:ACh selectivity coefficient of  $\sim$ 2.5 by our fixed cation interference method of calibration) and it is therefore possible that light-evoked ACh release could affect the potentials developed by a K<sup>+</sup>-selective microelectrode. To investigate this possibility, we made intraretinal measurements using ACh-selective microelectrodes. The ion-exchange resin used to make those electrodes has an ACh:K<sup>+</sup> selectivity coefficient of  $\sim$ 2.5 by our calibration method. Fig. 3 presents intraretinal ERGs and ACh-selective microelectrode responses recorded simultaneously at different retinal depths during a

microelectrode withdrawal in a rabbit eyecup. In this and six additional experiments, the light-evoked cation modulations detected by ACh-selective microelectrodes were similar but smaller than those recorded with  $K^+$ -selective electrodes (note the different gains in Figs. 2 and 3). The peak light-evoked responses measured with ACh-selective microelectrodes were two to three times smaller than were the peak responses measured with  $K^+$ -selective microelectrodes. There were no noticeable qualitative differences in the responses detected with the two types of ion-exchange resins. These results strongly suggest that intraretinal potentials developed by the ACh-selective microelectrodes represented their behaviors as poorly selective  $K^+$  electrodes and that synaptic release of ACh was not potentiometrically detected. We presume that the diminished size of the ACh-selective microelectrode responses (in millivolts) relative to those seen with  $K^+$ -selective microelectrodes is due primarily to poor membrane selectivity for



FIGURE 3. Simultaneous recordings of intraretinal ERGs and ACh-selective microelectrode responses evoked by diffuse light stimuli at different retinal depths, expressed as percent retinal depth, in a superfused rabbit eyecup. Diffuse light stimuli. Irradiance:  $1.0 \times 10^{-7}$  W/cm<sup>2</sup>.

K<sup>+</sup> over Na<sup>+</sup>. These results were not surprising in view of the fact that potentiometric measurement of ACh release, in vivo, has not yet been successfully accomplished in any biological preparation. After these experiments, we proceeded under the assumption that intraretinal K<sup>+</sup>-selective microelectrode measurements were not significantly influenced by endogenous ACh release.

## Light-evoked K<sup>+</sup><sub>o</sub> Decrease and the C-Wave

Both biochemical and electrophysiological studies in rod-dominated retinas have established that the retinal pigment epithelium (RPE) component of c-wave (RPE c-wave) is generated in response to a decrease in  $K_o^+$  (Noell, 1953; Brown and Wiesel, 1961; Steinberg et al., 1970, 1980; Steinberg, 1971; Oakley, 1975, 1977; Oakley and Green, 1976; Vogel, 1980). A light-evoked  $K_o^+$  decrease in distal

retina has now been reported in a number of  $K_o^+$ -selective microelectrode studies (Oakley, 1975, 1977; Oakley and Green, 1976; Tomita, 1976; Karwoski and Proenza, 1977, 1978; Olsen and Miller, 1977; Dick and Miller, 1978*a*, 1985; Kline et al., 1978; Vogel, 1980). During experiments in which a c-wave remained stable, parallel temporal and dynamic range properties of the  $K_o^+$  decrease and c-wave could be demonstrated, as illustrated in Fig. 4. Fig. 4A is a storage oscilloscope record of the ERG, intraretinal ERG, and  $K_o^+$  decrease evoked simultaneously by a 12-s diffuse light stimulus. Using a relatively low gain for



FIGURE 4. (A) Parallel time course of the light-evoked  $K_o^+$  decrease and the c-wave in rabbit. A photographic reproduction of a storage oscilloscope record of the simultaneously recorded ERG, intraretinal ERG, and the K<sup>+</sup> electrode response evoked by a 12-s diffuse light stimulus. Symmetry is seen between the time course and waveform of the  $K_o^+$  decrease, the c-wave, and the intraretinally recorded slow potential, which, in this case, includes both positive c-wave and positive slow PIII components. Irradiance:  $3.2 \times 10^{-8}$  W/cm<sup>2</sup>. (B) Intensity-response curves for the cwave and the light-evoked  $K_o^+$  decrease shown in A.

the intraretinal voltage trace reduced the resolution of the intraretinal b-wave. However, this allowed for simultaneous recording, which better revealed the symmetry of time course and waveform between the  $K_o^+$  decrease, the ERG cwave, and the intraretinally recorded slow potential, which, as the result of our recording configuration, includes both positive RPE c-wave and positive slow PIII components. The correspondence between the  $K_o^+$  decrease and the c-wave in the rabbit retina is consistent with previous findings in frog (Oakley, 1975, 1977; Oakley and Green, 1976; Vogel 1980) and cat (Steinberg et al., 1980). Fig. 4*B* illustrates the parallel intensity-response relationships for the ERG cwave and  $K_o^+$  decrease responses shown in Fig. 4*A*. Both responses show a dynamic range of ~5.0 log units, but the c-wave curve is shifted slightly to the right.

## Light-evoked K<sup>+</sup><sub>o</sub> Increases

Light-evoked  $K_o^+$  increases in the rabbit retina can be detected from close to the vitreal surface into the distal retina near, or beyond, the level of the OPL. We have been able to divide light-evoked  $K_o^+$  increases into distal and proximal phenomena on the basis of V vs. log I properties and their sensitivity to pharmacological agents.

V VS. LOG I MEASUREMENTS Fig. 5 presents a comparison of V vs.  $\log I$ 



FIGURE 5. A comparison of V vs. log I curves for the b-wave (solid lines, closed circles), the intraretinal b-wave (dashed lines, open circles), and the light-evoked  $K_o^+$  increase (dotted lines, open triangles) in distal vs. proximal retina of a superfused rabbit eyecup. In distal retina, the threshold for detection of the light-evoked  $K_o^+$  increase is higher than that of both b-waves. At higher stimulus intensities, the normalized amplitude of the distal  $K_o^+$  increase closely parallels that of both b-waves. In proximal retina, the  $K_o^+$  increase is not detected until after the b-wave recorded across the eye is clearly observed, and, at higher stimulus intensities, it shows no relationship to the b-wave. Diffuse light stimuli. Maximum irradiance (0.0 log): 1.0  $\times 10^{-3}$  W/cm<sup>2</sup>.

data for the b-wave, the intraretinally recorded b-wave, and light-evoked  $K_o^+$  increases recorded in distal vs. proximal levels of a rabbit eyecup during a single microelectrode withdrawal. In distal retina (~60% retinal depth in this experiment), the detection threshold for the  $K_o^+$  increase was 0.5 log units higher than the stimulus intensity level at which both b-waves (recorded intraretinally and across the eye) were clearly observed. At higher stimulus intensities, the behavior of the  $K_o^+$  increase closely paralleled that of the b-waves. In the proximal retina (~35% retinal depth in this case), the  $K_o^+$  increase was not detected until after the b-wave was clearly observed, and, at higher stimulus intensities, the proximal  $K_o^+$  response showed no relationship to the b-wave recorded either intraretinally or across the eye.

One property of the ERG b-wave seen in this preparation is a decline in amplitude as the stimulus intensity is increased beyond intermediate levels. This decline in the b-wave amplitude at high light intensities is not typical of the ERG measured in the intact rabbit (personal observation). The reason for this decline is not clear, although it may be due to a subtractive interaction between the b-wave and slow PIII responses. This high-intensity decline is evident whether the b-wave is recorded across the eye or intraretinally at any depth. Note, however, that the  $K_o^+$  increase measured in proximal retina showed neither saturation nor decline at light intensities above those that elicit a maximum b-wave. Intensity



FIGURE 6. Pharmacological properties of the ERG, intraretinal ERG, and lightevoked  $K_0^+$  modulations recorded in distal (upper section) vs. proximal (lower section) retina of a superfused rabbit eyecup during a single microelectrode withdrawal. The distal light-evoked  $K_0^+$  increase (arrow 1), illustrated at two gains, is enhanced during drug application (arrow 2). In contrast, proximal  $K_0^+$  increases at light onset and offset are largely abolished by the application of ethanol plus GABA. Enhancement of the b-wave occurs concurrently with these effects, as seen in both ERG records. Diffuse light stimuli. Irradiance:  $3.2 \times 10^{-7}$  W/cm<sup>2</sup>.

response data for distal vs. proximal  $K_o^+$  responses were obtained from nine different rabbit eyecups. In every case, the characteristics of distal and proximal  $K_o^+$  increases were similar to those illustrated in Fig. 5.

PHARMACOLOGICAL SEPARATION OF LIGHT-EVOKED  $K_o^+$  INCREASES Previous reports have shown that a number of pharmacological agents modify the light-responsive network of mudpuppy in a manner useful for discriminating between distal and proximal light-evoked  $K_o^+$  increases (Dick and Miller, 1978*a*; Dick, 1979; Shimazaki et al., 1983, 1984). We have found that the combination of 3% ethanol (EtOH) plus 3 mM  $\gamma$ -aminobutyric acid (GABA) is particularly effective for demonstrating distinct distal and proximal components of the lightevoked  $K_o^+$  increase in rabbit retina. Fig. 6 illustrates the effects of a 2-min application of 3% EtOH plus 3 mM GABA on the ERG, the intraretinal ERG, and K<sup>o</sup> modulations recorded in distal and proximal retina. In this experiment, a doubled-barreled K<sup>+</sup>-selective micropipette was initially positioned at the most distal level where a light-evoked K<sub>o</sub><sup>+</sup> increase could be detected (~65% retinal depth in this case) and control ERGs and Ko modulations were recorded. At such retinal levels, the  $K_{0}^{+}$  response is dominated by the light-evoked  $K_{0}^{+}$  decrease and the fast, transient K<sub>o</sub><sup>+</sup> increase is not optimally resolved. However, such a distal location was found to be desirable in these experiments in order to evaluate drug actions on the distal K<sup>+</sup><sub>o</sub> increase while minimizing possible contributions from more proximal sources of K<sup>+</sup> release. After obtaining control responses, the perfusate was switched to a Ringer's containing 3% EtOH plus 3 mM GABA and the light-evoked responses were recorded. 2 min after introducing the test agents, the control Ringer's was reintroduced and the ERG and  $K_{\circ}^{+}$  responses were again recorded to check for reversibility of the drug effects. This control/ test/return-to-control sequence is illustrated in the upper three horizontal rows of traces in Fig. 6. Immediately after those responses were obtained, the micropipette was withdrawn into the proximal retina near the level of the maximum  $K_{0}^{*}$  increase and the experiment was repeated, as illustrated in the lower half of Fig. 6. It is apparent that EtOH plus GABA affects the retina in a selective fashion. The distal K<sub>o</sub><sup>+</sup> increase (arrow 1), illustrated at two gains in Fig. 6, is enhanced during drug application (arrow 2). In contrast, the proximal  $K_{\circ}^{+}$  increase at light onset was reduced by  $\sim 86\%$  and the proximal K<sub>o</sub><sup>o</sup> increase at light offset was abolished. Applications of EtOH plus GABA always enhanced the distal  $K_{o}^{+}$ increase and b-wave, whereas they always depressed proximal K<sub>o</sub><sup>+</sup> increases. In 22 trials involving eight eyecup preparations, the mean EtOH-plus-GABAinduced effects upon the b-wave and intraretinal b-wave were increases to, respectively,  $265 \pm 55$  and  $252 \pm 60\%$  of control values. In 11 trials involving six evecup preparations, the mean EtOH-plus-GABA-induced effect upon the distal  $K_0^+$  increase was an increase to  $244 \pm 43\%$  of control values (repeated applications of drugs during a single microelectrode penetration to a distal or proximal retinal locus were not considered separate trials). In the same set of 11 trials, plus an additional five carried out in those same six eyecups, the mean EtOH-plus-GABA-induced effect on the light-evoked proximal K<sub>o</sub><sup>+</sup> increase was a reduction to  $16 \pm 5\%$  of control values.

The application of EtOH plus GABA is also associated with a reduction of the  $K_o^+$  decrease response. In 15 trials involving seven eyecups, the mean EtOH-plus-GABA-induced effect upon the  $K_o^+$  decrease was a reduction to 78 ± 8% of control values. Consequently, we do not know whether drug-induced enhancement of the distal  $K_o^+$  increase results from an actual increase in neuronally released  $K^+$  or whether it is secondary to a reduction in the  $K_o^+$  decrease response.

Enhancement of the b-wave induced by the application of 3% EtOH plus 3 mM GABA may result from one or more of several factors, including: (a) a change in the resisitive properties of the neural retina or pigment epithelium; (b) summation with an enhanced c-wave; (c) summation with a diminished PIII response; or (d) direct enhancement of the b-wave current-generating mechanism. To test the first of these possibilities, we applied current pulses (5  $\mu$ A, 1 s

duration) between the reference electrode and vitreous under control conditions and during the application of 3% EtOH plus 3 mM GABA. The voltage associated with these impulses was unaltered during drug application, which suggests that drug-induced ERG changes were not caused by changes in the extracellular resistance pathways. With no apparent change in the extracellular resistive pathways and a diminution of the light-evoked K<sup>+</sup><sub>o</sub> decrease, it is unlikely that cwave enchancement could contribute to the enhanced b-wave observed during exposure to EtOH plus GABA. Indeed, in the absence of apparent changes in the extracellular resistive pathways, one would predict a diminished K<sup>+</sup><sub>o</sub> decrease to be associated with a diminished c-wave, as illustrated in Fig. 7. The experiment



FIGURE 7. Pharmacological properties of the ERG, intraretinal ERG, and lightevoked  $K_o^+$  modulations recorded in distal vs. proximal retina of a superfused rabbit eyecup during a single microelectrode withdrawal. Control responses are shown in the left-hand column of traces; the effects of a 2-min application of 3% EtOH plus 3 mM GABA are shown in the right-hand column. During drug application: (a) bwaves were enhanced; (b) c-waves were reduced; (c) the light-evoked  $K_o^+$  decrease was reduced concomitantly with the appearance of a distal light-evoked  $K_o^+$  increase (arrow 1); and (d) proximal  $K_o^+$  increases at light onset and offset were markedly reduced. Diffuse light stimulation. Irradiance:  $1.0 \times 10^{-7}$  W/cm<sup>2</sup>.

shown in Fig. 7 was similar to the one shown in Fig. 6, except that the responses were obtained during a period in which the c-wave persisted in the eyecup. A double-barreled K<sup>+</sup>-selective microelectrode was initially positioned in the distal retina at 68% retinal depth, and control responses, including the illustrated ERG, were recorded as shown in the upper left-hand set of penwriter traces. The upper right-hand set of traces was recorded 2 min after introducing a perfusate containing 3% EtOH plus 3 mM GABA. After returning to a control perfusate (not illustrated), the microelectrode was withdrawn into the proximal retina at 24% retinal depth and a parallel set of control and experimental records was collected. The b-wave was markedly enhanced during drug applications,

whereas the c-wave and light-evoked  $K_0^+$  decrease were diminished. These results, and those of experiments such as those illustrated in Fig. 6, indicate that b-wave enhancement during exposure to EtOH plus GABA must result from summation with a reduced PIII response and/or direct augmentation of the b-wave current-generating mechanism.

Fig. 7 illustrates an additional phenomenon revealed by drug application. A light-evoked  $K_o^+$  increase was not seen under control conditions at 68% retinal depth. As presented earlier, this is typical of our findings in rabbit retina. However, a  $K_o^+$  increase at that retinal level was revealed during exposure to



FIGURE 8. The transretinal  $K_o^+$  gradient measured in a superfused rabbit eyecup during the experiment shown in Fig. 2A. The solid circles represent the resting (dark)  $K_o^+$  values measured at different retinal levels. The peak magnitudes of the light-evoked  $K_o^+$  increases and that of the maximum  $K_o^+$  decrease are displayed as solid lines located, respectively, above and below the retinal levels at which they were recorded. Scales are of both  $K^+$  activity (milliequivalents per liter) and  $K^+$ concentration (millimolar). Diffuse light stimuli. Irradiance:  $1.0 \times 10^{-7}$  W/cm<sup>2</sup>.

EtOH plus GABA (arrow 1). The appearance of this distal light-evoked  $K_o^+$  increase is most likely to have been associated with a diminished  $K_o^+$  decrease and/or augmented release of  $K^+$  from a nearby neuronal source. The distal  $K_o^+$  increase cannot have represented  $K^+$  release from proximal retina since both light- and dark-evoked proximal  $K_o^+$  increases were markedly reduced by the drug, as seen in the bottom pair of traces in Fig. 7.

## Transretinal K<sup>+</sup><sub>o</sub> Gradient

The extracellular environment of vertebrate retina is not uniform with respect to  $K_o^+$  (Oakley, 1975; Oakley and Green, 1976; Dick, 1979; Dick and Miller, 1985). In the dark-adapted state,  $K_o^+$  is maximum in the distal retina and progressively declines toward the vitreal surface. Transretinal  $K_o^+$  gradients

measured in the isolated rabbit eyecup have been in the range of 1.5-3.0 meq/liter (~2.0-4.0 mM). Fig. 8 illustrates the transretinal gradient measured during the depth profile study shown in Fig. 2A. In Fig. 8, the solid circles represent the resting (dark) K\_o<sup>+</sup> values measured at different retinal levels. The peak magnitudes of light-evoked K\_o<sup>+</sup> increases, and that of the maximum K\_o<sup>+</sup> decrease, are displayed as solid lines located, respectively, above and below the retinal levels at which they were recorded.

Most of the transretinal  $K_o^{\circ}$  gradients measured in rabbit retina were of the order of 2.0 meq/liter (~2.5 mM). The rabbit retina is avascular, and transretinal  $K_o^+$  gradients on the order of 1.5–3.0 meq/liter (~2.0–4.0 mM) have also been characterized in the avascular retinas of the frog (Oakley, 1975; Oakley and Green, 1976), mudpuppy, tiger salamander, and toad (Dick, 1979; Dick and Miller, 1985). In contrast, Steinberg and co-workers (1980) demonstrated a transretinal  $K_{e}^{+}$  gradient of 0.5 mM in the intact, anesthetized cat preparation. Unlike the rabbit, the cat possesses an extensive intraretinal circulation with capillaries extending to the level of the inner nuclear layer (Henkind, 1966). This inner retinal circulation may act as a  $K^+$  sink that opposes the accumulation of K<sup>+</sup> in distal retina and that may contribute to the differences in magnitude between  $K_0^+$  gradients measured in rabbit vs. cat. It is also possible that the high  $K_{o}^{+}$  levels measured in distal retina of the in vitro rabbit eyecup are significantly elevated over those in the intact rabbit retina. Progressive anoxic damage to choroidal and scleral tissues, located farthest from the oxygenated perfusate, could lead to K<sup>+</sup> accumulation on the basal (choroid-facing) side of the pigment epithelium. K<sup>+</sup> diffusing proximally from such damaged tissue could artificially elevate  $K_o^+$  levels within the subretinal space. We conclude that there is a significant transretinal K<sub>o</sub><sup>+</sup> gradient distributed across the rabbit retina, but that its magnitude under in vivo conditions is yet to be determined.

In the dark-adapted state, the transretinal  $K_0^+$  gradient runs downhill from the subretinal space to the vitreous. However, it is evident in Fig. 8 that the magnitude of the light-evoked  $K_0^+$  decrease may exceed the magnitude of the gradient. Consequently, depending upon the intensity and period of the light stimulus, the transretinal  $K_0^+$  gradient may be reversed and run downhill from the vitreous to the subretinal space during illumination. This phenomenon has previously been characterized in the intact cat retina (Steinberg et al., 1980), where the "reversed" gradient during illumination may considerably exceed the magnitude of the dark-adapted gradient.

Fig. 8 also illustrates that in this preparation, as in the intact cat retina (Steinberg et al., 1980), the peak magnitudes of light-evoked  $K_o^+$  decreases considerably exceed those of light-evoked  $K_o^+$  increases.

## Intracellular Müller Cell Recordings

We have obtained more than 24 intracellular recordings in rabbit retina from cells that are characterized by: (a) a resting membrane potential of -80 mV or more; (b) a light response that consists of a transient depolarization followed by a sustained hyperpolarization; and (c) an off response that consists of a transient depolarization followed by a slower return to the (dark) resting membrane potential. These cells were tentatively identified as Müller cells, and we attempted

to confirm the glial origin of the responses through intracellular fluorescent dye injections (Dacheux, 1977) and intracellular injections of HRP. We have recovered complete or partially stained Müller cells in 13 cases. Fig. 9 shows the intracellularly recorded light response of a Müller cell (Fig. 9A, upper trace) and a cross-section of that cell after HRP staining and histological recovery (Fig. 9B).



FIGURE 9. (A) Intracellularly recorded Müller cell responses (upper trace) and simultaneously recorded ERG (lower trace) from a superfused rabbit eyecup. The resting membrane potential of this Müller cell was approximately -85 mV. The flash duration of a diffuse white light stimulus is represented by horizontal bars beneath the Müller cell response and ERG records. The light response of this glial cell is typical of Müller cells in rabbit retina and consists of a transient depolarization followed by a sustained hyperpolarization. At light offset, the repolarization of this cell shows both an early, fast component and a later, slower return to the cells dark potential. The ERG (lower trace) shows a positive b-wave followed by a sustained, negative PIII component. At light offset, the transient, negative, notched off response is followed by a decay of the PIII component and a subsequent return of the ERG potential to the resting (dark) level. Diffuse light stimulus. Irradiance: 1.0  $\times 10^{-7}$  W/cm<sup>2</sup>. (B) The HRP-stained Müller cell from which the intracellular recording in A was obtained. Calibration bar: 25  $\mu$ m.

This cell had a resting membrane potential of -85 mV and its light response is typical of those that we have associated with Müller cells. Fig. 9A (lower trace) illustrates the ERG recorded simultaneously with the Müller cell response.

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#### DISCUSSION

The  $K_o^+$  changes reported in this study include a  $K_o^+$  decrease at light onset, which is maximal in the distal retina, and a  $K_o^+$  increase at light onset, which is separable into distal and proximal components. There is also a proximal  $K_o^+$  increase at light offset. This pattern of light- and dark-evoked  $K_o^+$  modulations is largely similar to that described previously in amphibia (Oakley, 1975; Oakley and Green, 1976; Tomita, 1976; Karwoski and Proenza, 1977, 1978, 1982; Dick and Miller, 1978*a*, 1985; Dick, 1979; Vogel, 1980; Karwoski et al., 1982; Shimazaki et al., 1983), with the exception that several studies of amphibian retina have not described a light-evoked  $K_o^+$  increase arising from a distal source (Oakley, 1975; Oakley and Green, 1976; Tomita, 1976; Karwoski and Proenza, 1977, 1978; Vogel, 1980).

There are two main differences between the depth profiles of light-evoked  $K_o^+$  activity in rabbit and amphibia. First, the maximum  $K_o^+$  decrease detected in the rabbit retina is 50–100% larger than that recorded in mudpuppy (Karwoski and Proenza, 1977, 1978; Dick and Miller, 1978*a*), toad (Dick, 1979; Dick and Miller, 1985), or frog (Oakley, 1975, 1977; Oakley and Green, 1976). Second, a  $K_o^+$  decrease is detected throughout the rabbit retina, whereas in amphibia, a  $K_o^+$  decrease can only be demonstrated in proximal retina through the use of long-duration light stimuli (Karwoski and Proenza, 1977; Dick, 1979). One factor that is likely to contribute to this second difference is that the rabbit retina has a measured thickness of only 100–180  $\mu$ m, whereas the amphibian retinas are generally 230–300  $\mu$ m thick. Therefore, diffusional forces may play a more prominent role in establishing a distribution of the  $K_o^+$  decrease that extends to proximal levels of the rabbit retina.

The pattern of intraretinal light-evoked  $K_o^+$  changes in rabbit is also similar to that reported in cat, particularly with respect to the  $K_o^+$  decrease (Steinberg et al., 1980). Steinberg and co-workers reported light-evoked  $K_o^+$  decreases that were of similar magnitude to those reported here and also extended through the proximal retina in cat. However, only limited comparisons can be drawn between the  $K_o^+$  increases in rabbit and cat. Steinberg et al. (1980) established that there is a light-evoked  $K_o^+$  increase in the cat retina, but they did not direct their investigation toward the question of whether there is more than one intraretinal source of light-evoked  $K^+$  release. In the cat retina, the  $K_o^+$  increase evoked with low-intensity light showed a sustained time course, which Steinberg and his colleagues related to the generation of the DC component of the ERG. This phenomenon was not observed in the present study. Additionally, a dark-evoked  $K_o^+$  increase was not observed in the above study of cat retina.

## Light-evoked K<sup>+</sup><sub>o</sub> Decrease

Our present results in rabbit retina are fully consistent with previous studies in cat (Steinberg et al., 1980), amphibia (Oakley, 1975, 1977; Oakley and Green, 1976; Tomita, 1976; Olsen and Miller, 1977; Karwoski and Proenza, 1977, 1978; Dick and Miller, 1978*a*, 1985; Vogel, 1980), and skate (Kline et al., 1978) in establishing that there is a light-evoked  $K_o^+$  decrease that is maximal in the

distal retina. In both frog (Oakley, 1975) and toad, the  $K_o^+$  decrease maximum has been localized to the level of the rod inner segments. In the present study, the level of the  $K_o^+$  decrease maximum has not been precisely established, although our results are consistent with the localization established by Oakley and co-workers. In addition, the correspondence that we report in rabbit retina between the temporal and intensity-response properties of the  $K_o^+$  decrease and c-wave further supports the view, originally proposed by Steinberg and Miller (1973), that a light-evoked  $K_o^+$  decrease initiates the RPE c-wave through a passive hyperpolarization of pigment epithelial cells.

The light-evoked  $K_o^+$  decrease is also proposed to passively hyperpolarize Müller cell processes, setting up a transretinal current detected as the slow PIII voltage (Faber, 1969; Zuckerman, 1973; Witkovsky et al., 1975; Karwoski and Proenza, 1977; Lurie and Marmor, 1980; Welinder et al., 1982; Dick and Miller, 1985). In the present study, temporal properties of the  $K_o^+$  decrease corresponded to slow PIII responses (Figs. 2, 3, and 6), whereas the Müller cell response in rabbit was shown to have a significant hyperpolarizing component whose development closely paralleled that of slow PIII (Fig. 9). Our results lend further support to the theory that slow PIII is a Müller cell–generated, second-order signal of photoreceptor activity.

#### Light-evoked K<sup>+</sup><sub>o</sub> Increases

We have shown that light-evoked  $K_o^+$  increases can be detected from near the vitreal surface to retinal levels that are distal to the OPL. This distribution is similar to that which we have reported in preceding studies of light-evoked  $K_o^+$  changes in amphibian retinas (Dick and Miller, 1978*a*, 1985). In the rabbit retina, as in amphibian retina, we have been able to differentiate distal vs. proximal regions of light-evoked  $K_o^+$  increases. This differentiation has been made on the basis of intensity-response properties and selective sensitivity to pharmacological agents. We conclude from these experiments that light-evoked  $K_o^+$  increases in distal vs. proximal retina are generated by predominantly different populations of retinal neurons. In addition, since our depth profile studies did not reveal spatially separate maxima for these distal and proximal  $K_o^+$  increases are made up of overlapping contributions from both distal and proximal sources of light-evoked  $K^+$  release.

#### Neuronal Origins of Light-evoked K<sup>+</sup><sub>o</sub> Increases

Studies of amphibian retina are in general agreement that proximal  $K_o^+$  increases originate from the activity of amacrine and ganglion cells (Oakley, 1975; Karwoski and Proenza, 1977, 1978, 1980; Karwoski et al., 1978; Dick and Miller 1978*a*, 1985; Dick, 1979; Vogel, 1980; Shimazaki et al., 1984). This laboratory evaluated the distal  $K_o^+$  increase in amphibia and concluded that it is primarily, if not exclusively, of depolarizing bipolar cell origin (Dick and Miller, 1978*a*, 1985; Dick, 1979). This conclusion was based largely on comparisons between the effects of various chemical agents upon intracellularly recorded neuronal responses, ERGs, and light-evoked  $K_o^+$  changes. Presently, we have far fewer intracellular recording data on the pharmacological sensitivities of rabbit vs. amphibian retinal neurons. Consequently, we cannot make direct comparisons between drug influences on neurons, ERG components, and K<sup>+</sup><sub>o</sub> changes in rabbit retina. However, the pharmacological sensitivities of field potentials and lightevoked  $K_{o}^{h}$  changes provide some measure of comparison between the neuronal networks of amphibian and rabbit retinas. For example, the ERG and proximal negative response in rabbit and mudpuppy retinas are known to be similarly affected by many substances, and the present study shows that EtOH plus GABA affects the ERG and K<sup>+</sup><sub>o</sub> activity changes of rabbit retina in the same way that it affects these responses in the mudpuppy. Such correspondences between these species suggest that common neuronal classes probably share similar mechanisms for light-response electrogenesis. Based on this hypothesis and the parallel action of EtOH plus GABA on rabbit and mudpuppy retinas, we suggest that in rabbit, as in amphibia, proximal  $K_{0}^{+}$  increases are primarily post-bipolar in origin, whereas the distal K<sup>+</sup><sub>o</sub> increase is probably generated by depolarizing bipolar cells. This model is strengthened by the observation that depolarizing bipolars make up the sole class of rabbit retinal neurons that are known to both depolarize at light onset and extend processes into the OPL.

## $K_o^+$ Increases and the B-Wave

Our present findings are consistent with several conditions of the Müller cell hypothesis of b-wave generation presented by Faber (1969) and Miller and Dowling (1970a, b). Faber's current source density analysis in rabbit retina concluded that the "active site" of b-wave generation lies in the OPL and that the Müller cell is the sole retinal element whose morphology is consistent with the spatial distribution of the b-wave current. Miller and Dowling (1970a, b) and Faber (1969) concluded that Müller cell processes in the outer (distal) retina must be passively depolarized in response to a local light-evoked  $K_0^{+}$  increase. The distal light-evoked  $K_{o}^{+}$  increase reported both previously (see Dick and Miller, 1985, and references cited therein) and presently fulfills the spatial requirement for the  $K_o^+$  increase predicted by the Müller cell hypothesis. In addition, the b-wave and distal K<sub>o</sub><sup>+</sup> increase show parallel intensity-response properties and a similar sensitivity to the pharmacological agents used in this study. We have also identified the Müller cell response in rabbit retina and have shown that this cell is depolarized at light onset. We conclude that our findings support a glial origin for the b-wave in rabbit retina. Our conclusion is consistent with those of a variety of studies whose results have supported the Müller cell theory (see Dick and Miller, 1985, and references cited therein). The bulk of investigations into the mechanism of b-wave generation have been carried out in lower vertebrate preparations. Recently, however, Welinder and co-workers (1982) looked at b-wave generation by examining the effects of  $\alpha$ -aminoadipate, a glial-specific toxin, on the ERG in rabbit retina. Those investigators also concurred that Müller cells must be critically involved in b-wave generation.

The contributions of proximal  $K_o^+$  increases to  $K^+$ -mediated, transglial current flow is not at all clear. No specific relationship between proximal  $K_o^+$  increases and the b-wave has been demonstrated in any species (Oakley, 1975; Karwoski and Proenza, 1978; Dick and Miller, 1978*a*, 1985; Vogel, 1980). We have shown that b-wave enhancement can occur concurrently with a marked depression of the proximal light-evoked  $K_o^+$  increase (Figs. 6 and 7; Dick and Miller, 1978*a*, 1985; Dick, 1979). This finding indicates that proximal  $K_o^+$  increases cannot be a primary factor in b-wave generation.

It is parsimonious to point out that alternative theories to the Müller cell hypothesis have been put forth to explain b-wave generation (Oakley, 1975; Vogel, 1980; Yanagida and Tomita, 1982). As we mentioned in the companion paper (Dick and Miller, 1985), a common theme among the alternative hypotheses is that depolarizing bipolar cell responses directly generate part, or all, of the b-wave. The methods used in the present study cannot differentiate between direct vs. indirect neuronal contributions to the ERG. However, there is no experimental support for the alternative theories, and the results of current source density analyses, experiments with glial-specific toxins, ontogenic studies, and comparisons between the latencies and dynamic ranges of neuronal responses vs. b-waves all argue against significant, direct depolarizing bipolar cell contributions to the b-wave (see Dick and Miller, 1985). In the companion paper (Dick and Miller, 1985), we proposed the following model of how the primary b-wave voltage may be generated. Depolarizing bipolar cell processes in the OPL generate a  $K^+$  efflux at light onset that can be detected as the distal  $K_o^+$  increase. This K<sup>+</sup><sub>o</sub> modulation depolarizes Müller cell processes and initiates a transretinal current associated with the b-wave. In this model, the b-wave would primarily represent a second-order signal of depolarizing bipolar cell activity. Our current findings in rabbit retina are fully consistent with such a model.

In summary, we have characterized three separable light-evoked  $K_o^+$  modulations in rabbit retina: a  $K_o^+$  decrease that is maximal in the distal retina and two  $K_o^+$  increases that appear to originate from sources in relatively distal vs. proximal retina. The properties of the  $K_o^+$  decrease are consistent with the concepts that it is related to the c-wave and slow PIII components of the ERG through K<sup>+</sup>mediated influences on, respectively, the retinal pigment epithelium and Müller cells. The properties of the distal light-evoked  $K_o^+$  increase are consistent with the concept that it is related to b-wave generation through a K<sup>+</sup>-mediated depolarization of Müller cell processes.

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#### REFERENCES

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Baum, G. 1970. An organic cation-selective electrode: potentiometric determination of acetylcholine activity. Anal. Lett. 3:105–111.

- Brown, K. T., and T. N. Wiesel. 1961. Analysis of the intraretinal electroretinogram in the intact cat eye. J. Physiol. (Lond.). 158:229-256.
- Dacheux, R. F. 1977. A physiological study of the ontological formation of synaptic interactions in the rabbit retina. Ph.D. Thesis. State University of New York, Buffalo, NY. 233 pp.
- Dacheux, R. F., M. Delmelle, R. F. Miller, and W. K. Noell. 1973. Isolated rabbit retina preparation suitable for intra- and extracellular analysis. *Fed. Proc.* 32:327. (Abstr.)
- Dick, E. 1979. Light- and dark-dependent extracellular K<sup>+</sup> activity modulations in the vertebrate retina: origins of electroretinographic components. Ph.D. Thesis. State University of New York, Buffalo, NY. 310 pp.
- Dick, E., and R. F. Miller. 1977. Extracellular potassium activity in the mudpuppy retina: its relationship to the b-wave of the ERG. Soc. Neurosci. Abstr. 3:557.
- Dick, E., and R. F. Miller. 1978a. Light-evoked potassium activity in mudpuppy retina: its relationship to the b-wave of the electroretinogram. Brain Res. 154:388-394.
- Dick, E., and R. F. Miller. 1978b. Contribution of the K<sup>+</sup>-mediated Müller cell responses to ERG components of E- and I-type retinas. *Invest. Ophthalmol. Visual Sci.* 17(ARVO Suppl.):262. (Abstr.)
- Dick, E., and R. F. Miller. 1985. Extracellular K<sup>+</sup> activity changes related to electroretinogram components. I. Amphibian (I-type) retinas. J. Gen. Physiol. 85:885-909.
- Faber, D. S. 1969. Analysis of the slow trans-retinal potentials in response to light. Ph.D. Thesis. State University of New York, Buffalo, NY. 303 pp.
- Granit, R. 1935. Two types of retinas and their electrical responses to intermittent stimuli in light and dark adaptation. J. Physiol. (Lond.). 85:421-438.
- Henkind, P. 1966. The retinal vascular system of the domestic cat. Exp. Eye. Res. 5:10-20.
- Karwoski, C. J., M. H. Criswell, and L. M. Proenza. 1978. Laminar separation of light-evoked K<sup>+</sup> flux and field potentials in frog retina. *Invest. Ophthalmol. Visual Sci.* 17:678-682.
- Karwoski, C. J., and L. M. Proenza. 1977. Relationship between Müller cell responses, a local transretinal potential, and potassium flux. J. Neurophysiol. (Bethesda). 40:244-259.
- Karwoski, C. J., and L. M. Proenza. 1978. Light-evoked changes in extracellular potassium concentration in mudpuppy retina. *Brain Res.* 142:515-530.
- Karwoski, C. J., and L. M. Proenza. 1980. Neurons, potassium, and glia in proximal retina of Necturus. J. Gen. Physiol. 75:141-162.
- Karwoski, C. J., and L. M. Proenza. 1982. Light-evoked K<sup>+</sup>-increases in amphibian retina. Invest. Ophthalmol. Visual Sci. 21(ARVO Suppl.):281. (Abstr.)
- Kline, R. P., H. Ripps, and J. E. Dowling. 1978. Generation of b-wave currents in the skate retina. Proc. Natl. Acad. Sci. USA. 75:5727-5731.
- Lurie, M., and M. F., Marmor. 1980. Similarities between the c-wave and slow PIII in the rabbit eye. Invest. Ophthalmol. Visual Sci. 19:1113-1117.
- Masland, R. H., and A. Ames. 1976. Responses to acetylcholine of ganglion cells in an isolated mammalian retina. J. Neurophysiol. (Bethesda). 39:1220-1235.
- Masland, R. H., and C. J. Livingstone. 1976. Effect of stimulation with light on synthesis and release of acetylcholine by an isolated mammalian retina. *J. Neurophysiol.* (*Bethesda*). 39:1210-1219.
- Massey, S. C., and M. J. Neal. 1979. The light-evoked release of acetylcholine from the rabbit retina in vivo and its inhibition by  $\gamma$ -aminobutyric acid. J. Neurochem. 32:1327–1329.
- Massey, S. C., and D. A. Redburn. 1982. A tonic γ-aminobutyric acid-mediated inhibition of cholinergic amacrine cells in rabbit retina. J. Neurosci. 2:1633-1643.
- Miller, R. F. 1973. Role of K<sup>+</sup> in generation of b-wave of electroretinogram. J. Neurophysiol. (Bethesda). 36:28-38.

- Miller, R. F., and J. E. Dowling. 1970a. Intracellular responses of the Müller (glial) cells of mudpuppy retina: their relation to b-wave of the electroretinogram. J. Neurophysiol. (Bethesda). 33:323-341.
- Miller, R. F., and J. E. Dowling. 1970b. A relationship between Müller cell slow potentials and the ERG b-wave. Int. Soc. Clin. Electroretinog. Symp. Pisa. 85-100.
- Newman, E. A. 1979. B-wave currents in the frog retina. Vision Res. 19:227-234.
- Newman, E. A. 1980. Current source-density analysis of the b-wave of frog retina. J. Neurophysiol. (Bethesda). 43:1355-1366.
- Newman, E. A., and L. L. Odette. 1984. Model of electroretinogram b-wave generation: a test of K<sup>+</sup> hypothesis. J. Neurophysiol. (Bethesda). 51:164-182.
- Noell, W. K. 1953. Studies on the electrophysiology and metabolism of the retina. Project No. 21-1201-004, Report No. 1. United States Air Force School of Aviation Medicine.
- Oakley, B. 1975. Measurement of light-induced transient changes in extracellular potassium ion concentration in the frog retina. Ph.D. Thesis. University of Michigan, Ann Arbor, MI. 173 pp.
- Oakley, B. 1977. Potassium and the photoreceptor-dependent pigment epithelial hyperpolarization. J. Gen. Physiol. 70:405-425.
- Oakley, B., and D. G. Green. 1976. Correlation of light-induced changes in retinal extracellular potassium concentration with c-wave of the electroretinogram. J. Neurophysiol. (Bethesda). 39:1117-1133.
- Olsen, J. S., and R. F. Miller. 1977. Spontaneous slow potentials and spreading depression in amphibian retina. J. Physiol. (Lond.). 40:752-767.
- Proenza, L. M., and J. A. Freeman. 1975. Light-evoked extracellular potentials of the *Necturus* retina: current source density analysis of the electroretinographic b-wave and the proximal negative response. *Soc. Neurosci. Abstr.* 1:104.
- Rager, G. 1979. The cellular origin of the b-wave in the electroretinogram—a developmental approach. J. Comp. Neurol. 188:225-244.
- Schmidt, R., and R. Steinberg. 1971. Rod-dependent intracellular responses to light recorded from the pigment epithelium of cat retina. J. Physiol. (Lond.). 217:71-91.
- Shimazaki, H., C. Karwoski, and L. Proenza. 1983. Pharmacological separation of Müller cells, [K<sup>+</sup>]<sub>o</sub>, and the b-wave in mudpuppy retina. *Invest. Ophthalmol. Visual Sci.* 22(ARVO Suppl.):220. (Abstr.)
- Shimazaki, H., C. J. Karwoski, and L. M. Proenza. 1984. Aspartate-induced dissociation of proximal from distal retinal activity in the mudpuppy. *Vision Res.* 24:587-595.
- Steinberg, R. 1971. Incremental responses to light recorded from pigment epithelial cells and horizontal cells of the cat retina. J. Physiol. (Lond.). 217:93-110.
- Steinberg, R., and S. S. Miller. 1973. Aspects of electrolyte transport in frog pigment epithelium. Exp. Eye Res. 16:365-372.
- Steinberg, R. H., B. Oakley II, and G. Niemeyer. 1980. Light-evoked changes in K<sup>+</sup><sub>o</sub> in the retina of the intact cat eye. *J. Neurophysiol.* (*Bethesda*). 44:897–921.
- Steinberg, R., R. Schmidt, and K. T. Brown. 1970. Intracellular responses to light from cat pigment epithelium: origin of the electroretinogram c-wave. *Nature (Lond.)*. 227:728–730.
- Tomita, T. 1976. Electrophysiological studies of retinal cell function. Invest. Ophthalmol. Visual Sci. 15:171-187.
- Vogel, D. A. 1980. Potassium release and ERG b-wave current flow in the frog retina. Ph.D. Thesis. University of Michigan, Ann Arbor, MI. 158 pp.
- Welinder, E., O. Textorius, and S. E. G. Nilsson. 1982. Effects of intravitreally injected DL-a-

aminoadipic acid on the c-wave of the D.C.-recorded electroretinogram in albino rabbits. Invest. Ophthalmol. Visual Sci. 23:240-245.

- Witkovsky, P., F. E. Dudek, and H. Ripps. 1975. Slow PIII component of the carp electroretinogram. J. Gen. Physiol. 65:119-134.
- Yanagida, T., and T. Tomita. 1982. Local potassium concentration changes in the retina and the electroretinographic (ERG) b-wave. *Brain Res.* 237:479-483.
- Zuckerman, R. 1973. Ionic analysis of photoreceptor membrane currents. J. Physiol. (Lond.). 235:333-354.