Delayed Basal Hyperpolarization of Cat Retinal Pigment Epithelium and Its Relation to the Fast Oscillation of the DC Electroretinogram

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ABSTRACT   Previous work has shown that the cat retinal pigment epithelium (RPE) is the source of two potential changes that follow the absorption of light by photoreceptors: a hyperpolarization of the apical membrane, peaking in 2–4 s, which leads to the RPE component of the electroretinogram (ERG) c-wave, and a depolarization of the basal membrane, peaking in 5 min, which leads to the light peak. This paper describes a new basal membrane response of intermediate time course, called the delayed basal hyperpolarization. Isolation of this response from other RPE potentials showed that with maintained illumination the hyperpolarization begins ~2 s after light onset, peaks in 20 s, and slowly ends as the membrane repolarizes over the next 60 s. The delayed basal hyperpolarization is very small for stimuli <4 s in duration and grows with duration, becoming ~15% as large as the preceding apical hyperpolarization with stimuli longer than 20 s. Extracellularly, this response contributes to the transepithelial potential (TEP) across the RPE. In response to light the TEP first rises to a peak, the c-wave, as the apical membrane hyperpolarizes. For stimuli longer than ~4 s, the decline of the TEP from the peak of the c-wave results partly from the recovery of apical membrane potential and partly from the delayed basal hyperpolarization. For long periods of illumination (300 s) the delayed basal hyperpolarization leads to a trough in the TEP between the c-wave and light peak. This trough is largely responsible for a corresponding trough in vitreal recordings, which has been called the "fast oscillation." The term "fast oscillation" has also been used to denote the sequence of potential changes resulting from repeated stimuli ~1 min in duration. In addition to the delayed basal hyperpolarization, such responses also contain a basal off-response, a delayed depolarization.

INTRODUCTION

Retinal pigment epithelial (RPE) cells have two distinct cell membranes—an

apical membrane, facing the photoreceptors, and a basal membrane, facing the choroid. Previous studies have shown that each membrane is the source of a unique electrical response that follows the absorption of light by the photoreceptors. The better understood of these is an apical membrane hyperpolarization that peaks 2–4 s after light onset in the cat (Steinberg et al., 1970) and is caused by a light-evoked decrease in the extracellular potassium concentration ($[K^+]_o$) in the subretinal space around the outer segments of the photoreceptors (e.g., Oakley and Green, 1976; Steinberg et al., 1980). The much slower response of the basal membrane has been described only recently (Griff and Steinberg, 1982; Linsenmeier and Steinberg, 1982), and is a depolarization that peaks ~5 min after the onset of illumination.

In the lizard *Gekko gekko*, Griff and Steinberg (1984) have described a third light-evoked change in membrane potential. This component is a delayed hyperpolarization of the basal membrane that occurs after the K^+-dependent hyperpolarization of the apical membrane, but before the slow depolarization of the basal membrane. This paper describes experiments that demonstrate the presence of a similar basal hyperpolarization in the cat RPE. We have also performed an analysis in cat to isolate the basal hyperpolarization from other RPE potential changes and have characterized some aspects of its dependence on stimulus conditions.

Another way to view the work to be described is in the context of the electroretinogram (ERG), since potentials originating in the RPE contribute to the signals observed in corneal or vitreal recordings. The apical hyperpolarization gives rise to the pigment epithelial component of the c-wave of the ERG (Steinberg et al., 1970), and the depolarization of the basal membrane gives rise to the light peak (Griff and Steinberg, 1982; Linsenmeier and Steinberg, 1982; Valeton and van Norren, 1982). Both the c-wave and the light peak can be recorded with DC amplification from the cornea of humans (Skoog, 1975; Täumer et al., 1976) and from the cornea or vitreous humor of many experimental animals (e.g., Kikawada, 1968; Gouras and Carr, 1965). Human and cat DC recordings also contain two events between the c-wave and light peak: one of these is a trough in the potential following the c-wave, called the "fast oscillation" (Täumer et al., 1976; Marmor and Lurie, 1979), and the other is a shoulder on the rising side of the light peak, called the "second c-wave" (Nikara et al., 1976). The cellular origin of these components of the DC ERG has not been defined, and some investigators regard the fast oscillation and the second c-wave as parts of the same event (Täumer et al., 1976). Here we will show that the fast oscillation is largely a result of the delayed hyperpolarization of the RPE basal membrane. The second c-wave will be shown to result from the addition of several previously defined events, rather than having a unique cellular origin.

METHODS

The methods used for preparation and recording from the intact cat eye have been reported previously (Steinberg et al., 1980; Linsenmeier and Steinberg, 1982) and will be summarized only briefly here. Experiments were performed on the right eye of adult cats, anesthetized during the experiment with urethane (200 mg/kg loading dose admin-

istered during surgery, followed by 20–30 mg/kg·h), and paralyzed with gallamine triethiodide. Animals were artificially ventilated at a rate and volume chosen to maintain arterial PO_2, PCO_2, and pH in the normal ranges. Some animals were decerebrated rather than urethane anesthetized. Bilateral lesions were made in the pontine reticular formation by applying radiofrequency current to electrodes lowered through the brain and positioned stereotaxically. The electrodes were two stainless-steel wires (exposed length ~2 mm) separated by 2 mm horizontally. The lesions, made at F1, occupied the area between L1.5 and L5, and H0 and H-4, as first described by Iwama and Jasper (1957). This lesion also roughly corresponds to the rostropontine decerebration of Batini et al. (1959), which put their cats in a state of permanent EEG sleep.

Vitreal recordings were made between a chlorided silver wire in the vitreous humor and a chlorided silver reference behind the eye (retrobulbar reference). Intraretinal and intracellular recordings were made with a glass microelectrode referred to the retrobulbar electrode. Potentials were amplified, filtered (DC to 50 Hz), displayed on a storage oscilloscope and chart recorder, and stored on magnetic tape. A PDP 11/03 computer (Digital Equipment Corp., Marlboro, MA) was used later to digitize and plot the data.

The stimulus was white light generated either by a fiberoptic illuminator and fiber bundle or by a dual-beam ophthalmoscope (Brown, 1964). The stimuli were made diffuse by placing a paper diffusing screen between the stimulator and the eye. Retinal illumination for the cat is given in log quanta $(507) \cdot deg^{-2} \cdot s^{-1}$, and in these units rod saturation is at ~8.2 (see Linsenmeier and Steinberg, 1982, for further details).

RESULTS

The Delayed Basal Hyperpolarization

The transepithelial potential (TEP) across the RPE can be recorded in vivo between a microelectrode in the subretinal space, just outside the RPE apical membrane, and a retrobulbar electrode. Fig. 1 A shows the light-evoked change in TEP during the initial 16 s of the response to a 60-s stimulus. After the small passive voltage drop of the b-wave across the RPE, the TEP increases to the peak of the c-wave and then decreases relatively slowly. The first problem was to identify the intracellular events responsible for these changes in TEP.

As previously discussed (Griff and Steinberg, 1984; also see Miller and Steinberg, 1977; Linsenmeier and Steinberg, 1983), a circuit analysis of the RPE indicates that a change in TEP can originate from changes in membrane potential (i.e., in the batteries) at either the apical or basal membrane. In general, a potential change at one membrane will also lead to a potential change at the opposite membrane, as a result of passive current flow (shunting). The potential change has the same polarity at both membranes, but is always smaller at the passively affected membrane. The observed change in TEP is the difference between the polarization of the two membranes.

These considerations allow us to use the intracellular recordings of Fig. 1 B to deduce the events responsible for the TEP changes in part A. The solid lines are the basal membrane potential, V_{ba}, recorded with a microelectrode in an RPE cell referred to the retrobulbar reference, and the apical membrane potential, V_{ap}, obtained by subtracting V_{ba} from the TEP (Linsenmeier and Steinberg, 1982). In Fig. 1 B both membranes hyperpolarized at the onset of illumination, but the apical membrane response was larger, which indicates that the increase

in TEP over the first 2 s of the response was caused by a hyperpolarization of the apical membrane (period 1). The smaller basal hyperpolarization during this time was presumably due to shunting of this potential (Miller and Steinberg, 1977; Oakley, 1977; Griff and Steinberg, 1982; Linsenmeier and Steinberg, 1982). As described in previous work on frog and cat, this apical hyperpolariza-
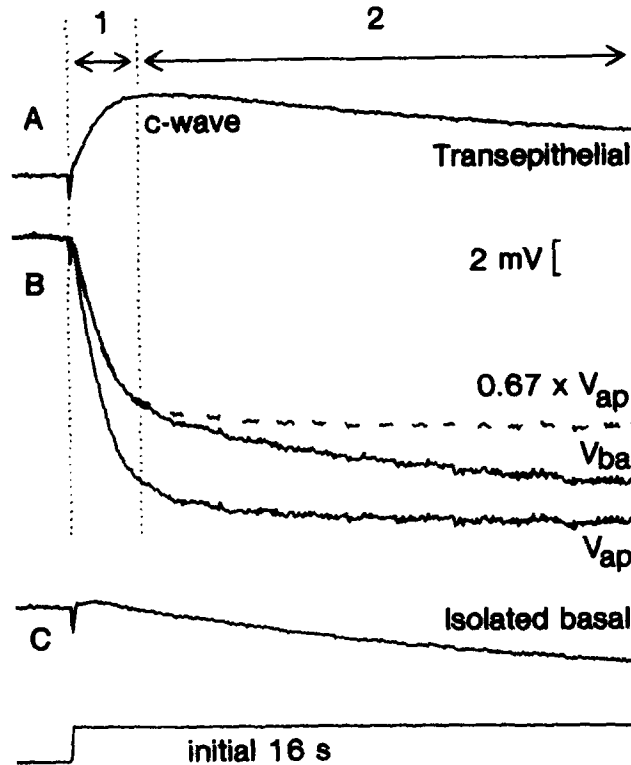

FIGURE 1.   Transepithelial and intracellular RPE recordings showing the initial 16 s of the response to a 60-s period of illumination at 9.3 log $q \cdot deg^{-2} \cdot s^{-1}$. (A) Transepithelial recording made between a microelectrode in the subretinal space and a retrobulbar reference electrode. (B) Apical (V_{ap}) and basal (V_{ba}) membrane potentials of an RPE cell (continuous lines), and a trace showing the apical membrane potential multiplied by a factor of 0.67 (interrupted line). (C) Computer subtraction of the scaled apical response from the basal response. A b-wave, caused by a passive voltage drop of current generated in the retina, appears in A and B. None of this voltage appears at the apical membrane, so the b-wave also appears in C. (Cat 36.)

tion was a response to a decrease in $[K^+]_o$ in the subretinal space (Oakley and Green, 1976; Oakley, 1977; Steinberg et al., 1980).

After the initial increase in TEP to the c-wave peak, the potential slowly declined (period 2). The intracellular recordings show that during the decline both membranes continued to hyperpolarize, but the basal membrane hyperpolarized more. Since a decrease in TEP could result from an apical depolarization, a basal hyperpolarization, or both (e.g., Griff and Steinberg, 1984), the simplest

explanation of these recordings is that the decrease in TEP resulted largely from a hyperpolarization that originated at the basal membrane.[1] As in the previous paper (Griff and Steinberg, 1984), this event is called the delayed basal hyperpolarization.

Isolation of the Delayed Basal Hyperpolarization

It is difficult to determine the precise magnitude and time course of basal events, since potential changes may continue to be generated at the apical membrane while the basal membrane is hyperpolarizing. These apical events will be shunted electrically to the basal membrane, causing passive iR drops that will add to voltage changes originating at the basal membrane. The basal events could be isolated relatively completely, however, by the following procedure. If no potential change originated at the basal membrane, then the response observed there would result only from electrical shunting of the apical response. In this case the basal membrane response would be a constant fraction of the apical membrane response, and by scaling V_{ap} one would be able to match V_{ba}. The dashed line in Fig. 1B shows a scaled version of V_{ap} ($0.67 \times V_{ap}$), which does superimpose on V_{ba} exactly for the first 2 s of the response, up to the peak of the c-wave. In other cells these two traces superimposed for as long as 4 s (Fig. 6). After this time (period 2), the basal membrane was more hyperpolarized than one would have expected on the basis of shunting of V_{ap} alone. The size and time course of the hyperpolarization generated at the basal membrane are given by the difference between the actual basal response and the basal response expected because of shunting (dashed line). Fig. 1C shows this difference, the delayed basal hyperpolarization, isolated from shunted apical membrane events.

For this analysis to be valid, it is necessary that the only potentials observed at the apical membrane be the ones generated there. Any potentials of basal origin shunted to the apical membrane would be scaled and subtracted in the analysis, distorting the apparent size of the delayed basal hyperpolarization. Fortunately, the apical membrane potential can be considered relatively pure in the cat, because the relative values of the membrane resistances (apical smaller than basal [Linsenmeier and Steinberg, 1983]) make passive iR drops large across the basal membrane, but small or undetectable across the apical membrane. Direct evidence that V_{ap} does not contain shunted basal responses is provided by recordings of the light peak, which is generated at the basal membrane and is shunted very little to the apical membrane (Linsenmeier and Steinberg, 1982).[2]

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[1] The alternative explanation for a decrease in TEP is a battery in the paracellular pathway that would polarize both membranes passively but in opposite directions. A change in this battery would appear as a hyperpolarization of the basal membrane and a depolarization of the apical membrane. Although an apical depolarization does not appear in Fig. 1, it was observed in some cells (Fig. 3). As discussed below, however, this depolarization almost certainly was generated at the apical membrane, rather than at a paracellular battery. Furthermore, a paracellular battery cannot explain the recordings in gecko (Griff and Steinberg, 1984).

[2] The analysis also assumes that the shunting in both directions is constant during the response. The relative membrane resistances in the cat actually appear to change in a manner similar to that in gecko (Griff and Steinberg, 1983), reducing basal to apical shunting and increasing apical to basal shunting. Taking this into account would make the delayed basal hyperpolarization appear slightly larger.

If V_{ap} does not contain shunted basal responses, then it should be determined solely by the light-evoked changes in $[K^+]_o$ in the subretinal space. Support for this conclusion is provided in Fig. 2, which compares a $[K^+]_o$ response evoked by 5 min of illumination, previously obtained by Steinberg et al. (1980), with an
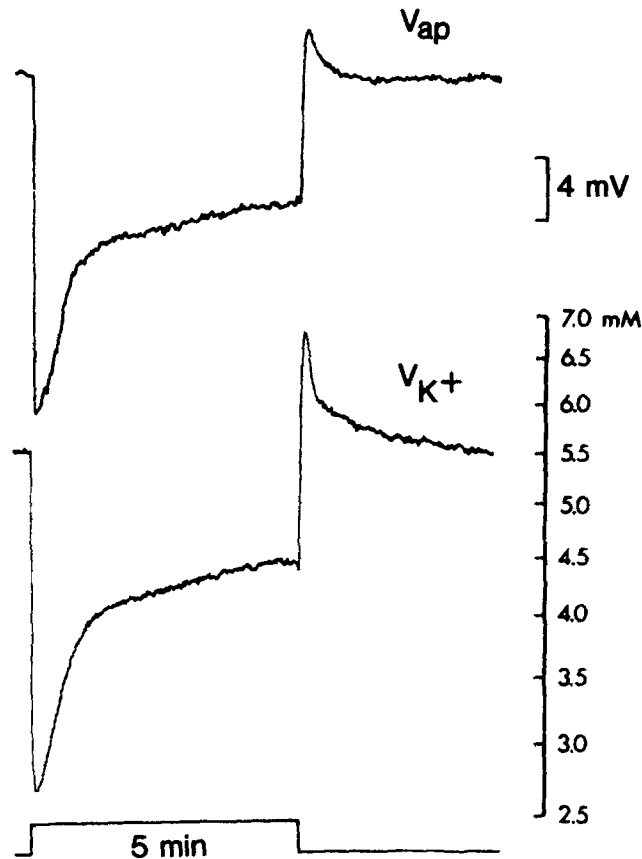

FIGURE 2. Apical membrane potential (V_{ap}) and subretinal space potassium concentration (V_{K^+}) in response to 300 s of illumination. The stimuli were similar, but not identical. V_{ap} was a response to diffuse white light at 9.3 log $q \cdot deg^{-2} \cdot s^{-1}$. (Cat 34.) V_{K^+} was a response to a large spot (75 deg^2) of 440-nm light at 8.1 log $q \cdot deg^{-2} \cdot s^{-1}$, from Steinberg et al. (1980), by permission of The American Physiological Society.

apical membrane response obtained in the current experiments. The match between these responses is remarkable: the apical membrane hyperpolarizes as $[K^+]_o$ decreases, and subsequently repolarizes as $[K^+]_o$ partially recovers toward the dark-adapted level.

In Fig. 1 we observed that the decline of the TEP c-wave from its peak was accompanied by a delayed basal membrane hyperpolarization. Fig. 2 shows clearly that the reaccumulation in $[K^+]_o$ beginning a few seconds after the onset

of light causes a depolarization (repolarization) of the apical membrane that should also produce a decline in TEP from the peak of the c-wave. What are the time courses and relative magnitudes of these two events?

Fig. 3 uses the same format as Fig. 1 to illustrate both membrane potentials over a duration sufficient to show the entire time course of the delayed basal
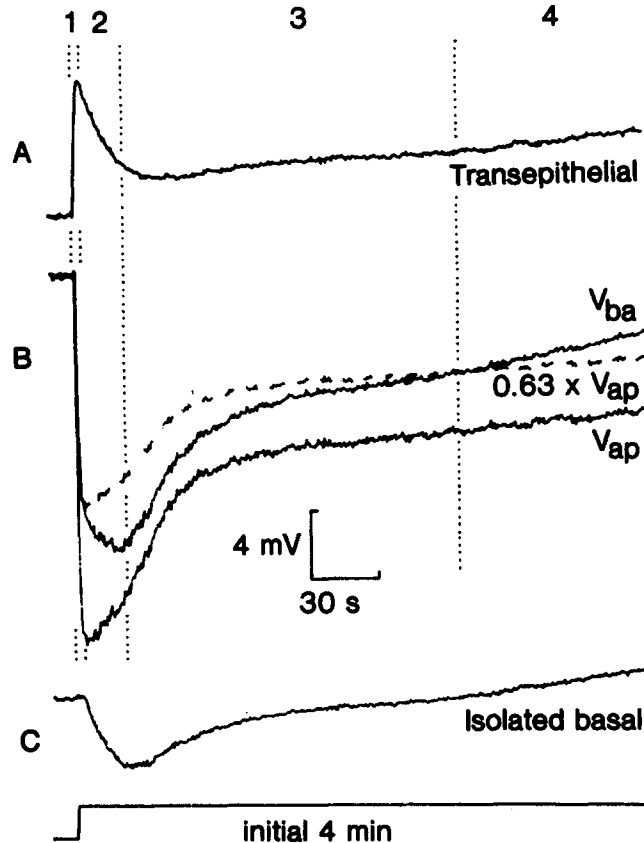
FIGURE 3. Transepithelial and intracellular RPE recordings showing the first 4 min of the response to a 5-min period of illumination with diffuse white light at 9.3 log $q \cdot deg^{-2} \cdot s^{-1}$. (A) Transepithelial recording, as in Fig. 1, but here the negative-going b-wave was lost because of the frequency of digitizing. (B) Apical, basal, and scaled apical membrane potentials of an RPE cell (same cell as Fig. 2). (C) Subtraction of the scaled apical response from the basal response, showing events originating at the basal membrane. This trace has been slightly retouched to remove a small positive-going artifact after the b-wave introduced by the technique used for subtraction. (Cat 34.)

hyperpolarization. These traces are the first 4 min of the response to a 5-min stimulus. Again, V_{ap} could be exactly scaled to V_{ba} over the first 2 s of the response (period 1), which suggests that only an apical hyperpolarization occurred during this time. The decrease in TEP (period 2) was accompanied by both a hyperpo-

larization of the basal membrane and a depolarization of the apical membrane. As in Fig. 1, the subtraction of the scaled V_{ap} from V_{ba} gives an estimate of the delayed basal hyperpolarization (Fig. 3C). Comparing the delayed basal response (Fig. 3C) with the apical response (Fig. 3B), we observe that both the apical and delayed basal hyperpolarizations peak and then return toward baseline, but with very different time courses. The delayed basal hyperpolarization was much slower, beginning approximately at the peak of the apical hyperpolarization, and itself peaking during the recovery phase of the apical membrane response, ~19 s after the onset of light. After its peak (period 3), the delayed basal hyperpolarization ended as the potential slowly returned toward baseline. Between ~80 and 160 s the potential changed very little. In this cell the voltage during this plateau was slightly below the baseline, but in other cells it was at or slightly above baseline. During period 4 the potential increased again as the basal membrane depolarized relative to its baseline, giving rise to the light peak.

The records of Fig. 3 allow us to assess the relative contributions of the delayed basal hyperpolarization and the apical repolarization to the decline of the TEP from its peak. The contribution of the apical event is the observed hyperpolarization at the apical membrane minus the amount of this hyperpolarization seen at the basal membrane due to shunting (i.e., $V_{ap} - 0.63 \times V_{ap}$). This accounts for only ~25% (1.2 mV) of the decline in TEP from the peak of the c-wave to the peak of the delayed basal hyperpolarization (period 2). The contribution of the basal event is given by the isolated basal trace (Fig. 3C). In this example, ~75% (3.7 mV) of the decline in TEP was contributed by the delayed basal hyperpolarization. After the peak of the delayed basal hyperpolarization (beginning of period 3), the TEP continued to decrease slightly. During this time apical and basal events work in opposite directions on the TEP: the apical repolarization decreasing, and the basal repolarization increasing, the TEP.

The relative effects of the apical repolarization and delayed basal hyperpolarization on the two membrane potentials varied. In some cells (Fig. 3) the delayed basal hyperpolarization was large enough that a hyperpolarization was observed at the basal membrane while the apical membrane repolarized. In other cells (Fig. 6) a depolarization was observed at both membranes, and the basal membrane hyperpolarized only relative to the potential that would have been expected on the basis of the shunted apical response. In these cases V_{ba} was dominated by the shunted apical repolarization rather than by the delayed basal hyperpolarization itself. The third type of response, in which both membranes hyperpolarized for 20 s (Fig. 1), was relatively rare. In these cases either the $[K^+]_o$ recovery was very slow, or else there was some basal to apical shunting (see Griff and Steinberg, 1984). All cells shared the property that the scaling of V_{ap} to V_{ba} failed after 2–4 s, and a subtraction always revealed a delayed basal hyperpolarization. The average value of the scaling factor was 0.69 (SD = 0.07; 34 responses in 15 cats), and the shape of the delayed basal hyperpolarization at or above rod saturation did not depend on the scaling factor.

Dependence on Stimulus Illumination

By using the scaling and subtraction analysis, we investigated the dependence of the delayed basal hyperpolarization on stimulus illumination. The basal hyper-

polarization decreased with decreasing illumination and became nearly undetectable at illuminations less than one log unit below rod saturation. This does not mean that it had a sharp threshold, however, since it appeared to be a constant proportion (~15%) of the preceding apical hyperpolarization. This is shown in Fig. 4, where the peak delayed basal hyperpolarization is plotted as a function of the peak apical hyperpolarization. These data can be fit well by a line passing near the origin, which suggests that both responses have a similar
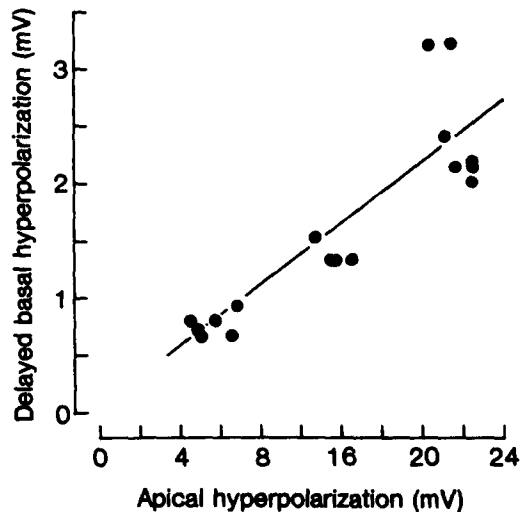

FIGURE 4.   Amplitude of the basal hyperpolarization as a function of the peak apical hyperpolarization for 17 responses in 12 cells. The stimuli used for these responses were 60–600 s in duration and between 7.3 and 8.3 $\log q \cdot \deg^{-2} \cdot s^{-1}$ (i.e., at or below rod saturation). One data point with a very large basal hyperpolarization (4.5 mV at an apical hyperpolarization of 16.7 mV) has been omitted. The regression line has a slope of 0.134 mV/mV and an intercept of 0.0068 mV.

dependence upon illumination, and that an apical hyperpolarization of some criterion amount is not required to initiate the basal event.

The small delayed basal responses at illuminations below rod saturation had slower times to peak (mean 36 s; SD = 9 s; $n = 10$) and rarely recovered during the 60-s periods of illumination used to elicit these responses.

Dependence on Stimulus Duration

Previously, both the increase and decrease in TEP that make up the c-wave have been thought to be apical membrane phenomena: a hyperpolarization followed by a repolarization (e.g., Steinberg et al., 1970). In response to the stimuli described so far, however, a delayed basal hyperpolarization also contributed to the decline in TEP. An important question, then, is whether a delayed basal hyperpolarization is always present, or whether at some stimulus durations the c-wave is in fact solely an apical membrane response. Fig. 5 shows the TEP and basal and scaled apical potentials in response to flashes of 2 and 4 s. For simplicity, the actual V_{ap} has been omitted. As before, the difference between the interrupted

and continuous lines represents the delayed basal hyperpolarization (subtraction not shown). For these short durations, a shunted apical response predicts the basal response reasonably well, although a very small deviation is apparent beginning at ~2 s. Here the decline of the TEP from the peak of the c-wave
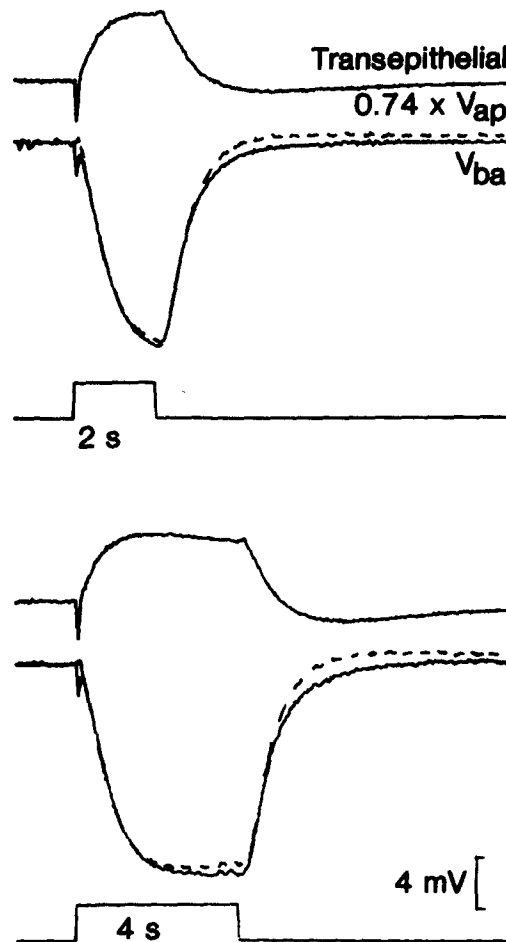

FIGURE 5.   The basal hyperpolarization in response to short flashes. The upper set of records shows transepithelial, basal, and scaled apical traces in response to a 2-s flash, and the lower set shows the same traces in response to a 4-s flash. The difference between the continuous and interrupted lines represents the events generated at the basal membrane. Both sets of responses at 8.3 log $q \cdot deg^{-2} \cdot s^{-1}$ from the same cell. (Cat 67.)

occurred almost entirely after the termination of the stimulus, and resulted largely from the apical membrane repolarization, rather than from a basal event. Thus, the TEP c-wave reflects essentially only apical membrane events for flashes shorter than ~4 s. Fig. 6 presents responses to longer flashes, 4–32 s in duration, from another cell. The delayed basal hyperpolarization began at ~4 s, and grew

in amplitude until stimulus duration reached 16 s. For these longer flashes the TEP declined during illumination, and the delayed basal hyperpolarization accounted for a substantial part of this decrease, bringing the TEP down more rapidly than the apical repolarization alone would have. Figs. 5 and 6, therefore,
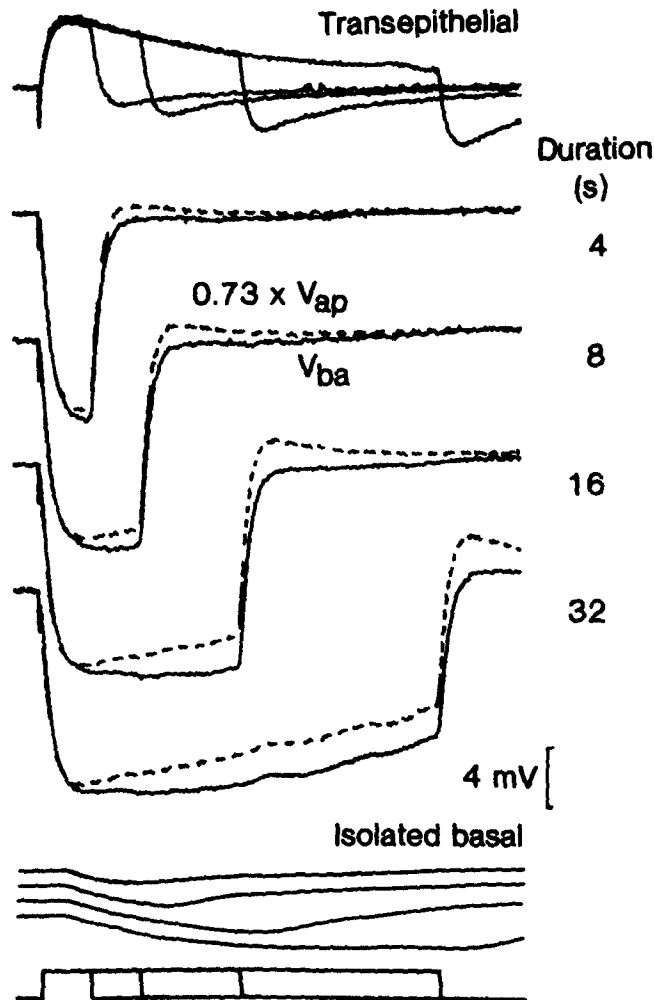

FIGURE 6. The basal hyperpolarization as a function of stimulus duration. At the top are superimposed transepithelial responses for each duration. Below this are the basal and scaled apical responses in response to stimuli of 4, 8, 16, and 32 s at 8.3 log q·deg^{-2}·s^{-1}. Subtractions of scaled apical from basal responses are shown at the bottom. Subtractions have been retraced from the original records to remove artifacts introduced by the subtraction technique. (Cat 71.)

show that the rapid recovery of TEP when illumination was terminated resulted from an apical membrane repolarization, whereas the slower fall of TEP during illumination resulted from both an apical repolarization and a delayed basal hyperpolarization.

Events at Light Offset

The complete sequence of events at light offset is illustrated best by the response to a 60-s stimulus (Fig. 7). Here again, for clarity, only the scaled V_{ap} is shown. The first event at light offset was a repolarization and overshoot of V_{ap}, as expected, since $[K^+]_o$ also overshoots (Steinberg et al., 1980; also see Fig. 2). The basal membrane repolarized more slowly, and a few seconds after the end of the stimulus V_{ba} was depolarized relative to the scaled V_{ap}. Thus, in cat, as in gecko
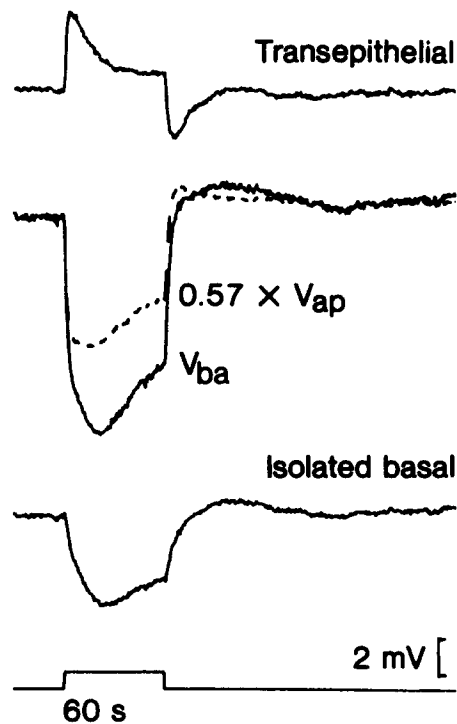

FIGURE 7. Transepithelial, intracellular, and isolated basal responses to a 60-s stimulus at 9.3 log q·deg^{-2}·s^{-1}. (Cat 37.)

(Griff and Steinberg, 1984), a delayed depolarization was generated at the basal membrane. The isolated basal trace in Fig. 7 shows that this delayed basal depolarization at light offset was essentially the mirror image of the delayed basal hyperpolarization at light onset.

At light offset, therefore, both apical and basal events contributed to the TEP. The apical repolarization (depolarization), overshoot, and recovery led to a TEP decrease, undershoot, and recovery. The delayed basal depolarization caused the TEP to recover faster from the undershoot than it otherwise would have, and produced a small positive-going "hump" in the TEP.

With stimuli longer than 60 s, the delayed basal depolarization at light offset was difficult to separate from the light peak, which is also a basal depolarization.

With stimuli shorter than 60 s, the delayed basal depolarization was difficult to detect, although it probably accounts for part of the isolated basal responses in Fig. 6. It is perhaps worth pointing out that the small discrepancies between the V_{ba} and scaled V_{ap} traces in Fig. 5 after light offset cannot be basal off-responses, but are instead small delayed basal on-responses (hyperpolarizations).

DISCUSSION

The Delayed Basal Hyperpolarization

Maintained illumination of the retina produces three separate and consecutive potential changes in the cat and gecko RPE. The earliest of these is the well-known hyperpolarization of the apical membrane in response to a decrease in $[K^+]_o$ in the subretinal space (Steinberg et al., 1970, 1980; Schmidt and Steinberg, 1971; Oakley and Green, 1976). As described here, at about the time when $[K^+]_o$ and the apical membrane potential have begun to recover, a delayed hyperpolarization of the basal membrane begins. The third and slowest change in membrane potential is another basal event, a depolarization that begins during or after the recovery from the preceding basal hyperpolarization (Griff and Steinberg, 1982; Linsenmeier and Steinberg, 1982).

RELATION TO PREVIOUS WORK AND THE C-WAVE There are two reasons why previous studies of the RPE have not revealed a basal hyperpolarization. First, much of this work had been performed on frog, which appears not to have this response (see Griff and Steinberg, 1984, for comparison). Second, in most of the earlier work on cat, relatively short flashes were used (Steinberg et al., 1970; Schmidt and Steinberg, 1971). As shown above, the basal hyperpolarization is small at flash durations less than ~4 s, and becomes maximal only at stimulus durations of 16–30 s. The effect of flash duration bears directly on our understanding of the origin of the RPE component of the c-wave. Previous work in cat with relatively short flashes indicated that the c-wave was generated completely by the apical hyperpolarization and subsequent repolarization in response to changes in $[K^+]_o$ in the subretinal space. When illumination is maintained for more than ~4 s, however, the basal hyperpolarization is responsible for part of the TEP decrease, and therefore for part of the c-wave.

ISOLATION OF THE BASAL MEMBRANE RESPONSE A major difficulty to be considered in describing an RPE membrane potential change is that, in addition to the voltage originating at the membrane being studied, there may be passive iR drops resulting from currents originating elsewhere. Current responsible for such passive voltage changes may originate at the opposite membrane of the RPE and be "shunted" through the paracellular junctions (e.g., Miller and Steinberg, 1977), or it may originate in the neural retina.

In the Results we accounted for the part of V_{ba} arising at the apical membrane, and this allowed us to isolate the delayed basal hyperpolarization. Passive voltages arising in the neural retina contribute less to V_{ba} than those shunted from the apical membrane, but they do reduce our ability to estimate the amplitude of the delayed basal hyperpolarization and should be considered. The most obvious retinal event producing a passive voltage drop across the RPE is the b-wave. It

appears almost entirely in V_{ba}, since basal membrane resistance is higher than apical, and also because our recordings of V_{ba} include passive voltage drops across the sclera. Of more consequence in the present context, however, is the contribution to RPE recordings from "slow PIII," the retinal component of the c-wave (Faber, 1969; Rodieck, 1972). This component is opposite in sign to the b-wave and would cause an inward (hyperpolarizing) current across the apical membrane and an outward (depolarizing) current across the basal membrane. Because of the relative membrane resistances, however, only the basal membrane component would be important.

It is difficult to determine the size of the passive voltage produced by slow PIII because the time courses of slow PIII and the TEP c-wave are so similar that there is no simple way to determine how much of the TEP c-wave is due to this passive voltage. If we use, instead, the b-wave as an index of the magnitude of passive iR drops across the RPE arising from retinal sources, then the maximum effect of slow PIII would be to reduce V_{ba} by ~25%. When we subtracted a potential having this maximum amplitude and the time course of slow PIII from V_{ba} before performing the scaling described in the Results, the major effect was to increase the factor used to scale the apical response (i.e., the potential shunted from apical to basal appeared to be larger). The subtraction of the scaled apical response from the basal response still revealed a delayed basal hyperpolarization that had essentially the same time course as the delayed basal hyperpolarization shown in the figures, but was up to 30% smaller.

Origin of Vitreal Potentials

At this point we can only speculate on the role of the delayed basal hyperpolarization in controlling or reflecting aspects of RPE cell function (see Griff and Steinberg, 1984), but we can use the data presented here to describe its contribution to slow components of the ERG. Fig. 8 shows a DC vitreal recording of a response to a stimulus 300 s in duration. After the c-wave there is a return to baseline, or to below baseline at higher illuminations (Fig. 9; also see Linsenmeier and Steinberg, 1982, Fig. 10), which we call the "fast oscillation," in accord with the nomenclature of Marmor and Lurie (1979). (The term "fast oscillation" is sometimes used in a different sense, as described below.) After this trough, the potential rises to a shoulder, the "second c-wave" (Nikara et al., 1976), and then continues to rise to the light peak. The peak voltage is reached at 5–6 min and then descends, whether or not illumination is maintained. The end of illumination is accompanied by a relatively rapid decrease in potential, an "off c-wave" (Noell, 1953; Faber, 1969; Steinberg et al., 1980), and often by a shoulder with about the same time course as the second c-wave. This recording is very similar to DC recordings made from the human eye (e.g., Täumer et al., 1976).

In general, vitreal potentials originate from algebraic interactions between the TEP and retinal components. This is shown in Fig. 9 with transretinal and TEP recordings that were obtained simultaneously with another vitreal recording. We are interested here only in the events between the c-wave and the light peak, so that only the first 4 min of the response to a 5-min stimulus is shown. The transretinal trace was recorded between a microelectrode in the subretinal space
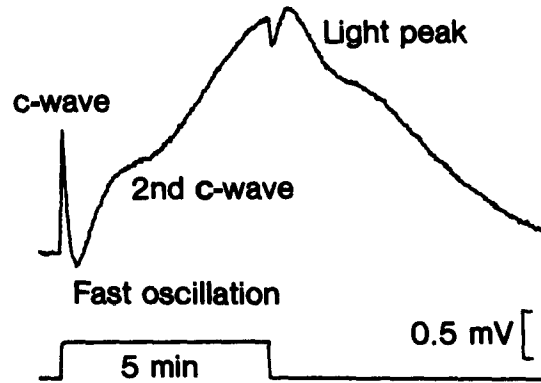
FIGURE 8. A vitreal DC ERG in response to a 300-s period of illumination at 9.3 log $q \cdot deg^{-2} \cdot s^{-1}$, showing components identified in previous studies. (Cat 64.)
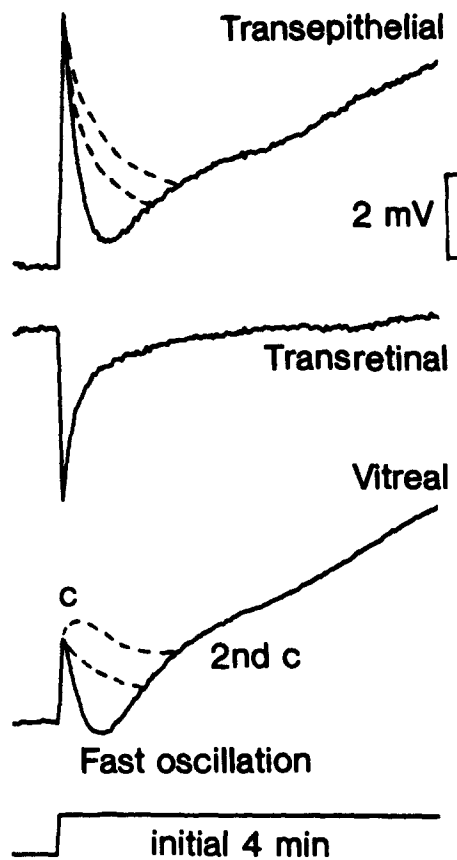

FIGURE 9. Transepithelial, transretinal, and vitreal recordings of the response to 300 s of illumination. The first 240 s of the responses are shown. The solid lines are actual recordings, and the dashed lines were synthesized responses as described in the text. Illumination 10.3 log $q \cdot deg^{-2} \cdot s^{-1}$. (Cat 34.)

and the vitreal electrode, and shows potentials of retinal origin, uncontaminated by RPE potentials (Rodieck and Ford, 1969; Linsenmeier and Steinberg, 1982). This potential has been inverted so that the vitreal electrode is positive. Observe, for example, that the vitreal c-wave amplitude is smaller than the amplitude of either of its components because the negative-going slow PIII component that dominates the transretinal recording adds to the positive-going c-wave in the TEP recording (Fig. 9, solid lines).

ORIGIN OF THE FAST OSCILLATION   The trough in the vitreal recording, the fast oscillation, is also formed from the addition of retinal and RPE potentials. Both the transretinal and TEP components descend toward the baseline after the c-wave peak. Their rates of return differ, however, because the faster-descending TEP is produced by two events, the K^+-dependent apical repolarization and the delayed basal hyperpolarization, whereas slow PIII presumably contains primarily a K^+-dependent component (Karwoski and Proenza, 1977; Steinberg et al., 1980). Since both the RPE and retinal potentials are changing during the vitreal fast oscillation, both must contribute to it. If, however, the delayed basal hyperpolarization did not bring the TEP down more rapidly, then this trough in the vitreal recording would be much smaller or absent.

The importance of the basal hyperpolarization in forming the fast oscillation can be shown by synthesizing a TEP response in which the basal hyperpolarization is absent, and adding it to the measured transretinal potential to obtain a new vitreal response. Two methods were used to synthesize this response. The first method assumed that in the absence of the basal hyperpolarization the time course of the TEP response would be determined solely by the apical membrane response to $[K^+]_o$. It would have the same peak height as the measured TEP response, since the basal hyperpolarization does not appear to begin until after the peak of the c-wave. To obtain this response we inverted and scaled the apical membrane response obtained from the intracellular recording that preceded the set of responses shown in Fig. 9. This is the upper dashed TEP trace. The second method was similar, but assumed that only part of the observed TEP response resulted from the apical membrane K^+ response and that the remainder resulted from the passive iR drop of slow PIII across the RPE (see above). Pickup of slow PIII was assumed to account for at most half of the peak amplitude of the observed TEP response. This is the lower dashed TEP trace. These two methods provide different synthesized TEP responses, because the apical membrane (or K^+) and slow PIII responses have slightly different time courses. The true TEP response in the absence of the basal hyperpolarization is probably between these extreme cases. The vitreal responses derived from the synthesized TEP responses are shown as dashed lines in Fig. 9. Observe that most of the decrease in the vitreal response after the c-wave is abolished by omitting the basal hyperpolarization. This is true even though the K^+-dependent part of the decrease in the TEP and slow PIII responses is still present. This analysis argues, then, that most of the fast oscillation in cat results from the delayed basal hyperpolarization. The similarity between cat and human recordings suggests that the same interpretation holds for the human response, which implies that a basal hyperpolarization must also be present in the human RPE.

ORIGIN OF THE SECOND C-WAVE   Clearly the events responsible for the second c-wave can only arise from the RPE, since the retinal potential has returned nearly to baseline at this time. There are two possibilities for the origin of the second c-wave. It could arise from an additional, discrete potential change in the RPE that we have not yet been able to isolate, or it could result from the interaction of previously defined events. The latter hypothesis appears to adequately account for the data. In particular, three events seem to create the second c-wave: (a) the end of the recovery from the delayed basal hyperpolarization; (b) the initiation of the basal depolarization (light peak); and (c) the maintained K^+-dependent hyperpolarization of the apical membrane. The basal events are shown in Fig. 3, and, as pointed out earlier, there is an interval between the end of the basal hyperpolarization and the onset of the light peak when very little potential change is generated at the basal membrane. This steady potential corresponds in time to the plateau seen in the vitreous. The vitreal potential is above the baseline throughout this time because the third event, the maintained apical hyperpolarization, keeps the TEP above the dark-adapted level. Thus, the shape of the second c-wave is determined by basal events, but the amplitude of the response at this time is due to the maintained apical hyperpolarization. If the recovery from the basal hyperpolarization and the beginning of the light peak were contiguous or overlapped in time, then one would not expect to see a second c-wave. This is exactly the situation in the gecko, which has a basal hyperpolarization and light peak, but no demonstrable interval between them, and no second c-wave in trans-tissue (the equivalent of vitreal) recordings (see Fig. 3 of Griff and Steinberg, 1982).

By using completely different methods, Täumer and co-workers (Hennig et al., 1974; Täumer et al., 1976) also concluded that the fast oscillation is a separable, distinct entity, whereas the second c-wave is not. On the basis of responses to sinusoidally modulated diffuse light, they separated the human DC ERG into three components: an "on-peak" (c-wave) component, a main oscillation (light peak) component, and a fast oscillation component. A difference between their interpretation and ours is that they felt that the fast oscillation in response to a step of light actually consists of more than one oscillation. The simplest interpretation of our recordings, however, is that the fast oscillation in response to long (5 min) stimuli is a single decrease and recovery of potential.

VITREAL RESPONSES TO BRIEFER STIMULI   We have been calling the trough between the c-wave and light peak the fast oscillation, but, as originally defined in human and animal recordings, the fast oscillation referred not just to this trough, but to the series of oscillations of potential produced by a series of stimuli (Kolder and Brecher, 1966; Kolder and North, 1966). The best stimulus was found to be ~1-min periods of light separated by 1 min of darkness, and each stimulus period evoked one cycle of the fast oscillation. The response to shorter periods of illumination is more complex, since both on- and off-responses are present. It is important, nevertheless, to analyze responses to 1-min stimuli, since this approximates the technique used for recording the fast oscillation in humans (Rohde et al., 1981).

Fig. 10 shows that the vitreal response to a 1-min stimulus is qualitatively

different from the response to a 5-min stimulus. There is a trough following the c-wave, but then, instead of a plateau, there is a second peak and decline of potential. The term "second c-wave" has been used for this peak, as well as for the plateau in Figs. 8 and 9. As we have seen, however, the second c-wave in response to long stimuli probably does not have a discrete cellular origin, so this
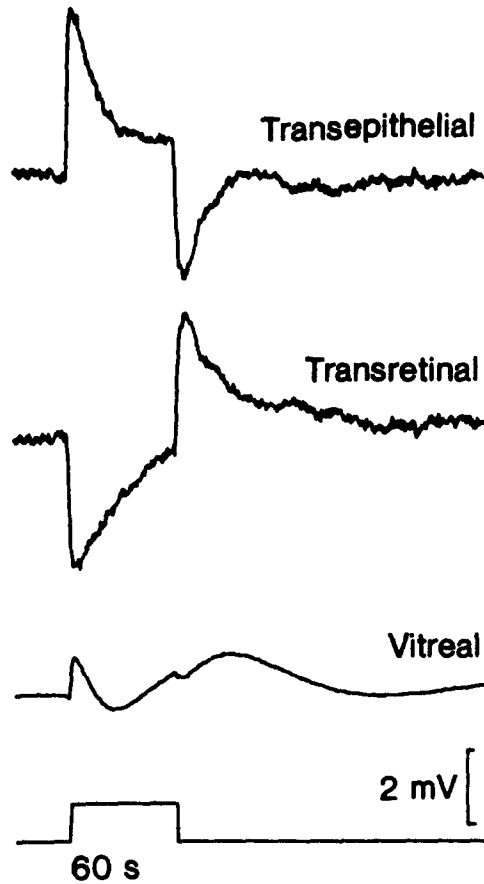

FIGURE 10.   Transepithelial, transretinal, and vitreal recordings of the response to 60 s illumination. This is the same transepithelial response as shown in Fig. 7.

term is not used here. We will call the second peak simply the "off-peak," although we do not propose that this be the generally accepted designation.

The events that lead to the off-peak can be deduced from the TEP and transretinal traces in Fig. 10, and these can be related to intracellular events. Immediately after the end of illumination the TEP declines and undershoots, and the transretinal potential increases and overshoots. These events have nearly the same magnitude, so only a small off c-wave is observed in the vitreal recording. Both components then return to baseline, but with different time

courses, since the TEP response contains a delayed basal depolarization (Fig. 7). The delayed basal depolarization dominates the vitreal response, since the apical events contributing to the TEP recovery and the recovery of the transretinal potential nearly cancel. Thus, a 60-s stimulus evokes the delayed basal hyperpolarization at light onset, producing a trough, and then the delayed basal depolarization at light offset, producing the off-peak. These appear at the vitreal level as one rather simple, nearly smooth wave, rather than the separate responses they actually are. Both basal events, therefore, contribute to each cycle of the fast oscillation evoked with relatively brief stimuli. The delayed depolarization is, of course, also present following longer stimuli, and accounts for the positivity after the off c-wave in Fig. 8. It seems that stimuli ∼1 min in duration maximize the fast oscillation not because they match the resonant frequency of a single event, but because they allow both on- and off-events at the basal membrane to develop in a way that leads to a deceptively simple vitreal or corneal response.

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