J. exp. Biol. (1981), **95**, 75–92
With 7 figures
Printed in Great Britain

IONIC AND POSSIBLE METABOLIC INTERACTIONS BETWEEN SENSORY NEURONES AND GLIAL CELLS IN THE RETINA OF THE HONEYBEE DRONE

BY J. A. COLES AND M. TSACOPOULOS
Experimental Ophthalmology Laboratory, 22 rue Alcide Jentzer, 1211 Geneva 4, Switzerland and Department of Physiology, Geneva University School of Medicine

SUMMARY

This is a review paper that includes original calculations and figures. The drone retina is composed of two essentially uniform populations of cells, the photoreceptors and the glial cells. The photoreceptors contain many mitochondria but no glycogen has been detected; the glial cells contain much glycogen and very few mitochondria. The oxygen consumption of the photoreceptors in the dark is 20 μl min^{-1} per g of retinal tissue and in response to a single flash of light there is an extra consumption that reaches a maximum of 40 μl min^{-1} per g. In addition, light stimulation of the photoreceptors leads to changes in the glycogen metabolism of the glial cells, and to movements of K^+. Measurements with intracellular K^+-sensitive micro-electrodes showed that during light stimulation with a series of flashes the K^+ activity (a_K) in the photoreceptors fell by an average of 27% while in the glial cells a_K rose by an amount that is estimated to correspond to most of the quantity of K^+ lost by the photoreceptors. The relative contributions to the clearance of extracellular K^+ of extracellular diffusion, spatial buffering and possible net K^+ uptake by glial cells are discussed.

I. INTRODUCTION

Each of the various preparations that have been used for studying glial function has features appropriate for certain kinds of investigation. For example, in this volume, Orkand, Orkand & Tang (1981) describe a preparation containing only glial cells that can be obtained by axotomising *Necturus* optic nerve; and Treherne & Schofield (1981) report work on the well-developed blood–brain barrier formed by peripheral neuroglia in the cockroach central nervous system. The interest of the drone retina seems to us to lie in a histology that suggests a very marked compartmentation of energy metabolism between glial cells and sensory neurones; in the uniform and extensive structure that makes feasible quantitative studies of such functions as oxygen consumption; and in the fact that a direct demonstration has been made of a large flux of potassium ions into the glial cells during quasi-physiological stimulation of the photoreceptor cells.

II. ANATOMY

Nearly all the volume of the retina of the compound eye of the drone is made up of only two types of cells; the *large retinula cells*, which are photoreceptors, and the *outer pigment cells*, which, in the electron-microscope study of Perrelet (1970), are considered to be glial cells. The photoreceptors contain visual pigment and respond directly to light. They are about 400 μm long and are clustered in groups of six so that in cross-section each group (or *retinula*) appears as a rosette about 20 μm in diameter (Fig. 2). The visual pigment is located on tightly packed microvilli that constitute the *rhabdom*, a structure about 2 by 6 μm that runs down the centre of the retinula. Ranged close to the plasma membrane are numerous mitochondria, whose oxygen supply in the intact animal is provided by the tracheoles that run through the proximal half of the retina parallel to and between the retinulas. Apart from the extracellular clefts, which probably make up about 5% of the total volume of the retina (Coles & Tsacopoulos, 1979; cf. Shaw, 1977, 1978), the rest of the space between the retinulas is filled by the pigment cells. About 30 of these surround each retinula and send finlike processes between the photoreceptors towards, but not reaching, the rhabdom; the whole functional unit is known as an ommatidium. In contrast to the photoreceptors, the outer pigment cells contain very few mitochondria, but large quantities of glycogen particles (Perrelet, 1970). Also, only the pigment cells stain with the periodic acid–Schiff reaction, which normally detects glycogen (see Pearse, 1968; A. Perrelet & V. Evêquoz, personal communication). These observations suggest that most of the aerobic metabolism is confined to the photoreceptors, while carbohydrate stores appear to be mainly in the pigment cells. In some mammalian nervous tissue a similar, but less extreme, compartmentation is found (O'Connor, 1977) and there is evidence for compartmentation of certain other metabolic functions, such as amino acid synthesis (see Hertz, 1979).

II. BASIC ELECTROPHYSIOLOGY

Most experiments on the drone retina have been done on the *cut head preparation* (Baumann, 1968). A slice is removed from the caudal aspect of the isolated head to expose layers of ommatidia corresponding to the large dorsal facets (Van Praagh, Ribi, Wehrhahn & Wittmann, 1980). The head is then fixed in the floor of a chamber and superfused with an oxygenated Ringer solution (Fig. 1). Light stimulation is made perpendicular to the cut surface and, except near the cornea, attenuation is less than 0·3 log units at a depth of 300 μm. The visual pigment (unlike that of vertebrates) does not bleach when it is photo-isomerized and, provided that the spectral composition of the light is kept constant, the quantity of pigment in the active form does not change (Bertrand, Fuortes & Muri, 1979).

Three classes of electrical response can be recorded with a glass micropipette in response to intense flashes of light (in the order of 10 mJ/cm^2). The extracellular potentials are predominantly negative-going, have a complex waveform and an amplitude of as much as 15 mV (see Baumann, 1974, and Gardner-Medwin, Coles & Tsacopoulos, 1981). The response recorded from within a photoreceptor is depolarizing and has an amplitude of at least 50 mV, and it arises because light causes an

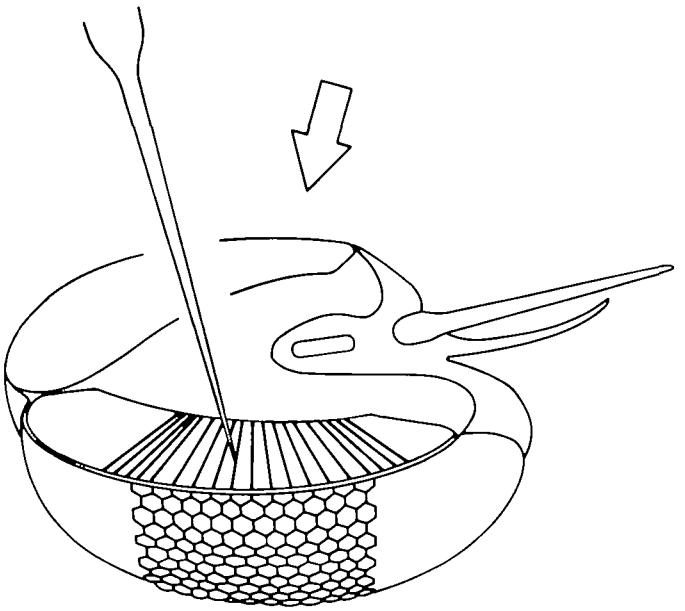

Fig. 1. Scheme of the cut head preparation. The back of the head has been removed by a slice made parallel to a layer of ommatidia (radial lines) and perpendicular to the corneal facets. The head is fixed with wax in the floor of a chamber and superfused with Ringer solution. The diffuse stimulating light (arrow) is incident from above, perpendicular to the physiological direction, and electrophysiological recordings are made by advancing a micropipette into the retina as shown.

increase in the conductance of the membrane to sodium ions (Fulpius & Baumann, 1969). Concomitant with the response of the photoreceptors, the outer pigment cells also undergo a transient depolarization whose maximum amplitude is usually 10–20 mV. This depolarization reaches its peak more slowly than does the receptor potential of the photoreceptors, and has a slower decay (Bertrand, 1974). It seems to be analogous to the response recorded by Orkand, Nicholls & Kuffler (1966) in the glial cells of *Necturus* optic nerve and which they attributed to the increase in extracellular K^+ concentration caused by nerve impulses. This explanation can account reasonably for the time course of the response of the drone pigment cell to a single light flash, and also for the effect on its amplitude of changing the K^+ concentration in the superfusate (Bertrand, 1974). Hence, on morphological, histological and electrophysiological grounds, the outer pigment cells can be identified as glial cells.

The glial cells would also depolarize after a light flash if there were electrical junctions between the glial cells and the photoreceptors and, since we will later be discussing the possibility of a transfer of substances between the glial cells and the photoreceptors, it seems appropriate to list the observations concerning the absence of junctions between the two classes of cell: (1) When the fluorescent dye Lucifer Yellow is injected into a photoreceptor, it passes into thé other large photoreceptors of the retinula, which are known to be electrically coupled (Shaw, 1969), but the dye is not seen in glial cells (A. Perrelet, unpublished). (2) Although gap junctions have been observed histologically between cells of the same type (between photoreceptors, or

between outer pigment cells) they have not been observed between the different classes of cell (Perrelet, 1970, and personal communication). (3) As described in Section V, the intracellular K^+ activities measured by Coles & Tsacopoulos (1979) were significantly different in photoreceptors and glial cells.

We conclude that there are few, if any, junctions between photoreceptors and glial cells. A general review of this question for other tissues can be found in Varon & Somjen, (1979).

IV. METABOLISM

(1) *Oxygen consumption*

The drone retina cannot function without oxygen. If the Ringer solution superfusing the cut head preparation is replaced by one lacking oxygen, then the anaerobic metabolism of the photoreceptors is insufficient to maintain the membrane potential in the dark (M. Tsacopoulos & A. Mauro, in preparation); with light stimulation, anoxia causes complete depolarization within 3 min (Baumann & Mauro, 1973). These observations suggest that the energy metabolism of the photoreceptors is predominantly aerobic, and since nearly all the mitochondria of the retina are in the photoreceptors, the oxygen consumption should be a measure of the rate of the photoreceptor energy metabolism. Knowledge of this should provide an indication of the necessary size of stores of metabolic substrate (Section IV. 2) and of the energy available for sustaining ion fluxes (Section VI).

Tsacopoulos, Poitry & Borsellino (1980, 1981) have shown that (provided the superfusion is well arranged) the diffusion of oxygen into the retina of the cut head occurs essentially in one dimension, perpendicular to the plane of the cut. They further showed that oxygen consumption was independent of P_{O_2} down to a small value (less than 20 torr). With this information they could calculate the steady-state oxygen consumption from measurements of P_{O_2} made with an oxygen microelectrode (tip diameter less than 2 μm) at different depths in the tissue and in the bath. The oxygen consumption they found in the dark at 23 °C was 20 μl min^{-1} per g of tissue, which may be compared to the value of 40 μl min^{-1} per g of tissue found for the isolated retina of the worker bee (with its smaller cells) by Autrum & Hamdorf (1964) (see Tsacopoulos *et al.* (1981) for the conversion of the units). In the drone cut head preparation the oxygen entering through the cut surface is sufficient to maintain a constant oxygen consumption down to a depth of about 460 μm in the dark, and about half of this when the photoreceptors are stimulated by a series of light flashes.

As shown in Fig. 3, a single light flash evokes a change in P_{O_2} within the superfused retina that is readily measurable with an oxygen microelectrode (Tsacopoulos & Lehmenkühler, 1977; Tsacopoulos, 1978). In a preparation oxygenated all the way through (in practice, a slice of retina 300 μm thick) the light-induced change in oxygen consumption can be calculated from the P_{O_2} by the method of discrete Fourier transforms (Poitry & Tsacopoulos, 1981). The extra consumption reaches a peak very rapidly (2–3 s) after the flash and the total extra consumption is about 12 μl per g of tissue for a flash of 40 mJ cm^{-2}. Comparable measurements exist only for frog striated muscle (Mahler, 1978) where the kinetics are found to be much slower.

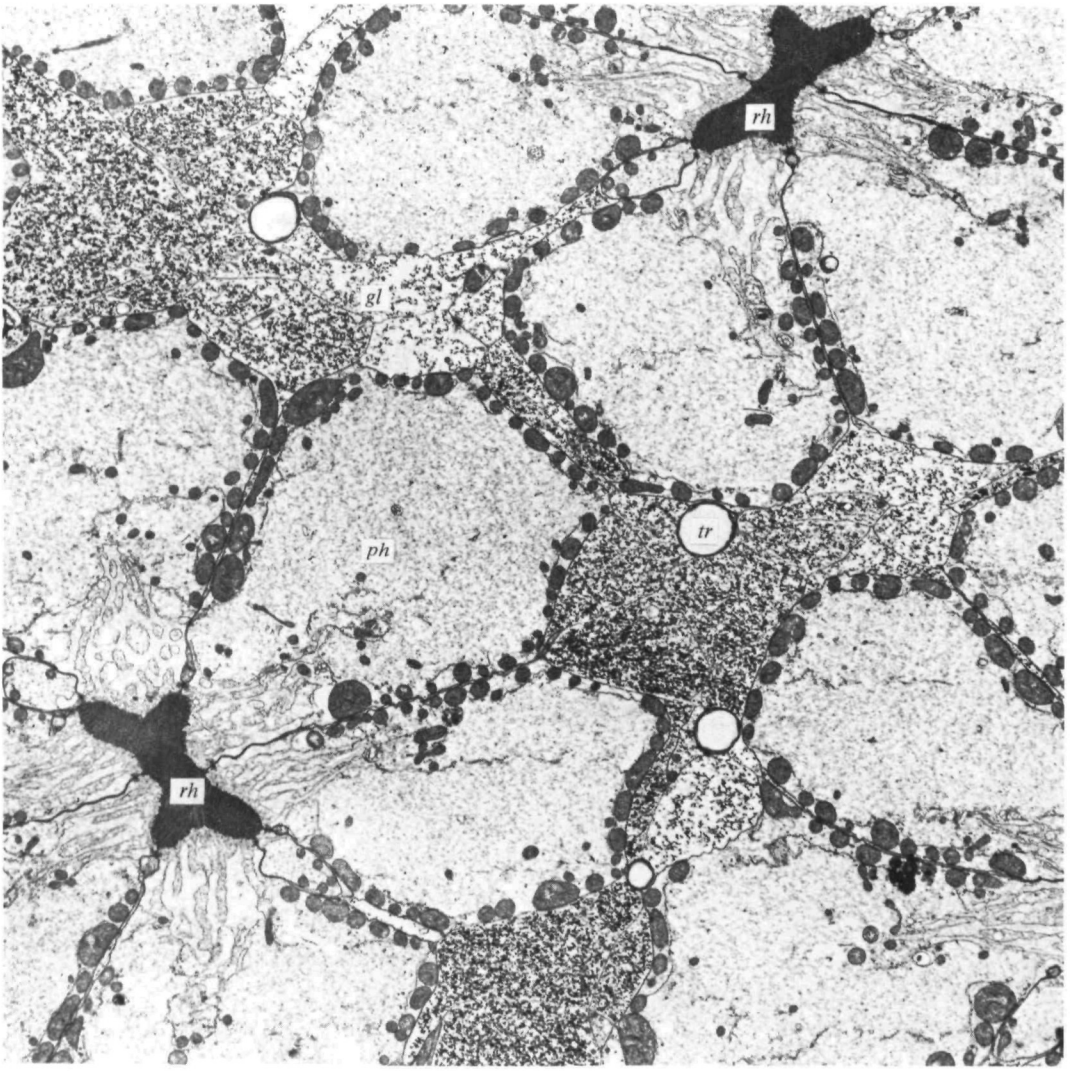

Fig. 2. Section through a part of the compound eye of the honeybee drone, parallel to the surface of the cornea. Parts of four ommatidia are shown, each consisting of a rosette of photoreceptor cells (*ph*) associated with glial cells (outer pigment cells; *gl*). The visual pigment of each photoreceptor cell is located in the membranes of microvilli at the centre of the ommatidium: the juxtaposed arrays of microvilli constitute the rhabdom (*rh*). Each ommatidium has six, large, functionally identical, photoreceptor cells that contain numerous mitochondria situated close to the cell membrane. The glial cells contain very few mitochondria but are packed with glycogen particles; fin-like processes extend from the glia between the photoreceptors almost to the rhabdom. The white circles (*tr*) are the tracheoles that ventilate the tissue. The two rhabdoms in the Plate are about 30 μm apart. This section is closer to the basal membrane than the cornea: distally, the glial cells are larger. Other features, including small photoreceptor cells (not labelled) are described by Perrelet (1970). Photograph by courtesy of Dr A. Perrelet.

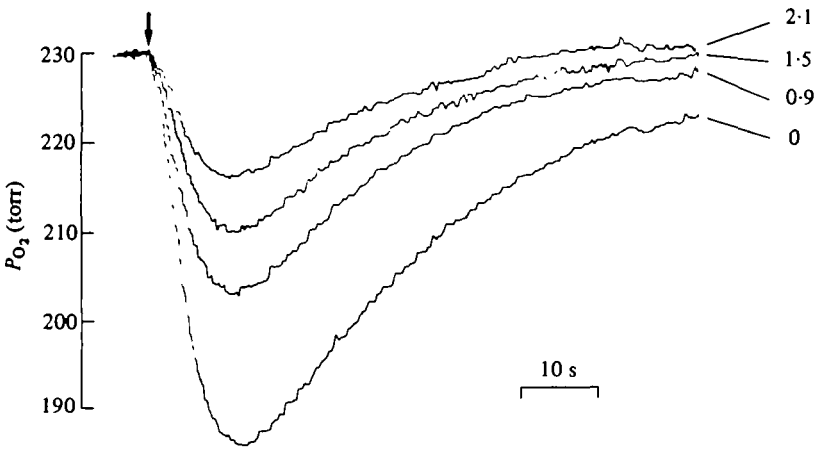

Fig. 3. Changes in P_{O_2} evoked by a brief flash. The head of a drone was embedded in wax and the back of the head sliced off to expose the ommatidia. Because the wax and the cornea are impermeable, oxygen could enter the retina only through the cut surface. The superfusing Ringer solution was oxygenated so that the P_{O_2} in the bulk of the bath was 650 torr; the P_{O_2} at the surface of the retina was 350 torr. A double-barrelled P_{O_2} microelectrode (Tsacopoulos & Lehmenkühler, 1977) was inserted in the retina to a depth of 50 μm. The local P_{O_2} in the dark had a constant value of 230 torr. The figure shows how the local P_{O_2} changed when the retina was stimulated with a 20 ms flash of white light (arrow). Each trace corresponds to a different light intensity and the optical densities of the filters used are shown on the right. For each intensity four individual responses were averaged. Note that to calculate the oxygen consumption from records such as these, more information and a fairly elaborate calculation are necessary (Poitry & Tsacopoulos, 1981).

(2) *What is the source of metabolic substrate?*

The cut-head preparation gives electrical responses to light stimulation for at least 12 h while superfused with a Ringer solution that contains no glucose or other metabolic substrate (F. Baumann & M. Tsacopoulos, unpublished). The oxygen consumption in the dark does not change detectably for about 6 h of this superfusion (Tsacopoulos *et al.* 1981) and light flashes continue to evoke rapid increases in consumption (Tsacopoulos, 1978). It seems clear that the retina is drawing on an internal store of substrate. We estimate that during 12 h of superfusion with intermittent stimulation the average oxygen consumption will be about 40 μl min^{-1} per g of tissue. If the substrate were carbohydrate, as is the case for the flight muscles of the bee (Jongbloed & Wiersma, 1935), then 54 μg min^{-1} per g of tissue would be oxidized, or about 38 mg per g of tissue in 12 h. The tempting hypothesis that the photoreceptors draw on the glycogen store in the glial cells has not been rigorously tested experimentally, but there is clear evidence that when the photoreceptors are stimulated by light the glycogen metabolism is modified. The experiment is the following one (Evêquoz, Deshusses & Tsacopoulos, 1978; Tsacopoulos & Evêquoz, 1980). One eye of a drone is occluded with opaque paint and the other eye is stimulated with bright flashes of light for 30 min. A bolus of radioactive glucose is then injected into the heart, and the light stimulation is continued. At a chosen time (5–60 min) after the injection the head is frozen and it is found that the glycogen of the stimulated eye has a higher radioactivity than that of the unstimulated eye, although the total

quantity of glycogen does not change significantly. Hence, *in vivo*, stimulation of the photoreceptors can lead to an increase in the turnover of the glycogen in the eye, presumably in the glial cells. Some signal, in the broadest sense of the word, must pass from the stimulated photoreceptor to the glial cell. We do not know what the relevant signal is, but a mechanism involving potassium seems to be a possibility. Salem, Hammerschlag, Bracho & Orkand (1975) have shown that the metabolism of glucose in glial cells of *Necturus* optic nerve can be stimulated by increased extracellular K^+ concentration and, as we describe in Section V, in the drone retina there do appear to be substantial movements of K^+ ions at the onset of photostimulation.

V. MEASUREMENT OF POTASSIUM ACTIVITY

Double-barrelled potassium-sensitive microelectrodes of the liquid-membrane type (Walker, 1971) can be made fine enough to give stable recordings from both the photoreceptors and the glial cells of the drone retina (Coles & Tsacopoulos, 1979). The electrodes measure K^+ activity (a_K) rather than concentration and to estimate the latter we assume that the ratio of the two (the activity coefficient) is the same as in the bee Ringer solution (0·70). We consider particularly the transition from darkness to the quasi-steady state produced by stimulation with a train of light flashes each 20 ms long and presented at 1 Hz.

(1) *Photoreceptors*

A typical record is shown in Fig. 4 and averages of results obtained by Coles & Tsacopoulos (1979) are given in Table 1. At the onset of the photostimulation a_K fell by an average of 27%, with a half time of 30 s. *A priori* this change might be due to an efflux of K^+ ions from the cell, an increase in the proportion of intracellular K^+ that is bound, or to an influx of water. The first of these hypotheses seems to be consistent with all the available experimental data and it is the only one we consider further. It is supported by the demonstration of a light-induced efflux of K^+ from invertebrate photoreceptors with radioactive tracers, in *Limulus* ventral nerve (Holt & Brown, 1972) and in crayfish (Stieve & Hartung, 1977). Further, in *Limulus* ventral photoreceptor there is a voltage-dependent K^+ conductance (Millecchia & Mauro, 1969; Pepose & Lisman, 1978) that would tend to facilitate such an efflux. In the drone photoreceptors, the change in a_K seems strikingly large, but if allowance is made for the large surface-to-volume ratio of the cells (1·8 μm^{-1}, due mainly to the microvilli) and for the long duration (50–100 ms) of the receptor potentials induced by the light flashes, then the estimated flux of ions per unit area of surface membrane is not very different from that measured for the squid giant axon during a nerve impulse.

(2) *Extracellular space: the need for a K^+ sink*

At depths of 20–150 μm from the surface of the cut head preparation Coles & Tsacopoulos (1979) found that at the onset of the photo-stimulation the extracellular a_K increased to a maximum and then declined to a plateau that was, on average, 3·5 mM above the value in the dark (Table 1). Fig. 5A shows an extracellular recording made

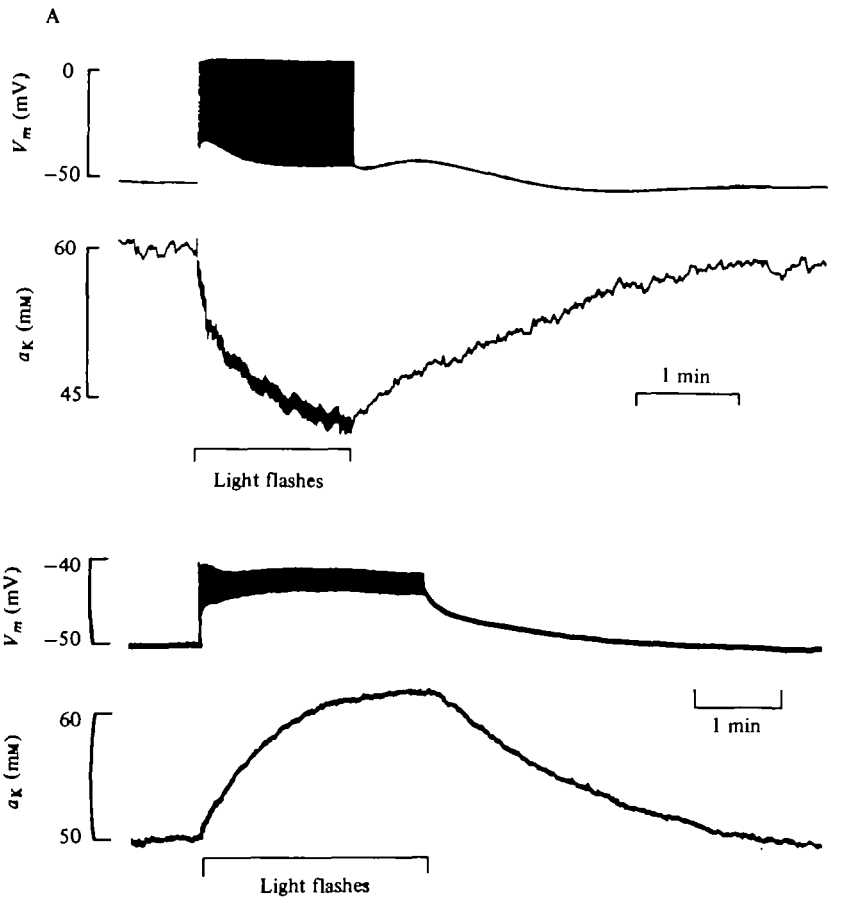

Fig. 4 Changes in intracellular K^+ activity. (A) Recording from a photoreceptor with a double-barrelled K^+-sensitive microelectrode (Coles & Tsacopoulos, 1977). The upper trace is the membrane potential recorded by the reference barrel and shows receptor potentials evoked by a train of light flashes (20 ms, about 0·1 W cm^{-2}) presented 1/s. The after depolarization is described by Baumann & Hadjilazaro (1972). The lower trace is the difference in potential between the two barrels: it is labelled with a K^+ activity scale derived from the calibration curve of this particular electrode. See Coles & Tsacopoulos (1979) for more details. (B) Recording from a glial cell under similar conditions. The electrical responses to the individual flashes appear fused.

Table 1. *Potassium activities and membrane potentials*

	V_m (mV)	a_K (dark) (mM)	Δa_K (mM)	E_K (dark) (mV)
Photoreceptors (n = 11)	−54·0	79	−21·5	−64·1
S.E. of mean	(0·8)	8	2·9	5·3
E.C. space (n = 22)	—	6·3	+ 3·5	—
S.E. of mean	—	0·7	—	—
Glial cells (n = 11)	−53·1	52	+14·0	−53·5
S.E. of mean	(0·9)	4	1·6	4·8

Summary of results from Coles & Tsacopoulos (1979). Only photoreceptors and glial cells with dark membrane potentials more negative than −50 mV are included. Note the relation between membrane potential, V_m, and potassium equilibrium potential, E_K. Δa_K is the change in a_K induced by stimulation with a series of light flashes: for extracellular space it refers to the plateau value rather than the maximum.

under different, but better defined, conditions: a precise depth in a fully oxygenated slice of retina. During the photostimulation the K^+ voltage signal increased and at the end it transiently undershot the dark level. With the aid of the calibration curve of the electrode the K^+ concentration was calculated for the first 80 s of the stimulation and this is shown as the solid line in Fig. 5B: the maximum concentration is about 20 mM. Since we know the efflux of K^+ from the photoreceptors, we can make assumptions about its fate in the extracellular space, calculate the predicted change in extracellular K^+ concentration, and test the assumptions by comparing the predictions with the experimental measurement.

The analysis is greatly simplified if we approximate the time course of the change in the K^+ concentration in the photoreceptors by an exponential:

$$\Delta K_i(t) = \Delta K_i(\infty)(1 - \exp(-t/\tau)), \quad (1)$$

where ΔK_i is the change in intracellular concentration at time t, $\Delta K_i(\infty)$ is the change when a steady state is reached and τ is the time constant. $\Delta K_i(\infty)$ is estimated to be $-30{\cdot}7$ mM and τ is 45 s (with large standard errors: see Table 1 and Coles & Tsacopoulos, 1979). As a first case we predict how the K^+ concentration in the extracellular space would increase if there were no diffusion or other process tending to clear it. This was shown by the dotted line in Fig. 5B, which makes it evident that some kind of clearance must occur. We next consider diffusion to the bath through the extracellular clefts. The physical parameters involved have been discussed by Gardner-Medwin (1980): it is necessary to know the extracellular space fraction, α, which we take to be 5% (Coles & Tsacopoulos, 1979) and the tortuosity factor, λ, which we take to have the value measured in vertebrate brain (1·6; Nicholson, Phillips & Gardner-Medwin, 1979, and references therein). The prediction for the change in extracellular K^+ concentration as a function of time at the centre of a slice of retina 300 μm thick and responding to light all the way through is shown as the dashed line in Fig. 5B (the calculation being outlined in the Legend). The calculated concentration has a maximum value more than three times the measured one, and it seems clear that there must be another sink for K^+ from the extracellular space, in addition to the bath. The only evident one is the glia and in the next section we describe measurements of intracellular a_K in these cells.

(3) *Glial cells*

The magnitudes of the resting potentials of the glial cells, measured with the reference barrel of a K^+ electrode, were about 50 mV, considerably lower than those of glial cells in most other preparations (see, for example, Somjen, 1975; Bracho, Orkand & Orkand, 1975). However, neither Bertrand (1974) nor Coles & Tsacopoulos (1979) were able to record larger resting potentials with fine, single-barrelled electrodes either in the cut-head preparation or in the retina *in situ*. As found by Nicholls & Kuffler (1964, 1965) for glial cells in the leech, and predicted by Kuffler, Nicholls & Orkand (1966) for those in *Necturus* optic nerve, a_K in the drone glial cells in the dark coincided (within experimental variability) with that corresponding to Nernst equilibrium (Table 1). During photostimulation a_K rises, as shown in the recording in Fig. 4B. The mean increase was 14 mM, from a dark level of 52 mM; the half time of the rise was variable, but not obviously different from that of the corresponding fall in the photoreceptors.

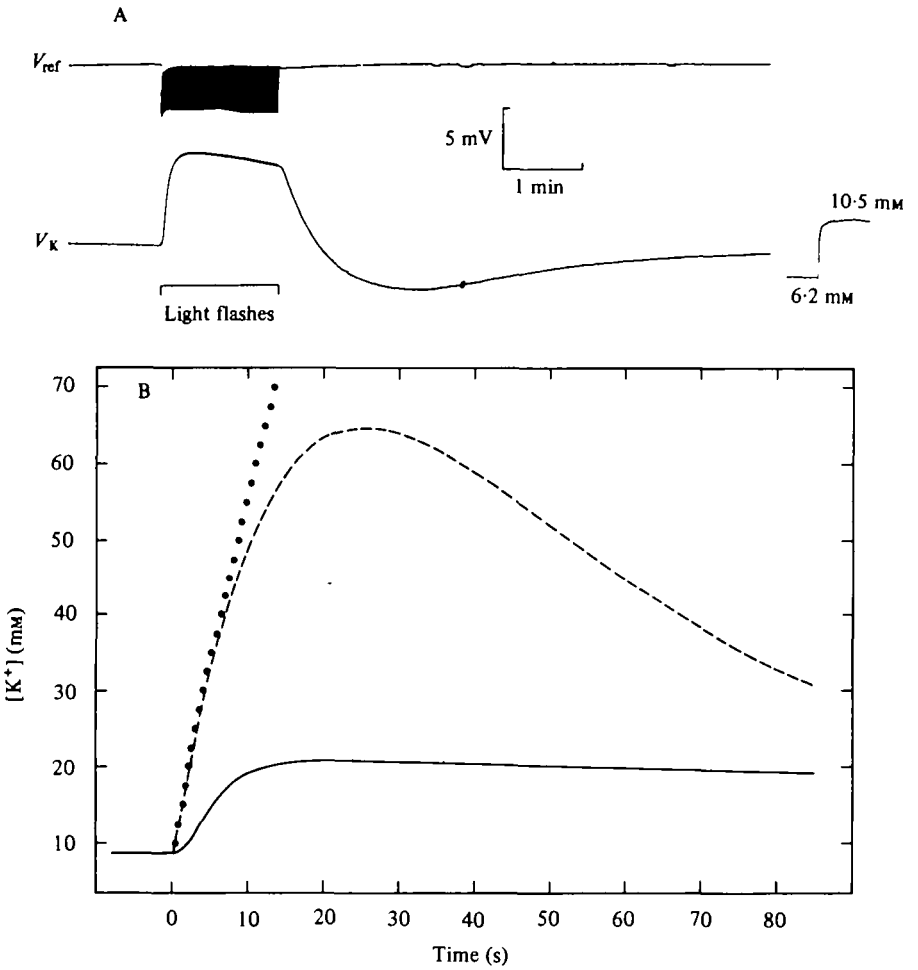

Fig. 5. Light-induced changes in K^+ concentration in the extracellular space.

(A) *Sample recording.* A double barrelled K^+ electrode was constructed with a large bevelled tip (2·5 μm at the base of the bevel). The sensor, a gift from Dr D. Ammann, had the composition (by weight): valinomycin, 5 %, K tetra (*p*-chlorophenyl) borate, 2 %; 2,3-dimethylnitrobenzene, 93 %. The electrode was advanced obliquely to a depth of 150 μm into a slice of drone retina about 500 μm thick, superfused on both faces with a Ringer solution containing 6·2 mg ion/l K^+. Measurements of intracellular receptor potentials and tissue P_{O_2} have shown that under these conditions, all the tissue is adequately oxygenated. The electrode initially recorded a high K^+ concentration, but after 43 min, when the record begins, it had fallen to a fairly steady value. As in Fig. 4, the upper trace shows the electrical response to a series of 90 light flashes and the lower trace is the difference in potential between the two barrels. The short section to the right shows the response of the electrode to a solution change in a calibration chamber (see fig. 15 in Coles & Tsacopoulos, 1979): the K^+ concentration was increased from 6·2 to 10·5 mM.

(B) *A comparison of an observed light-induced change in extracellular K^+ concentration with two hypothetical cases.* The solid line is the K^+ concentration derived from the ion signal in (A). The light stimulation begins at time zero. The two other curves are calculated on the assumption that the K^+ concentration in all the photoreceptors in the slice decreases exponentially according to equation (1):

Case 1 (dotted line): no diffusion or other clearance process. Coles & Tsacopoulos (1979) estimate that the volume of the extracellular space is 5/38 times that of the photoreceptors. Hence, K^+ concentration in the extracellular space will increase 38/5 times more quickly

Mori, Miller & Tomita (1976) recorded an increase in intracellular a_K in the glial cell (Müller cell) of the vertebrate retina during spreading depression, but Coles & Tsacopoulos, 1979, appears to be the first report of an increase in a_K in a glial cell during quasi-physiological stimulation of a tissue, so it is appropriate to consider the validity of these results. (1) Cells gave repeated, closely reproducible responses to light stimulation for as long as the dark membrane potential remained constant (a maximum of 25 min). (2) The change in the glial cells was an increase of a_K, while that in the photoreceptors was a decrease. If both responses are artifacts of some kind of cell damage, then the difference in sign seems somewhat surprising. (3) A drawback of the K^+ sensor used in the intracellular electrodes (Corning 477317) is that it is highly sensitive also to quaternary ammonium ions, including acetylcholine. However, in the extracellular space, at least, no differences have been observed between the light-induced ion signals recorded with this sensor or with other sensors more highly selective for K^+ (see Fig. 5A). (4) But the strongest argument for the validity of the results in the cut-head preparation is the self-consistency of the results: this is described in the next section.

(4) *Different routes of K^+ clearance from the extracellular space*

Some of the potassium that is released by the photoreceptors diffuses through the extracellular clefts to the bath or, in the cut-head preparation, to the deep inactive layers of the retina. We feel that, at present, the uncertainties in the values of the parameters make an elaborate calculation unjustified, but a rough estimate can be made on the basis of an extracellular K^+ concentration gradient such as that in Fig. 6. We calculate that at a distance of 150 μm from the bath, probably less than 20% of the K^+ that leaves the photoreceptors is cleared by diffusion through the extracellular space. The total quantity of additional K^+ that remains in the extracellular space is also comparatively small (because both the K^+ concentration and the volume are small). Is the quantity that enters the glial cells sufficient to account for the rest?

---

than it decreases in the photoreceptors (equation 1). The extracellular K^+ concentration quickly rises to absurdly high values.

Case 2 (dashed line): diffusion through the extracellular clefts. The one-dimensional diffusion equation in the extracellular space is:

$$\frac{\partial}{\partial t} K_o(x,t) = F(t) + \frac{D}{\lambda^2} \frac{\partial^2 K_o}{\partial x^2}$$

where $K_o(x,t)$ is the extracellular concentration, D is the diffusion coefficient of K^+ in the extracellular fluid, λ is the tortuosity factor and $F(t)$ is the flux of K^+ into the extracellular space per unit volume of extracellular space. We take $D = 1{\cdot}5 \times 10^{-5}$ cm^2 s^{-1} and $\lambda^2 = 2{\cdot}5$ (see, for example, Gardner-Medwin, 1980). $F(t)$ is given by:

$$F(t) = -(38/5) \times d/dt\,(\Delta K_i)$$

where $\Delta K_i(t)$ is the change in concentration in the photoreceptors (equation 1) and, as for Case 1, 38/5 is the ratio of the volume of the photoreceptors to that of the extracellular space. A simple analytical solution is obtained if we consider $K_o(t)$ at the centre of a plane parallel slice of retina with both faces rapidly superfused with Ringer solution (Carslaw & Jaeger, 1959; equation 12·6 (20)). The curve in the figure was kindly calculated by Mr S. Poitry for a slice 300 μm thick.

We conclude from this figure that extracellular diffusion alone is quite inadequate to account for the K^+ clearance under these conditions.

Coles & Tsacopoulos (1979) estimated that the total volume of the outer pigment cells in the retina was about 1·5 times the volumes of the photoreceptors. If the increase in a_K in the glial cells (14 mM) is multiplied by this factor the value obtained is close to the decrease in a_K measured in the photoreceptors (21·5 mM, see Table 1). Hence, within the uncertainties of the values, all the potassium released by the photoreceptors is reasonably accounted for.

(5) *The entry of potassium into the glial cells: spatial buffering*

The different mechanisms by which potassium might enter cells have been reviewed by Gardner-Medwin (1980). One such mechanism, the 'spatial buffering' proposed by Orkand *et al.* (1966), appears to make a major contribution to the entry of potassium into the glial cells in the experiments just described on the drone retina, and we discuss this first.

Spatial buffering is a process by which an increase in the quantity of K^+ in the extracellular space in a limited region of tissue is spread over a larger volume by passive movement of K^+ through cells; it operates when an unexcited cell, or an electrically coupled syncytium of such cells, extends from the region of high extracellular K^+ concentration to regions of lower concentration. The mechanism works best if the surface membranes are selectively permeable to K^+ and hence glial cells are likely to be more effective than neurones, but for generality we call them 'transfer cells' (Gardner-Medwin, 1981*b*). The net K^+ flux across any part of the surface membrane of a transfer cell is zero when the membrane potential is at the Nernst equilibrium potential for K^+. If the extracellular K^+ concentration rises, as normally occurs in a region of tissue where neurones are active, then K^+ will enter the transfer cells. If all parts of the cell membrane were confronted by the same increased K^+ concentration then the entry of only a very small quantity, enough to increase the intracellular concentration by a few μmoles/l, would suffice to depolarize the membrane capacitance to the new Nernst equilibrium potential. However, parts of the transfer cell (or the syncytium) extend to regions where the extracellular K^+ concentration has not increased and, by electrical coupling, the membrane potential in the active region is prevented from reaching the new Nernst equilibrium. Consequently, K^+ continues to enter the cells in the active region. Conversely, the membranes in the inactive region are *depolarized* by the electrical coupling and here there is an efflux of K^+. Electrical current, carried by unspecified ions, flows intracellularly through the transfer cell syncytium from the active region to the inactive region to maintain the transmembrane K^+ fluxes and there are necessarily return current loops in the extracellular space. These extracellular currents are associated with extracellular potential gradients, and measurement of these gradients can provide a basis for estimating the magnitude of the spatial buffering of K^+ (Gardner-Medwin, Gibson & Willshaw, 1979). The circumstances in which spatial buffering makes an appreciable contribution to clearance of raised extracellular K^+ concentration are being investigated in a number of laboratories (e.g. Gardner-Medwin, 1977, 1980, 1981*a*; Nicholson *et al.* 1979; Dietzel, Heinemann, Hofmeier & Lux, 1980).

An estimate of the contribution of spatial buffering to the K^+ movements in the drone cut head preparation has been made by Gardner-Medwin *et al.* (1981). Extracellular K^+ concentrations and also extracellular potentials were measured at different

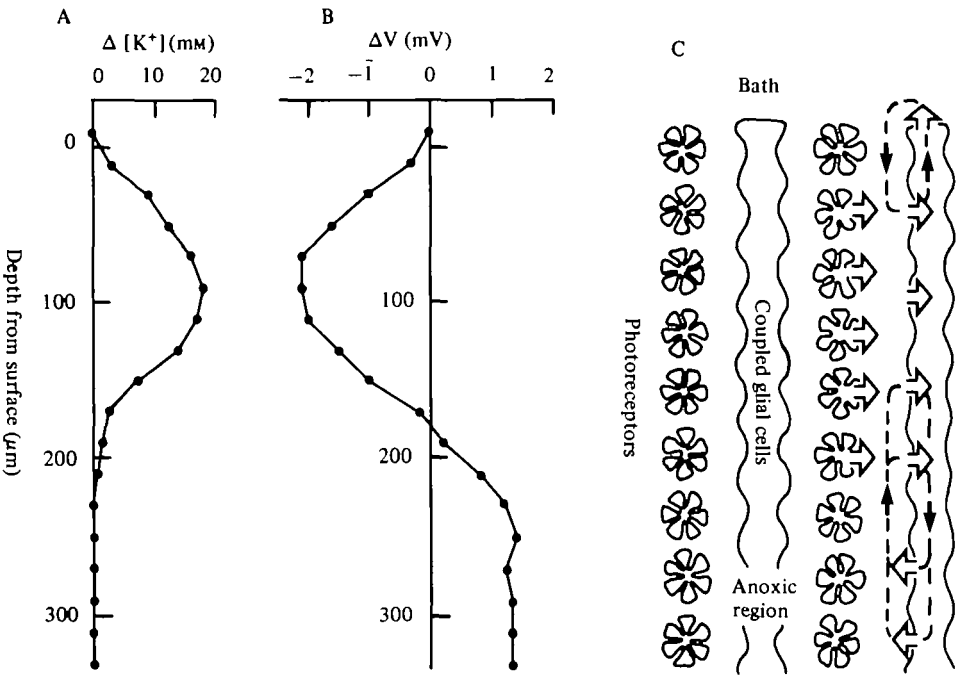

Fig. 6. Extracellular gradients and spatial buffering. A double-barrelled K^+-sensitive micropipette was advanced 400 μm into the retina of the cut-head preparation and then withdrawn in steps. At each depth a train of 20 flashes was presented, 1/s, and extracellular K^+ and voltage signals similar to those in Fig. 5A were recorded. The change in K^+ concentration from the dark level 5 s after the onset of stimulation is plotted in (A). The slow component of the voltage response described by Gardner-Medwin *et al.* (1981) was also measured and is plotted in (B). (C) A schematic interpretation of the results. The diagram represents a section of the retina drawn on the same scale as the vertical axes of the graphs (A) and (B). The photoreceptors are shown as rosette-like clusters. The glial cells, coupled by gap junctions, actually fill most of the intervening space (see Fig. 2), but for schematic purposes they are shown as syncytial slabs running vertically between columns of retinulas. The volume of the extracellular space is greatly exaggerated. Broken lines and black arrows show the directions of current flow and the white arrows show the K^+ flux across the photoreceptor and glial cell membranes during stimulation. Redrawn from fig. 2 of Gardner-Medwin *et al.* (1981).

distances from the superfused surface of the retina. During light stimulation the photoreceptors released K^+ into the extracellular space in an active layer some 200 μm thick (Fig. 6A).The extracellular potential evoked by a light flash in this region was resolved into two components, a rapid one, corresponding to current that entered the photoreceptors and left through their axons, and a slower one corresponding to a current that was deduced to enter the glial cells. Measurement of the slow component at different depths (Fig. 6B) showed that this current flowed in two loops. It entered the glial cells in the active region and left either close to the bath or in the deep anoxic layers where the photoreceptors did not respond to light. The current loops were completed through the extracellular space as shown in the diagram in Fig. 6C. The changes in intracellular a_K measured in the glial cells (Section V. 3) fit with the idea that the current entering the glial cells is carried by K^+ ions.

In other tissues where spatial buffering has been studied, both neurones and glial syncytia extend over distances of several hundred microns and it has not been entire

certain which cell type subserved the spatial buffering: in the drone retina it is unambiguously the glia, at least in the direction perpendicular to the axes of the ommatidia, since the photoreceptors in adjacent retinulas are isolated from each other.

To estimate the *magnitude* of the spatial buffering current in the drone retina Gardner-Medwin *et al.* (1981) made calculations that assumed a value of the extracellular space fraction, α, of 5%, and of the tortuosity factor, λ, of 1·6 (see Section V. 2). It was concluded that within the uncertainties of the calculation, the ionic current that entered the glial cells was sufficient to account for the dispersal of the K^+ released by the photoreceptors. (Alternatively, we note that this is independent evidence, not depending on intracellular recordings, that the photoreceptors lose a substantial fraction of their potassium during stimulation.)

In most cells, K^+ is thought to be the principle diffusible ion in the cytoplasm so that within the glial syncytium the current will be carried mainly by K^+, and the quantity of K^+ that accumulates in the glial cells in the active region should be much less than the quantity lost from the excited cells. In the drone retina the two quantities appear to be of similar magnitudes. A possible explanation for this is that the free K^+ (74 mg ion/l) represents only a small fraction of the total osmotically active particles (587 mg species/l) and might have a transport number roughly in this proportion (74/587) in the cytoplasm and in the intercellular junctions. This hypothesis requires that most of the other particles be electrically charged, and their nature is not obvious; it is desirable that the composition of the cytoplasm be studied further.

(6) *Are there additional mechanisms by which K^+ enters the glial cells?*

In those tissues in which extracellular potassium dispersal has been analysed most fully, the most satisfactory explanation of the data seems to be that over distances of more than a few hundred microns potassium is cleared mainly by spatial buffering and partly by net uptake into cells (Gardner-Medwin, 1981*a*). The analysis just described shows that in the drone cut-head preparation spatial buffering apparently plays a major role, but it is not precise enough to decide whether or not there are significant contributions from other processes, such as, for example, entry of K^+ together with Cl^-, or Na^+/K^+ exchange. There, are indeed, observations, which we now summarize, that suggest that perhaps the latter process might occur.

Firstly, the photoreceptors in the cut drone head continue to respond to light stimulation for up to 12 h when the Ringer solution contains no sodium (Fulpius & Baumann, 1969) and the removal of calcium as well does not abolish the response (J. A. Coles, unpublished). Fulpius & Baumann (1969) suggested that the glia might contain a store of sodium which could be released to prevent depletion in the extracellular space. Secondly, spatial buffering in mammalian brain is associated with changes in the volume of extracellular space (Dietzel *et al.* 1980), but in the drone retina the volume changes appear to be smaller than would be predicted (Coles *et al.* 1981). Thirdly, Tsacopoulos & Coles (1978) found that after superfusing the retina with Ringer solution that contained strophanthidin-k, a specific inhibitor of (Na^+,K^+)-ATPase, (2×10^{-5} M for 2 min) the light-induced changes in glial a_K were smaller than in the control. This treatment with strophanthidin caused no detectable modification of the

light-induced changes in a_K in the photoreceptors nor, paradoxically, in the extracellular space.

These observations are inconclusive, but they do suggest that it would be worthwhile to continue to investigate the mechanism by which K^+ enters the glial cells. In doing this, it may prove necessary to take a less simplistic view of the extracellular space, since there is evidence in other species that the activity coefficients for cations may be much less than in the Ringer solution (Treherne & Schofield, 1981) and that the osmolarity may vary (Kraig & Nicholson, 1978).

VI. A COMPARISON OF OXYGEN CONSUMPTION AND THE ESTIMATED ENERGY COST OF THE LIGHT-INDUCED ION MOVEMENTS

In the photoreceptors, light stimulation activates channels in the surface membrane that admit a current carried mainly by sodium ions (Section III). This sodium must be pumped out of the cell again and the work required is one evident use of the ATP that will be produced by the aerobic metabolism. We now use the results obtained with K^+-sensitive microelectrodes to make a tentative estimate of the quantity of ATP consumed by the extra work of the pump. If the cell is considered before and after a receptor potential, when the membrane has repolarized, it is clear that the electric charge carried into the cell by the Na^+ must be balanced by other charge movements. A small contribution is made by an inward movement of Cl^- ions (Fulpius & Baumann, 1966) and some current passes down the photoreceptor axon (Gardner-Medwin *et al.* 1981), But, presumably, most of the charge is balanced by the outward movement of K^+. Hence, we infer that the fluxes of K^+ across the surface membranes of the photoreceptors are rather smaller than the fluxes of Na^+. However, in order to make an approximate calculation, we assume that there is a one-for-one exchange of Na^+ for K^+.

During stimulation with a series of light flashes, the photoreceptor reaches a quasi-steady state in which the Na^+ that enters the cell after a flash is pumped out again before the next one. Concomitantly there must be fluctuations in the intracellular K^+ concentration. Although corresponding fluctuations can be detected in the extracellular space with an ion-selective microelectrode, fine, intracellular electrodes, such as that used for the recording in Fig. 4A, cannot follow the small, rapid (1 Hz) fluctuations in intracellular a_K; so in order to estimate the Na^+ influx per flash we consider the initial rate of change of intracellular K^+ at the onset of photostimulation. At the very beginning of the stimulation there will be no additional Na^+ in the cell to be pumped out, i.e. the only significant Na^+ flux will be *into* the cell, and, hence, the rate of change of the intracellular K^+ concentration will be a measure of this flux. The value given by equation (1) is 0·7 mM s^{-1}. Coles & Tsacopoulos (1979) estimated that the photoreceptors occupy about 38% of the volume of the retina, so this concentration change is equivalent to 0·27 μmol per flash per g of tissue. In excitable tissues the sodium pump usually hydrolyses one ATP molecule for roughly three Na^+ ions transported (see Glynn & Karlish, 1975), so the estimated requirement for the drone retina is $0{\cdot}27/3 = 0{\cdot}09$ μmoles ATP per flash per g of tissue. The extra oxygen consumption in the steady state during stimulation with a series of flashes

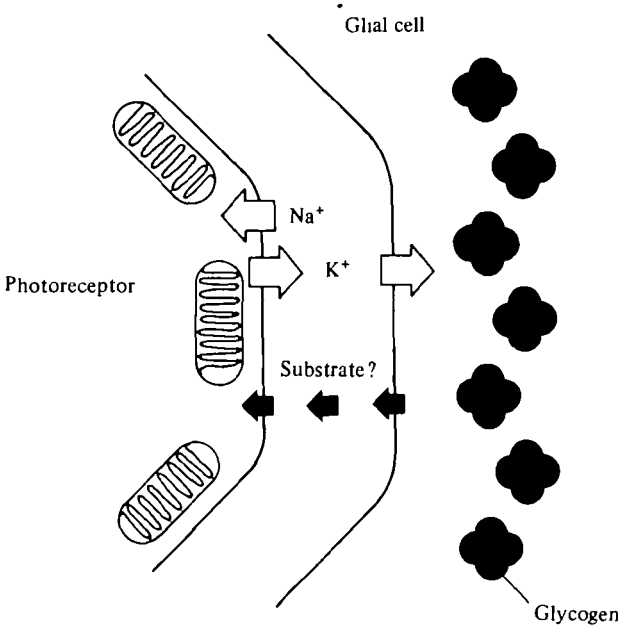

Fig. 7. Summary of the conclusions. When rhodopsin molecules in the surface membrane of the photoreceptor cell (on left) are photoisomerized, membrane channels open and Na^+ ions enter (Fulpius & Baumann, 1969). Simultaneously, K^+ ions leave the photoreceptor and most of them enter the glial cells (Coles & Tsacopoulos, 1979). The arrival of this K^+, or some other, unknown, signal, stimulates glycogen metabolism (Tsacopoulos & Evêquoz, 1980). An unidentified substrate is probably supplied by the glial cells to maintain the metabolic activity of the mitochondria in the photoreceptors.

about the same intensity is 0·040 ml min^{-1} per g of tissue (Tsacopoulos *et al.* 1980). This is equivalent to 0·030 μmole of oxygen per g of tissue per flash. If the substrate of the photoreceptor metabolism were glucose, then consumption of one molecule of oxygen would correspond to the production of six molecules of ATP (see, for example, Stryer, 1975), i.e. $6 \times 0{\cdot}03 = 0{\cdot}18$ μmole of ATP per g of tissue. Hence, there seems to be ample ATP to sustain the observed ion fluxes in the photoreceptors.

In the glial cells the K^+ fluxes associated with spatial buffering will be driven entirely by the changes in extracellular K^+ concentration. In addition, the anaerobic breakdown of glycogen into a substrate that can be transferred to the photoreceptors (glucose or pyruvate are *a priori* possibilities) would be associated with the production of ATP that could be used to pump K^+ if, in addition to spatial buffering, there is a net uptake during activity (Section V. 6).

VII. CONCLUSIONS

The results demonstrate the intimate dependence of the photoreceptors on the glial cells, firstly, for preventing large changes in extracellular K^+ concentration, and, secondly, for the possible supply of metabolic substrate. This is summarized in Fig. 7. The fractional changes in intracellular a_K in the photoreceptors and the glial cells might be large enough to affect the functioning of these cells and it is perhaps note-

worthy that the surface-to-volume ratio of the photoreceptors is less than that of cylinder 2 μm in diameter: dendrites and axons in vertebrate nervous tissue are often finer than this and it seems possible that significant changes in intracellular a_K may occur in them too.

Two lines of enquiry seem evident: (1) By what mechanism, in addition to spatial buffering, does potassium enter the glial cells? (2) What is the identity of the metabolic substrate for the photoreceptors? How is it transported from the glial cells? And by what signal does photoreceptor activity affect glycogenolysis in the glia?

We thank Dr F. Baumann and other colleagues in Geneva for helpful discussion, and also Drs A. R. Gardner-Medwin & C. J. Karwoski for commenting on an early draft of the manuscript. This work was supported in part by the Swiss National Fund for Scientific Research, grant no. 3.399.78, and the Kernen Foundation.

REFERENCES

AUTRUM, H. & HAMDORF, K. (1964). Der Sauerstoffverbrauch des Bienenauges in Abhängigkeit von der Temperatur bei Belichtung und im Dunkeln. *Z. vergl. Physiol.* **48**, 266–269.

BAUMANN, F. (1968). Slow and spike potentials recorded from retinula cells of the honeybee drone in response to light. *J. gen. Physiol.* **52**, 855–875.

BAUMANN, F. (1974). Electrophysiological properties of the honeybee retina. In *The Compound Eye and Vision of Insects.* (ed. G. A. Horridge), pp. 53–74. Oxford: Clarendon Press.

BAUMANN, F. & HADJILAZARO, B. (1972). A depolarizing aftereffect of intense light in the drone visual receptor. *Vision Res.* **12**, 17–31.

BAUMANN, F. & MAURO, A. (1973). Effects of hypoxia on the change in membrane conductance evoked by illumination in arthropod photoreceptors. *Nature, New Biol.* **244**, 146–148.

BERTRAND, D. (1974). Etude des propriétés électrophysiologiques des cellules pigmentaires de la rétine du faux-bourbon (*Apis mellifera*). Thesis no. 1650, University of Geneva.

BERTRAND, D., FUORTES, G. & MURI, R. (1979). Pigment transformation and electrical responses in retinula cells of drone, *Apis mellifera* ♂. *J. Physiol., Lond.* **296**, 431–441.

BRACHO, H., ORKAND, P. M. & ORKAND, R. K. (1975). A further study of the fine structure and membrane properties of neuroglia in the optic nerve of *Necturus*. *J. Neurobiol.* **6**, 395–410.

CARSLAW, H. S. & JAEGER, J. C. (1959). *Conduction of Heat in Solids*, 2nd ed. London: Oxford University Press.

COLES, J. A. & TSACOPOULOS, M. (1977). A method of making fine double-barrelled potassium-sensitive micro-electrodes for intracellular recording. *J. Physiol., Lond.* **270**, 13–14P.

COLES, J. A. & TSAGOPOULOS, M. (1979). K^+ activity in photoreceptors, glial cells and extracellular space in the drone retina: changes during photostimulation. *J. Physiol., Lond.* **290**, 525–549.

COLES, J. A., TSACOPOULOS, M., RABINEAU, P. & GARDNER-MEDWIN, A. R. (1981). Movement of potassium into glial cells in the retina of the drone, *Apis mellifera*, during photostimulation. In *Ion-selective Microelectrodes and Their Use in Excitable Tissues* (ed. E. Sykova). New York: Plenum.

DIETZEL, I., HEINEMANN, U., HOFMEIER, G. & LUX, H. D. (1980). Transient changes in the size of the extracellular space in the sensorimotor cortex of cats in relation to stimulus induced changes in potassium concentration. *Expl Brain Res.* **40**, 432–439.

EVÊQUOZ, V., DESHUSSES, J. & TSACOPOULOS, M. (1978). The effect of photostimulation on the glycogen turnover in the retina of the honey-bee drone. *Experientia* **34**, 897.

FULPIUS, B. & BAUMANN, F. (1966). Influence du Na^+, du Ca^{++}, du K^+ et du Cl^- sur le potentiel récepteur de la cellule rétinienne du faux-bourdon. *Helv. Physiol. Pharmacol. Acta* **24**, C86–87.

FULPIUS, B. & BAUMANN, F. (1969). Effects of sodium, potassium, and calcium ions on slow and spike potentials in single photoreceptor cells. *J. gen. Physiol.* **53**, 451–561.

GARDNER-MEDWIN, A. R. (1977). The migration of potassium produced by electric current through brain tissue. *J. Physiol., Lond.* **269**, 32–33P.

GARDNER-MEDWIN, A. R. (1980). Membrane transport and solute migration affecting the brain cell microenvironment. In Nicholson, C., *Dynamics of the Brain Cell Microenvironment. Neurosciences Research Program Bulletin* **18**, 208–226.

GARDNER-MEDWIN, A. R. (1981*a*). The role of cells in the dispersal of brain extracellular K^+. In *Ion-selective Microelectrodes and Their Use in Excitable Tissues* (ed. E. Sykova). New York: Plenu

GARDNER-MEDWIN, A. R. (1981*b*). Possible roles of vertebrate neuroglia in potassium dynamics, spreading depression and migraine. *J. exp. Biol.* **95**, 111–127.

GARDNER-MEDWIN, A. R., COLES, J. A. & TSACOPOULOS, M. (1981). Clearance of extracellular potassium: evidence for spatial buffering by glial cells in the retina of the drone. *Brain Res.* **209**, 452–457.

GARDNER-MEDWIN, A. R., GIBSON, J. L. & WILLSHAW, D. J. (1979), The mechanism of potassium dispersal in brain tissue. *J. Physiol., Lond.* **293**, 37–38*P*

GLYNN, I. M. & KARLISH, S. J. D. (1975). The sodium pump. *A. Rev. Physiol.* **37**, 13–53.

HERTZ, L. (1979). Functional interactions between neurons and astrocytes. I. Turnover and metabolism of putative amino acid transmitters. *Prog. Neurobiol.* **13**, 277–323.

HOLT, C. E. & BROWN, J. E. (1972). Ion fluxes in photoreception in *Limulus polyphemus* ventral eye. *Biochim. biophys. Acta*, **274**, 140–157.

JONGBLOED, J. & WIERSMA, C. A. G. (1935). Der Stoffwechsel der Honigbiene während des Fliegens. *Z. vergl. Physiol.* **21**, 519–533.

KRAIG, R. P. & NICHOLSON, C. (1978). Extracellular ionic variations during spreading depression. *Neuroscience* **3**, 1045–1059.

KUFFLER, S. W., NICHOLLS, J. G. & ORKAND, R. K. (1966). Physiological properties of glial cells in the central nervous system of Amphibia. *J. Neurophysiol.* **29**, 768–787.

MAHLER, M. (1978). Kinetics of oxygen consumption after a single isometric tetanus of frog sartorius muscle at 20 °C. *J. gen. Physiol.* **71**, 559–580.

MILLECCHIA, R. & MAURO, A. (1969). The ventral photoreceptor cells of *Limulus*. III. A voltage-clamp study. *J. gen. Physiol.* **54**, 331–351.

MORI, S., MILLER, W. H. & TOMITA, T. (1976). Microelectrode study of spreading depression (SD) in frog retina – Müller cell activity and K^+ during SD. *Jap. J. Physiol.* **26**, 219–233.

NICHOLLS, J. G. & KUFFLER, S. W. (1964). Extracellular space as a pathway for exchange between blood and neurons in the central nervous system of the leech: ionic composition of glial cells and neurons. *J. Neurophysiol.* **27**, 645–671.

NICHOLLS, J. G. & KUFFLER, S. W. (1965). Na and K content of glial cells and neurons determined by flame photometry in the central nervous system of the leech. *J. Neurophysiol.* **28**, 519–525.

NICHOLSON, C., PHILLIPS, J. M. & GARDNER-MEDWIN, A. R. (1979). Diffusion from an iontophoretic point source in the brain: role of tortuosity and volume fraction. *Brain Res.* **169**, 580–584.

O'CONNOR, M. J. (1977). Origin of labile NADH tissue fluorescence. In *Oxygen and Physiological Function* (ed. F. F. Jöbsis), Dallas Professional Information Library, pp. 90–99.

ORKAND, R. K., ORKAND, P. M. & TANG, C-M. (1981). Membrane properties of neuroglia in the optic nerve of *Necturus*. *J. exp. Biol.* **95**, 49–59.

ORKAND, R. K., NICHOLLS, J. G. & KUFFLER, S. W. (1966). Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of Amphibia. *J. Neurophysiol.* **29**, 788–806.

PEARSE, A. G. E. (1968). *Histochemistry, Theoretical and Applied*, 3rd ed. vol. 1, pp. 307–322, 366. Boston: Little Brown.

PEPOSE, J. S. & LISMAN, J. E. (1978). Voltage-sensitive potassium channels in *Limulus* ventral photoreceptors. *J. gen. Physiol.* **71**, 101–120.

PERRELET, A. (1970). The fine structure of the retina of the honeybee drone. *Z. Zellforsch. mikrosk. Anat.* **108**, 530–562.

POITRY, S. & TSACOPOULOS, M. (1981). Kinetics of oxygen consumption after a single flash of light in photoreceptors of the drone (*Apis mellifera*). *Association for Research in Vision and Ophthalmology. Abstracts of Spring Meeting*, p. 180.

SALEM, R. D., HAMMERSCHLAG, R., BRACHO, H. & ORKAND, R. K. (1975). Influence of potassium ions on accumulation and metabolism of ^{14}C glucose by glial cells. *Brain Res.* **86**, 499–503.

SHAW, S. R. (1969). Interreceptor coupling in ommatidia of drone honeybee and locust compound eyes. *Vision Res.* **9**, 999–1029.

SHAW, S. R. (1977). Restricted diffusion and extracellular space in the insect retina. *J. comp. Physiol.* **113** 257–282.

SHAW, S. R. (1978). The extracellular space and blood-eye barrier in an insect retina: an ultrastructural study. *Cell Tiss. Res.* **188**, 35–61.

SOMJEN, G. G. (1975). Electrophysiology of neuroglia. *A. Rev. Physiol.* **37**, 163–190.

STIEVE, H. & HARTUNG, K. (1977). Kinetics of ^{42}K and ^{86}Rb loss from the crayfish retina in the dark and the effect of light on the rate of isotope loss. *Biochim. biophys. Acta* **465**, 634–649.

STRYER, L. (1975). *Biochemistry*, p. 344. San Francisco: W. H. Freeman.

TREHERNE, J. E. & SCHOFIELD, P. K. (1981). Ionic homeostasis of the brain microenvironment in insects. *J. exp. Biol.* **95**, 61–73.

TSACOPOULOS, M. (1978). Micro-electrode recordings of an increase in O_2 consumption by drone photoreceptors induced by photostimulation. *Association for Research in Vision and Ophthalmology. Abstracts of Spring Meeting*, pp. 239–240.

TSACOPOULOS, M. & COLES, J. A. (1978). Inhibition by strophanthidin of the uptake of potassium by pigment cells in the drone retina. *Experientia* **34**, 903–904.

TSACOPOULOS, M. & EVEQUOZ, V. (1980). L'effet de la stimulation photique sur le métabolisme du glycogène intrarétinien. *Klin. Mbl. Augenheilk.* **176**, 519–521.

TSACOPOULOS, M. & LEHMENKÜHLER, A. (1977). A double-barrelled Pt-microelectrode for simultaneous measurement of P_{O_2} and bioelectrical activity in excitable tissues. *Experientia* **33**, 1337–1338.

TSACOPOULOS, M., POITRY, S. & BORSELLINO, A. (1980). Oxygen consumption by drone photoreceptors in darkness and during repetitive stimulation with light flashes. *Experientia* **36**, 702.

TSACOPOULOS, M., POITRY, S. & BORSELLINO, A. (1981). Diffusion and consumption of oyxgen in the superfused retina of the drone (*Apis mellifera*) in darkness. *J. gen. Physiol.* (In the Press.)

VAN PRAAGH, J. P., RIBI, W., WEHRHAHN, C. & WITTMANN, D. (1980). Drone bees fixate the queen with the dorsal frontal part of their compound eyes. *J. comp. Physiol.* **136**, 263–266.

VARON, S. S. & SOMJEN, G. G. (1979). Neuron-glia interactions. *Neurosci. Res. Progr. Bull.* **17**, 1–239.

WALKER, J. L. JR (1971). Ion specific liquid ion exchanger microelectrodes. *Analyt. Chem.* **43**, 89A–92A.