Transient Adaptation and Sensitization in the Retina of *Necturus*

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ABSTRACT Responses to repetitive stimulation were monitored at several retinal levels in the eyecup of the mudpuppy *Necturus maculosus*. When alternating sequences of low-intensity small and large spots were presented, two effects were found, which could be localized to the proximal retina: (*a*) response decrement (RD), in which, after the first small spot response, subsequent small spot responses are decreased in amplitude and (*b*) transient response enhancement (TRE), in which the first small spot response after a large spot sequence is larger than preceding or subsequent small spot responses. RD and TRE are absent or weak in sustained on or off responses (horizontal and bipolar cells, and ON and OFF ganglion cell post-stimulus time histograms (PSTH) but are particularly well developed in the on/off responses of the proximal retina (proximal negative response, *M*-wave, PSTHs of ON/OFF ganglion cells, and intracellular responses from on/off neurons and Müller cells). RD and TRE appear to arise from a stimulus-evoked slow depolarization in on/off neurons that interacts with the amplitude of succeeding responses. We conclude that RD and TRE are a form of neural adaptation that is largely specific to the on/off channels of the proximal retina.

INTRODUCTION

The sensitivity of the visual system is largely established within the retina and depends strongly on spatial and temporal variables associated with background and test illlumination, as well as on the absolute level of background illumination. The visual effects of these variables are thought to be mediated partly by both photochemical and neural mechanisms within receptor cells and partly by neural interactions occurring in the postreceptoral retinal network (see, for example, reviews by Dowling [1978] and Enroth-Cugell [1978]). The various effects mediated proximal to photoreceptor cells have been referred to by several names (e.g., "network adaptation," Green et al. [1975]; "contrast gain control," Shapley and Victor [1978 and 1979]; "neural" or "field" adaptation, Dowling [1978]; "adaptation pool," Rushton [1965]), and it is known that background levels of illumination (e.g., Green et al. [1975]; Dowling and Ripps [1977]), background diameter (e.g., Westheimer [1965]), and temporal variables (e.g., Teller et al. [1971]; Enoch [1978]) can exert adaptive and/or sensitizing effects.

The effects of background illumination on increment thresholds are well

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known and need not be reiterated here (see Dowling [1978]). Such effects, as well as the influence of background diameter, have generally been studied in the steady state; that is steady background fields of various diameters are studied with regard to their effects on the threshold of small, centrally placed test stimuli. Temporal variables are also of some importance, but they, and particularly spatiotemporal interactions, have not been explored as extensively. In many sensory systems, it is known that when a stimulus is repeatedly presented, the first response to the stimulus is found to be greater than subsequent responses. In the visual system, this response decrement phenomenon was first described in some of the earliest recordings of the electroretinogram (ERG; Piper, 1911; Creed and Granit, 1933) and subsequently in the spike discharges from ganglion cells (Enroth, 1952) and in the response of Müller cells (Miller, 1973). Moreover, in monkey retina, Brown and Watanabe (1965) found that repetitive stimulation decreased the *b*-wave of the ERG to a greater extent than the isolated *a*-wave, prompting them to suggest that a neural stage of adaptation lies between the receptors and the *b*-wave generators.

On the other hand, with regard to repetitive stimulation in which large and small spot sequences are alternated, it is found that the first small spot response after the large spot sequence is larger than the small spot responses that preceded the large spot sequence (Kaneko and Hashimoto, 1969; Proenza and Burkhardt, 1973; Karwoski and Proenza, 1977). This transient response enhancement is notable because it cannot have a photopigment origin because the same amount of light strikes the photoreceptors under the test spot when the large or small spots are presented. Thus, the enhanced response that follows the large spot sequence and the subsequent response decrement to repeated small spot stimuli must represent a form of neural adaptation.

This paper is an attempt to further specify the mechanisms of response decrement (RD) and transient response enhancement (TRE) by monitoring responses to repetitive stimulation at several retinal levels. We conclude that with stimulation not too far above threshold progressive contributions to RD and TRE are made at various retinal loci, but that the major source of these phenomena lies in on/off neurons of the proximal retina, in which a slowly decaying depolarization evoked by the first stimulus interacts with the amplitude of succeeding responses.

METHODS

The preparation, optical system, electrodes, and general experimental procedure have been described in detail elsewhere (Karwoski and Proenza, 1978). Briefly, glass micropipettes and conventional electronic amplification were used to record intracellular responses and extracellular field potentials in eyecups of the mudpuppy *Necturus maculosus*, to which a drop of Ringer's solution was added to retard drying. Extracellular spike activity was recorded with Insl-X-coated (Insl-X Co., Ossining, N. Y.) tungsten microelectrodes, whose impedance at 1,000 Hz was 5–20 MΩ. A diffuse background of white light at 0.19 lm/m^2 was always present, and stimulating flashes were superimposed on this.

Procedure

The electrode was lowered into the retina while the position of a 0.25-mm stimulating spot was carefully adjusted until a response of maximum amplitude was obtained. Then, the stimulus sequence illustrated in Fig. 1 was presented. This sequence was essentially a square-wave flicker; both flash duration and interstimulus interval were 1 s. As shown in Fig. 1, the diameter of the first stimulus (A) and the next three stimuli (B) was 0.25 mm, which we refer to as small spot stimulation. The next four stimuli (C) covered the whole eye and are referred to as full field, or large spot, stimulation. The diameter of the next stimulus (D) and the last three stimuli (E) was again 0.25 mm.

The following terminology is used when responses to this stimulus sequence are described. If the response to the first small (0.25-mm) stimulus is greater than the subsequent responses to this stimulus (i.e., if $A > B$), then the response is said to show "response decrement" (RD). If the response to the large spot stimulation is less than that to the small spot (i.e., if $B > C$), then the response shows "surround antagonism." Finally, if the response to the first small spot after the large spot series is larger than

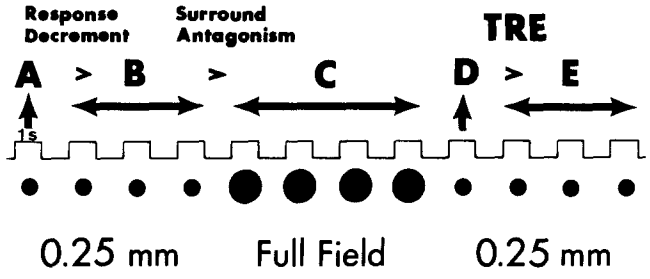

FIGURE 1. The standard stimulus sequence.

subsequent responses to the small spot (i.e., if $D > E$), then the response is said to exhibit "transient response enhancement" (TRE). Note that TRE is specified in relation to a "second" response decrement (i.e., if $D > E$, then TRE); however, if $B = E$ (as found in this study), then $D > E$ is equivalent to saying $D > B$, the relationship that prompts the term TRE. For simplicity, this paper addresses RD ($A > B$) and TRE ($D > E$).

Data Display

Responses to the full stimulus sequence were drawn by a polygraph (Grass Instrument Co., Quincy, Mass.) (bandpass: DC, 75 Hz) on curvilinear paper. Single responses from the sequence were amplified and drawn by an X-Y plotter driven by a Nicolet 1072 Computer (Nicolet Instrument Corp., Madison, Wis.) For spike activity, post-stimulus time histograms (PSTH) were constructed as previously described (Karwoski, 1978). In short, spike-triggered pulses were smoothed through an RC network (decay time, 20 ms) and then summed by computer and plotted by the X-Y plotter. PSTHs were formed with from 8 to 16 repetitions of the stimulus sequence.

RESULTS

Proximal Field Potentials

In the top part of Fig. 2, the standard stimulus sequence is reproduced, and field potential responses recorded in the proximal retina are displayed above it. To the small (0.25-mm) stimulus, the initial, negative-going, transient responses, best developed at light onset, are the proximal negative response (PNR) (Burkhardt, 1970; Proenza and Burkhardt, 1973), and the slower, negative-going components are the *M*-wave (Karwoski and Proenza, 1977). The specific relationships between responses (i.e., $A > B =$ RD; $D > E =$ TRE) can be more readily perceived in the bottom half of Fig. 2, where responses *A*, *B*, *D*, and *E* from a different retina are amplified. Because the PNR and *M*-wave both are larger to the first small spot (*A*) than to subsequent

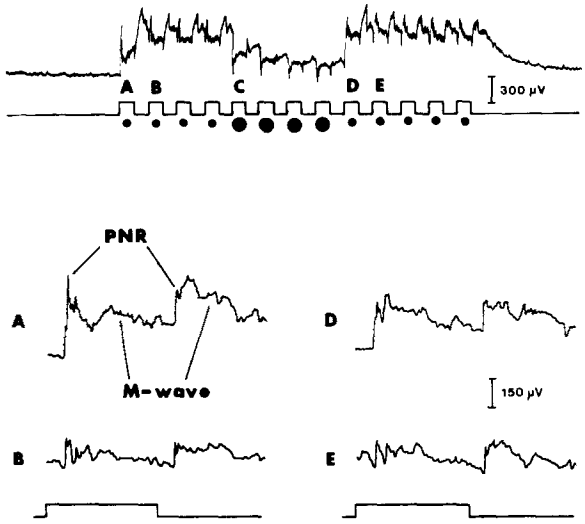

FIGURE 2. Field potentials (predominantly the PNR and *M* wave) recorded in the proximal retina. (*Upper trace*) Response to the standard stimulus sequence. (*Lower four traces*) Responses from a different retina to single light flashes within a standard stimulus sequence. Negative is up in all figures displaying field potentials.

ones (*B*), these responses show RD. Likewise, because the responses to the first small spot (*D*) after the larger spot series are larger than subsequent responses (*E*), the PNR and *M*-wave also show TRE. In response to the large spot (*C*), the PNR and *M*-wave show their characteristic complexity (Burkhardt, 1970; Proenza and Burkhardt, 1973; Karwoski and Proenza, 1977), but these features will not be further considered here.

TRE has been reported from experiments in which related stimulus paradigms were used (Kaneko and Hashimoto, 1969; Proenza and Burkhardt, 1973; Karwoski and Proenza, 1977). This phenomenon is important because it cannot have a photopigment origin inasmuch as the same amount of light struck the receptors illuminated by the 0.25-mm spot when either the large or the small spots were presented (i.e., the intensity and rate of flash presentation

were the same throughout; only the diameter was changed). Thus, the decrease in amplitude of responses after the transiently enhanced response indicates a form of neural adaptation.

Intracellular Neuron Responses

After these basic observations of RD and TRE in proximal field potentials, and in an attempt to examine their underlying mechanisms, a survey of the intracellular response properties of retinal cell types was undertaken.

DEPOLARIZING ON/OFF NEURONS 24 recordings were obtained from neurons that produced depolarizing on/off responses. Because it is difficult to specify with functional criteria whether these responses arise from amacrine or ganglion cells, we conservatively refer to these responses as arising from on/off neurons (see Karwoski and Proenza [1980]).

Responses from 15 on/off neurons were tested with the standard stimulus sequence, and two of these are shown in Fig. 3. These responses show RD, surround antagonism, and TRE. In both cells, RD appears to occur, at least

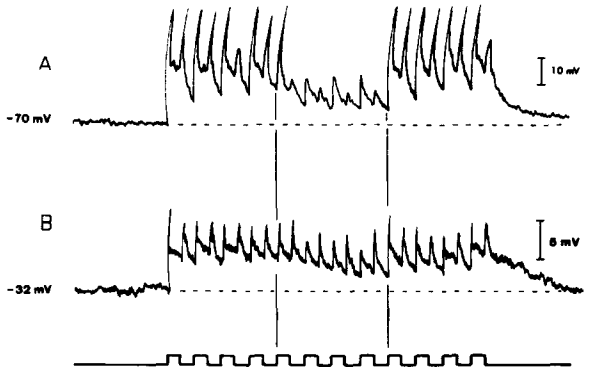

FIGURE 3. Intracellular responses of two on/off neurons to the standard stimulus sequence. Resting membrane potentials are shown to the left of each trace. Positive is up in all figures displaying intracellular responses.

in part, because the first small spot response evokes a slowly decaying depolarizing component that subtracts from the amplitude of the second small spot response. However, in the cell of Fig. 3 *B*, as in about half of the others, a second factor contributing to RD is that peak amplitude actually decreases.

Fig. 3 also shows that, during large spot stimulation, response amplitude decreases, and the mean potential shifts toward the resting level. Then, during the second series of small spots, the response shows TRE. Hence, when the membrane potential has been shifted toward the resting level, whether by lack of stimulation or by large spot stimulation, the following small spot response will be relatively large. Subsequent small spot responses will have decreased amplitude, since repeated small spot stimulation shifts the membrane potential away from the prevailing level. Thus, RD and TRE are seen to have a similar underlying mechanism.

HYPERPOLARIZING ON/OFF RESPONSES A large number of neurons that

generated hyperpolarizing on/off responses were also recorded. These responses usually showed RD, TRE, and, as previously demonstrated (Karwoski and Proenza, 1977), surround antagonism. Hyperpolarizing on/off responses are thought to be enhanced IPSPs arising from damaged, chronically depolarized ganglion cells and generated in large part by input from depolarizing on/off amacrine cells (Miller and Dacheux, 1976; Werblin, 1977; Wunk and Werblin, 1979). The present results are consistent with this model because depolarizing on/off responses, at least some of which likely arise from amacrine cells (see above), also exhibit RD, TRE, and surround antagonism.

HORIZONTAL CELLS Responses of 2 of the 18 horizontal cells recorded are shown in the top half of Fig. 4. As in all responses assigned to horizontal

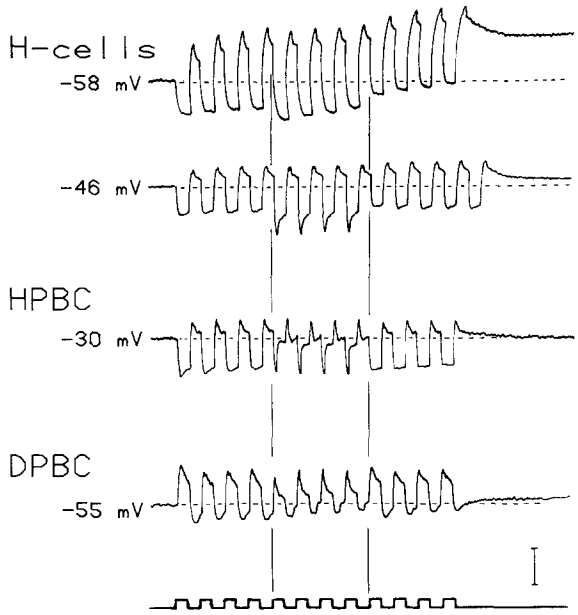

FIGURE 4. Intracellular responses of two horizontal cells (*H cells*), one hyperpolarizing bipolar cell (*HPBC*), and one depolarizing bipolar cell (*DPBC*) to the standard stimulus sequence. Calibration in lower right corner: for H cells, 10 mV; for HPBC, 16 mV; for DPBC, 8 mV. Resting potentials are shown to the left of each trace; the H cells are hyperpolarized several millivolts by the maintained background illumination.

cells, the hyperpolarizing response to large spot stimulation is greater than the response to the 0.25-mm spot. To the specific stimulus paradigm used in these experiments, horizontal cell responses did not show appreciable RD or TRE. On the contrary, as either small spot series proceeds, response amplitude can be seen to increase. This occurs in part because, at stimulus offset, a slowly decaying depolarizing component arises in all horizontal cells, and this component adds to the amplitude of the next hyperpolarizing response. The topmost cell in Fig. 4 showed one of the largest depolarizing shifts. The resting potential of this cell was stable for over 1 h, and it regularly took more than

5 min for the potential to return to resting level at the end of the standard stimulus sequence. Because receptors are the major input to horizontal cells, these data strongly suggest that both RD and TRE do not arise from mechanisms at the level of the photoreceptors. Rather, these effects must arise from neural interactions proximal to the horizontal cells.

BIPOLAR CELLS Responses from bipolar cells were identified by the criteria of Werblin and Dowling (1969). Four were obtained from hyperpolarizing bipolar cells (HPBC) and five from depolarizing bipolar cells (DPBC). Responses from both of these cell types are shown in the lower half of Fig. 4. The magnitude of RD and TRE in bipolar cells depends somewhat on how the responses are measured: if the difference in potential from the onset of each stimulus flash to the peak of the on response is considered, RD and TRE are nonexistent; however, if the difference between resting membrane potential and peak of the on response is measured, RD and TRE are present, although weak (see also Table II). It should be emphasized, however, that regardless of the measurement strategy employed, the bipolar cell data suggest that the strong RD and TRE seen in the PNR and on/off neurons arises predominantly from interactions in the inner plexiform layer.

DEPOLARIZING CELLS Seven depolarizing responses were obtained which, unlike those typical of DPBCs, generally had higher frequency components (with occasional spikes; Fig. 5 *A*) and sometimes small, depolarizing off responses (arrows in Fig. 5 *B*; see also Fig. 5 in Saito et al. [1979]). Because, during electrode penetrations, three of these were recorded immediately before horizontal cells, it is unlikely that many of these arise from ON ganglion cells with an injured spiking mechanism. However, their identification is uncertain, and, following the lead of Dacheux et al. (1979), we will refer to these cells of the inner nuclear layer as depolarizing cells (DPC). Responses of two DPCs to the stimulus sequence are shown in Fig. 5. These, as did all DPCs, show weak RD, surround antagonism, and weak TRE.

If it is assumed that these responses are recorded from cells postsynaptic to bipolar cells, then these results suggest that the strong RD and TRE seen in the PNR and on/off neurons depends on the response properties of on/off neurons, and does not depend on the dynamics of transmitter release by bipolar cells nor on the activity of sustained neurons in the proximal retina. This suggestion is further supported by the following data obtained with ganglion cell spike discharges.

Spike Responses

RD and TRE were studied in ganglion cells primarily via recordings of extracellular spike discharges, because these provide more stable recordings than can usually be obtained intracellularly from ganglion cells. The spike responses were classified by the criteria of Karwoski and Burkhardt (1976), and PSTHs to the standard stimulus sequence were developed. Because on/off neurons likely provide relatively weak input to ON and OFF ganglion cells (Miller and Dacheux, 1976; Naka, 1977), it was expected that RD and TRE, if present in these cells, might be weaker than in ON/OFF cells.

ON CELLS Representative PSTHs from two ON cells are shown in the top two traces of Fig. 6. All six ON cells tested showed relatively weak RD, surround antagonism, and weak TRE. Two other aspects of ON-cell responses merit comment here: (*a*) The two topmost response sequences shown in Fig. 6 are not necessarily from "transient" and "sustained" ON-cell types, respectively, since, when stimulus intensity was increased by 1 log unit, the upper (relatively transient) response became quite sustained. (*b*) Some ON-cell PSTHs revealed the presence of a weak, but definite, off response, usually consisting of one to two spikes; this will be discussed in greater detail elsewhere.[1]

OFF CELLS OFF-cell PSTHs are shown in the lowest two records of Fig. 6. Eight Off cells were tested and these usually showed weak RD, surround

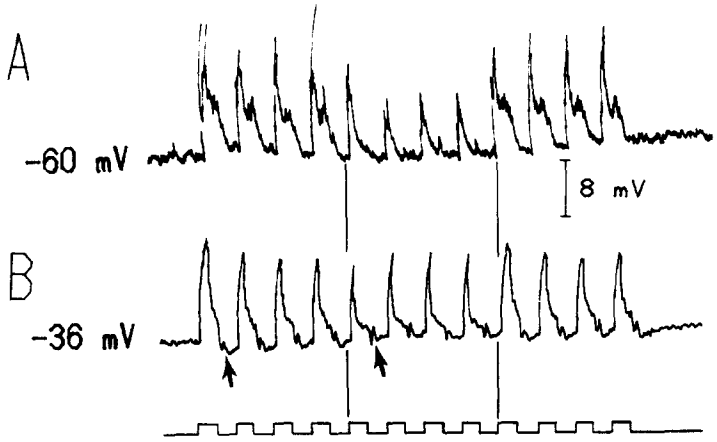

FIGURE 5. Intracellular responses of depolarizing cells to the standard stimulus sequence.

antagonism (though relatively weak with this paradigm), and weak TRE. Occasionally, some OFF cells showed some long latency spikes at light onset; two groups of these are pointed out by arrows in one of the OFF cells in Fig. 6.

ON/OFF CELLS The middle two PSTHs in Fig. 6 are from ON/OFF cells. The on response of the upper cell, and both the on and the off response of the lower cell, illustrate a frequently encountered extreme form of RD and TRE, namely, spikes appeared only to the first one or two stimuli in both small spot series. RD and TRE were also present, but weak, in the off response of the upper cell. The on and off responses of the two ON/OFF cells in Fig. 6 are representative of ON/OFF cells in that these responses typically showed either moderate RD and TRE, or the extreme form; few cells showed responses of intermediate magnitude. This wide variance in the magnitudes of RD and TRE may be the result of nothing more than natural variation in the responses of ON/OFF cells, but it may also indicate subtypes of on/off neurons, possibly

[1] Manuscript in preparation.

amacrine and ganglion cells. RD has previously been described in frog ON/OFF-cell spike responses (Morrison, 1979).

Quantitative comparisons of the magnitudes of RD and TRE in spike responses were made by computing a ratio: mean PSTH peak amplitude of the last three responses in a small spot series divided by the amplitude of the first response. For example, a cell that showed no RD would have a ratio of 1.00, since all responses would have the same amplitude. A cell in which subsequent responses were not present (e.g., the on and off responses of the lower ON/OFF cell of Fig. 6) would have a ratio of 0.0.

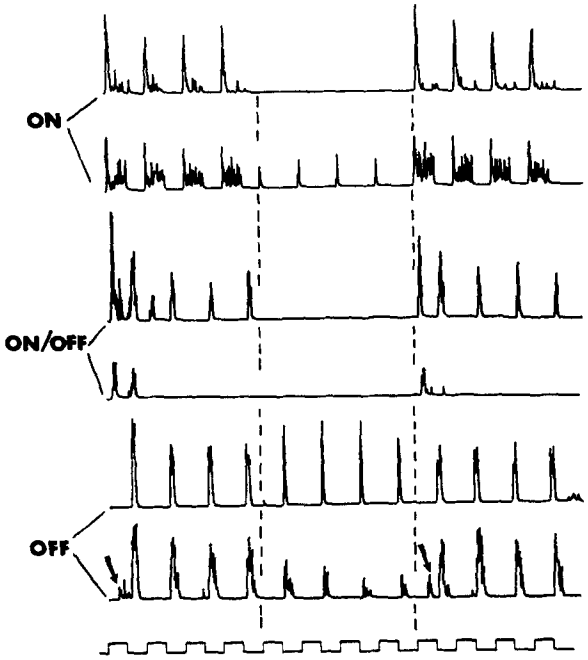

FIGURE 6. PSTHs developed from extracellularly recorded spike activity. (*Top two traces*) ON cells. (*Middle two traces*) ON/OFF cells. (*Bottom two traces*) OFF cells.

Table I shows the results of this analysis. Because the assumption of normal distributions did not seem warranted, especially for the responses of ON/OFF cells, the statistical significance of these mean differences was assessed with a nonparametric test, the Kruskall-Wallis "analysis of variance" (Noether [1976], p. 175). For RD, $H = 15.2$ ($P < 0.003$); for TRE, $H = 13.4$ ($P < 0.005$). A multiple comparisons test (Noether [1976], p. 179) showed that RD and TRE were stronger in the on responses of ON/OFF cells than in ON cells and stronger in the off response of ON/OFF cells than in OFF cells. All four of these comparisons were significant at the 0.05 level, except for the RD of the off responses ($P < 0.10$). (The main effects were on responses of ON/OFF cells vs. off responses of OFF cells, $P < 0.01$.) Thus, because both ON and OFF cells show weak RD and TRE, as compared with the on and off responses of ON/OFF cells, the suggestion that these phenomena are primarily organized within the on/off channel is supported.

In the PNR (Fig. 2) and in intracellular reponses from on/off neurons (Fig. 3), RD and TRE reliably are better developed in the on response than in the off response. The strength of these effects were also compared in the PSTHs of on and off responses of extracellularly recorded ON/OFF cells by means of the Wilcoxon signed rank test for paired comparisons (Noether [1976], p. 139). For RD, the on response showed a stronger effect than the off response ($P < 0.01$), but the difference for TRE was not statistically significant ($P < 0.10$).

One possible explanation for the increased RD and TRE in ON/OFF-cell spikes is that these cells were subjected to twice as many excitatory stimulus events per unit of time (stimulus onset and offset), and their response mechanism simply cannot resolve this higher temporal detail. This possibility was explored by testing two ON cells with the normal (1 s on and 1 s off) square-wave stimulus sequence and then retesting these with a special (0.5 s on and 0.5 s off) square-wave sequence. The latter sequence yielded twice as many responses per unit of time, but the RD and TRE ratios were affected <10%. Thus, RD and TRE seem to be the result of qualitatively different mechanisms in these two cell types.

TABLE I

SUMMARY TABLE OF RD AND TRE IN THE PSTHs OF on AND off RESPONSES OF ON/OFF CELLS AND OF ON CELLS AND OFF CELLS

	ON/OFF cells			
	On response	Off response	ON cells	OFF cells
Response decrement				
Number of cells	16	13	6	8
Ratio range	0–0.70	0–0.87	0.52–0.85	0.38–1.60
Mean ratio ± SD	0.25±0.26	0.41±0.36	0.74±0.09	0.86±0.34
Transient response enhancement				
Number of cells	15	13	6	8
Ratio range	0–1.00	0–1.00	0.49–1.00	0.36–1.30
Mean ratio ± SD	0.30±0.31	0.37±0.38	0.82±0.19	0.90±0.30

Müller Cells

The responses of 18 Müller cells, identified by the criteria of Miller and Dowling (1970) and Karwoski and Proenza (1977 and 1980) were also tested. Responses of one Müller cell to the standard stimulus sequence are shown in Fig. 7 *A*. Like all Müller cells tested this one shows strong RD, surround antagonism, and TRE. It is possible that RD and TRE could arise because the K^+ that depolarizes Müller cells may be liberated by neurons which themselves show RD and TRE, and, therefore, their K^+ release (and subsequent Müller cell depolarization) would show RD and TRE.

That this is likely the case is suggested by a model that we have recently presented (Karwoski and Proenza, 1980) for the relationship between neural and glial responses. This model is based on the following assumptions: (*a*) K^+ release is positively related to the instantaneous amplitude of the on/off

neuron response, and this K^+ accumulates (is integrated) in extracellular space. (*b*) Clearance mechanisms (with approximately exponential time-courses) act on K^+ accumulating in extracellular space so that steady-state conditions are restored. (*c*) Müller-cell responses are proportional to changes

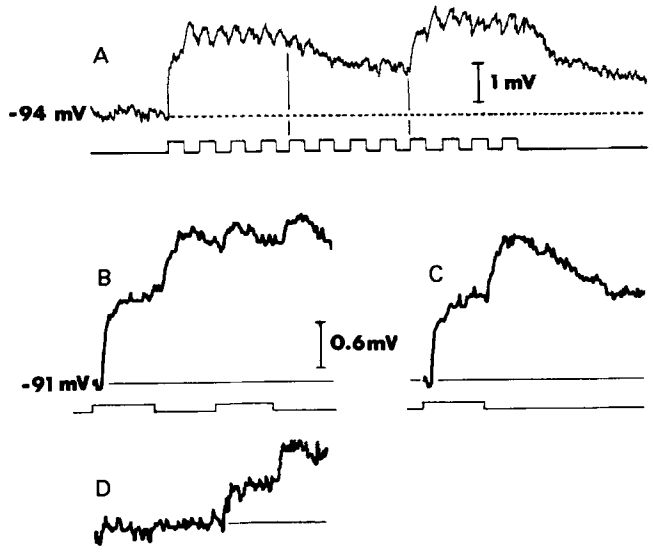

FIGURE 7. Intracellular Müller cell responses. (*A*) Response to the standard stimulus sequence. (*B*) Response to two 0.25-mm flashes (the first two stimuli of the standard sequence). (*C*) Response to one 0.25-mm flash. (*D*) Trace *C* subtracted from trace *B*.

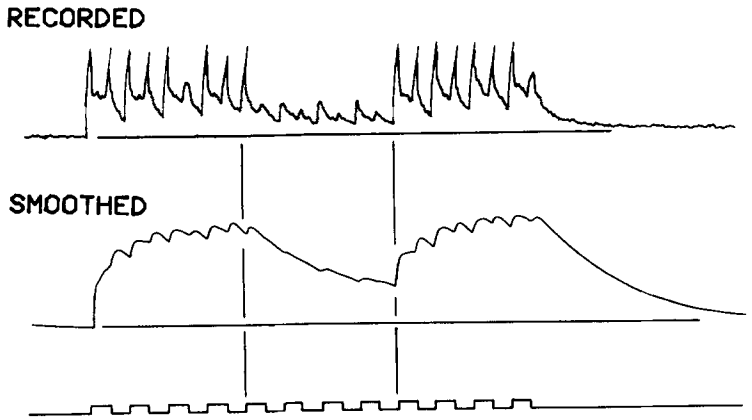

FIGURE 8. (*Top*) Intracellular response from on/off neuron to the standard stimulus sequence. (*Bottom*) Same response but after having been passed through a low-pass filter.

in $[K^+]_o$. These transformations were approximated by playing the neural responses through an appropriate low-pass filter (i.e., "smoothing" the response; Karwoski and Proenza, 1980). Fig. 8 (*bottom*) shows a similarly "smoothed" response of a depolarizing on/off neuron to the standard stimulus

sequence; Fig. 8 (*top*) shows the unfiltered (i.e., recorded) response, which is the same as that shown in Fig. 3 *A*. The smoothed response has a number of similarities to Müller cell responses (see Fig. 7 *A*), including (*a*) the general shape of the waveforms; (*b*) the sustained depolarizing shift during the smaller spot flicker, with a trend back toward resting potential during the large spot flicker; and, importantly, (*c*) the magnitudes of the RD and TRE. These results are thus consistent with the model presented above and with previous results in which Müller-cell and K^+ responses were in agreement with those modeled from measured on/off-neuron responses (Karwoski and Proenza, 1980).

The RD and TRE apparent in Müller-cell responses and light-evoked K^+ increase in the proximal retina (Karwoski and Proenza, 1977 and 1978) may also have any of the following alternative explanations, for all of which it can be assumed that the magnitude of K^+ release is actually invariant for each small spot presentation:

(*a*) The decay of the response to the first small spot could interact subtractively with the rising portion of the second response, leading to a response of depressed amplitude. To assess the magnitude of this effect, Müller-cell responses were obtained to two cycles (Fig. 7 *B*) and one cycle (Fig. 7 *C*) of the small spot flicker, and then the latter sequence was subtracted from the former, leaving the second response uninfluenced by the decay of the first response (Fig. 7 *D*). In Fig. 7 *B*, the first on response (ON-1) is about 1.1 mV and the second on response (ON-2) is 0.3 mV. In Fig. 7 *D*, the second on response after subtraction (ON-2S) is 0.5 mV. The raw RD (ON-1 minus ON-2) is 0.8 mV, and when corrected (ON-1 minus ON-2S) is 0.6 mV. Thus, 25% (1.0 − 0.6/0.8) of the RD is attributable to the decay of the first response subtractively interacting with the amplitude of the second on response. Calculations of the magnitude of this interaction were performed on five Müller-cell responses, resulting in means for the RD of 19%, and of 43% for TRE.

(*b*) If Müller cells behave as K^+ electrodes whose potential is related to log $[K^+]_o$ (but cf. Karwoski and Proenza [1980]), the response to the second small spot would be depressed because it begins when $[K^+]_o$ is elevated as a result of the first response (Miller, 1973). To assess the magnitude of this effect, we determined the average values of ON-1 for five Müller cells and found them to equal 1.2 mV. Then, using calculation procedures discused by Karwoski and Proenza (1977), we obtained the following results: The 1.2-mV response corresponds to a $\Delta[K^+]_o$ of 0.141 mM. If this same K^+ increase generates ON-2S, at the start of which $[K^+]_o$ has increased by 0.141 mM, then the ON-2S response should equal 1.125 mV. This value is smaller than ON-1, and, thus, the Nernstian behavior of Müller cells will contribute to RD. However, the expected decrement (1.2 mV − 1.125 mV = 0.075 mV) can only account for ~11 % (0.075/0.7) of the decrement (1.2 mV − 0.5 mV = 0.7 mV) observed remaining after the subtractive correction discussed above. Calculations to determine the contribution of the Nernstian relation to TRE were also made. In this case, the magnitude of ON-1 in five cells averaged 0.67 mV, and ON-

1 began at a higher $[K^+]_o$: 3.141 mM. ON-2S remains ~0.5 mV. Because, from Nernstian considerations, ON-2S should have been reduced to only 0.65 mV, Nernstian behavior can account for only ~12% of the response decrement after the transiently enhanced response.

(*c*) Interactions of extracellular K^+ increases and decreases generated by neurons, which themselves show little or no RD and TRE, could contribute to these phenomena in the extracellular K^+ response and, thus, in Müller-cell responses. However, even if such interactions do occur, they would probably be a minor factor in the present results, because there is evidence that Müller-cell depolarizing responses are generated primarily by K^+ released from on/off neurons (Karwoski and Proenza, 1978 and 1980), which themselves show RD and TRE.

In summary, the strong RD and TRE seen in Müller cells seems clearly related to the RD and TRE seen in on/off neurons, although a small contribution from on and off neurons cannot be excluded at this time. Additional contributions are also made by subtractive interaction between succeeding responses and by Nernstian effects (i.e., the logarithmic relation between membrane potential and $[K^+]_o$).

b-wave

Because it appears that RD and TRE arise largely from interactions in the inner plexiform layer, it is of interest to determine whether the *b*-wave of the ERG exhibits these phenomena. The local ERG (LERG), recorded in the distal retina, was used in this case because the ERG recorded transretinally was found to be of negligible amplitude in response to the small spot flashes of the standard stimulus sequence. A typical LERG sequence is shown in the top part of Fig. 9, where it can be seen that the *b*-wave exhibits RD and greatly increased amplitude to large spot stimuli. However, by comparing responses to small spot stimuli *D* and *E*, it is difficult to determine whether TRE is present because response amplitude is low and because the responses are superimposed on the rapidly decaying portions of previous responses. To assess the importance of the latter effect on the responses to stimuli *D* and *E*, a large spot series followed by no small spots was presented, and this series was subtracted from the appropriate portion of the standard stimulus sequence, resulting in the lower left trace of Fig. 9. A large spot series followed by one small spot was also presented, and this series was subtracted from the standard stimulus sequence, resulting in the trace in the lower right of Fig. 9. The accuracy of the subtraction procedure may be assessed by observing, during the first stimuli in both lower traces of Fig. 9, the horizontal slope of the traces and the small amplitudes of the responses. When these corrected responses to stimuli *D* and *E* are compared, TRE is present if amplitude is measured from baseline to the peak of the *b*-wave, but not present if measured from the peak of the *a*-wave to the peak of the *b*-wave. Inasmuch as all retinal responses that show strong RD also show TRE, one could make the assumption that the *b*-wave, which shows strong RD, should also show TRE, in which case the former measurement strategy would be appropriate. The *b*-wave measurements entered in Table II were obtained with this same strategy.

DISCUSSION

Response Decrement

The main results of this study are quantitatively summarized in Table II, which presents the degree of RD seen in the various retinal responses. Degree of RD is specified as a ratio for each response type as in Table I: the mean amplitude of the last three responses in a small spot flicker series divided by the peak amplitude of the first response. Amplitudes of the last three responses in a series were calculated in two different ways: (*a*) "baseline-to-peak amplitude," in which the difference between the preseries potential level is subtracted from peak response level and (*b*) simple "response amplitude," in which the difference between the potential just before stimulus onset (or offset)

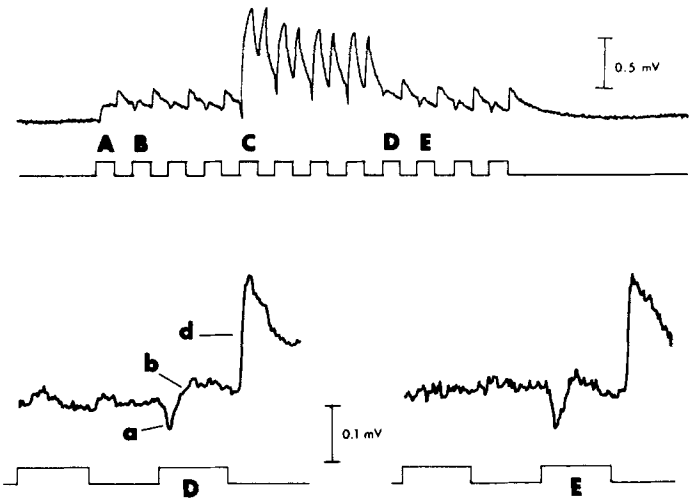

FIGURE 9. Local electroretinogram (LERG) to the standard stimulus sequence (upper trace). Lower traces are described in the text. *a*, *b*, and *d* in the lower traces refer to components of the LERG: *a*-wave, *b*-wave, and *d*-wave.

is subtracted from peak amplitudes of the response to the stimulus onset (or offset). Thus, in Table II, most cell types have four ratios associated with them: degree of RD calculated in two ways (using both "baseline-to-peak" and "response" amplitudes) for the changes in potential at stimulus onset (ON response) and offset (OFF response).

Inspection of Table II, in conjunction with the data presented in Results, suggests the following trends and conclusions:

(*a*) Regardless of how measured, there is little RD visible in the retinal cells that repond in primarily "on" or "off" fashion. However, the degree of RD in these responses appears to increase as one proceeds proximally, and is maximum in the PSTHs of ganglion cells. Thus, some contribution to RD may be made at every synapse.

(*b*) When "response amplitude" is considered, the on components of all "on/off"-type responses show strong RD (ratios $\leq$ 0.50), as compared with

"on"-type responses (ratios ≥ 0.74). Therefore, a factor unique to the on/off channel, and arising in the proximal retina, is a major contributor to RD. This factor appears to be that in on/off neurons a slowly decaying depolarization evoked by a small, well-centered stimulus flash subtracts from the amplitude of succeeding evoked responses. Werblin (1972) and Werblin and Thibos (1978) have shown that a spinning "windmill" stimulus does not affect

TABLE II

MEAN RESPONSE DECREMENT RATIOS PLUS OR MINUS STANDARD DEVIATION OF VARIOUS RESPONSE TYPES

		Light onset		Light offset	
Response types	*n*	Baseline-to-peak amplitude	Response amplitude	Baseline-to-peak amplitude	Response amplitude
"ON" or "OFF" cells					
Horizontal cell	9	0.93±0.06	1.33±0.22*	1.26±0.15*	1.05±0.06
HPBC	4	0.85±0.07	1.01±0.04	1.09±0.09	1.00±0.02
DPBC	5	0.85±0.13	0.96±0.19	0.92±0.08	1.02±0.17
DPC	5	0.80±0.05	0.86±0.08	—	—
ON cell (PSTH)	6	0.74±0.09	0.74±0.09	—	—
OFF cell (PSTH)	8	—	—	0.86±0.34	0.86±0.34
"ON/OFF" response types					
on/off neuron (intracellular)	15	0.76±0.19	0.50±0.27‡	0.91±0.15	0.82±0.23
ON/OFF cell (PSTH)	13	0.25±0.26‡	0.25±0.26‡	0.41±0.36‡	0.41±0.36‡
PNR	7	0.91±0.11	0.46±0.11‡	0.89±0.10	0.73±0.12
Müller cell	7	1.43±0.22*	0.22±0.07‡	1.03±0.05	0.59±0.09
M-wave	7	1.20 (?)*	<0.30 (?)‡	1.00 (?)	0.7 (?)
LERG					
b-wave	5	1.04±0.16	0.26±0.05‡	—	—
d-wave	5	—	—	0.89±0.08	1.02±0.02

n is the number of cells, or, in the case of field potentials, the number of preparations. DPC off responses were difficult to measure, so are not included. The same PSTH amplitudes are entered under both measurement methods. Because the interaction of the PNR with the *M*-wave made amplitude determinations of the latter uncertain, *M*-wave ratios should be considered rough approximations. These entries are followed by a question mark.

* Ratios ≥1.20.

‡ Ratios ≤0.50.

bipolar cell responses, but in on/off amacrine cells elicits a sustained depolarization that subtracts from the amplitude of succeeding responses. This windmill effect, and the RD and TRE of the present paper, thus appear similar in that they both may arise because of a sustained, stimulus-generated depolarization of amacrine cells. Such a sustained membrane depolarization, as elsewhere in the nervous system, might also result in decreased synaptic efficacy of these cells.

(*c*) Our results largely support the relationship between the various on/off-

type responses that we have discussed elsewhere (Karwoski and Proenza, 1980). Specifically, on/off neurons in the proximal retina depolarize to light and release K^+ that depolarizes Müller cells, whose extracellular currents generate the *M*-wave. Furthermore, on/off neurons generate extracellular currents recorded as the PNR. This latter idea is strengthened by the close quantitative relation between the behavior of the intracellular responses of on/off neurons and the PNR and by how both of these differ from bipolar cells and from PSTHs of ON ganglion cells (Table II; see also Karwoski and Proenza [1978]).

However, because RD and TRE were usually significantly greater in the PSTHs, a remaining question is, what is the relation between intracellular responses of on/off neurons (presumaby amacrine and/or ganglion cells) and PSTHs of on/off spiking cells? A definitive resolution of this discrepancy cannot be provided until the specific identities of the cells producing the intracellular responses and extracellular spike discharges are known. The large variance in the RD ratios for both of these responses is notable, however, and would be compatible with more than one cell type generating these responses, e.g., possibly both amacrine and ganglion cells, possibly with subtypes, generating both on/off intracellular and extracellular spike responses.

(*d*) For on/off-type responses, when "response amplitude" is measured, the on responses show stronger RD than the off responses. This probably occurs because in on/off neurons, the first response to the stimulus sequence, an on response, has a sustained component that subtracts from the amplitude of the first off response. The result is that all succeeding responses, whether on or off responses, are depressed about equally relative to the first on response.

(*e*) When one or the other form of measurement is considered, Müller-cell responses, the *M*-wave, and the negative-going *b*-wave, show similar RD ratios. This prompts the suggestion that a portion of the negative-going *b*-wave, evoked by a small spot only 1–2 log units above threshold in the light-adapted retina of the mudpuppy eyecup, may perhaps be generated by Müller-cell responses evoked by K^+ released from neurons of the proximal retina. However, alternative explanations are possible. For example, the response of the distal K^+ increase (Dick and Miller, 1978; Kline et al., 1978) to the stimulus sequence would be particularly interesting, but we continue to find this response both elusive and too labile to be worked with reliably (Karwoski et al., 1979).

When response amplitude was measured, the negative-going *d*-wave of the distal retina showed no RD, unlike Müller-cell responses and the *M*-wave. Perhaps this difference exists because a portion of the *d*-wave arises from the decay of the *a*-wave rather than from the Müller-cell off response. The receptors, which generate the *a*-wave (e.g., Brown [1968]), probably show minimal RD, because neurons immediately postsynaptic to them (horizontal and bipolar cells) show minimal RD.

Transient Response Enhancement

A series of large spot flashes causes the next response to a small flashed spot to be larger than subsequent responses—TRE. The magnitude of this effect

was typically less than the enhancement of the response to the first small spot flash occurring after a period of no stimulation. Both RD and TRE, however, appear to originate primarily in a similar type of neural interaction in the proximal retina. Specifically, the intracellular potential of on/off neurons approaches the resting membrane potential after a period of no stimulation or of large spot flashes, thus allowing the amplitude of the next on response to a small spot to be relatively large compared with the amplitude of subsequent responses to the small spot. A psychophysical effect, which seems analogous to TRE and, thus, might also originate largely from interactions in the proximal retina, has been described by Teller et al. (1971).

With regard to both RD and TRE, we find that $[K^+]_o$, extracellular potential, and Müller-cell membrane potential parallel the changes seen in on/off neurons (see also Karwoski and Proenza [1977, 1978, and 1980]). It is difficult, however, to determine whether the potential changes seen in on/off neurons are the result or cause of these other changes. Our model for the release and clearance of K^+ suggests that these variables should indeed follow closely from the observed responses of on/off neurons, but feedback effects cannot be ruled out. Thus, though we might suggest, as have Dowling and Ripps (1976) with respect to network adaptation, that modulations in the levels of $[K^+]_o$ might lead to these adaptive and sensitizing effects, at this time it seems more parsimonious to attribute them to the observed changes in neuronal membrane potential. However, regardless of the particular mechanism involved, our results clearly show that some aspects of postreceptoral adaptation are specific to the proximal retina.

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