Neurons, Potassium, and Glia in Proximal Retina of *Necturus*

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ABSTRACT Light-evoked K^+ flux and intracellular Müller (glial) cell and on/off-neuron responses were recorded from the proximal retina of *Necturus* in eyecups from which the vitreous was not drained. On/off-responses, probably arising from amacrine cells, showed an initial transient and a sustained component that always exhibited surround antagonism. Müller cell responses were small but otherwise similar to those recorded in eyecups drained of vitreous. The proximal K^+ increase and Müller cell responses had identical decay times, and on some occasions the latency and rise time of the K^+ increase nearly matched Müller cell responses, indicating that the recorded K^+ responses were not always appreciably degraded by electrode "dead space." The spatiotemporal distribution of the K^+ increase showed that both diffusion and active reuptake play important roles in K^+ clearance. The relationship between on/off-neuron responses and the K^+ increase was modelled by assuming that (*a*) K^+ release is positively related to the instantaneous amplitude of the neural response, and (*b*) K^+ accumulating in extracellular space is cleared via mechanisms with approximately exponential time-courses. These two processes were approximated by low-pass filtering the on/off-neuron responses, resulting in modelled responses that match the wave form and time-course of the K^+ increase and behave quantitatively like the K^+ increase to changes in stimulus intensity and diameter. Thus, on/off-neurons are probably a primary source of the proximal light-evoked K^+ increase that depolarizes glial cells to generate the M-wave.

INTRODUCTION

In recent years, considerable attention has been paid to the relationship between light-evoked neural responses, K^+ flux, Müller (glial) cell responses, and field potentials in the vertebrate retina. These results show that extracellular levels of K^+ are differentially altered throughout the retina and correspondingly impinge upon different regions of the Müller cell's membrane. More specifically, in the *distal* retina a prominent photoreceptor-induced K^+ decrease generates, through its hyperpolarizing effects on pigment epithelial and Müller cells, the *c*-wave and slow PIII components of the electroretinogram (ERG), respectively (Oakley, 1975; Oakley and Green, 1976). Also in the distal retina, a transient K^+ increase has been reported that may largely depend on depolarizing bipolar cells and generate, through its depolarizing

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effects on Müller cells, the *b*-wave of the ERG (Dick and Miller, 1978; Kline et al., 1978). In the *proximal* retina, a prominent K^+ increase has been recorded (Oakley, 1975). On/off-neurons (probably amacrine cells) may be the primary contributors to this K^+ increase (Karwoski and Proenza, 1978 *b*), which in turn depolarizes Müller cells, whose associated extracellular currents give rise to the M-wave (Karwoski and Proenza, 1977 *a*).

With regard to the scheme of events in the proximal retina, however, two problems may be raised. First, on/off-neurons, when compared to Müller cell responses and the proximal K^+ increase, appear to behave dissimilarly with respect to response duration, intensity-response characteristics, and the presence of surround antagonism (e.g., Kaneko and Hashimoto, 1969; Werblin and Copenhagen, 1974; Naka and Ohtsuka, 1975; Murakami and Shimoda, 1977). Second, in mudpuppy, Müller cell responses and the M-wave have previously been studied in eyecups drained of vitreous humor (Miller and Dowling, 1970; Karwoski and Proenza, 1977 *a*), whereas the proximal K^+ increase and amacrine cell responses have been primarily studied in eyecups perfused or not drained of vitreous (Miller and Dacheux, 1976; Werblin, 1977; Dick and Miller, 1978; Karwoski and Proenza, 1978 *b*). It could be argued that responses obtained from eyecups drained of vitreous are unrepresentative, since evoked K^+ accumulates in the proximal retina and could thus yield anomalous K^+, glial, and/or neural responses.

In the present paper, therefore, basic responses of on/off-neurons are reexamined to determine if they can be considered a major source of the proximal K^+ increase. Müller cell responses and the M-wave in normal eyecups are also recorded and compared to previous results from drained eyecups. Together, these observations clarify a number of the links in the schema relating neural responses, K^+ flux, glial responses, and field potentials in the proximal retina. In addition, a model is presented to describe the relationship between neural and K^+ responses recorded in the proximal retina.

METHODS

Preparation

Mudpuppy (*Necturus maculosus*) eyecups were dissected and prepared for recording as previously described (Proenza and Burkhardt, 1973), except that a drop of amphibian Ringer's solution (Grant, 1977) was placed in the eyecup to retard drying. Such eyecups produce field potentials and spike responses that are reasonably stable for at least 5 h and appear very similar to responses recorded from perfused eyecups (Grant, 1977) or live animals.[1]

Electrodes

Intracellular records were obtained with fine micropipettes filled with 3 M K-acetate (DC resistance: 100-200 MΩ). Micropipettes used for recording field potentials were broken to about 1 μm, filled with 120 mM NaCl and 2.5 mM KCl, and had DC resistances of 50-100 MΩ. Double-barreled K^+-sensitive microelectrodes measured 1-3 μm across the tips and were prepared as previously described (Karwoski and Proenza, 1978 *b*).

[1] Fowlkes, D. Unpublished observations in this laboratory.

Light Stimulation

Details of the optical system have been described elsewhere (Karwoski and Proenza, 1977 *a*). A diffuse background of 0.02 lm/m^2 was always present. Stimulating flashes were superimposed upon this background. In the figures and text, stimulus intensity is given by an arbitrary logarithmic notation where 100 lm/m^2 equals 10.0. Unless noted otherwise, stimulus intensity is about 2 log units above threshold.

RESULTS

Basic Properties of On/Off-Neuron Responses

GENERAL PROPERTIES Samples of intracellular on/off-response types encountered in the mudpuppy retina are shown in Fig. 1. The first column

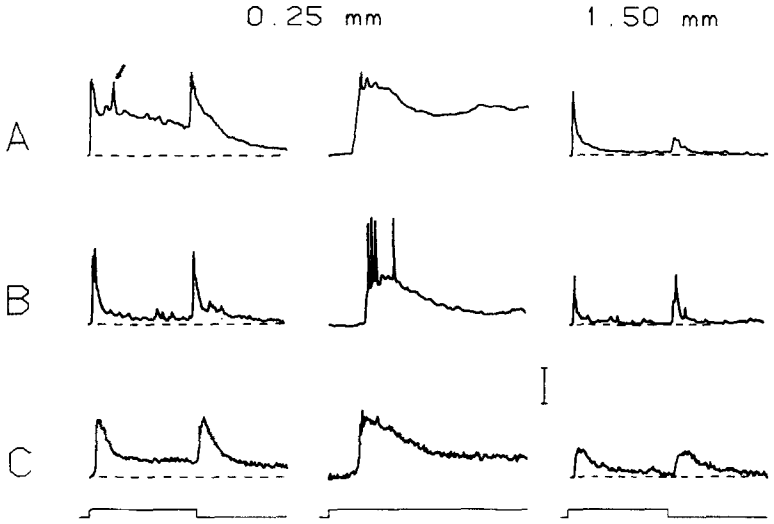

FIGURE 1. Intracellular responses of three on/off-neurons (A, B, and C). Responses in the left column are to a 0.25-mm stimulus. Stimulus duration is 4 s for A and B and 2 s for C. The first 0.5 s of these responses are shown in the middle column. Stimulus in the right column is the same as for the left, except its diameter is 1.50 mm. Calibration: (A) 19 mV; (B) 28 mV; (C) 7 mV. Resting potentials: (A) -64 mV; (B) -62 mV; (C) -38 mV.

shows responses of three cells to a well-centered 0.25-mm spot. All these responses show prominent phasic depolarizations at light onset and at offset, and a relatively sustained component of smaller amplitude that lasts as long as the stimulus is present. As previously reported by Werblin (1977), the phasic depolarization usually occurs only once at light onset and once at offset, but an instance where it occurs a second time during light onset is pointed out by the arrow over the response of cell A.

The first 500 ms of the on-responses of these waveforms are expanded in the second column of Fig. 1. Here, it can be seen that cells A and C show higher

frequency components near the peak of the phasic depolarization, and cell B shows clear spike activity.

The third column of Fig. 1 shows the responses of these cells to a large diameter (1.5 mm) stimulus of the same intensity. In all on/off-neurons, the large diameter stimulus greatly depressed the sustained component. The phasic components were usually minimally affected (13 of 24 cells; e.g., Fig. 1 A). However, when the phasic components were also affected (e.g., Fig. 1 B and C), they generally were decreased relatively less than the sustained components.

DEPENDENCE ON MEMBRANE POTENTIAL The responses of on/off-neurons were noted to be critically dependent on level of polarization of the cell. When cells were relatively depolarized, presumably due to injury inflicted by the recording electrode, action potentials often disappeared, the amplitudes of the depolarizing components were usually altered, and hyperpolarizing compo-

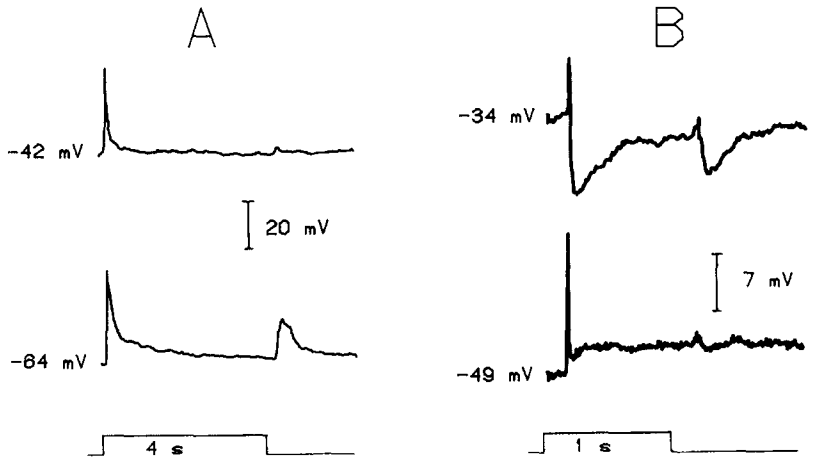

FIGURE 2. Responses of two on/off-neurons (A and B) obtained when their resting potentials were at different values. Stimulus diameter: (A) 1.00 mm; (B) 0.25 mm. See text for explanation of other details.

nents often appeared (Karwoski and Proenza, 1977 *b*). Two commonly encountered examples are shown in Fig. 2. Here it can be seen that cell A might have been classified as a "transient on-cell" when it was relatively depolarized at −42 mV. However, when the membrane potential shifted to −64 mV, the response now shows both on- and off-depolarizing components. Again in Fig. 2, cell B, when relatively depolarized at −34 mV, showed on/off-depolarizing components followed by large hyperpolarizing components. However, when its membrane potential shifted to −49 mV, the hyperpolarizing components disappeared, and the response became that of a typical on/off-depolarizing neuron (the depolarizing off-response was strongly developed to a 4-s stimulus, but not to the 1-s stimulus shown in Fig. 2 B).

STIMULUS INTENSITY The initial transient and later sustained portions of the on/off-neuron response behave differently as stimulus intensity is in-

creased. Fig. 3 shows the responses of an on/off-neuron to 0.25-mm stimuli of increasing intensity. As stimulus intensity increases, the amplitude of the initial transient component of the on-response increases. But as reported by Werblin and Copenhagen (1974), this component has a dynamic range of no more than 1 log unit, its amplitude remaining constant at higher intensities. Unlike the initial transient, the later sustained component of the on-response increases over several log units and, in the cell of Fig. 3, is best developed about 3 log units above threshold. The intensity dependence of these components of the on/off-neuron response will be considered again later.

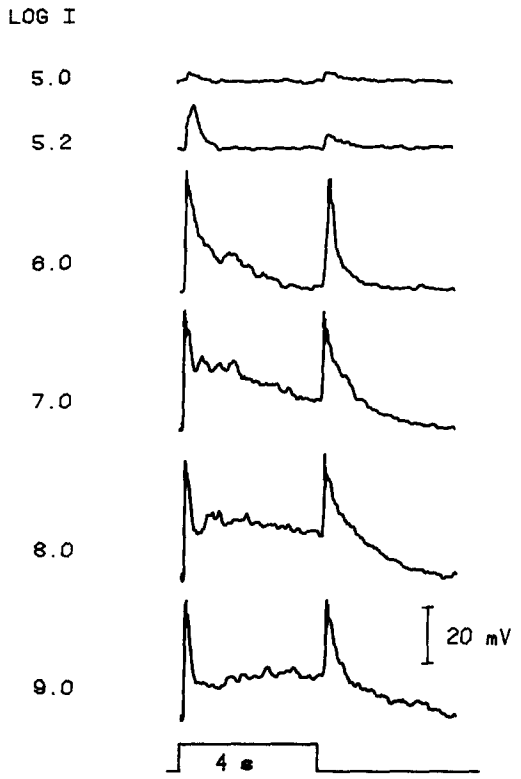

FIGURE 3. Responses of an on/off-neuron to stimuli of various intensities.

CELLULAR ORIGIN It is difficult to determine with certainty whether these responses arise from on/off-amacrine cells or on/off-ganglion cells. Like amacrine cells, and unlike ganglion cells (Werblin and Copenhagen, 1974; Miller and Dacheux, 1976), these cells show large excitatory postsynaptic potentials (EPSPs) up to 40 mV) and no trace of hyperpolarizing components when the resting potential is large. However, like ganglion cells, and unlike amacrine cells (Werblin and Copenhagen, 1974; Werblin, 1977), these cells always showed surround antagonism and often had several large spikes. Since most of the responses had an initial, phasic, regenerative component, charac-

teristic of amacrine cells (Werblin, 1977), a majority of these responses probably did arise from amacrines. The finding that horizontal cells were often penetrated immediately after on/off-neurons further supports this suggestion. For the purposes of the present paper, however, these responses will subsequently be referred to as simply arising from "on/off-neurons."

Müller Cell Responses

Responses were recorded from 36 Müller cells in eyecups not drained of vitreous humor. The responses of one of these cells to a 0.25-mm stimulus of increasing intensity are shown in Fig. 4, column A. Like the sustained component of the on/off-neuron response, the dynamic range of these responses is at least 2 log units, and response amplitude decreases at the highest intensity. The responses in column B are those of another cell to a small and large spot, respectively, and, like the sustained component of the on/off-neuron response, show a clear surround antagonism.

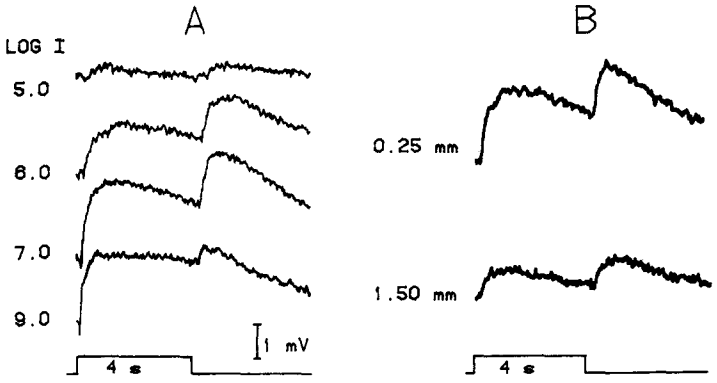

FIGURE 4. Intracellular responses of Müller cells from eyecups not drained of vitreous humor. Column A shows responses of a cell to a 0.25-mm stimulus of various intensities. Column B shows responses of another cell to stimuli of different diameters.

These responses of Müller cells recorded from undrained eyes are basically similar to those recorded in eyecups from which most of the vitreous humor was drained (Miller and Dowling, 1970; Karwoski and Proenza, 1977 *a*). Two differences were noted. First, in undrained eyecups, maximum Müller cell response amplitudes (measured from base line to the peak of the off-component) were never more than 4 mV, and often only 1 mV. This compares to responses in drained eyes of commonly 5-10 mV, and sometimes as much as 15 mV. The cells in the undrained eyes had stable resting potentials of −85 to −100 mV, which are comparable to those found in drained eyes. On several occasions, after low amplitude Müller cell responses were obtained in an undrained eye, the same eye was then drained of vitreous and the electrode reinserted, whereupon responses of 6 mV or more were readily recorded. A second, but minor, difference was in the size of the summation areas. In

drained eyes, responses were maximum to 0.5-mm stimuli (Karwoski and Proenza, 1977 *a*), whereas in undrained eyes, there was a tendency for responses to be maximum to a 0.25-mm stimulus ($n = 12$ cells, $t = 1.6$, $P < 0.07$).

Comparison of the K^+ Increase to Müller Cell Responses

The basic properties of light-evoked changes in $[K^+]_o$ recorded from eyecups not drained of vitreous humor have been rather extensively described (Oakley and Green, 1976; Dick and Miller, 1978; Karwoski et al., 1978; Karwoski and Proenza, 1978 *b*; Kline et al., 1978), and various suggestions have been made about possible relations between K^+ responses, Müller cell responses, and field potentials. In order to incorporate this evidence into a model, the recorded K^+ responses must be assumed to bear some relation to those changes in K^+ actually occurring in the intercellular clefts. As yet, however, ther has been little attempt to quantitatively determine this relation in the retina. Elsewhere in the nervous system, electrodes with 1–3-μm tip diameters have been estimated to produce around their tip a "dead space" of 10-15 μm through which substances must diffuse before being detected (Herz et al., 1969). Moreover, it has been calculated that this dead space could introduce a diffusion delay of up to 70 ms (Lux and Neher, 1973), which would also act to slow the time-course and decrease the peak of transient K^+ responses recorded with K^+-selective microelectrodes. On the other hand, since glial cells may be assumed to respond to neurally released K^+ with a negligible diffusion delay, the time-course and amplitude of their change in intracellular potential should give a good estimate of the true time-course and amplitude of the change in $[K^+]_o$ occurring in the intercellular clefts.

LATENCY As in previous work (Karwoski and Proenza, 1977 *a*), the latency of the Müller cell response was found to nearly equal that of the proximal negative response (PNR), a field potential recorded in the proximal retina (Burkhardt, 1970; Proenza and Burkhardt, 1973) and also within most Müller cells (e.g., Fig. 4: initial sharp negative deflections in the responses in column A; see also Karwoski and Proenza, 1977 *a*). This correspondence of latencies is very useful, since K^+ responses were not usually obtained in the same preparations as Müller cell recordings, but were obtained simultaneously with the PNR. Thus, the latency of the PNR (now used as an index of Müller cell latency) could be directly compared to the latency of the proximal K^+ increase that was obtained in the same preparation with identical stimuli. PNR latency was found to always be less than the latency of the K^+ increase. The difference in latencies varied widely from < 10 ms (4 of 28 electrode penetrations) to over 200 ms. Recordings where the latency difference was greater than ~ 100 ms (16 of 28 electrode penetrations) were discounted since this indicated an unacceptably large diffusion delay.

Fig. 5 (bottom row) shows one of the short latency K^+ increases recorded in the proximal retina. The same stimulus evoked the field potentials, shown in the upper three rows, which were recorded from various retinal depths. By comparing the expanded responses displayed in the right column, it is apparent that the latency of the K^+ increase is nearly as short as that of the

PNR and of the *b*-wave. This finding does not necessarily imply any functional relation between the proximal K^+ increase and the *b*-wave, but it does provide one indication that some recorded K^+ responses are nearly as fast as field potentials and Müller cell responses, and thus the electrode dead space in these cases may be insignificant.

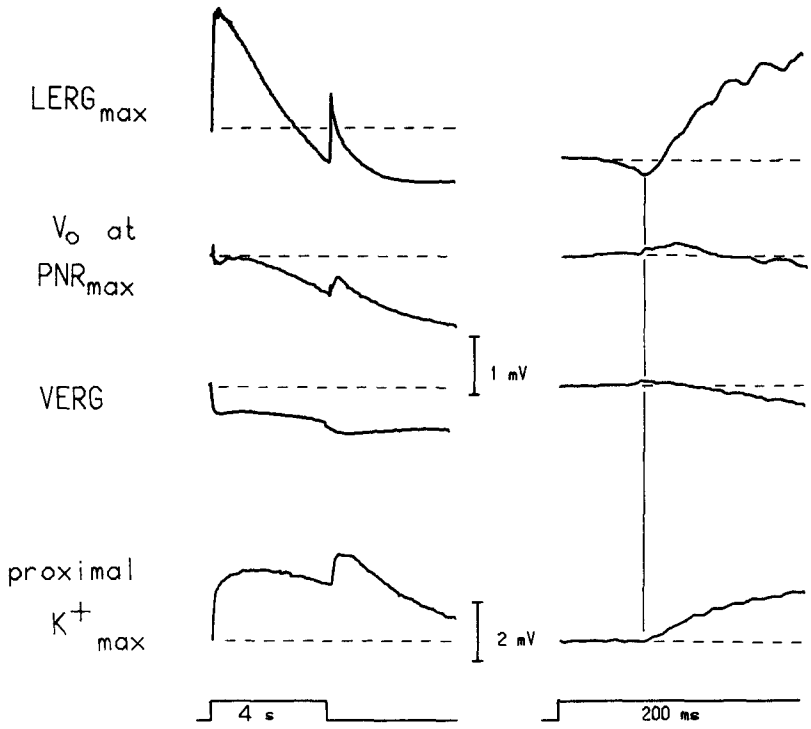

FIGURE 5. The top three rows show field potentials recorded from the distal retina at the level of the maximum negative *b*-wave ($LERG_{max}$), from the proximal retina at the level where both the PNR and K^+ increase are maximum (PNR_{max}), and from the vitreous humor (VERG). The bottom row shows the proximal K^+ increase recorded at the same depth as the maximum PNR shown in the third row. Stimulus diameter: 1.5 mm; intensity: 9.0 log. Negative is up in the traces of field potentials. In the right column, the first 200 ms of the responses displayed in the left column are expanded. Here, the response of $LERG_{max}$ is seen to consist of the positive-going *a*-wave followed by the negative *b*-wave with superimposed oscillations. Response at PNR_{max} shows a PNR, but not well developed due to the large diameter stimulus. Response recorded in the vitreous humor (VERG) consists of the negative *a*-wave followed by the positive *b*-wave.

RISE TIME Another index of the effects of dead space can be obtained by comparing rise times of glial and K^+ responses. Like the data of Futamachi and Pedley (1976) in cortex, glial responses in the retina were usually found to be faster than the proximal K^+ increase: the half-rise time (time for the

response to reach one half its maximum amplitude) of Müller cells ranged from 80 to 210 ms; those of the K^+ increase averaged >250 ms, but were comparable to Müller cells in five preparations—i.e., < 150 ms. These data suggest that with 1–3-μm electrodes, dead space will often cause the recorded K^+ responses to be appreciably slower than the true changes in $[K^+]_o$, but on fortuitous occasions the recorded responses may be negligibly affected.

DECAY TIME The third aspect of Müller cell and K^+ responses compared was the time it took for these responses to decrease to half their maximum amplitude (half-decay time). Half-decay time of the off-response was measured for six Müller cells and six K^+ increases. Mean decay times were not significantly different, and were between 2 and 3 s in all cases. A similarity in decay times of glial and K^+ responses was also observed by Futamachi and Pedley (1976).

AMPLITUDE A fourth way to assess whether the recorded K^+ responses are degraded by dead space is to calculate if the K^+ response is large enough to account for the amplitude of the Müller cell response. Amplitudes of the K^+ increases, recorded at the retinal depth at which they were maximum, and of Müller cell responses were determined to standard stimuli. The on-component of the K^+ increase averaged ~ 0.6 mM in amplitude, and in some preparations was as high as 1.2 mM. If Müller cells, like other glial cells, respond as nearly perfect K^+ electrodes (Kuffler et al., 1966; Miller, 1973; Lothman and Somjen, 1975), then, with a resting $[K^+]_o$ of 2.8 mM (Oakley and Green, 1976; Karwoski and Proenza, 1978 *b*), increases in $[K^+]_o$ of 0.6–1.0 mM should generate Müller cell-depolarizing responses with amplitudes of ~ 5–9 mV. As discussed above, however, Müller cell responses in eyecups not drained of vitreous humor were small. In 36 cells, the maximum amplitude of the on-component averaged only 1.8 mV, and the largest one recorded was 3.2 mV. These results indicate that the amplitude of the Müller cell response cannot be used to assess whether the measured K^+ increase is degraded by dead space. This is perhaps the case because Müller cells extend over a considerable distance and their membranes are exposed to nonuniform changes in K^+. Other considerations will be treated in the Discussion.

Mechanisms of K^+ Clearance

The decay time of the K^+ increase can also be used to analyze mechanisms of clearance of K^+ from extracellular space if two conditions are met. First, the slowing effects of electrode dead space must be negligible compared to the time-course of the decay. This seems true since, as shown above, K^+ decay time was similar to glial decay time, even in recordings where K^+ rise time was significantly slowed. Moreover, all results were verified in some of the preparations in which rise time of the K^+ increase was as fast as glial response rise time. Second, no K^+ additional to that released by resting neurons must be liberated during the period of the response decay. To maximize the probability of this situation, large-diameter light flashes ~ 2 log units above threshold were presented. Such stimuli evoke from on/off-neurons (see above) and from ganglion cells (Karwoski and Burkhardt, 1976) brief responses which

cease within ~ 1 s from light offset. Thus, if it is assumed that negligible additional K^+ is released from neurons 0.5 s after cessation of their postsynaptic potentials, then the decay of the K^+ increase evoked by this stimulus should provide a good index of the time-course of clearance processes, when the decay is measured beginning 1.5 s after stimulus offset.

At 1.5 s after stimulus offset, the proximal K^+ increase showed a maximum amplitude at the level of the inner plexiform layer, and decreasing amplitude proximal or distal or this location. A typical distribution is shown by the solid circles in Fig. 6, where retinal depth is normalized so that 0 μm represents that depth in the retina where the amplitude of the proximal K^+ increase was maximum. Small deviations from symmetry sometimes occurred if the light-evoked increase or decrease in K^+ in the distal retina was particularly pronounced, but the greatest effects were seen at depths > 60 μm distal to the center of the distribution. Also, a light-evoked K^+ increase was observed more

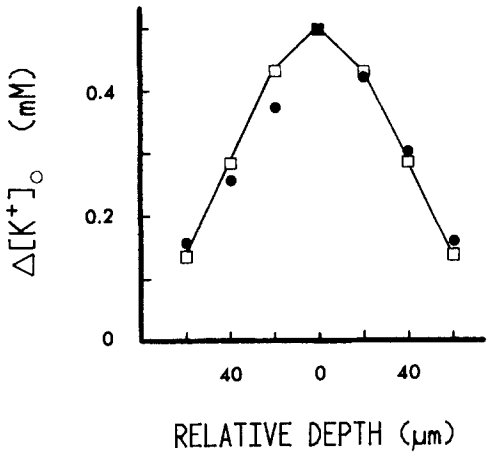

FIGURE 6. (●) Amplitude of proximal K^+ increase measured at various retinal depths 1.5 s after stimulus offset. Depth of maximum K^+ increase normalized to 0 μm. (□) Theoretical distribution calculated, as described in the text, to match the measured values.

proximally on some occasions, possibly at the level of optic nerve axons. This latter increase, however, was always small and created negligible asymmetry when measurement began 1.5 s after stimulus offset.

The next task was to generate a theoretical distribution similar to the filled circles (●) of Fig. 6 and then to calculate how this distribution would be degraded by diffusion. The distribution was approximated by first beginning with a plane source of finite (20 μm) thickness (rectilinear distribution) and then calculating how it would be degraded in time by diffusion (Crank, 1956: p. 13). The thickness of 20 μm was chosen since this is the approximate thickness of the inner plexiform layer, from which the majority of the proximal K^+ is likely released (Karwoski et al., 1978). A diffusion constant of 2.3×10^{-6} cm^2/s was assumed, as recently determined in amphibian tectum,[2] although

[2] Freeman, J. A. Personal communication.

the use of slightly higher values of this constant as found in mammalian brain (Lux and Neher, 1973; Fisher et al., 1976) minimally affects the results. A time during the degradation process of the rectilinear distribution was determined at which its shape closely matched the measured distribution, and this time was called "time zero." The subsequent decay of this distribution from time zero was followed and, when compared to the measured decay in $[K^+]_o$ at the various depths, it was found that the calculated decay rate was always too slow to match the measured rate. Thus, it would appear that in addition to diffusion, active reuptake mechanisms may also contribute to K^+ clearance from retinal extracellular space. Accordingly, the diffusion equation was modified to include a term for reuptake (Jacobs, 1935; and see Appendix). For a first approximation, it was assumed that reuptake followed a simple exponential, although there is evidence that it may occasionally be more complex (e.g., Lux and Neher, 1973; Vern et al., 1977). A value for the reuptake coefficient could then be determined that would result in both a theoretical distribution that matched the measured distribution, and one which also resulted in a closer match of the calculated and measured decay rates.

The open squares (□) connected by lines in Fig. 6 show points calculated from the degradation of the initial 20-μm-thick rectilinear distribution at 3 s (time zero). A reuptake coefficient of 0.1/s was used. Use of other reuptake coefficients also results in calculated values that closely match the measured values if the time zero is altered. However, values of the reuptake coefficient had to be near 0.1 for the subsequent decay of the calculated distribution to match reasonably well that of the measured distribution. Typical results are shown in Fig. 7. Here, the measured (●) and calculated (□) values of K^+ shown at time zero are simply transposed from Fig. 6. The decays of these values in time are illustrated at various distances from the center of the distribution (depth: 0 μm). The calculated curves fit the measured values reasonably well, although the match is not perfect. In eight experiments, the best value of the reuptake coefficient averaged $0.13 \pm 0.05\sigma$.

The relative contributions of diffusion and reuptake to the clearance process can be determined by comparing measured decay rates with calculated decay rates for diffusion alone, reuptake alone, and the combined function. In Fig. 8, the measured (●) and calculated (□) values of the K^+-decay at 0 μm are again replotted (transposed from the lowest graph of Fig. 7) and compared to (*a*) the decay of the distribution by diffusion alone (◇) and (*b*) the decay of the distribution by reuptake alone (○). These calculations were performed in five other animals, and at 5 s, the ratio of the clearance due to diffusion vs. clearance due to reuptake ranged from ~ 0.5 to 1.5. It thus seems reasonable to conclude that both diffusion and reuptake play significant roles in the clearance of light-evoked K^+ in the proximal retina of the mudpuppy eyecup.

A Model for Release and Clearance of K^+

It seems likely that on/off-neurons contribute to the proximal K^+ increase because (*a*) the K^+ increase and the PNR reach maximum amplitude at the same retinal depth (Karwoski and Proenza, 1978 *b*), and on/off-neurons

probably generate the PNR (Burkhardt, 1970; Karwoski and Proenza, 1978 *a*), and (*b*) the depolarizing responses of on/off-neurons are the largest in the retina (Werblin and Dowling, 1969; Miller and Dacheux, 1976), and neural K^+ release is probably directly related to the level of depolarization. Yet, a cursory examination of the waveform and properties of the on/off-neuron

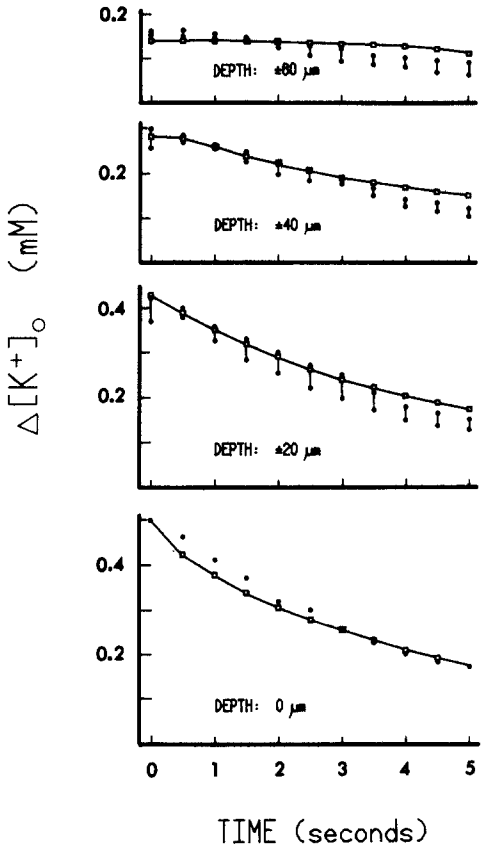

FIGURE 7. Measured (●) and calculated (□) changes in $[K^+]_o$ beginning 1.5 s (time zero in the figure) after stimulus offset. Bottom graph shows these curves at the depth where the $\Delta[K^+]_o$ is maximum. Upper graphs show these curves determined at various distances from the depth of maximum response. For example, at ±20 μm, the responses measured at 20 μm proximal and distal to the depth of maximum K^+ increase are both plotted, yielding two measured points (●) at each time.

response suggests that they are quite different from those of the K^+ increase, so that it is not readily apparent how the two could be quantitatively related. In an attempt to resolve any apparent discrepancies, a model has been developed to represent a plausible linkage between the on/off neural response and the K^+ increase. The model has two assumptions:

(*a*) K^+ release is positively related to the instantaneous amplitude of the on/off-neuron response and K^+ accumulates (is integrated) in extracellular space.

It is known that the response of on/off-neurons is associated with a conductance increase (Nelson, 1973; Werblin, 1977), which would seem most likely to be an increase in sodium conductance. If potassium conductance (g_K) were constant during this response, the K^+ efflux (I_K) would be linearly related to response amplitude. However, if g_K increases during the response, which is plausible given that the reversal potential of the response may be somewhat less than the sodium equilibrium potential (Nelson, 1973; Werblin, 1977), then, from Ohm's law, I_K would be proportional to the product of g_K and response amplitude, $V_m - E_K$ (where

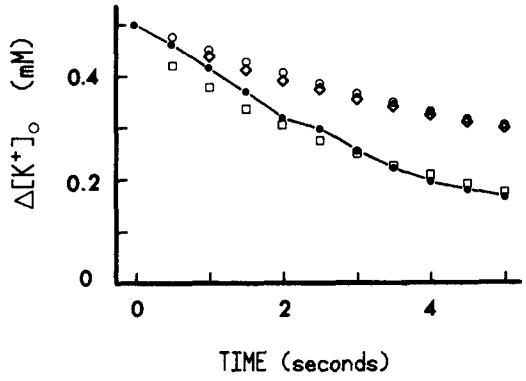

FIGURE 8. Filled circles (●) and open squares (□) are same points as in Fig. 7, lower graph. Open diamonds (◇) show the decay, calculated at 0 μm from the distribution of Fig. 6, caused by diffusion without reuptake. Open circles (○) show how, in the absence of diffusion, a substance would decay due to reuptake with a coefficient of 0.1/s.

V_m is intracellular potential and E_K is potassium equilibrium potential), and the curve relating I_K to response amplitude would be nonlinear—positively accelerated.

(*b*) Clearance mechanisms (with an exponential time-course) act on K^+ accumulating in extracellular space so that steady-state values are restored.

The clearance of K^+ in the eyecup preparation is probably accomplished by (1) diffusion, via extracellular clefts and/or glia and (2) active reuptake, by neurons and/or glia. Evidence was presented above (Figs. 6-8) that both of these mechanisms may play major roles, and that the clearance process has a half-decay time of 2-3 s. Fig. 8 also shows that, over a period of many seconds, both processes can be described fairly well by simple exponential decay functions. Thus, regardless of the exact contribution of reuptake and diffusion to K^+ clearance, the clearance may be considered, to a first approximation, a simple exponential process.

Applications of the Model

Since the stages of integration and simultaneous exponential decay can be modelled electronically by means of a low-pass filter, then, if the assumptions of the model are correct, playing the neural responses through a low-pass filter should result in waveforms similar to the proximal K^+ increase. A simple low-

pass filter would be sufficient to generate "good" modelled responses if K^+ efflux were linearly related to response amplitude and if the clearance processes followed a single exponential function. However, to the degree that K^+ clearance is nonmonoexponential (Vern et al., 1977), or that a nonlinear relationship exists between K^+ efflux and response amplitude, a linear low-pass filter might be inadequate since the decay time of the recorded response would be longer than the rise time. To test these possibilities, responses of on/off-neurons were evoked with a variety of stimulus conditions, and these responses, both before and after various modes of low-pass filtering, were compared to the K^+ increases and Müller cell responses evoked with identical stimuli.

WAVEFORM Passing on/off neural responses through a nonlinear, low-pass filter with a half-rise time of 650 ms and half-decay time of 3 s yielded "smoothed" waveforms which were found to provide the best match with regard to all aspects of K^+ and Müller cell responses. However, it should be noted that altering the half-decay time of the filter within a fairly wide range of values (bracketing the linear case) primarily affects the match during the decay phase of the response and has essentially no effect on the quantitative comparisons developed below.

Fig. 9 (left column) shows the responses of an on/off-neuron to stimuli of various diameters. These same responses, when played through the nonlinear low-pass filter, yield the modelled responses shown in the right column and labeled "smoothed responses." As described in Fig. 1, the neural response to the 0.25-mm spot has sharply rising phasic components at light onset and offset and, also, a relatively sustained phase that lasts as long as the stimulus is on. The smoothed responses, which have only slow components at light onset and offset, have a general time-course similar to that of the proximal K^+ increase (Karwoski and Proenza, 1978 *b*).

SURROUND ANTAGONISM The proximal K^+ increase also shows surround antagonism (Karwoski and Proenza, 1978 *b*; Kline et al., 1978), a property that was previously hard to reconcile with an on/off amacrine cell source, since these neurons are generally not reported to possess surround antagonism. Fig. 9 shows that the smoothed responses exhibit surround antagonism even when the peak response of the on/off-neurons does not. Moreover, the surround antagonism seen in the smoothed responses has a magnitude similar to that seen in the proximal K^+ increase and Müller cell responses (Fig. 4 and see also Karwoski and Proenza, 1977 *a*; 1978 *b*). Thus, results from the model also suggest that much of the recorded K^+ increase results from K^+ released during the slower portions of the on/off-neuron response.

INTENSITY Some of the intensity-response relationships introduced in Figs. 3 and 4 are shown graphically in Fig. 10. In this figure, response amplitudes are normalized and the curves are shifted laterally to compensate for slight differences in sensitivity across preparations. In the top graph, the filled symbols represent peak response amplitudes of three on/off-neurons and the open symbols represent the peak amplitudes of these same responses after smoothing. Curves were fitted by eye to both sets of points. The dynamic range of the smooth responses (2-3 log units) is greater than that of the

recorded responses (0.5 log or less). At very high intensities, the amplitudes of the smoothed responses decrease, whereas peak amplitudes of the neural responses remain unchanged. The curve for the smoothed responses is reproduced in the lower part of Fig. 10, where the amplitudes of Müller cell responses (filled symbols) and the proximal K^+ increase (open symbols) to various stimulus intensities are also plotted. It can be seen that the curve for the smoothed neural responses describes the behavior of the Müller cell and K^+ responses quite well.

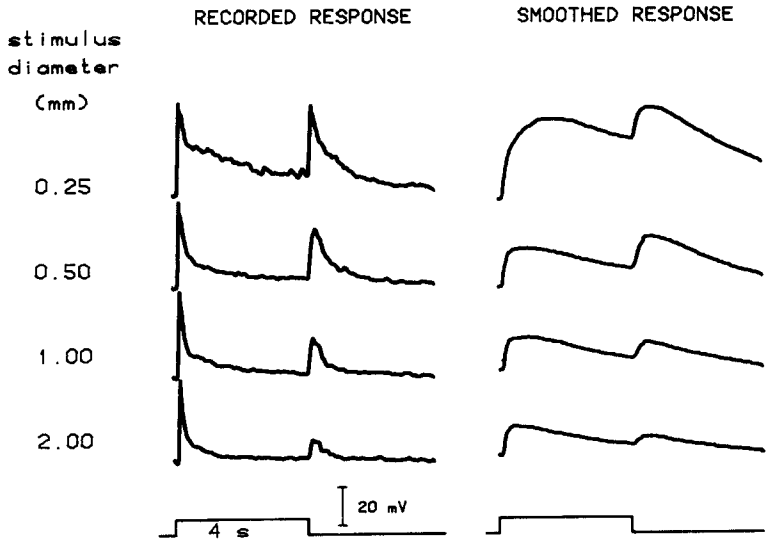

FIGURE 9. Recorded and smoothed responses of an on/off-neuron to stimuli of various diameters.

DISCUSSION

The data presented in this paper, together with findings summarized in the Introduction, suggest the outline of events shown in Fig. 11. Light evokes retinal activity that is transmitted to the proximal retina where on/off-responses are generated. K^+ is released into extracellular space by on/off-neurons and is then cleared via mechanisms with approximately an exponential time-course. These processes can be modelled by low-pass filtering the response of the on/off-neuron, which results in the "smoothed neural response" shown in Fig. 11. To the left of this modelled response is an actual K^+ increase recorded from the inner plexiform layer. This K^+ increase in turn induces a Müller cell depolarization, as shown at the bottom of Fig. 11. The extracellular currents associated with these cellular responses also generate field potentials: on/off-neurons generate the PNR, and Müller cells generate the M-wave. These two potentials sum in the proximal retina and result in the extracellularly recorded response shown in the right of Fig. 11.

In a previous paper (Karwoski and Proenza, 1977*a*), evidence was presented

for a similar set of events in the proximal retina of eyecups drained of vitreous humor. The present results, however, go beyond earlier work in demonstrating that several properties of the proximal K^+ increase can be quantitatively related to the activity of on/off-neurons. In addition, these results constitute

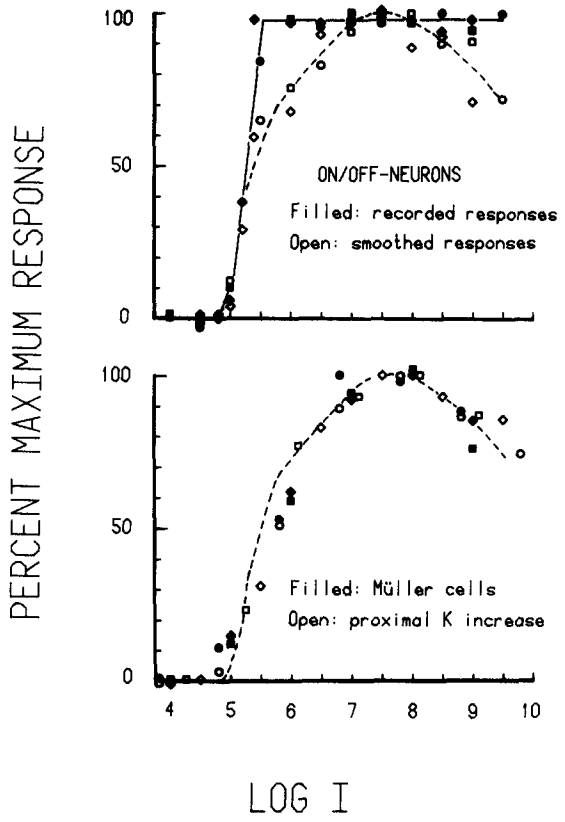

FIGURE 10. Normalized peak amplitudes of various responses to 0.25-mm stimuli of increasing intensities. Upper graph shows amplitudes of the recorded responses of three on/off-neurons (filled symbols) and of these same responses when smoothed (open symbols). Lower graph shows amplitudes of Müller cell responses (filled symbols) and the proximal K^+ increase (open symbols). The solid curve in the upper graph was fitted by eye to the recorded neural responses. The dashed line in the upper graph was similarly fitted to the smoothed responses and is also reproduced in the lower graph.

the first definite recordings of Müller cell responses and the M-wave from eyecups not drained of vitreous humor; they further suggest that some contribution to the M-wave may also be made by the extracellular currents of on/off-neurons, since these cells have a sustained component of appreciable amplitude in their response.

On/Off-Neurons

On/off-neurons with large resting potentials always showed a sustained component to their response and never showed hyperpolarizing components. However, when the resting potential of these cells shifted in the depolarizing direction, the sustained component sometimes disappeared resulting in a response that could then be described as "transient on," and in other cases hyperpolarizing components also appeared at light onset and offset. These results raise the question of what is the "normal" resting potential of intact

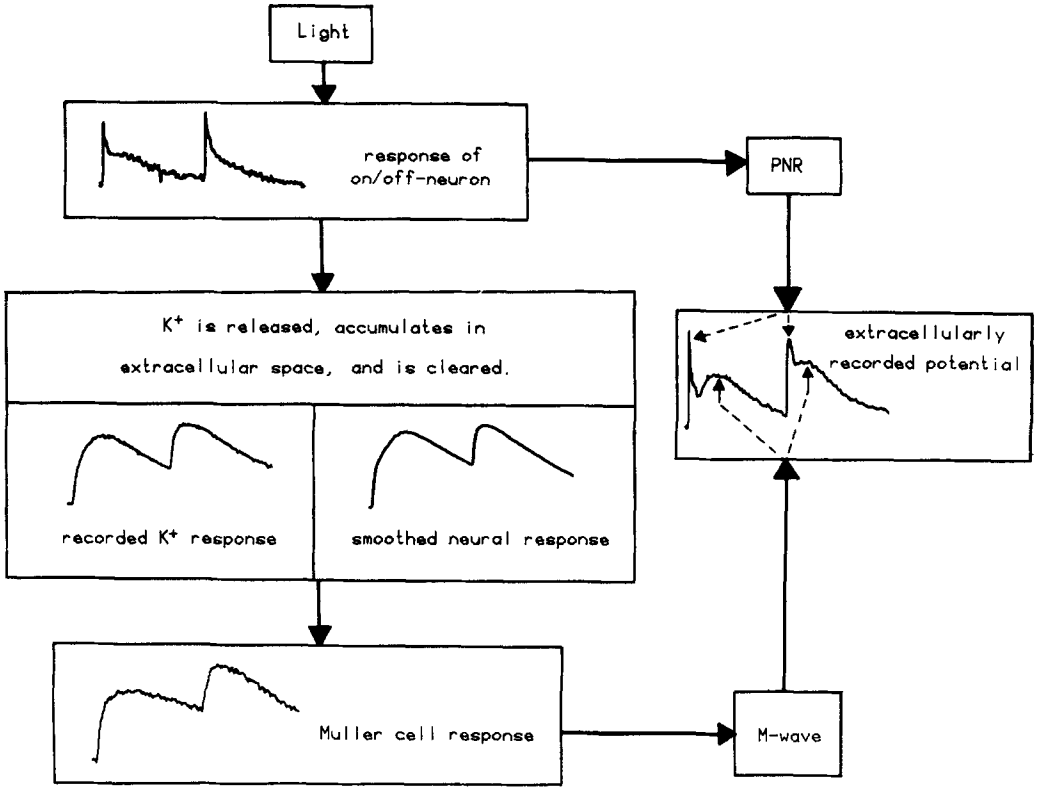

FIGURE 11. Summary of events in the proximal retina of the mudpuppy. See text for details.

on/off-neurons in the mudpuppy retina. We would argue that, under the present recording conditions, the "healthiest" cells have resting potentials of ~ -55 to -70 mV (6 of the 24 studied in the present series). In support of this suggestion is the observation that, in these cells, spikes appeared on the EPSPs only when the membrane potential hyperpolarized to > -55 mV. We have found, as have other authors (e.g., Werblin and Dowling, 1969; Nelson, 1973), resting potentials of most neurons in the proximal retina to be between -40 and -10 mV. However, the results of the present work prompt us to suggest that such cells might be chronically depolarized from injury inflicted by the

recording electrode, and thus that their true resting potentials may well lie more negative, closer in line with those of neurons elsewhere in the nervous system.

On/off-neurons, particularly on/off amacrine cells, have not generally been reported to possess antagonistic surrounds. The only previous report of this property appears to be that of Matsumoto (1975) who showed surround antagonism in one on/off amacrine cell in the frog retina; however, observations in mudpuppy also indicate that the responses of some on/off amacrine cells are weaker to large-diameter stimuli than to small spots.[3] In the present study, surround antagonism was found to be a property of all on/off-neurons when attention was focused on the sustained components of the responses. Since it is almost certain that at least some of these responses, and probably a majority, arose from amacrine cells (see Results), it appears that surround antagonism should be considered a general characteristic of on/off amacrine cell responses of the mudpuppy.

Müller Cells

Both the decreased amplitude and decreased summation area of Müller cell responses in undrained eyes are consistent with the idea that the vitreous humor can act as a K^+ sink, an idea suggested because a K^+ increase can only be recorded in the vitreous after most of it has been drained off (Mori et al., 1976; Karwoski and Proenza, 1977*a*). When the vitreous humor is drained, K^+ released in the proximal retina may build to a higher level, resulting in larger Müller cell responses. Moreover, diffusion is limited to the lateral and distal dimensions, which would make the summation area larger.

With regard to the quantitative relationships between the amplitudes of the recorded K^+ response and Müller cell responses, there are several reasons why a simple Nernstian relationship might not be apparent in the proximal retina, as it is elsewhere in the nervous system (Somjen, 1979). (*a*) Müller cell recordings might have been obtained from damaged cells. This is unlikely, because cells typically had resting potentials of -85 to -100 mV that were often stable for over 1 h. (*b*) The response of K^+ electrodes may in large part be determined by fluxes of substances other than K^+ (Kuramoto et al., 1978). However, the magnitude of this problem has yet to be assessed in the retina. (*c*) A hyperpolarization generated by the distal K^+ decrease may subtract from the amplitude of the Müller cell depolarizing response generated by the proximal K^+ increase. This is also unlikely, since at light offset the decay of the hyperpolarizing response should add to the depolarizing off-component of the K^+ increase, but Müller cell off-responses were similarly smaller than predicted by the K^+ off-response. (*d*) The space constant of the Müller cell may be short, so that Müller cell responses would be large when measured in the inner plexiform layer where the K^+ increase is large, but would have decreased amplitude at locations distal or proximal to this. Since Müller cells are largest at the inner limiting membrane where their end-feet are located and in the inner nuclear layer where their somas are, most recordings are

[3] Miller, R. F. Unpublished observations.

likely obtained from one of these levels where only small responses would thus be recorded. (*e*) The space constant of Müller cells might, on the other hand, be long, in which case the resting potential of the cell, when measured in the inner retina, would be partly biased by the higher resting concentration of K^+ in the distal retina. Increases in $[K^+]_o$ in the inner retina would then produce smaller Müller cell responses than predicted by the Nernst equation (Dick and Miller, 1978). At this time, without detailed knowledge of the electrical properties of Müller cell membranes or of the spatiotemporal quantitative relationships for both the distal increases and decreases in $[K^+]_o$, it is difficult to decide between some of the more plausible of these explanations.

The time-course of Müller cell responses was usually found to be somewhat faster than that of the proximal K^+ increase. Evidence was presented that this was often due in large part to electrode dead space. Another possibility, suggested by Kline et al. (1978), is that if the Müller cell has a large space constant, then the recorded response could have a component generated by the distal K^+ increase, which has a faster rise time than the proximal increase. This would only be the case, however, if the distal K^+ increase is sufficiently large relative to the proximal increase to affect significantly the Müller cell response that is recorded in the proximal retina, but this is not yet known in mudpuppy (Dick and Miller, 1978).

Clearance Mechanisms

The contributions of diffusion and active reuptake to K^+ clearance have been estimated from spatiotemporal distributions of increases in $[K^+]_o$ induced by stimulation of the central nervous system (Vern et al., 1977; Cordingley and Somjen, 1978) and retina[4] of mammals. These K^+ increases have sufficient spatial extent, and their clearance half-times are so short, that diffusion seems to be an unimportant mechanism in the clearance process. If, for a first approximation, the K^+ clearance in mammals is assumed to follow a simple exponential function, then reuptake coefficients, calculated from data presented in the above papers, range from about 0.5 to greater than 1.0. On the other hand, in amphibian tectum[2] and retina (present paper), diffusion was found to be a rather important clearance mechanism. This appears to have two causes: (*a*) in both of these amphibian systems, the released K^+ is initially confined to a narrow region; and (*b*) the reuptake coefficient seems lower—0.04 in tectum and 0.13 in retina.

Conclusion

The results discussed in this paper suggest that the response properties of on/off-neurons are such that these cells can indeed serve as a primary source of the light-evoked proximal increase in $[K^+]_o$. Low-pass filtering the responses of on/off-neurons results in modelled responses which resemble K^+ responses both in waveform and in their behavior to various stimulus conditions. Related models have recently been used to link the light-evoked decrease of $[K^+]_o$ in the distal retina to intracellular receptor responses (Matsuura et al.,

[4] Daley, M. L. Manuscript submitted for publication.

1978; Oakley et al., 1979), and it will be of interest to see if these or similar operations will also serve as a basis for modelling neural-induced changes in $[K^+]_o$ elsewhere in the nervous system.

Note Added in Proof Gardner-Medwin (e.g., 1979. *J. Physiol. (Lond.).* **284:** 37P–38P) and Nicholson, Phillips, and Gardner-Medwin (1979. *Brain Res.* **169:**580–584) have recently presented strong evidence for the importance of transcellular K^+ movement in clearing excess K^+ from extracellular space. If applicable to the retina, this mechanism would constitute part of the process we designated as "active reuptake." Our conclusion that diffusion through extracellular clefts is important in the clearance of raised $[K^+]_o$ would be unaltered. However, our calculated active reuptake coefficient would now be reinterpreted as an "uptake" coefficient that includes K^+ cleared via *passive* uptake into glia (primarily), as well as via *active* uptake into neurons and glia.

APPENDIX

Diffusion from an extended source of limited extent can be described by the following equation from Crank (1956; p. 13):

$$C = (\tfrac{1}{2})C_0 \left\{ \operatorname{erf} \frac{h - x}{2\,(Dt)^{1/2}} + \operatorname{erf} \frac{h + x}{2\,(Dt)^{1/2}} \right\}, \quad (1)$$

where C is the concentration of the diffusing substance, C_0 is the initial concentration of the diffusing substance, D is the diffusion constant, t is time, and C_0 is initially confined in the region $-h < x < +h$. However, if another process (e.g., active reuptake) is simultaneously removing a constant proportion of the diffusing substance, then the right-hand side of Eq. 1 is multiplied by e^{-kt}, where e is the base of the natural logarithms and k is the reuptake coefficient (Jacobs, 1935; p. 142).

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