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POTASSIUM ACTIVITY IN PHOTORECEPTORS, GLIAL CELLS AND EXTRACELLULAR SPACE IN THE DRONE RETINA: CHANGES DURING PHOTOSTIMULATION

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SUMMARY

1. A double-barrelled potassium-sensitive micro-electrode was developed that was fine enough to record intracellular electrical potentials and potassium activities (a_K) in the drone retina.

2. a_K was measured in the photoreceptor cells, in the pigment (glial) cells, and in the extracellular space, in the superfused, cut, retina. The effect of photostimulation was studied: 20 msec light flashes, intense enough to evoke receptor potentials of maximum amplitude were presented, 1/sec, in a train lasting about 2 min.

3. In photoreceptors with membrane potentials $\geqslant$ 50 mV, a_K in the dark was 79 mM, S.D. = 27 mM, n = 11. During photostimulation a_K fell by 21·5 $\pm$ 9·5 mM with a half-time of 30 $\pm$ 22 sec. (A tentative conversion from activities to free concentrations can be made by taking the activity coefficient as 0·70, its value in the Ringer solution.)

4. In pigment cells with membrane potentials $\geqslant$ 50 mV, a_K in the dark was 52 mM, S.D. = 13 mM, n = 11. During photostimulation a_K increased by 14 $\pm$ 5 mM.

5. In the extracellular space a_K increased during photostimulation with a mean half-time of less than 1·3 sec to a maximum (mean value 14 mM, S.D. = 8·4 mM, n = 22), and then fell to a plateau.

6. It is estimated from the anatomy that the photoreceptors occupy approximately 38 % of the total volume of the retina, the pigment cells 57 %, and the extracellular space 5 %. Hence, it seems possible that during photostimulation nearly all the net loss of potassium from the photoreceptors is temporarily stored in the pigment cells.

7. Recordings were made in the extracellular space of the intact animal by passing the electrode through a hole in the cornea. The mean a_K in the dark was 7·7 mM, S.E. = 0·4 mM, n = 22. In the superfused retina, a_K in the dark was 6·3 mM, S.E. = 0·7 mM, n = 22, even though a_K in the Ringer solution was 2·2 mM. Increasing the a_K of the Ringer solution to 7·0 mM had no apparent effect on a_K in the extracellular space at depths greater than 20 μm.

8. In the intact animal the amplitude and time course of the change in extracellular a_K evoked by the standard pattern of photostimulation were within the range observed in the superfused preparation.

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9. For most of the potassium-sensitive micro-electrodes an ion-exchanger based on potassium tetra-*p*-chlorophenylborate was used. This ion-exchanger is sensitive to acetylcholine, so further recordings were made in extracellular space with micro-electrodes based on valinomycin which were insensitive to acetylcholine. The results showed that the measurements of a_K were not detectably contaminated by a response to acetylcholine.

10. It is suggested that when the glial cells take up potassium during photostimulation they simultaneously lose sodium. These ion movements can be summarized as tending to maintain homeostasis of a_K and a_{Na} in extracellular space.

INTRODUCTION

The retina of the compound eye of the drone, *Apis mellifera*, is composed of two essentially uniform cell populations: the photoreceptor or retinula cells and the pigment cells. Light entering a corneal facet is focussed on a rhabdom which forms the axis of an ommatidium about 400 μm long. The rhabdom is composed of microvilli contributed almost entirely by six large, symmetrically arranged, photoreceptor cells with diameters up to at least 10 μm (Perrelet & Baumann, 1969; see Fig. 1). Pigment cells, which have the histological characteristics of glial cells, envelop the photoreceptors, sending fin-like processes between them as far as the rhabdom (Perrelet, 1970). Absorption of light in the rhabdom causes an increase in the sodium conductance of the photoreceptor cell membrane which results in a depolarizing receptor potential. This, in the drone, has an action potential or spike on the leading edge (Naka & Eguchi, 1962; Baumann, 1968; Fulpius & Baumann, 1969; see Fig. 2). In the pigment cells a smaller and slower depolarization is observed (Bertrand, 1974). As in the glial cells of *Necturus* optic nerve and cat brain, this depolarization can be explained as caused by the increase in potassium concentration in the extracellular space that results from neuronal activity (Bertrand, 1974; Orkand, Nicholls & Kuffler, 1966; see Lothman & Somjen, 1975, for other references). In drone pigment cells the depolarization following a single flash of light can be as large as 30 mV: Bertrand (1974) deduced that to produce such a depolarization the extracellular potassium concentration must increase by at least 30 mM.

An efflux of potassium from an invertebrate photoreceptor during light stimulation was detected by a direct method by Holt & Brown (1972) who used radioactive tracers in the ventral eye of *Limulus*. Similar results in other invertebrate species are described by Stieve & Hartung (1977). We report measurements of changes in intracellular potassium activity (a_K) in drone photoreceptors by means of intracellular potassium-sensitive micro-electrodes; during photostimulation, a_K decreases by up to at least 25%. We also describe measurements of a_K in extracellular space and in the pigment cells and will argue that nearly all the reduction in a_K in the photoreceptors can be accounted for by a corresponding increase in the pigment cells. The main direction of this work, then, is towards demonstrating that in this tissue there is an important movement of potassium from photoreceptors to glial cells during quasi-physiological stimulation. The measurements of a_K in the extracellular space go further and suggest that extracellular a_K is largely controlled by transmembrane ion fluxes.

The interpretation, implicit above, that the observed changes in a_K are due to transmembrane fluxes of potassium, is not the only possible one. There might, for example, be a redistribution of potassium between bound and free states, or movement of water. However, transmembrane potassium fluxes appear to offer the simplest explanation of the present results and this hypothesis is assumed throughout.

METHODS

Preparations. Drones were obtained locally in summer and from Ms E. Coe, P.O. Box 2772, Tucson, Arizona 85702, in winter. They were kept, mainly in darkness, at 30 °C, for up to 3 weeks before use. For most experiments the superfused retina preparation described by Fulpius & Baumann (1969) was used. The head was cut off and a slice made close to the posterior surface and parallel to the axes of the ommatidia (Fig. 1). The cut head was pinned in a chamber with the exposed ommatidia uppermost and superfused with oxygenated Ringer solution, composition: NaCl, 280 mM; KCl, 3·2 mM; $CaCl_2$, 1·8 mM; Tris buffer, 10 mM, pH 7·4. Some recordings were made in the intact drone, which was placed in a hole in a perspex block so that it head protruded and was immobilized with Histoacryl Blau (B. Braun, Melsungen, W. Germany). A piece of dorsal cornea about 500 μm across was cut out and the micro-electrode was inserted obliquely through the hole. The indifferent electrode was a micropipette inserted in a similar hole in the non-stimulated eye. The experiments were made at 21–23 °C.

Fabrication of the potassium-sensitive micro-electrodes. These were of the liquid membrane type in which a column of potassium-selective ligand solution (resin) is contained in the open tip of one barrel of a double-barrelled glass micropipette. The second barrel was filled with an electrolyte solution, usually 0·3 M-lithium acetate or 0·3 M-sodium formate, and used to record electrical potentials. More details of the fabrication, calibration and testing of the electrodes are given in Appendix 1.

Electrical arrangements. The head stage voltage followers were based on Teledyne Philbrick 1029 or 1035.02 operational amplifiers and had capacity neutralization (see Fig. 16*A*). The potential of the reference barrel was subtracted from that of the active barrel to give the potassium signal. The potassium signal and the electrical potential were recorded at two different sensitivities on a four channel curvilinear chart recorder; transients were usually recorded also on an oscilloscope or a Hewlett Packard 7402A pen-recorder.

Calibration of the electrodes. Most of the experiments were done with potassium micro-electrodes that contained Corning resin 477317. This does not have perfect selectivity for potassium over sodium. To take account of this, the calibration solutions were modifications of the Ringer solution in which varying fractions of the sodium chloride had been replaced by equimolar quantities of potassium chloride. The molar activity coefficient, y, in the calibration solutions is taken as 0·702 (Staples, 1971). A calibration curve was plotted for each micro-electrode (Fig. 14) and used to obtain from the electrical potassium signal the corresponding potassium activity.

Light stimulation. Light from a 150 W xenon arc was passed through glass optics and led by a fibre optics bundle to about 7 mm from the cut surface of the retina. The intensity at the surface of the retina was about 0·1 W mm^{-2} and the standard flashes were 20 msec long. To illuminate the retina *in situ*, a lens was attached to the end of the fibre optics bundle so that the light converged approximately towards the centre of the drone's head. When the electrode was in a recording site, the position of the light guide was adjusted by trial and error so that the maximum electrical response to photostimulation was obtained.

Estimation of cell volumes. It was necessary to make an approximate estimate of the relative total volumes of the photoreceptors and the pigment cells in the retina. Dr Alain Perrelet kindly lent us some mounted sections of drone retina. Areas of three sections were selected, projected on card and the boundaries between photoreceptors and pigment cells traced. Each area was a transverse section through the ommatidia, cut slightly obliquely so that sections of both the corneal and basal ends were included. The areas of card corresponding to the two cell types (about 100 ommatidia in all) were cut out and weighed.

RESULTS

The double-barrelled potassium micro-electrode was brought up to the cut surface of the retina, roughly perpendicular to the axes of the ommatidia (Fig. 1), and advanced slowly while light flashes were presented once per second. Recordings were made in the dorsal retina at depths in the range 10–100 μm from the cut surface. In this layer of tissue, oxygenation appears to be adequate (M. Tsacopoulos, in preparation) and the stimulating light, incident from above, is attenuated by only about 0·2 log unit (R. Muri, personal communication). Hence, in the plane per-

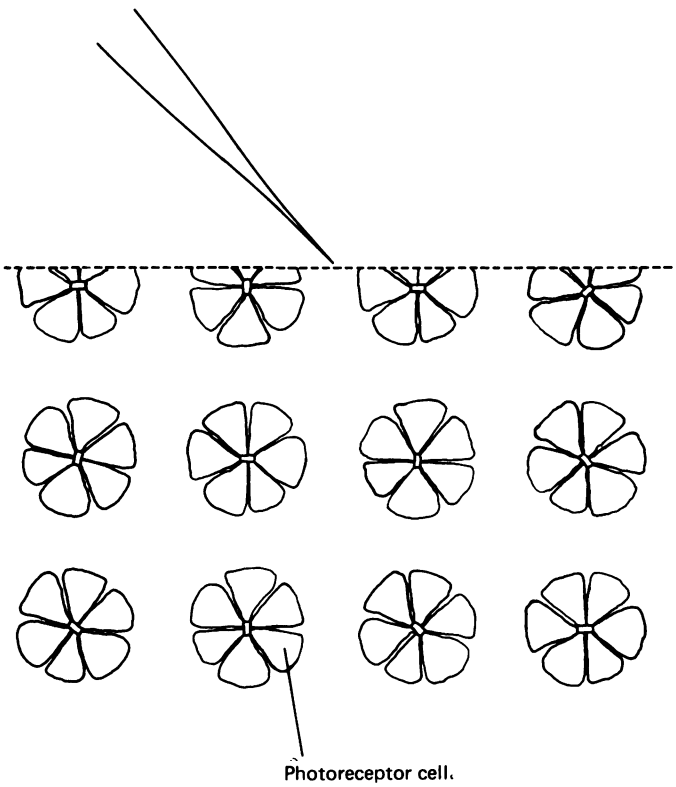

Fig. 1. Schematic section through the preparation. The micro-electrode is shown approaching the cut surface of the retina through the Ringer solution. Each flower-like structure represents the six large photoreceptor (retinula) cells that constitute an ommatidium, diameter about 20 μm. Light is absorbed in the rectangular rhabdom at the centre of each ommatidium. The space between the photoreceptors is filled with glial (pigment) cells.

pendicular to the axes of the ommatidia the system appears to repeat uniformly. If the coefficient of diffusion of potassium in the extracellular space were similar to the value in squid axoplasm then a sudden release of potassium from, say, the rhabdoms would be distributed at a uniform concentration round the pigment cells within a few tens of msec (see Bertrand, 1974). Three, clearly distinct, classes of electrical response were obtained, and, as described below for each class in turn,

these were ascribed to recordings from within photoreceptor cells, from within pigment cells, and from extracellular space. Values given for potassium activities (a_K) can be converted tentatively to free concentrations by dividing by 0·70, as explained in Appendix 1.

Potassium activity (a_K) in photoreceptors

Electrical responses of the form shown in Fig. 2*A* were ascribed to photoreceptors: there is a postive-going receptor potential with a spike on its rising phase (Baumann, 1968). If the amplitude of the receptor potential was more than 45 mV and the resting

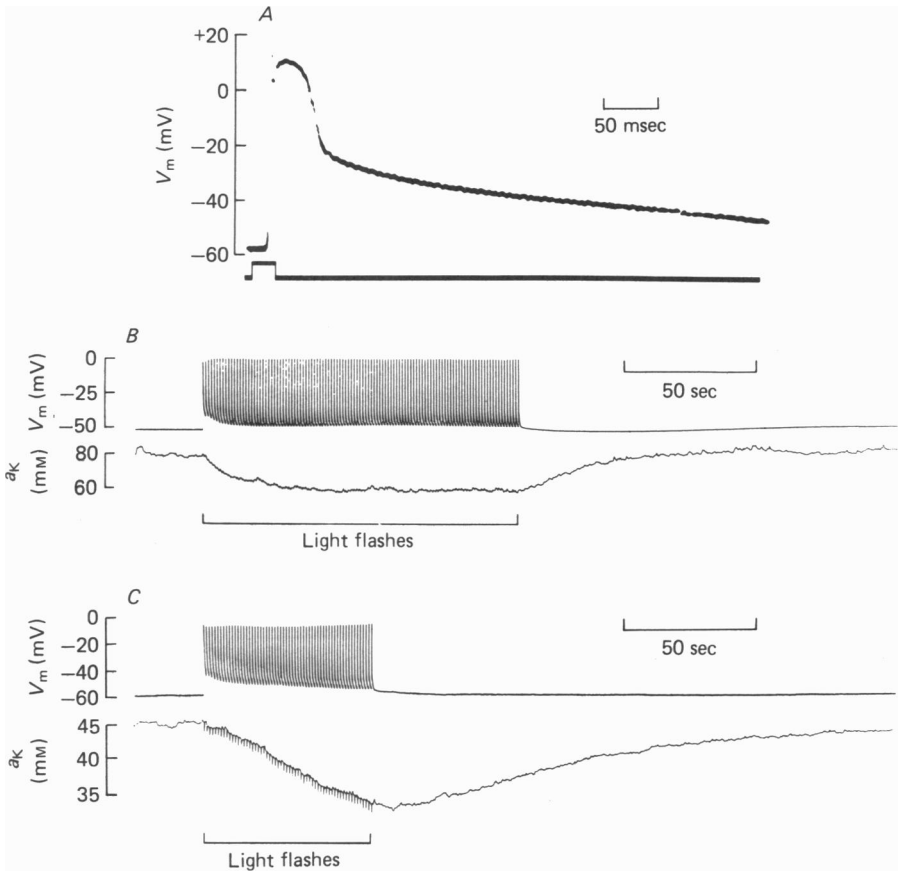

Fig. 2. Responses from photoreceptors. *A*, an intracellular receptor potential recorded by the reference barrel of a potassium micro-electrode. The deflexion on the lower trace indicates the time of the light stimulus. Only the tip of the spike on the rising phase of the receptor potential is visible. *B* and *C*, responses from two photoreceptors on a much slower time scale than in *A*. In each case the upper trace is the electrical potential recorded by the reference barrel of a double-barrelled potassium micro-electrode. During the time indicated by the bar, light flashes were presented, 1/sec. On this time scale, the receptor potential, which is similar to that in *A*, appears as a vertical line. The lower trace is the potassium signal, i.e. the difference in potential between the two barrels of the micro-electrode. The ionic activity scale shown on the left was obtained from the calibration curve of each electrode.

potential was stable, a repeating cycle of photostimulation was begun that consisted of a dark period, usually lasting about 3 min, followed by a train of flashes, 1/sec, usually for 2 min. The energy of each flash was sufficient to give a receptor potential of nearly saturating amplitude. An example of the effect of such stimulation is shown in Fig. 2*B*. The upper trace shows the electrical response recorded by the reference barrel of the micro-electrode and the lower trace shows the potential difference between the two barrels, i.e. the potassium signal. It is seen that a_K decreased during photostimulation to reach state with a half time of 10 sec and recovered, more slowly,

TABLE 1. Photoreceptors

	In the dark		In response to light		
Cell no.	V_m (mV)	a_K (mM)	Δa_K (mM)	$\Delta a_K/a_K$	$t_{\frac{1}{2}}$ (sec)
18	57	77·2	−21·8	−0·28	52
20	50	68·8	−23·9	−0·35	12
23	54	101·8	−21·1	−0·21	13
25	55	132·8	−31·6	−0·24	14
26	51	115·1	−42·8	−0·37	32
29	54	68·5	−13·0	−0·19	84
32	51	80·0	−24·5	−0·31	10
37	53	68·5	−18·5	−0·27	30
38	55	55·5	−17·5	−0·32	30
38b	56	60·5	−11·0	−0·18	25
41	58	45·2	−10·7	−0·24	35
Mean	54	79	−21·5	−0·27	31
S.D. of an entry		27	9·5	0·064	22
S.E. of mean		8	2·9	0·02	6

Summary of results for the eleven photoreceptor recordings with resting membrane potential $\geqslant 50$ mV. Δa_K in column 4 is the change in a_K produced by a prolonged train of the standard flashes in the 'best' response of the cell, i.e. the response for which the resting value of a_K was stable and at its highest. In Column 5 this value is expressed as a fraction of the resting value $t_{\frac{1}{2}}$ in column 6 is the time for the potassium signal to fall half-way to its steady state during photostimulation. For cell 41 the values in columns 4 and 6 are estimates obtained by extrapolation of the record. The coefficient of correlation of Δa_K and a_K is 0·82 which is significant with $F < 0{\cdot}01$.

in the dark. The phenomenon could be repeated for as long as the electrode remained in the cell, up to an hour. Fig. 2*C* is a similar recording from another photoreceptor cell. This example, in which a_K fell more slowly, is shown to illustrate the variability of time course from one cell to another. Recordings were obtained from eleven photoreceptors that had resting potentials of 50 mV or more. For each cell, a_K was estimated at the beginning and at the end of a stimulation period by means of the electrode calibration curve. The half-time of the fall in the potassium signal was also measured. These data are shown in Table 1: it is seen that in all these cells, photostimulation caused a fall in a_K and that the average decrease was 27 %. In photoreceptors with membrane potentials between 35 and 50 mV, photostimulation usually caused a fall in a_K in the same way, but sometimes the first effect was a small transient increase.

Potassium activity in the pigment cells

Electrical responses of the form shown in Fig. 3*A* were ascribed to pigment cells. There is no spike on the rising phase and the peak of the response occurs about 50 msec after the beginning of the flash, compared to 30 msec for a photoreceptor. The membrane potential returns to the dark level relatively slowly (with a mean half time of 121 msec in a sample of 11 cells) so that during photostimulation at the standard frequency of one flash per second the potential remains well above the dark level, as shown in the upper traces in Fig. 3*B*, *C*. A further test, made in some

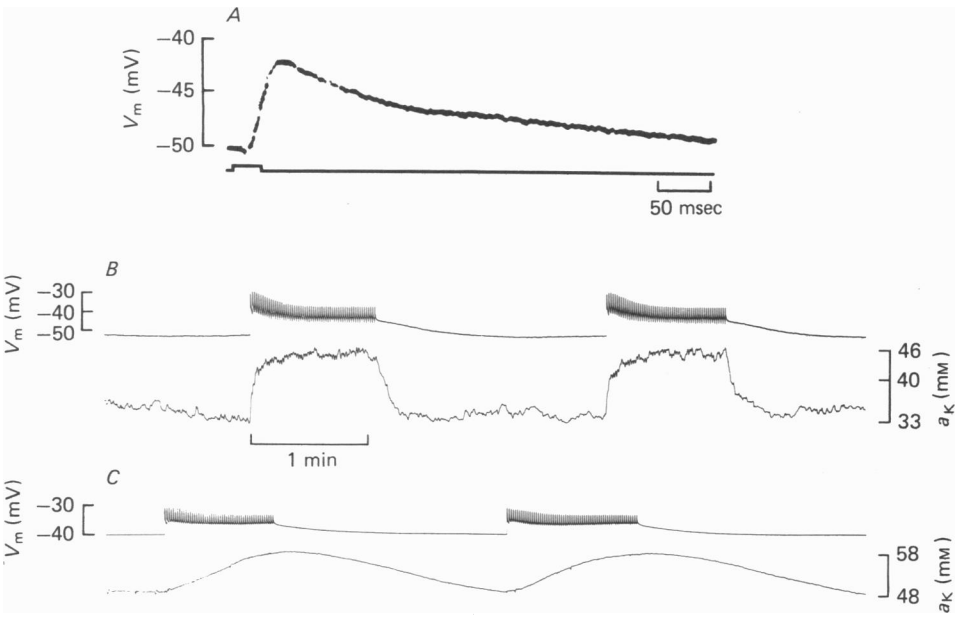

Fig. 3. Responses from pigment cells. *A*, the light-induced depolarization of a pigment cell recorded intracellularly by the reference barrel of a double-barrelled potassium micro-electrode. The deflexion on the lower trace indicates the time of the light stimulus. Compared to the response of a photoreceptor (trace *A*) the pigment cell depolarization has a small amplitude and a slow time course. *B* and *C*, responses from two pigment cells on a much slower time scale than in *A*. In each case the upper trace is the electrical potential recorded by the reference barrel of a double-barrelled potassium micro-electrode: responses to trains of flashes (2 trains per record) can be seen. The lower trace is the potassium signal: intracellular potassium activity (a_K) increased during photo-stimulation.

cases, was to present flashes of increasing intensity: on a graph of response amplitude *versus* stimulus energy, the responses of the photoreceptors saturate well before those of the pigment cells (Bertrand, 1974). It was possible, therefore, to distinguish the response of a pigment cell from that of a damaged photoreceptor.

Fig. 4. is a scatter diagram showing data obtained with potassium micro-electrodes in pigment cells. The measured membrane potential in the dark, V_m, is plotted against $\log_{10}(a_K)$. It is seen that the maximum V_m was 59 mV and that the mean value was less than 50 mV. Glial cells in most of the other species that have been studied have

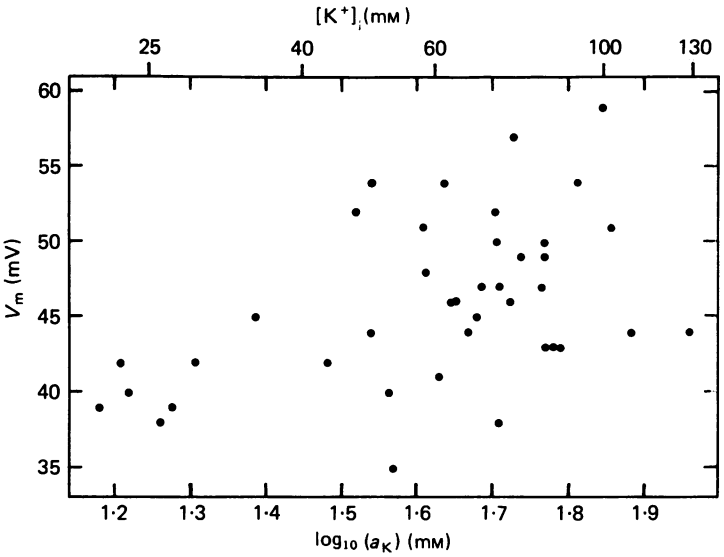

Fig. 4. Scatter diagram of the dark membrane potential, V_m, of 40 pigment cells plotted against the logarithm of the measured intracellular K^+ activity, a_K. The top horizontal scale gives an approximate conversion of activities to free concentrations on the assumption that the intracellular activity coefficient of K^+ is equal to that in the Ringer solution ($y = 0{\cdot}702$). The correlation coefficient of V_m and $\log_{10}(a_K)$ is 0·47 which is significant with $P < 0{\cdot}01$.

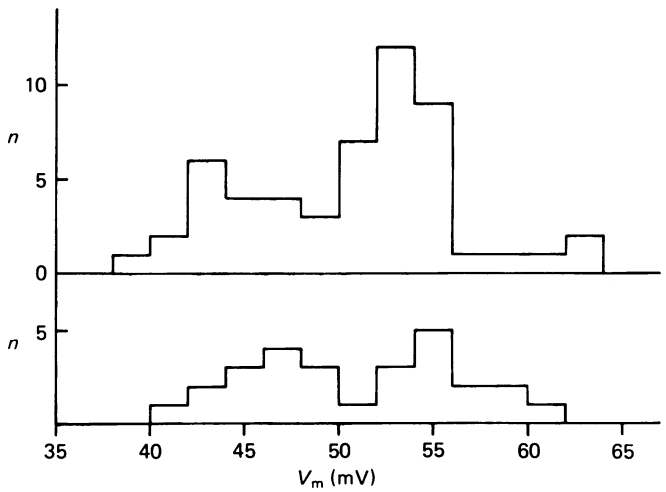

Fig. 5. Histograms of dark membrane potentials of pigment cells measured with single-barrelled micro-electrodes, resistances $\approx$ 20 MΩ. For the upper histogram, recordings were made in the isolated head preparation with the retina superfused, as for nearly all the other results in the paper. For the lower histogram recordings were made by passing the micro-electrode through a small hole in the cornea of the immobilized head of the intact drone.

considerably greater resting potentials, about 75 mV in the central nervuos system of the leach and 90 mV in vertebrates (summarized in Kuffler & Nicholls, 1976). Were the resting potentials of Fig. 4 low because the cells were damaged, either by being impaled with potassium micro-electrodes or as a result of the dissection? Control experiments that we now describe strongly suggest that damage did not greatly reduce V_m. Resting potentials were measured with fine, single-barrelled micro-electrodes in a sample of pigment cells in the superfused retina and in another sample in the eye *in vivo*. The results are presented in Fig. 5. The two populations are indistinguishable: for each, the estimated mean value of V_m was 51 mV and the S.D.

TABLE 2. Pigment cells

	In the dark		In response to light			
Cell no.	V_m (mV)	a_K (mM)	ΔV_m (mM)	Δa_K (mM)	$\Delta a_K/a_K$	$t_{\frac{1}{2}}$ (sec)
20b	50	50·9	11	+10·9	+0·21	8
23	54	43·5	17	+20·4	+0·47	54
33	51	40·7	14·5	+19·7	+0·48	4
37	50	59·3	16	+12·3	+0·21	48
39	52	50·5	11	+4·9	+0·10	16
50	57	53·5	35	+21·5	+0·40	2
52	51	72·0	15	+9·5	+0·13	32
53	59	70·2	15	+14·8	+0·21	45
55	52	33·2	22	+12·9	+0·39	1·5
56	54	34·8	16	+9·5	+0·27	20
63a	54	65·0	20	+17·5	+0·27	6
Mean	53	52	17·5	+14·0	+0·28	21
S.D. of an entry		13	6·6	5·3	0·13	20
S.E. of mean		4	2·0	1·6	0·04	6

Summary of the results for the eleven pigment cells with resting potentials $\geqslant$ 50 mV. The symbols have the same meanings as in Table 1; there is, in addition, ΔV_m, which is the depolarization induced by the first flash.

5 mV. This agreement suggests that in the superfused preparation the membrane potential is little affected either by the presence of a superficial layer of sliced cells or by the proximity of our Ringer solution. Further, the approximate symmetry of the histograms and the observed stability of the intracellular recordings suggest that 51 mV may not be a serious underestimate of the mean physiological resting potential. A similar low mean value, 46 mV, has previously been recorded in these cells (Bertrand, 1974), and a resting potential of only 39 mV has been reported for the Schwann cells surrounding the squid giant axon (Villegas, 1972).

Changes in pigment cell a_K during photostimulation. In response to the standard pattern of photostimulation all the cells identified as pigment cells showed an increase in a_K, as illustrated by the two examples in Fig. 3*B*, *C*. We have selected for tabulation data from the eleven cells that had $V_m \geqslant 50$ mV and these are shown in Table 2.

Although, as described above, our estimate of the mean physiological resting potential is only 51 mV, it seemed better to describe the cells with the greatest resting potentials for the following reasons. (1) Although they may represent a tail of the biological distribution curve they probably suffer from less than the average amount

of damage. (2) The mean V_m for this sample of pigment cells is, as it happens, close to the mean for the photoreceptors of Table 1, so that comparison of the two groups is more direct.

As seen in Table 2, the mean a_K in the dark was 52 mM, significantly less than in the photoreceptors, and during photostimulation a_K increased by 14 mM. There is a significant correlation ($P < 0{\cdot}05$) between the amplitude of the depolarization evoked by the first flash (ΔV_m in the Table) and Δa_K, the total increase in a_K during the train of flashes. This supports the idea that both phenomena are provoked by the increase in $[K^+]_0$. The time course of the increase in a_K, as for the decrease in a_K in the photoreceptors, varied widely from one cell to another. No significant

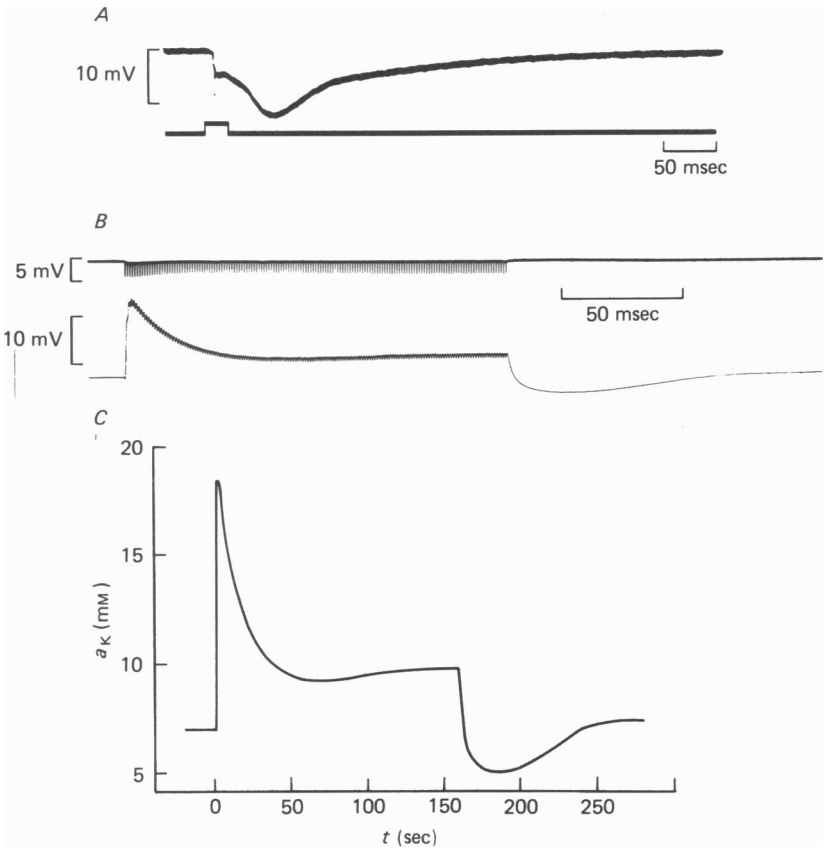

Fig. 6. Responses from extracellular space. *A*, an extracellular receptor potential recorded with the reference barrel of a potassium micro-electrode. The deflexion on the lower trace indicates the time of the stimulus. The tip of the small inverted image of the photoreceptor spike is just visible. At most of the extracellular recording sites the receptor potential amplitude was smaller than the one shown. *B*, the upper trace shows the electrical potential and the lower trace the potassium signal at an extracellular site. The time of the photostimulation with the standard train of flashes is evident from the receptor potentials on the upper trace. *C*, by using the calibration curve for the potassium micro-electrode the potassium activities a_K corresponding to the potassium signal in *B*, were calculated and are plotted here.

correlation was detected between the half time of the increase ($t_{\frac{1}{2}}$ in Table 2) and either a_K in the dark or the total increase (Δa_K in Table 2).

We have shown that, in the two main types of cell in the drone retina, photostimulation causes large fractional changes in a_K. If these changes are caused by transmembrane fluxes of potassium then the potassium must flow through the extracellular space and we expect to record at least transient changes in a_K there.

Potassium activity in the extracellular space

Recording sites where the apparent resting potential was less than about 2 mV were classed as extracellular. The electrical response to a light flash was a negative-going potential with an amplitude rarely greater than 10 mV: an example is shown in Fig. 6*A*. As the electrode was advanced from the bath into the retina, there was always an apparent increase in a_K even before there was a receptor potential of detectable amplitude. The behaviour remained the same throughout the time (up to 8 hr) that the preparations were used, although extracellular recordings were

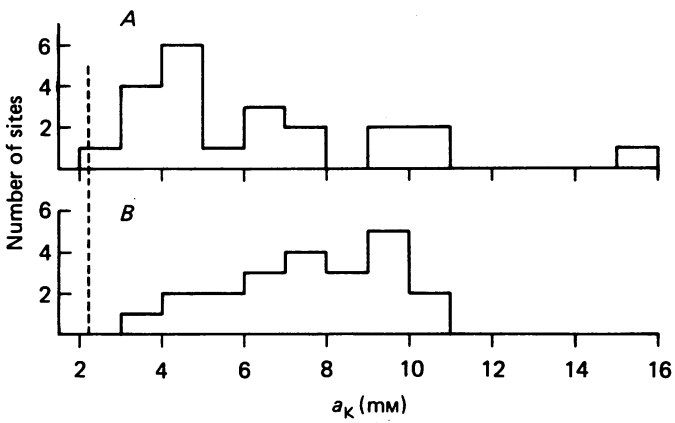

Fig. 7. Extracellular a_K in the dark. Histogram *A* shows steady state values obtained for a_K in the cut, superfused retina. Histogram *B* shows values obtained in the retina *in vivo*. The dotted line indicates the value of a_K in the standard Ringer solution.

rarer in older preparations. If the extracellular clefts in the superfused retina have the width observed in electron micrographs, about 15 nm (A. Perrelet, personal communication), then the introduction of a micro-electrode with a tip diameter of more than 200 nm must necessarily disturb the neighbouring cells. We expected that cells would be damaged and release potassium so that in the extracellular space a_K would initially be elevated and then fall as the potassium reached a steady state, for example, by diffusing to the bath. In only nine of the twenty-seven recordings examined was such an initial elevation observed. In most cases the potassium signal came immediately to its steady value; if there was a drift it was usually in the direction of an *increase* in a_K and was not more than 2–3 mV in 30 min. We conclude that there is no evidence of any systematic error caused by leakage of potassium from damaged cells.

Extracellular a_K in the dark. The values obtained for a_K in the superfused retina in the dark at twenty-two recording sites are shown in Fig. 7*A*. The mean was

6·3 mM, S.E. = 0·7 mM. This is nearly 3 times the value in the superfusate, 2·2 mM. We also measured a_K in the extracellular space in the retina *in vivo*, passing the micro-electrode through a hole in the cornea. Recording sites were selected at which a_K was stable in the dark. The values obtained are shown in the lower histogram in Fig. 7. The mean value was 7·7 mM, S.E. = 0·4 mM, n = 22: this is not significantly different from the value in the retina superfused with a Ringer with a_K = 2·2 mM. When a Ringer solution with a_K = 7·0 mM was used, no change in extracellular a_K (or the electrophysiology) was observed, except in recording sites less than 20 μm from the cut surface.

Changes in extracellular a_K caused by photostimulation. The effect of photostimulation on a_K in the superfused retina is illustrated by the lower trace in Fig. 6*B*. The potassium signal was measured at intervals along this record and, by means of the calibration curve for the electrode, the corresponding concentrations were found.

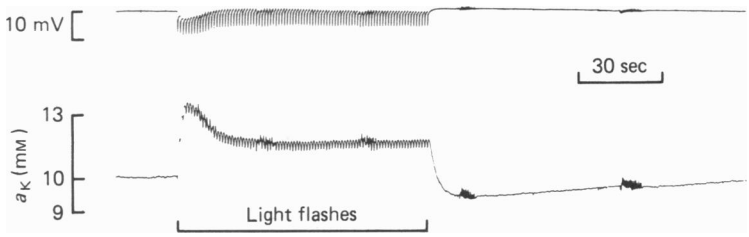

Fig. 8. Extracellular recording from the retina *in situ*. As for Fig. 6*B*, the upper trace is the electrical potential and the lower trace is the potassium signal. Pulses of movement artifact, produced by the animal's respiratory movements, are seen.

These were plotted and gave the curve shown in Fig. 6*C*. The potassium signal in Fig. 6*B* rises to a maximum with a half-time of less than one second. Since many of the potassium electrodes had response times of this order, the maximum of the signal underestimates the true maximum a_K. The mean of the apparent maxima was 14·0 mM, S.D. = 8·4 mM, n = 22; the extreme value was 41·6 mM (a concentration of 59·5 mM). After the maximum, the potassium signal declined to a plateau that was maintained for the duration of the stimulation (mean a_K = 9·8 mM, S.D. = 4·9 mM), and after the end of the stimulation a_K transiently undershot the resting level. The mean response is shown schematically in Fig. 10.

In the superfused retina it seemed possible that changes in extracellular a_K might be modified by diffusion of potassium to the Ringer solution at the cut surface. We therefore made recordings in the retina *in vivo*. There was some variability in the form of the potassium response (that may, in part, have been due to a failure to illuminate a large number of ommatidia uniformly through the cornea) but, in most sites, as in example of Fig. 8, a_K showed a transient, a plateau and an undershoot within the range observed in the superfused retina. We conclude that the form of the extracellular potassium response in the superfused retina was not significantly modified by diffusion to the bath.

Another conceivable source of error could also be investigated in the extracellular space. The ligand solution used in most of the potassium-selective electrodes, Corning

477317, is much more sensitive to acetylcholine than to potassium, the selectivity ratio being about 500. Oehme & Simon (1976) have developed another ligand solution, based on valinomycin, for which the preferences are reversed and the selectivity ratio of acetylcholine to potassium is only about 1/220. As explained in Appendix 1, the high resistivity of this mixture made it unsuitable for use in intracellular microelectrodes, but in slightly larger electrodes it could be used for extracellular recordings. An example is shown in Fig. 9: the form of the response to photostimulation is within the range observed with the Corning resin. We conclude that the main contributor to the potassium signal is indeed potassium.

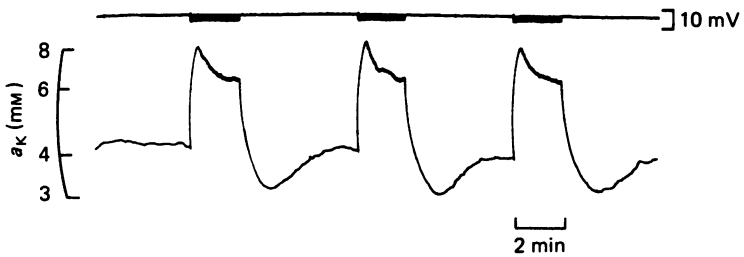

Fig. 9. Recordings from an extracellular site in the superfused retina made with a double-barrelled potassium micro-electrode. The active barrel contained a potassium-selective ligand solution based on valinomycin. The upper trace is the electrical response to photostimulation with trains of flashes. The lower trace is the potassium signal. Because of its high resistance, the response time of the electrode was slow. Because of its high selectivity for potassium over sodium, the relation between potassium signal and log (a_K) is linear to lower concentrations than is the case for a potassium electrode made with Corning resin 477317. Hence, the potassium signal undershoots the baseline at the end of the stimulation more markedly than in recordings made with the usual electrode as, for example, in that shown in Fig. 6*B*.

DISCUSSION

Potassium fluxes. In the drone retina we have observed that during photostimulation there is a decrease in a_K in the photoreceptors. Both classical electrophysiology (Frankenhaeuser & Hodgkin, 1956; Bertrand, 1974) and direct experiments on other invertebrate photoreceptors (Holt & Brown, 1972; Stieve & Hartung, 1977) argue that during the receptor potential there should be an efflux of potassium ions. If we assume that this is the only cause of the decrease in a_K then our results enable us to estimate the net flow of potassium ions across the cell membrane. During the first few flashes of a train, the photoreceptors lost potassium at a rate that caused the intracellular concentration to fall by at least 1 mM per flash. In the example of Fig. 2*B* the rate was 2·4 mM per flash. If the volume and surface of the cell have the values estimated in Appendix 2 then it follows that the transmembrane flux density was 10^6 ions μm^{-2} $flash^{-1}$. This is about 50 times the amount lost during an action potential in squid giant axon (Keynes, 1951; Keynes & Lewis, 1951; Hinke, 1961). However, it is not surprising that the flux per flash in the photoreceptor should be comparatively large because, during each of the first few receptor potentials recorded from a dark-adapted eye, the photoreceptor cell membrane remains depolarized for some 100 msec compared to the 1–2 msec of a nerve action potential (Baumann,

1968). In photoreceptors of *Balanus*, Brown (1976) has measured light-induced changes in a_{Na} that were smaller and slower than the changes in a_K in the drone: 6 min of continuous illumination caused a_{Na} to increase by not more than 5 mM.

Where does the potassium go when it leaves the photoreceptors? Since we have estimates of the changes in *concentration* in the three compartments of the retina we can, by making estimates of the relative volumes, calculate the changes in *quantity*. If the extracellular clefts have a width of 15 nm then the volume of the extracellular space will be only about 5 % of the total volume of the photoreceptors (see Appendix 2). The observed mean increase in $[K^+]_0$ during the plateau of the response to photo-

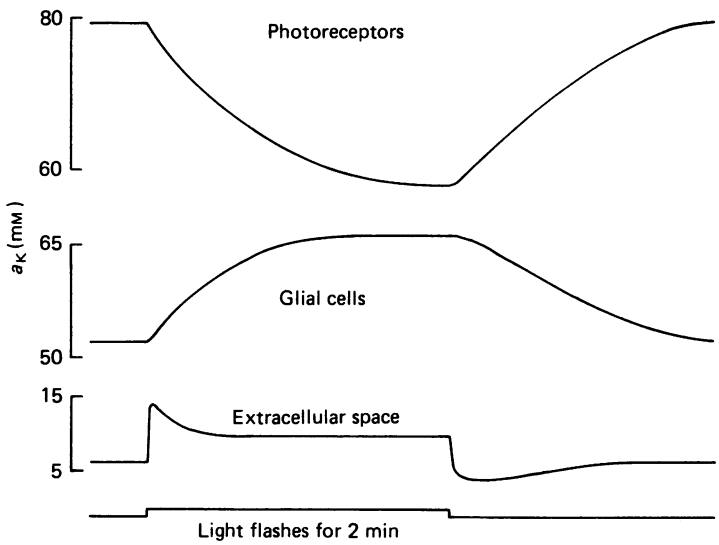

Fig. 10. Schematic summary of the changes in a_K. The curves are intended to illustrate the main features of the responses to the standard pattern of photostimulation in the photoreceptors, the glial cells and the extracellular space. The values drawn for the dark levels and the extreme values are the means of those observed for each class of recording site. Note that there was considerable variation in the time courses for individual cells (see Tables 1 and 2).

stimulation was 5·1 mM. This corresponds to a quantity less than 1 % of that lost from the photoreceptors. The ratio of the volume occupied by the pigment cells and the photoreceptors was estimated as described in the Methods and found to be about 1·5, the pigment cells being larger. The mean decrease in a_K in the photoreceptors was 21·5 mM and the mean increase in the pigment cells was 14·0 mM (see Fig. 10). When the ratio of the activities is multiplied by the ratio of the volumes it is seen that the uptake by the pigment cells is sufficient to account for all the loss from the photoreceptors. We conclude that the system appears to be a closed one: during the first minute or two of photostimulation there is a net loss of potassium ions from the photoreceptors and, apart from the 1 % that remains in the extracellular space, all this potassium is taken up by the pigment cells. There is no evidence of diffusion to the bath.

Extracellular potassium activity. In the recording sites classed as extracellular the

mean a_K in the dark was measured as 6·3 mM, nearly three times the value in the standard Ringer solution. The change in potassium signal (the potential difference between the two barrels) as the electrode was advanced from the bath to the extracellular recording site was 8–15 mV, depending on the calibration curve of the particular electrode. The potential change recorded by the reference barrel was quite consistently 1–2 mV negative to the bath: it did not show the variability that would be expected of an artifactual tip potential. We conclude that it is unlikely that more than a small part of the potassium signal was caused by a change in the tip potential of the reference barrel. We have already argued, in the Results, that the extracellular potassium signal did not behave in the way that would be expected if there were an important leakage of potassium from damaged cells. We assume tentatively, that the potassium concentration in the extracellular space really is considerably higher than in the bath.

Can this raised a_K be accounted for by a passive mechanism? The physical chemistry of the extracellular space would be expected to increase a_K in two closely related ways. First, the existence of a Donnan equilibrium across the cell membrane will cause an accumulation of cations in the vicinity (see, e.g., Overbeek, 1956). Secondly, if the extracellular space contains fixed negative charges these will produce a further accumulation of cations. If these mechanisms were the only cause of the increase in a_K above the value in the bath, then the ratio a_K/a_{Na} in the extracellular space should be proportional to (and almost equal to) a_K/a_{Na} in the bath. Hence if $a_K/a_{Na} \ll 1$ an increase of a_K in the bath should produce a proportional increase in a_K in the extracellular space. In preliminary experiments in which the bath a_K was increased to 7 mM only very small increases in extracellular a_K were observed. I.e. extracellular a_K was not proportional to bath a_K (over this range of activities, cf. Bertrand, 1974). This suggests that the distribution of potassium ions between the bath and the extracellular space is not passive. During photostimulation the quantity of potassium that leaves the photoreceptors and enters the glial cells is more than 100 times greater than the quantity estimated to be present in the dark in the extracellular space. This illustrates how large are the intracellular stores of potassium compared to the amount needed to produce the observed concentration in the extracellular space. Hence, if the extracellular diffusion pathway to the bath is relatively restricted then the intracellular stores (or active transport through the cells) might be sufficient to maintain the extracellular a_K higher than in the bath. An intracellular store of *sodium* has already been postulated by Fulpius & Baumann (1969) in order to explain their observation that the drone receptor potential persisted despite prolonged superfusion with sodium-free Ringer solution. In another insect preparation, the nerve cord of the cockroach, Treherne and coworkers have amassed evidence that extra-axonal sodium concentration is controlled by sodium transport by the perineural or glial elements or both (see Treherne, 1975, and Schofield & Treherne, 1978, for references). Fig. 11 is a recording of extracellular a_K in the drone retina in response to a long pulse of illumination. It is seen that during the illumination a_K fell well below the dark level. It is difficult to see how this would happen if there were a homeostatic mechanism that acted primarily to maintain a constant a_K. Perhaps the observed changes in a_K are in part a secondary consequence of a system that maintains sodium homoeostasis.

Are potassium ions in equilibrium across the cell membranes? If we assume the

validity of our measurements of a_K we can compare the Nernst equilibrium potential E_K, with the observed membrane potential V_m. In the photoreceptors the mean values in the dark were: $E_K = -64{\cdot}1$ mV, S.E. = 5·3 mV; $V_m = -54{\cdot}0$ mV, S.E. = 0·8 mV. The difference is typical of a nerve cell. In the glial cells $E_K = -53{\cdot}5 \pm 4{\cdot}8$ mV and $V_m = -53{\cdot}1 \pm 0{\cdot}9$ mV. The potassium appears to be in equilibrium, as Kuffler *et al.* (1966) predicted for the glial cells of *Necturus* optic nerve. The surface membranes of *Necturus* glial cells and, to a slightly lesser degree, those of the other glial cells that have been studied appear to be very selectively permeable to potassium

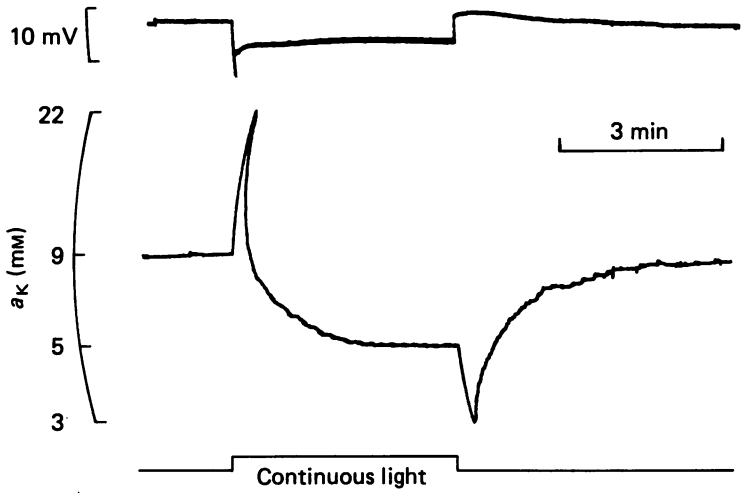

Fig. 11. Extracellular recording of a response to continuous illumination of a part of the retina *in situ*. As for Fig. 6*B*, the upper trace is the electrical potential and the lower trace is the potassium signal. This recording was made in collaboration with Mr S. Levy.

(Kuffler & Nicholls, 1976). In many preparations nervous activity causes a rise in potassium concentration on one side of a sheet of glial cells and potassium can then diffuse passively in one side of the glial cells and out of the other (e.g. Trachtenberg & Pollen, 1970; Gardner-Medwin, 1977). In the present experiments on the drone retina the situation was different. More than a thousand ommatidia were photostimulated almost uniformly and within a few tens of msec of a release of potassium from the photoreceptors the potassium presumably diffused through the extracellular space so that there was a uniform concentration on all sides of the glial cells (Bertrand, 1974). If the glial membrane behaved like a 'perfect potassium electrode' (Kuffler & Nicholls, 1976) then the membrane would depolarize to the extent given by the Nernst equilibrium potential: since the potassium would be in equilibrium it would not move across the membrane (beyond the minute amount necessary to change the charge on the membrane capacitance). But our results show that in drone retinal glial cells potassium *does* appear to move across the surface membrane. We conclude that in order to maintain electrical neutrality some other ion must also cross the membrane. This is seen particularly clearly after the termination of photostimulation. At this time intraglial a_K is raised beyond the dark level while extracellular a_K undershoots the dark level, i.e. the concentration gradient is greater than in the dark.

And yet the membrane is *depolarized* with respect to the dark level. The observations of Fulpius & Baumann (1969) on the drone and Treherne and coworkers (e.g. Schofield & Treherne, 1978) on cockroach nerve cord suggest that perhaps this is because the efflux of potassium is balanced by an influx of sodium.

We thank Mr J.-L. Munoz and Mr S. Levy for assistance and Drs F. Baumann, A. R. Gardner-Medwin, A. Mauro and P. Tissot for helpful discussion. Supported by SNSF grants 3.634.75 and 3.709.76.

APPENDIX 1

The fabrication and testing of the double-barelled potassium-sensitive micro-electrodes

The liquid membrane potassium-sensitive micro-electrode, developed by Walker (1971), has a column of ion-specific ligand solution in the open tip of a glass micropipette. The surface of glass is hydrophilic and normally hydrated (see Holland, 1964).

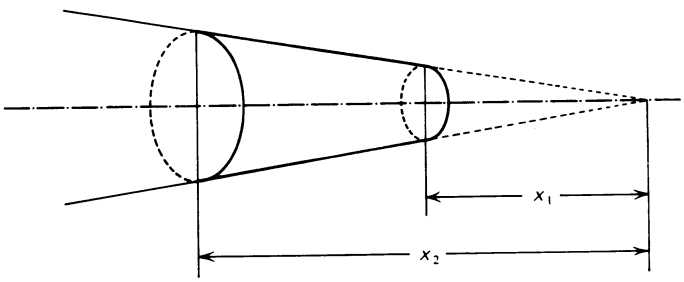

Fig. 12. Diagram of the tip of the single-barrelled potassium micro-electrode for defining the geometrical parameters in eqns. (1) and (2). The part of the micropipette containing the ligand solution is considered as the frustum of a cone and is shown in heavy lines. The tip of the electrode is distant x_1 from the apex of the cone and the column of ligand solution extends to a distance x_2.

If the interior surface of the micropipette were untreated two problems would arise: the column of hydrophobic ligand solution would tend to be displaced by water, and the leak conductance along the hydrated layer of the glass surface would tend to short-circuit the liquid membrane e.m.f. The latter effect becomes more important if the tip diameter is small, so that the ratio of perimeter to cross-section is large, or if a ligand solution of high resistivity is used. If the column of ligand solution has the form of a frustum of a cone, as in Fig. 12, then its resistance, which is approximately in series with its e.m.f., will be

$$R_s = \rho/\pi \left(\frac{x_1}{a}\right)^2 \left(\frac{1}{x_1} - \frac{1}{x_2}\right), \tag{1}$$

where ρ is the resistivity of the ligand solution, a is the internal radius of the micropipette tip and x_1 and x_2 define the length of the column as shown in Fig. 12. The approximation has been made that when current flows the isopotential surfaces are planar: this is justified provided the half angle of the cone is small ($< 10°$). The leak resistance, R_p, along the surface of the glass in contact with this cone is given by

$$R_p = \sigma/2\pi \left[\left(\frac{x_1}{a}\right)^2 + 1\right]^{\frac{1}{2}} \ln (x_2/x_1), \quad (2)$$

where σ is the surface resistivity. If the half angle of the taper of the cone is 5°, a is 0·1 μm and x_2 is 500 μm, then

$$R_p = 11{\cdot}1\sigma. \quad (3)$$

For an untreated glass surface, σ is of the order of 10^8 Ω. With this value, $R_p = 1{\cdot}11 \times 10^9$ Ω. But it is found experimentally that R_s for such an electrode is about 10^{11} Ω, so it is necessary to increase σ by a factor of at least 10^4. This can be done by treating the hydrated glass surface with a chlorosilane which reacts with the water and polymerizes to form a hydrophobic layer of silicone (Norton, 1944). σ increases to some 10^{12} Ω, so that for the model electrode in question, $R_p = 1{\cdot}11 \times 10^{13}$ Ω, and the leak current is negligible. The first recordings in the present work were obtained with electrodes treated in this way by means of the third of the methods described by Lux & Neher (1973). According to Norton, if the reaction goes to completion the silicone film tends to be about 0·2 μm thick, so that for tip diameters of less than 0·4 μm the reaction must be carefully controlled. In our hands, more success was obtained when the chlorosilane was applied in a dry atmosphere; presumably the silane binds directly to the hydroxyl groups of the glass itself as described by Holland (1964, p. 386).

Micropipettes were pulled from borosilicate theta capillary and bevelled minimally on a machine by Stähli (2542 Pieterlen, Switzerland). We found it useful to bevel, not so much to decrease the resistance of the electrode, but because with unbevelled micropipettes the ligand solution often passed from the active to the reference barrel. The taper of the micropipette was heated to 200 °C in a nitrogen atmosphere in an apparatus described previously (Coles & Tsacopoulos, 1977). Usually, but not necessarily, the barrel that had been lowermost during the bevelling was chosen to be the active one. A fine hypodermic needle was inserted into the back of it and dry nitrogen was passed through the needle for 5 min to dry the glass, then dichlorodimethylsilane vapour, carried by nitrogen, was passed for 10 min. The vapour diffused to the tip of the active barrel: silanization of the reference barrel was prevented by forcing a stream of nitrogen out of its tip. Excess silane was flushed with nitrogen from the active barrel for 2 min and the pipette was kept at 200 °C for a further 2 min to allow any residual silane to react with the glass. The times were not critical. A ligand solution, usually one based on potassium tetra-*p*-chlorophenylborate (Corning 477317; Baum & Wise, 1971) was injected into the active barrel from the back to form a column some 5 mm long. An electrolyte solution (see below) was injected into the reference barrel. If there were bubbles in the resin column the pipette was placed in a vacuum for 1 min. Electrical contact with the back of the resin column was made through 0·1 M-potassium chloride and a chlorided silver wire. For the reference barrel the following electrolyte solutions were tried: bee Ringer solution (see Methods), 4 M-sodium chloride, 3 M-potassium chloride, 0·3 M-lithium acetate, 0·3 M-sodium formate. Of these, the 3 M-potassium chloride had a clear effect on the measurements of a_K. Of the other solutions, the lithium acetate had the smallest junction potentials in test solutions (in agreement with Oehme, 1977). When an intracellular recording was made with an electrode containing lithium in the reference barrel there was no evidence of a change in the rate of uptake of potassium.

Subtraction of the reference potential. The potassium signal was the potential difference between the active barrel and the reference barrel of the micro-electrode. To check that changes in the electrical potential at the tip of the electrode had no effect on the potassium signal, a double-barrelled potassium electrode and also a conventional micro-electrode were inserted into one of the large photoreceptor cells in the ventral eye of *Limulus* using techniques described by Lisman & Brown (1971). When a pulse of current was passed through the single micro-electrode, a potential

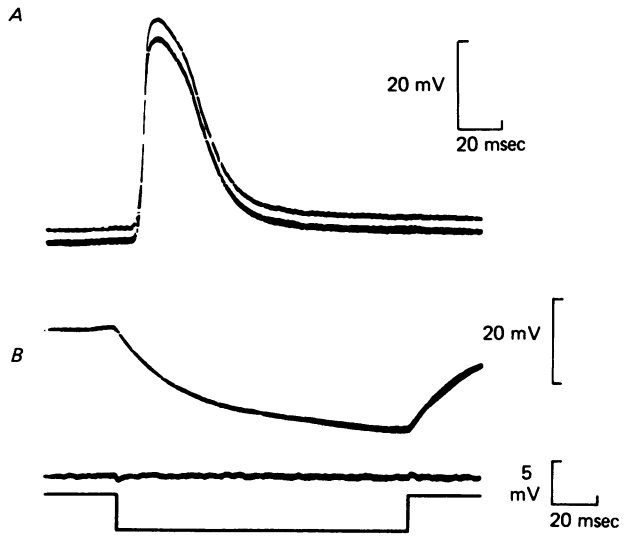

Fig. 13. *A*, a receptor potential recorded from a photoreceptor cell of *Limulus* ventral eye in response to a flash of light. The cell was impaled by a conventional single-barrelled micro-pipette and also a double-barrelled potassium-sensitive micro-electrode. The single micro-electrode and the reference barrel of the potassium electrode and the reference barrel of the potassium electrode recorded identical electrical changes. One of the two traces was displaced vertically so that both could be seen clearly. *B*, immediately after the response shown in *A* a current pulse (10 n*A*) was passed through the single electrode. Top trace: signal from the reference barrel. Middle trace: potassium signal; only small transient electrical artifacts are seen. Bottom line: current monitor.

change was recorded by the reference barrel but, by suitably adjusting the positive capacitative feedback of the headstage amplifiers, electrical artifacts could be almost completely removed from the potassium signal (Fig. 13*B*).

Calibration of the potassium micro-electrodes

Corning resin 477317, which was used for most of the experiments, does not have perfect selectivity for potassium over sodium so that the potassium signal was affected by the presence of sodium. The values of a_{Na} in the cells and extracellular space of the tissue were not known: for the purpose of making a correction it was assumed that the sum of the concentrations of sodium and potassium ions was constant and equal to the value in the Ringer solution,

$$[Na^+]+[K^+] = [C^+], \tag{4}$$

where $[C^+]$ is a constant. The appropriate calibration solutions then consisted of

bee Ringer solution in which varying fractions of the sodium chloride had been replaced by equimolar quantities of potassium chloride. Calibration responses of four electrodes are shown in Fig. 14. The solid line is a form of the Nicolsky equation (Nicolsky, 1937):

$$E = E_0 + RT/F \ln ([K^+] + k\,[Na^+]). \quad (5)$$

E is the potassium signal and E_0 is a constant chosen so that the curves coincide at $[K^+] = 100$ mM. The selectivity factor, k, for Na^+, is taken as 1/70, close to the value obtained by Oehme & Simon (1976). All the calibration solutions have the same ionic strength so, according to the Debye-Hückel theory, the activity coefficient of K^+ should be the same in all of them and equal to that of K^+ in a solution of potassium chloride of the same ionic strength. The value of the molar activity coefficient, y, is taken as 0·702 from National Bureau of Standards tables (Staples, 1971).

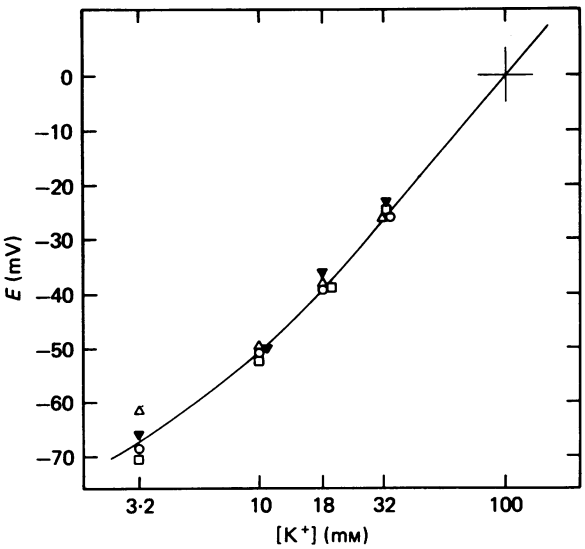

Fig. 14. Typical calibration data from the potassium micro-electrodes. Bee Ringer solution and four modifications of it containing the potassium concentrations shown on the abscissa were made to flow past the electrode (see Fig. 15). The observed potassium potentials, relative to that for $[K^+] = 100$ mM, are plotted in the y direction. Each type of symbol corresponds to one of four sample micro-electrodes that were used for intracellular recording. The continuous line corresponds to eqn. (5): $E = E_0 + RT/F \ln ([K^+] + 1/70[Na^+])$; E_0 was chosen so that $E = 0$ when $[K^+] = 100$ mM.

Available evidence would suggest that in the cells the sum of the sodium and potassium concentrations is less than in the Ringer solution (see, e.g., Brown, 1976, for barnacle photoreceptors) and, further, the activity coefficient of the intracellular sodium may be smaller than in the Ringer solution. On account of these two factors, our calibration method may have led to an underestimate of intracellular a_K. But the error is probably small compared to the standard deviations of the intracellular measurements. For example, eqn. (5) predicts that if $[K^+]$ were 100 mM then changing $[Na^+]$ by as much as 100 mM would lead to an error of only 1·4 %. In the extracellular

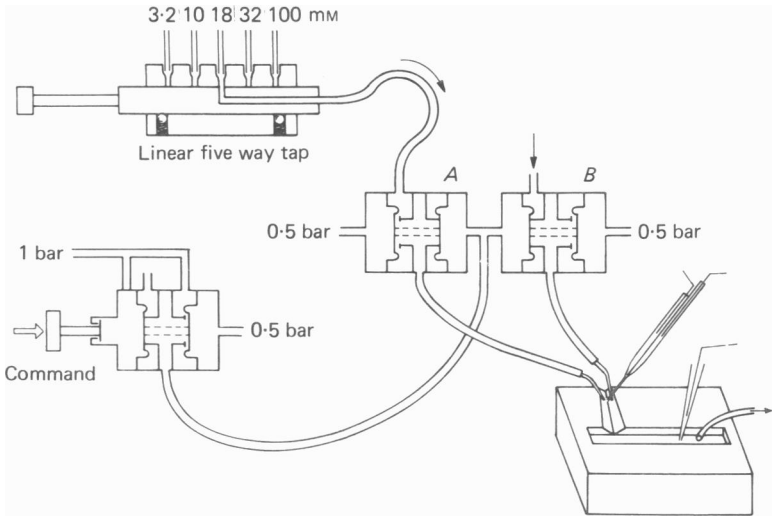

Fig. 15. Apparatus for changing calibration solutions. Part of the system was designed so that any one of five calibration solutions could be selected in order to measure the steady state potassium potential: for this purpose a rapid change was unnecessary. In addition, in order to measure the response time of the electrode, the selected solution could be replaced, with a dead time of msec, by another solution chosen at the beginning of the procedure (usually containing 32 mM-potassium).

For measurement of the steady state potential a solution was selected by means of the five way tap shown upper left in the Figure. The solution flowed to the calibration bath by way of pneumatic valve *A* (Dreloba 0·10 00 24 18 double membrane relay; VEB Reglerwerk Dresden, 806 Dresden, Grosshainer Str. 1–5, East Germany). For steady-state measurements valve *A* was kept constantly open. The solution flowed through a 12 gauge hypodermic needle with a tapered tip into the end of a groove, 1 mm wide, in the surface of a Perspex block. The groove led to a bath that was evacuated at a rate adjusted to maintain a small rivulet of solution in the groove. An agar bridge to a chlorided silver wire was situated downstream. The tip of the potassium electrode was positioned in the beginning of the rivulet, within 100 μm of the tip of the hypodermic needle. To make a rapid solution change, valve *A* was closed and valve *B* opened simultaneously by a pneumatic signal from the remote command valve (Dreloba VTV). The solution that flowed through valve B was brought close to the potassium electrode by a second hypodermic needle so as to keep the dead volume small. The pneumatic valves were connected in a manner devised by Mr J.-L. Munoz that we believe to be novel. Two sources of compressed air were used, at 0·5 bar and 1 bar. An upper limit for the time taken for completion of a solution change is given by the lower trace in Fig. 16*B*.

space, where $a_{Na} > a_K$, the error introduced by assuming an incorrect value for a_{Na} is greater. At the outset of the present work it was assumed that, in the dark, extracellular a_{Na} would be equal to that in the Ringer solution. The observation that the apparent a_K in the extracellular space is higher than that in the Ringer solution tends to question this assumption. However, a high extracellular a_{Na} could not reasonably be the sole explanation for the high apparent a_K: the necessary sodium concentration would have to be more than 400 mM.

Experience showed that an electrode that was stable for 15 min was usually stable for two or three days, so calibrations were made immediately after any

successful recording. The results were discarded if the electrode responded to a change in $[K^+]$ from 3·2 to 100 mM with a potential change of less than 45 mV or if the base line drifted more than 2 mV in 30 min.

The response times of the potassium electrodes. An apparatus, shown in Fig. 15, was constructed for rapidly changing calibration solutions so that the response times of the electrodes could be determined routinely during the calibration procedure. The electrodes used responded to solution changes with a potential change that reached

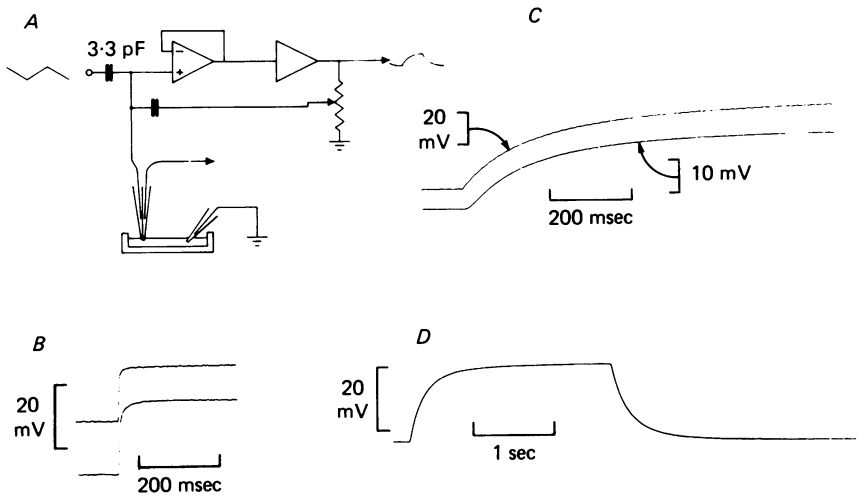

Fig. 16. Tests on the response time of the potassium signal. *A*, the conventional electrical arrangement used for measuring the electrical response time of the amplifier input circuit when connected to a potassium micro-electrode. A triangular-wave voltage was applied to a capacitor (3·3 pF) so as to pass square-wave current to the input of the voltage follower (Teledyne Philbrick 1035.02). The signal was amplified 25 times and a fraction of it was fed back through a capacitor to partially compensate the effect of the input capacitance. The upper traces in *B* and *C* were obtained using this circuit. *B*, responses of a potassium micro-electrode with a large tip (diameter 2 μm). The upper trace shows the response to a current step applied as described in *A*. The lower trace is the response to a rapid change of the solution to one with a higher potassium concentration in the apparatus of Fig. 15. $[K^+]$ was increased from 10 to 32 mM. *C*, responses as for *B* but recorded with a potassium micro-electrode suitable for intracellular recording. The resistance of the references barrel was 65 MΩ and of the active barrel $2{\cdot}7 \times 10^{10}$ Ω. *D*, the lower trace in *C* continued on a slower time scale.

90% of its final value within about 1·5 sec. The apparatus also enabled us to show that the delay was mainly attributable to the time constant of the input circuit, i.e. the source resistance times the input capacitance. The lower trace in Fig. 16*B* shows the response to a solution change of a potassium micro-electrode with a large tip (diameter $\approx$ 2 μm). The potential came to 90% of its final value in about 25 msec. This is an upper limit for the time taken for the solution to change and also for the potassium ions to reach a steady state distribution in the region of the interface between the resin and the solution. The lower trace in Fig. 16*C* shows the response to a solution change of an electrode suitable for intracellular recording. The resistance of the active barrel was $2{\cdot}7 \times 10^{10}$ Ω; the time constant of the response was 175 msec.

The upper trace is the response obtained when a step of current was fed into the back of the electrode as described in the legend. The response time in this case should depend mainly on the input time constant of the circuit and the effectiveness of the input capacity compensation. The similarity of the upper and lower traces shows that the response time to a change in potassium concentration depended mainly on these same factors and not, for example, on diffusion processes at the membrane surface. The final steady value of the potassium signal was reached only after a creep lasting 2–3 sec, as shown in Fig. 16*D*.

Valinomycin-filled electrodes. In addition to having an imperfect selectivity for K^+ over Na^+, Corning resin 477317 has a great sensitivity to acetylcholine and certain other lipophilic cations (Oehme & Simon, 1976). Electrodes in which valinomycin is the ion-selective ligand are relatively insensitive to acetylcholine but have a very high resistance. The resistance can be reduced with little loss of selectivity by the addition of potassium tetra-*p*-chlorophenylporate (Oehme & Simon, 1976; Oehme, 1977). The resistivity of this solution is still greater than that of the Corning resin (Oehme, 1977) and electrodes fine enough for intracellular recording had an unusably high resistance. However, some electrodes with slightly larger tips (0·6–0·8 μm) were made and used for recording extracellularly (Fig. 9). The valinomycin was obtained from Sigma, St Louis, and the tetra-*p*-phenylborate was kindly provided by Dr P. Müller.

APPENDIX 2

The volume and surface area of a drone photoreceptor

Volume. The diameter of the ommatidial group of photoreceptors is 20 ± 2 μm (Perrelet, 1970, Fig. 3), and the length in the dorsal retina is about 400 μm. The volume of one photoreceptor is 1/6 of the volume of the ommatidium = $1{\cdot}2 \times 10^4$ μm^3.

Surface area. The surface area is calculated in two parts: the rhabdomere, and the rest of the surface. The rest of the surface is approximately a right equilateral triangular prism length 400 μm and side 10 μm. This has a surface area of $1{\cdot}2 \times 10^4$ μm^2. The rhabdom has a mean cross section of about 2 μm × 5 μm. The microvilli have an external diameter of 0·08 μm. They are packed in layers 0·08 μm between centres. In one layer the total length of the microvilli is $2 \times 5/0{\cdot}08$ μm. In the whole rhabdom, length 400 μm, the number of layers is 400/0·08. The surface area per unit length of microvillus ($\pi \times$ diameter) = $\pi \times 0{\cdot}08$ μm^2. Hence the total surface area of the microvilli in the rhabdom can be calculated and divided by 6 to give the area corresponding to one cell. The value is $2{\cdot}6 \times 10^4$ μm^2. Adding the value for the rest of the surface, the total surface area is $3{\cdot}8 \times 10^4$ μm^2.

Volume of the extracellular space. If the extracellular clefts have a mean width of 15 nm then the volume of the clefts bounding a photoreceptor (including the spaces between the microvilli) is 15 nm × (the surface area of the cell). This gives $5{\cdot}7 \times 10^2$ μm^3, which is 4·8 % of the estimated volume of the cell. To express the total volume of the extracellular space in the retina relative to the total volume of the photoreceptors small corrections to this percentage should be made: to account for clefts shared by two photoreceptors the percentage should be reduced and to account for clefts shared by two pigment cells the percentage should be increased. We conclude

that the total volume of the extracellular space is about 5% of the volume of the photoreceptors, in agreement with the value found by Shaw (1978) in locust retina.

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