MODIFICATION OF POTASSIUM MOVEMENT THROUGH THE RETINA OF THE DRONE (*APIS MELLIFERA♂*) BY GLIAL UPTAKE

By J. A. COLES and R. K. ORKAND*

From the Experimental Ophthalmology Laboratory, 22, rue Alcide-Jentzer, 1211 Genève 4 and the Department of Physiology, Geneva University, Switzerland

SUMMARY

1. Intracellular recordings were made in photoreceptors and glial cells (outer pigment cells) of the superfused cut head of the honey-bee drone (*Apis mellifera♂*). When the $[K^+]$ in the superfusate was abruptly increased from 3·2 mM to 17·9 mM both photoreceptors and glial cells depolarized.

2. The time course of the depolarization of the photoreceptors was slower with increasing depth from the surface. Half time of depolarization was plotted against depth^2: this graph was compatible with the arrival of K^+ being exclusively by diffusion through the extracellular clefts. However, as we then showed, this interpretation is inadequate.

3. The time course of depolarization of the glial cells was almost the same at all depths. This indicates that they are electrically coupled. Consequently, current-mediated K^+ flux (spatial buffering) through glial cells will contribute to the transport of K^+ through the tissue: K^+ ions enter the glial syncytium in the region of high external potassium concentration, $[K^+]_o$, and an equivalent quantity of K^+ ions leave in regions of low $[K^+]_o$.

4. Intracellular K^+ activity (a^i_K) was measured with double-barrelled K^+-sensitive micro-electrodes in slices of retina superfused on both faces. When $[K^+]$ in the superfusate was increased from 7·5 mM to 17·9 mM an increase in a^i_K was observed in glial cells at all depths in the slice (initial rate 1·7 mM min^{-1}, s.e. of the mean = 0·2 mM min^{-1}), but there was little increase in the photoreceptors ($0·3 \pm 0·2$ mM min^{-1}). The increase in a^i_K in glial cells near the centre of the slice could not have been caused by spatial buffering; it presumably resulted from net uptake.

5. We conclude that when $[K^+]$ is increased at the surface of this tissue, the build up of K^+ in the extracellular clefts depends on extracellular diffusion, spatial buffering and net uptake. The latter two processes, which have opposing effects, involve about 10 times as much K^+ as the first. This is in rough agreement with less direct experiments on mammalian brain (Gardner-Medwin, 1977, 1983b).

INTRODUCTION

The K^+ released by neurones during activity can modify the activity of these and other, neighbouring neurones (Baylor & Nicholls, 1969; Syková & Orkand, 1980;

* Permanent address: Department of Physiology and Pharmacology, School of Dental Medicine, and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA. U.S.A.

Yarom & Spira, 1982). Consequently, it is of interest to know how extracellular [K$^+$] is kept within limits and brought back to its resting level. Several routes of K$^+$ clearance are conceivable: diffusion through extracellular clefts, rapid re-uptake into the active neurones, temporary storage in other cells (inactive neurones or glia), or a current-mediated entry into cells that is associated with an efflux in another part of the tissue where the extracellular [K$^+$] is lower. The last process was named 'spatial buffering' by Orkand, Nicholls & Kuffler (1966) and is discussed in detail elsewhere (Gardner-Medwin, 1980, 1983b; Dietzel, Heinemann, Hofmeier & Lux, 1980, 1982; Coles & Tsacopoulos, 1981).

To study K$^+$ clearance, experiments have been done on mammalian brain in which either extracellular [K$^+$] or extracellular electrical fields were measured and manipulated (Lux & Neher, 1973; Fisher, Pedley & Prince, 1976; Gardner-Medwin, 1977, 1983a; Gardner-Medwin & Nicholson, 1983). However, alternative interpretations of the results have been proposed (Gardner-Medwin, 1981, 1983b). The question is amenable to more direct investigation in the retina of the honey-bee drone, a tissue that has a number of practical advantages, notably that intracellular K$^+$ activity (a_K^i) can be measured (Coles & Tsacopoulos, 1979).

In the drone retina a natural stimulus, light, causes a fall in a_K^i in photoreceptor cells and a corresponding increase in the glial cells (Coles & Tsacopoulos, 1979). With light stimulation, extracellular electrical fields are set up that were interpreted by Gardner-Medwin, Coles & Tsacopoulos (1981) as being due to the currents associated with spatial buffering by the glial cells. Calculations suggested that a major part of the increase in glial a_K^i could be due solely to spatial buffering, but a contribution from net uptake could not be excluded (see Coles & Tsacopoulos, 1981). Net uptake has been reported in mouse oligodendrocytes in culture (Kettenmann, Sonnhof & Schachner, 1983): its possible occurrence in glial cells of intact drone retina is a main question addressed by the present paper.

METHODS

The methods are an extension of those of Coles & Tsacopoulos, 1979.

Anatomy

Between the distal dioptric apparatus and the proximal basal membrane, the drone retina is composed essentially of only two kinds of cells. The *large photoreceptor cells* are about 400 μm long and 10 μm across and are grouped in clusters of six called retinulae (see, e.g. Perrelet, 1970). The six cells within a retinula are electrically coupled (Shaw, 1969), but the retinulae are not directly coupled one to another. The axons from the photoreceptors pass through the electrically resistant basal membrane (Shaw, 1978), and continue in sheathed bundles to synapse in the brain. Since the part of the drone eye that we use has a radius of some 3000 μm the retinulae within 100 or 200 μm of each other are approximately parallel. Between the retinulae lie glial cells, known as outer pigment cells. Gap junctions between glial cells are observed in electron micrographs (Perrelet, 1970), but it is not clear whether these occur only between the glial cells within the functional unit centred on a retinula (the ommatidium) or whether there are junctions between glia in neighbouring ommatidia: the present paper will have something to say about this. There are no blood vessels in the retina, and, *in vivo*, oxygen is delivered by way of tracheoles that pass through the basal membrane and run parallel to the retinulae.

Preparations

Honey-bee drones were obtained from Mr Nathan Merin, Chicun Amal, Hadera, Israel, and kept in dim light for up to 3 weeks, fed by workers who were supplied with sucrose and water. The head

was cut off and placed, anterior side down, in melted wax at 37 °C (Eicosan, Fluka, Buchs, CH 9470) in a well in the top of a stainless-steel cylinder 8 mm in diameter. The cylinder was then rapidly cooled on ice. The back of the head was sliced off with a razor blade to expose a layer of ommatidia, as shown in Pl. 1 of Bertrand, Fuortes & Muri, 1979. The cylinder was then placed in a well in the floor of a Perspex chamber and superfused with oxygenated Ringer solution (see below). This is the 'cut-head' preparation.

A second preparation, a slice of retina, was also used. The head was mounted on a stainless-steel cylinder as before but with a shallower well. A first superficial cut was made and then a second that cut the cornea 500–900 μm deeper. The resulting slice was held by a pin, cemented to a weight, so that it lay on the floor of a perfusion chamber as shown in Fig. 1. The retinas were positioned over a channel in the floor of the chamber so that both faces were exposed to the Ringer solution. The delay between the switching of the valve in the perfusion line and the change in the composition of the bulk of the bath solution by 90 % was about 15 sec.

Solutions

The Ringer solution used for the cut-head preparation was oxygenated and contained (mM); NaCl, 270; KCl, 3·2; $CaCl_2$, 1·6; $MgCl_2$, 10; MOPS buffer, 10; pH adjusted to 7·3 with NaOH. $MgCl_2$ was added to the Ringer solution used previously (Coles & Tsacopoulos, 1979) in an attempt to prolong the viability of the preparation.
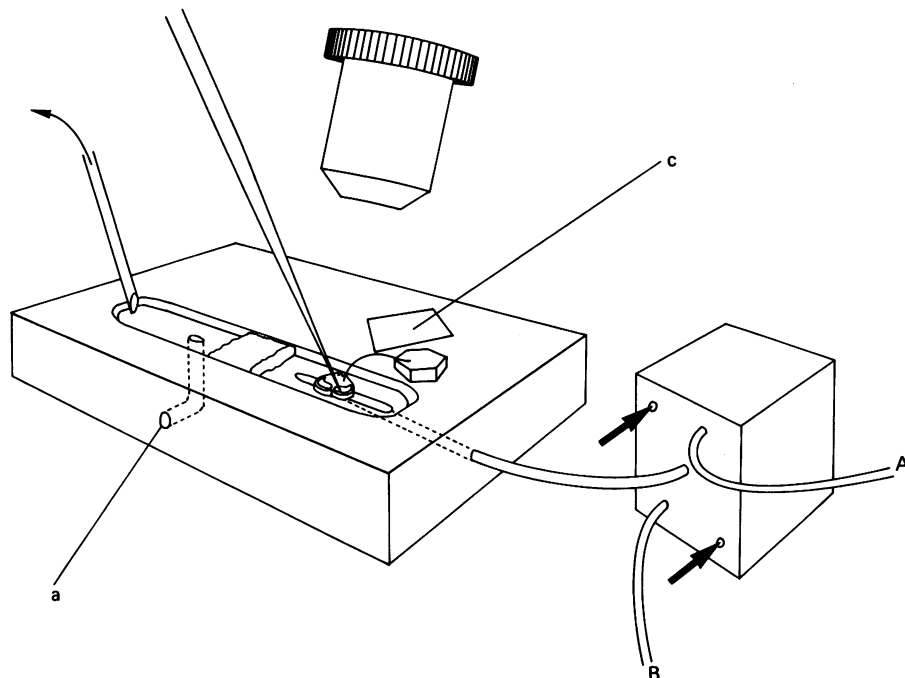

Fig. 1. Perfusion chamber. Oxygenated solutions entered through tubes A or B. The selection was made by applying compressed air, as indicated by the arrows, at one or other of the control inlets of a pneumatic valve (Dreloba, 806 Dresden; model 2 143 04:1800). The perfusion chamber was milled in a block of Perspex. The solution entered from the floor of the chamber in a channel of decreasing depth, and the slice of drone head bridged the channel so that at least one of the retinas was perfused on both faces. The slice was held down by a wire cemented to a hexagonal weight. The micro-electrode is shown entering from the upper left. A wad of tissue paper in the middle of the chamber damped vibration from the suction tube. An agar bridge, a, led to an Ag/AgCl electrode. K+-sensitive electrodes were calibrated with a system similar to that in Fig. 15 of Coles & Tsacopoulos, 1979, in the area c. The perfusion chamber was mounted on the mechanical stage from a microscope so that it could be moved relative to the stimulating light beam incident through the objective (× 3·5).

In experiments in which 15 mM-NaCl was replaced by 10 mM-MgCl$_2$ the photoreceptor membrane potential consistently hyperpolarized. The effect was more apparent just after light stimulation, in which case the membrane hyperpolarized about 5 mV more than in the control. There was no obvious change in the receptor potential itself. These results suggest that Mg^{2+} increases membrane resistance, and we thought it beneficial to include it in the Ringer solution.

Intracellular recordings from glial cells in slices of drone retina have not been reported previously. Our initial attempts yielded glial resting potentials of 15–40 mV, well below those found in the cut-head preparation (Coles & Tsacopoulos, 1979). We tried several modifications of the Ringer solution and the version finally used (cf. Baumann, 1968) contained (mM); NaCl, 200; KCl, 7·5; CaCl$_2$, 1·6; MgCl$_2$, 10; NaHCO$_3$, 10; bubbled with 95 % O$_2$, 5 % CO$_2$, pH 7·1. Furthermore, immediately after the slice was cut, it was superfused for 5–10 min with Ringer solution containing 16 mM-CaCl$_2$ and 10 mM-MOPS as buffer. The high Ca presumably hastened the sealing of glial cells damaged during the cutting (Delèze, 1970). In the high K$^+$ solutions NaCl was replaced by KCl. All experiments were done at 21–23 °C.

K$^+$-sensitive micro-electrodes

Theta capillaries were bought from several suppliers and lengths were examined end-on under a compound microscope. Only batches in which the partition was sealed into the wall with a not more than barely visible seam were used, since tests showed that if there was a clearly visible fissure then inter-barrel resistance was low. Micropipettes were made on a horizontal solenoid puller with the following operating sequence: heat plus weak pull, until there was an elongation set at about 5 mm; delay and/or air jet on heating filament; strong pull. The solenoid was turned on and off with a hexfet (IRF 232). The active barrel was silanized in the apparatus shown in Coles & Tsacopoulos, 1977, at 250 °C for 5–7 min (Deyhimi & Coles, 1982) using (dimethylamino)-trimethylsilane (Fluka). A K$^+$-ion exchanger sensor solution, similar to that described by Oehme & Simon (1976) was made from 3 % by weight K tetra-*p*-chlorophenylborate in 2,6-dimethylnitrobenzene (Fluka). In a few experiments, a sensor based on valinomycin (Calbiochem) was used (Wuhrmann, Ineichen, Riesen-Willi & Lezzi, 1979). The sensor was injected into the taper of the active barrel, and the reference barrel was filled with a solution containing 0·1 M-KCl and 1 M-Li acetate (Oehme, 1977). When the reference barrel had filled to the tip by capillarity, a chlorided silver wire was sealed into it with Shellac. If the active barrel had not filled spontaneously, the micro-electrode was placed, tip downwards, in a chamber that was connected to a vacuum pump for about 1 min. Any remaining bubbles in the resin column dissolved in about an hour. The active barrel was back-filled with Ringer solution. Some of the electrodes were used in this state, but it was found that often the K signal could be improved by bevelling the tip, or breaking it by plunging the electrode into the exposed drone brain; sometimes impalements made with tips as large as 0·8 μm in diameter gave membrane potentials more negative than those made with unbroken tips. The electrodes were calibrated immediately after any successful recording as described by Coles & Tsacopoulos (1979). The activity coefficient of K$^+$ in the calibration solutions was taken to be 0·70 (Staples, 1971).

Recording arrangements

The double-barrelled K$^+$ electrode was clipped to a head-stage consisting of two conventional operational amplifier voltage followers in parallel (either Teledyne-Philbrick 1035-02 or Analog Devices AD 515L). Capacity neutralization was adjusted when the electrode was in a cell so that the electrical artifact on the K signal was close to minimum. The valinomycin electrodes had high resistances (e.g. 8×10^{11} Ω) so when one was used its active barrel was connected directly to the input pin of the operational amplifier and the capacity neutralization was removed from the circuit. When only membrane potential was recorded, micropipettes filled with 3 M-KCl were used. The depth of the electrode tip in the retina was determined with the aid of a helical potentiometer connected to the fine drive of the manipulator (Leitz). The signals were recorded on a chart recorder that attenuated the receptor potential by less than 2 %. The recordings were photocopied: for some figures, faint traces of rapid upstrokes were darkened with a pen.

Light stimulation

Light from a xenon lamp was focused by glass optics to a spot that covered the whole retina. The unattenuated photon flux measured with a photodiode (OSD 100-1, Centronic, Croydon CR9 0BG, U.K.) was 2×10^{10} photons μm^{-2} sec^{-1}. All light flashes were of 20 msec duration.

RESULTS

Transfer of K+ through the tissue: changes in extracellular concentration

We did an experiment analogous to that of Fisher *et al.* (1976) in which [K+] in the Ringer solution flowing over the surface of the preparation was abruptly increased and the time course of the change in [K+] in the extracellular space at different depths was followed. Instead of using extracellular K+-sensitive micro-electrodes we chose to use the membrane potentials of the photoreceptors as indicators of extracellular [K+] (Frankenhaeuser & Hodgkin, 1956; Nicholls & Kuffler, 1964). The drone photoreceptors lend themselves particularly well to this technique because their axes lie roughly parallel to the exposed surface of the retina and the clusters of photoreceptors (retinulae) are not electrically coupled (see *Anatomy*). The electrical length constant of the photoreceptors is greater than their anatomical length (Carreras, 1978), so the recorded membrane potential will be determined mainly by that major part of the cell which is relatively undisturbed by the electrode impalement. In addition, the results for the photoreceptors serve as a control for similar experiments on the glial cells. In this series of experiments, we chose to use the cut-head preparation, rather than a slice of retina, because there was less chance that the tissue would be distorted in the dissection.

Fig. 2 shows intracellular recordings made while bath [K+] was increased from 3·2 mM to 17·9 mM. Record *A* is from a superficial photoreceptor, 22 μm from the surface. As found by Fulpius & Baumann (1969), the amplitude of the depolarization is less than that predicted by the Nernst relation for the change in bath [K+]. This is probably due in part to the membrane properties of the photoreceptors and partly to the fact that in the cut-head preparation, the deepest cells are anoxic (Tsacopoulos, Poitry & Borsellino (1981). These deep cells appear to release K+, and [K+] everywhere in the extracellular space is more than 3·2 mM (Gardner-Medwin *et al.* 1981). Record *B* is from a deep photoreceptor, 204 μm from the surface. The depolarization was slower and had a smaller amplitude than that in *A*: this difference is possible because the retinulae are not electrically coupled. The smaller amplitude was presumably because of higher extracellular [K+], and because we will later compare the photoreceptors with the glial cells we note that the difference is significant. For the ten photoreceptors within 50 μm of the surface, the mean depolarization was 8·8 mV, s.E. of the mean = 1·2 mV, while for the seventeen photoreceptors deeper than 165 μm the mean depolarization was 3·6 $\pm$ 0·3 mV.

Because of the standing gradient of [K+] in the extracellular space, a complete analysis of the depolarizations seems difficult. However, diffusion fluxes simply superpose, and it is instructive to make an approximate comparison between the increasing slowness of the depolarizations with depth and the arrival of K+ in the extracellular space that would occur if the only route were by diffusion through the extracellular clefts. To do this, the time from the switching of the pneumatic valve (see Methods) until the depolarization reached half its nearly constant value at 5 min was measured for each cell, and the results were plotted with filled circles in Fig. 3. We then fitted the data with the standard diffusion equation:

$$\Delta C(z, t) = \Delta C_0 \operatorname{erfc} \{\lambda z (4Dt)^{-\frac{1}{2}}\}, \tag{1}$$
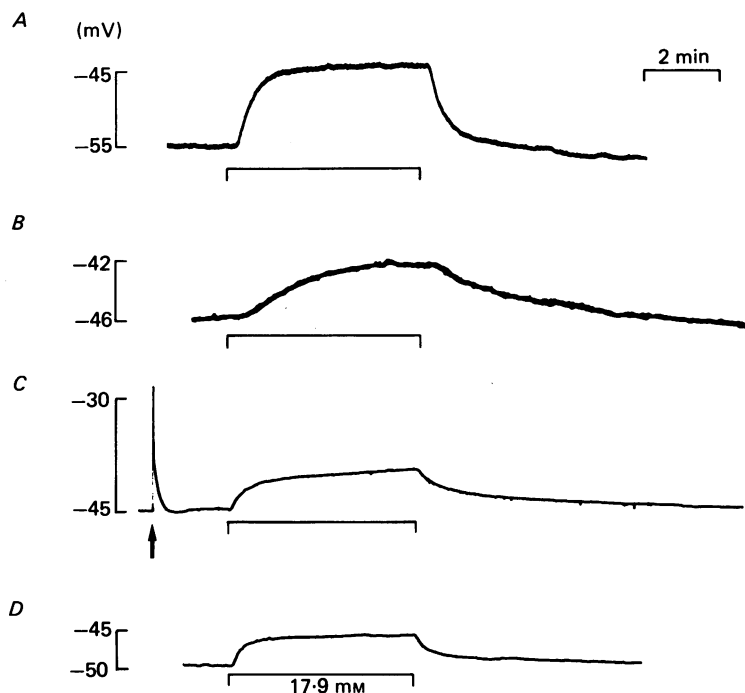
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Fig. 2. The time course of depolarization. Each trace is an intracellular recording from a different cell in the cut-head preparation. The scales on the left show the membrane potentials in mV. At the beginning of the time indicated by the horizontal bar, the valve was operated to switch the superfusate from Ringer solution with a [K$^+$] of 3·2 mM to one with 17·9 mM. A, superficial photoreceptor, depth 22 μm. B, deep photoreceptor, depth 204 μm (note the change in voltage gain). C, superficial glial cell, depth 14 μm. At the time indicated by the arrow, the photoreceptors were stimulated with an unattenuated 20 msec light flash. D, deep glial cell (from the same retina as C), depth 140 μm. Record C was obtained 105 min after D, at which time the glial membrane potentials had fallen by about 4 mV in this preparation.

where $\Delta C(z, t)$ is the change in concentration in the extracellular space at depth z, ΔC_0 is the step change in concentration in the superfusate that occurs at time $t = 0$, D is the diffusion coefficient of K in bulk solution and λ is the factor that takes account of the tortuosity and varicosity of the extracellular spaces (see, e.g. Harris & Burn, 1949). Since the depolarizations are small compared to the coefficient in the Nernst equation, we take it that the time to half depolarization is approximately equal to the time for half the concentration change (see, e.g. Dionne, 1976). Hence,

$$t_{\frac{1}{2}} = k + (\lambda z)^2 (0 \cdot 911 D)^{-1}, \tag{2}$$

where k is a constant that partially corrects for the time taken for the solution to pass from the pneumatic valve to the surface of the retina. A least-mean-squares fit of eqn. 2 to the data in Fig. 3 (continuous line) gives: $\lambda^2/(0 \cdot 911 D) = (2 \cdot 0 \pm 0 \cdot 3) \times 10^{-3}\ \mu\text{m}^{-2}\ \text{sec}^{-1}$. In the extracellular fluid, where the total salt concentration is about 0·3 M and the temperature is 21–23 °C, D is about $1 \cdot 7 \times 10^3\ \mu\text{m}^{-2}\ \text{sec}^{-1}$ (Harned & Nuttall, 1949). Hence, $\lambda = 1 \cdot 8 \pm 0 \cdot 2$. By coincidence, this is within the range of values of λ that have been measured in mammalian nervous

tissue (1.4–2·3, Patlak & Fenstermacher, 1975; Nicholson & Phillips, 1981), so the results are what might be expected if K+ moved through the drone retina exclusively by diffusion through the extracellular clefts. However, there is already evidence that this is not the case, and that substantial amounts of K+ cross the glial membranes (Gardner-Medwin *et al.* 1981; Coles, 1981). The way this can occur while at the same time the rate of arrival of K+ in the extracellular clefts is similar to that predicted for exclusively extracellular diffusion is the subject of the rest of this paper.
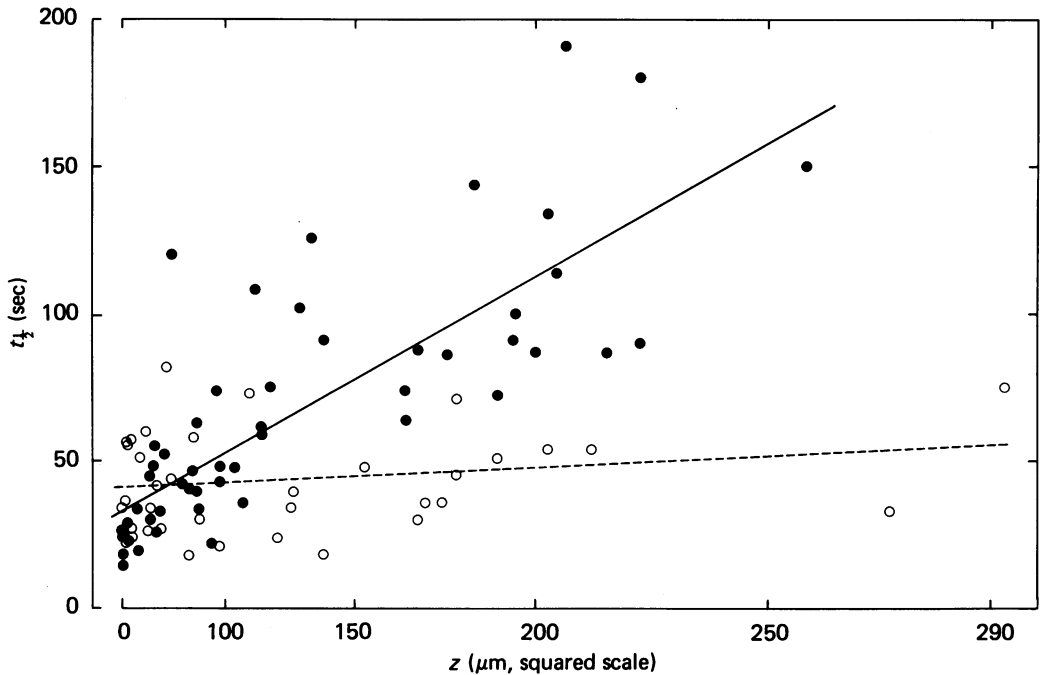

Fig. 3. Time for depolarization as a function of depth in the retina. The abscissa gives the depth in microns (squared scale) of the electrode tip from the surface of the retina. The ordinate indicates the half-time for depolarization as defined in the text. Filled circles: photoreceptors ($V_m \leqq -40$ mV); open circles: glial cells ($V_m \leqq -35$ mV). The least-square linear-regression lines have been drawn in. If $t_{\frac{1}{2}} = a + bz^2$, then for the photoreceptors (continuous line) $b = 2·0 \times 10^{-3} \mu m^{-2} sec^{-1}$, s.d. $= 0·3 \times 10^{-3} \mu m^{-2} sec^{-1}$; correlation coefficient $r = 0·79$. For the glial cells, $b = 1·7 \times 10^{-4} \mu m^{-2} sec^{-1}$, s.d. $= 1·3 \times 10^{-4} \mu m^{-2} sec^{-1}$ (i.e. the slope is not significantly different from zero). For $z \leqq 50 \mu m$, the mean $t_{\frac{1}{2}}$ for photoreceptors is 28 sec, s.e. of the mean = 4 sec; and for glial cells it is 47 sec, s.e. of the mean = 4 sec.

Depolarization of glial cells. Experiments similar to those just described for photoreceptors were also done on glial cells. Recordings from superficial and deep glial cells are shown in Figs. 2*C* and *D*. In Fig. 2*C* an arrow indicates the time at which the photoreceptors were stimulated by a 20 msec flash of light. The ensuing depolarization of the glial cell is greater than that caused by increasing [K+] in the superfusate to 17·9 mM. This suggests that this concentration is within the physiological range, in agreement with measurements of extracellular [K+] (Coles & Tsacopoulos, 1979). The half times of depolarization are plotted as a function of depth by open circles in Fig. 3. In contrast to the photoreceptors, the glial cells depolarize

with half times that do not increase significantly with depth; this is physiological evidence that they are electrically coupled. As is to be expected, in the superficial layers the glial cells took longer to depolarize than the photoreceptors did, because their membrane potential depends also on the extracellular [K$^+$] deep into the tissue. For the same reason, it is not surprising that the amplitude of the glial depolarization was small. The mean value was 3·7 mV (S.E. of the mean = 0·2 mV, $N = 48$) and, unlike the photoreceptors, it did not vary significantly with depth. Finally, we observed that in a given preparation membrane potentials recorded from glial cells were closely similar, whereas this was not true for the photoreceptors.

Is there net uptake of K$^+$ by cells?

The experiments just described indicate that the glial cells are electrically coupled and so confirm one of the assumptions made by Gardner-Medwin *et al.* (1981) when they suggested that spatial buffering occurs in this preparation and should speed up the movement of K$^+$ through the tissue. However, the results also suggest that [K$^+$] approaches its new value in the deep extracellular space no more quickly than it would do if the transfer occurred exclusively by diffusion through the extracellular clefts, i.e. there is a process that tends to counteract the contribution of spatial buffering to the K$^+$ movement: this might be net uptake of K$^+$ into cells. It has already been shown that in the cut-head preparation a_K^i in the glial cells (but not the photoreceptors) increases when bath [K$^+$] is increased (Coles, 1981). But this is not a sufficient demonstration of net uptake. In parts of a glial syncytium where spatial buffering causes an entry of K$^+$ into the cells, there is necessarily an increase in a_K^i (the 'transport number effect' of Barry & Hope, 1969), and in drone glial cells, in which K$^+$ appears to make up only about $\frac{1}{6}$ of the osmolarity, this increase might, *a priori*, be large enough to account for all the observed increase in a_K^i (Gardner-Medwin *et al.* 1981). We therefore designed experiments in which pure spatial buffering could only cause a *decrease* in a_K^i in the cells recorded from. This is the case when spatial buffering causes K$^+$ to leave glia, and it must occur at the centre of a slice of retina superfused on both faces with increased [K$^+$], as follows from the ion fluxes indicated in Fig. 9C. We describe first some general properties of the slice preparation.

It is possible to conceive of systems in which a current-mediated entry of K$^+$ in one part of a syncytium leads to an increase in a_K^i throughout. For example, if the membranes are permeable only to K$^+$ in the region of raised [K$^+$]$_0$ but permeable also to Cl$^-$ in the remote region where [K$^+$]$_0$ is not raised, then all the inward current would be carried by K$^+$ but some of the outward current would be carried by Cl$^-$ and so more K$^+$ would enter than had left. Since in making slices of drone retina the superficial layers of glial cells are cut (or at least torn apart) such a heterogeneity of membrane properties might be possible. To show that this is not the case, in the experiments that follow we set up gradients of extracellular [K$^+$] in two directions: with light stimulation from the centre of the slice towards the surface (Fig. 9B) and with raised bath [K$^+$] from the surface towards the centre (Fig. 9C).

Slices of retina

Base-line K$^+$ activities. Fig. 4 shows that the apparent a_K^i measured with the ion-exchanger electrodes in the glial cells appeared to change little during the 4 hr or so during which normal receptor potentials could be elicited from the photoreceptors. a_K^i also appeared to be independent of the depth of the cell from the surface, but did tend to be greater in cells with more negative membrane potentials: in the five glial

cells with $V_m \leqq -55$ mV (the most negative was -61.5 mV) the mean value of a_K^i was 92 mM, S.E. of the mean = 8.6 mM. There was no sign either that a_K^i in the photoreceptors changed with time or with depth from the surface. For the three photoreceptors with the most negative membrane potentials ($V_m \leqq -50$ mV), $a_K^i = 87$ mM, S.E. of the mean = 14 mM.
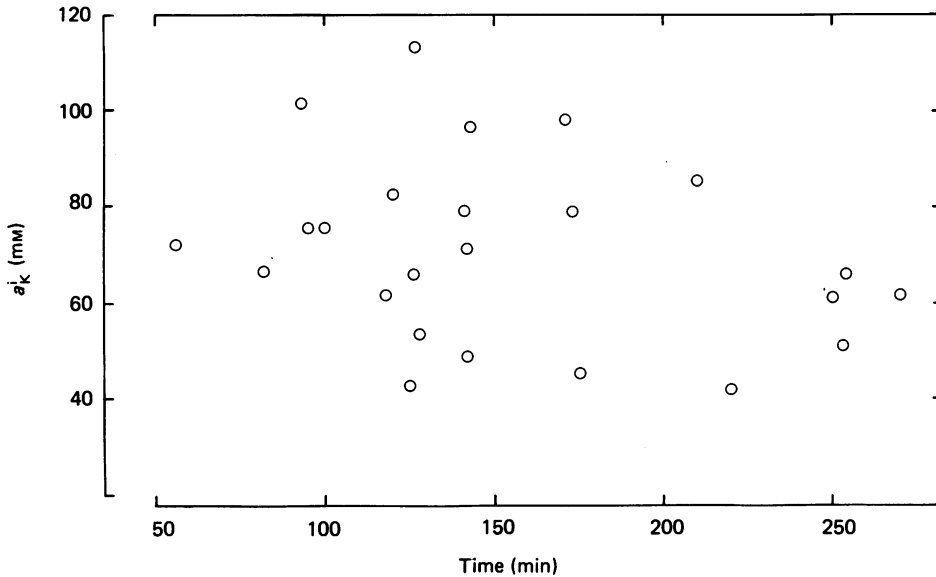

Fig. 4. a_K^i in glial cells in slices. Measurements from glial cells with $V_m \leqq -45$ mV are plotted as a function of time after dissection. These experiments suggest that some slices survived for more than $4\frac{1}{2}$ hr with little loss of K from the glial cells.

The ion exchanger in the electrode is sensitive not only to K^+, but also to compounds that may be present in cells, such as acetylcholine (Scholer & Simon, 1972), so these values may be over-estimates of the true a_K^i. Electrodes made with a sensor based on valinomycin are more selective (Oehme & Simon, 1976), but, in our hands, have an inconveniently high resistance (greater than $10^{11} \Omega$). Nevertheless, some measurements were made. For glial cells with V_m in the range -45 to -50.5 mV, $a_K^i = 56.3$ mM, S.E. of the mean = 2.1 mM, $N = 8$. For cells with the same range of V_m, the ion exchanger electrodes gave $a_K^i = 70.6$ mM, S.E. of the mean = 5.6 mM, $N = 11$. The difference is significant with $0.1 > P > 0.05$. This difference is comparable to that found in *Chironomus* salivary gland (Wuhrmann *et al.* 1979), but less than that in frog spinal cord (Bührle & Sonnhof, 1981). It has previously been suggested that ion exchanger electrodes detect a substance other than K^+ in the extracellular space of the drone retina (Coles, Tsacopoulos, Rabineau & Gardner-Medwin, 1981), but that this does not change greatly during light stimulation (Coles & Tsacopoulos, 1979).

Effect of increasing bath [K^+] on intracellular a_K

Photoreceptors. Fig. 5 shows a recording from a photoreceptor made with a double barrelled K^+-sensitive micro-electrode. When the bath [K^+] was raised from 7.5 to

17·9 mM (i.e. a_K was increased from 5·25 to 12·5 mM) a_K^i changed very little. However, when the photoreceptor was stimulated by a train of light flashes (filled bar) it reversibly lost about a quarter of its a_K^i, as described previously for the cut-head preparation (Coles & Tsacopoulos, 1979). A similarly modest uptake when extra-cellular [K$^+$] was raised was observed in four other photoreceptors with $V_m \leqq -45$ mV (Fig. 7A). In addition, in eleven photoreceptors in the cut-head preparation, when [K$^+$] in the superfusate was increased from 3·2 to 17·9 mM, the rate of change of a_K^i was in every case less than 0·6 mM min^{-1}; eight of these cells were referred to in Coles, 1981.
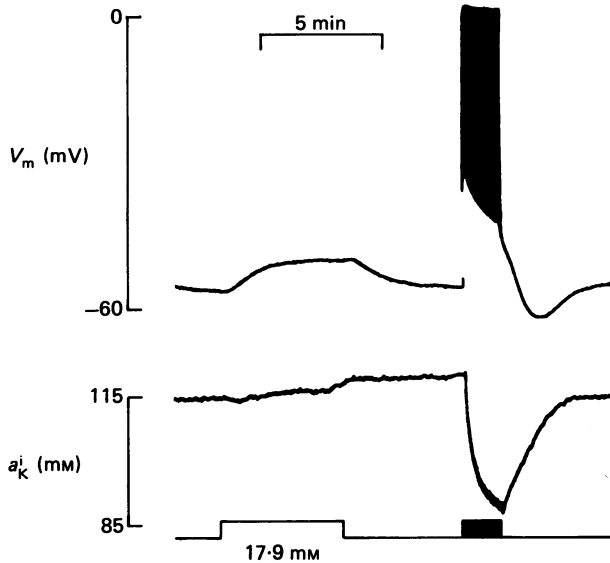

Fig. 5. K$^+$ activity in a photoreceptor. Recording obtained with a double-barrelled K$^+$-sensitive micro-electrode from a photoreceptor cell 63 μm from the surface. The upper trace shows the membrane potential recorded by the reference barrel. The lower trace is the difference in potential between the two barrels expressed in terms of a_K^i. The step in the bottom line indicates the time during which [K$^+$] in the Ringer solution was increased from 7·5 to 17·9 mM. The cell depolarized, but intracellular a_K changed very little. During the time indicated by the filled bar the retina was stimulated with ninety unattenuated light flashes, each of which elicited a receptor potential; a_K^i decreased and then recovered.

Glial cells. Fig. 6A shows a recording from a glial cell. In contrast to the photoreceptors, either an increase in bath [K$^+$] or light stimulation of the photo-receptors caused a marked increase in a_K^i. The mean rate of increase of a_K^i over the 3 min during which the rate of increase was greatest was measured for cells with $V_m \leqq -45$ mV. This quantity, which we call da_K/dt, is plotted with open circles as a function of the depth of the recording site in Fig. 7A. High values of da_K/dt are seen for deep cells as well as superficial ones. Further, as shown in Fig. 7B, a rapid uptake of K by the glial cells was not associated with a low initial activity. In most of the cells, a_K^i was still rising at the end of the approximately 5 min of superfusion with the high K solution: the mean value of the increase in a_K^i at this time was 9·1 mM, s.e. of the mean = 1·6 mM.

Five measurements were also made with valinomycin electrodes and a recording

is shown in Fig. 6B. The mean value of da_K/dt was 1·6 mM min^{-1}, S.E. of the mean = 0·1 mM min^{-1}. Responses measured with ion-exchanger electrodes in glial cells with the same range of V_m (-47.5 to -53.5 mV) were not detectably different; the mean value of da_K/dt was 1·4 mM min^{-1}, S.E. of the mean = 0·3 mM min^{-1}, $N = 11$.

Glial cells near the centres of slices. The cell in Fig. 8 was near the centre of a slice, 280 μm from the upper surface. When [K]$_o$ increased, a_K^i increased as in more superficial cells and in other deep cells as summarized in Fig. 7A.
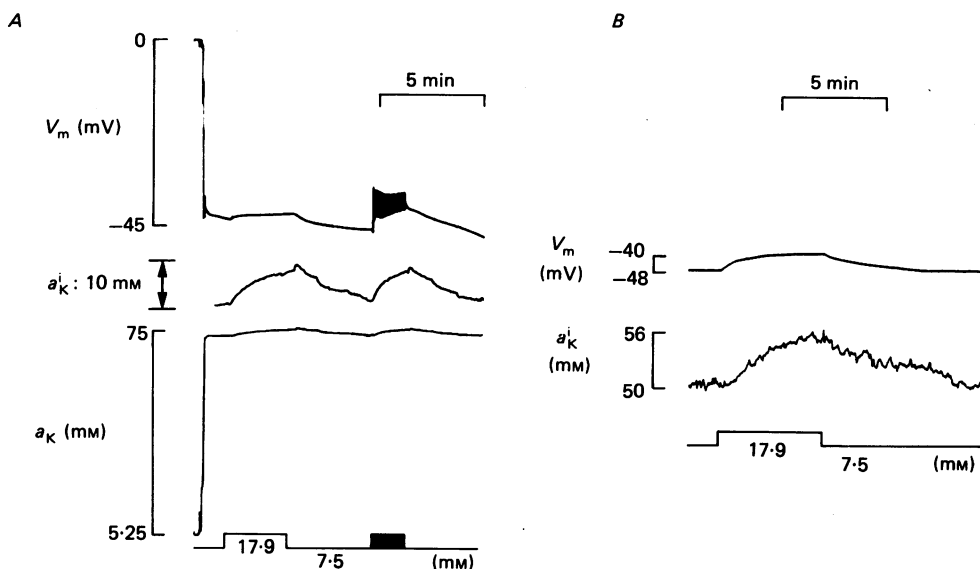

Fig. 6. a_K^i in a glial cell. A, recordings analogous to those in Fig. 5 but from a glial cell, 12 μm from the surface of a slice. The upper trace is the potential and it begins with the penetration of the cell. This is followed by a depolarization in response to an increase in bath [K$^+$] from 7·5 to 17·9 mM (step in bottom line) and then a series of depolarizations when the photoreceptors were stimulated with light flashes (filled block on bottom line). The third trace shows a_K recorded at low sensitivity; note that in the bath, where [K$^+$] is 7·5 mM, a_K is 5·25 mM. The middle trace shows a_K^i at a higher sensitivity. An increase in extracellular a_K, either by bath application, or by release from the photoreceptors, caused glial a_K^i to increase. B, response to an increase in bath [K$^+$] recorded with a valinomycin electrode. The cell was about 7 μm from the surface.

DISCUSSION

The homogeneity of the glial membranes

The conditions under which we have measured changes in glial a_K^i are illustrated in Fig. 9B and C. During light stimulation, the photoreceptors released K$^+$ into the extracellular clefts throughout the thickness of the slice, and spatial buffer currents would have been set up as shown in Fig. 9B. Current entered the glial cells in the middle of the slice and left near the surface; at both of these sites we observed increases in glial a_K^i. In the converse experiment (Fig. 9C), K$^+$ gradients were set up in the reverse directions so that current entered glial cells at the surface of the slice and left in the centre. Again, increases in a_K^i were observed in both superficial and deep glial cells. Because of this symmetry, we propose that the glial membranes have
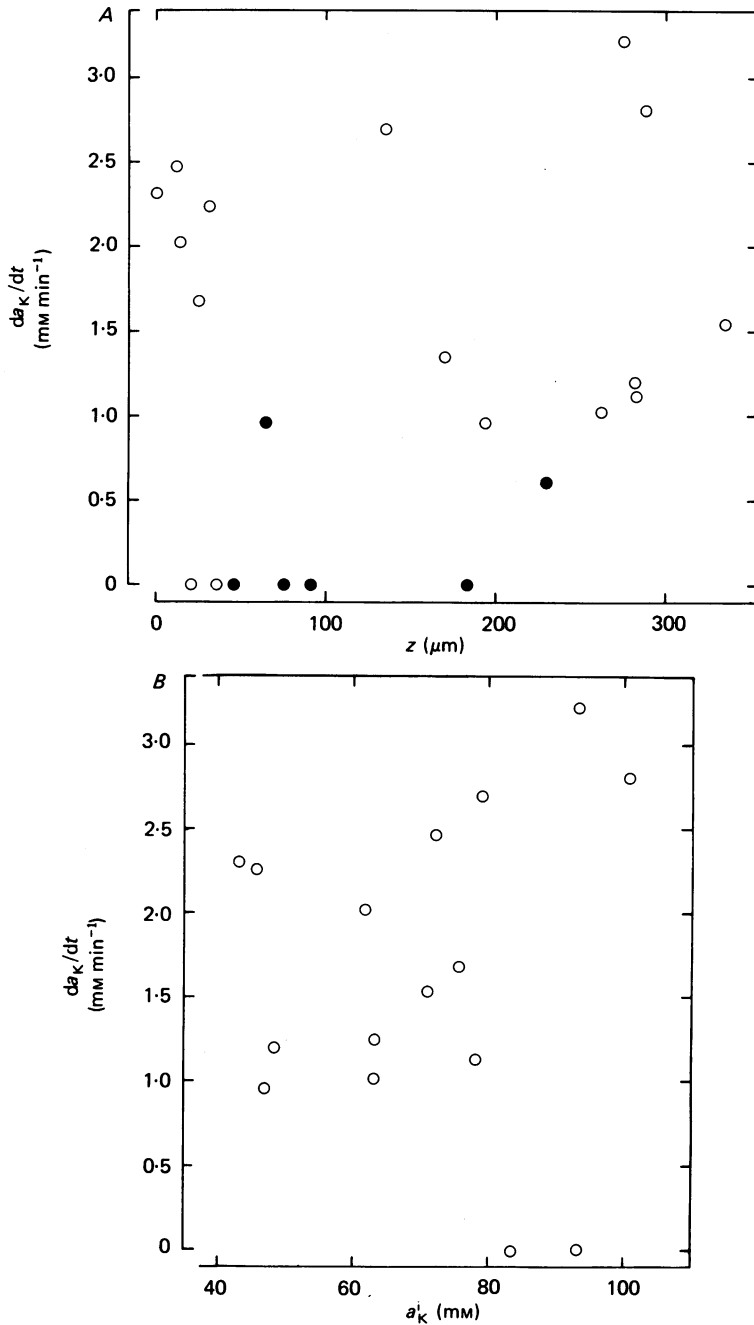
Fig. 7. Rate of increase of a_K^i in retinal slices when $[K^+]_o$ was increased. Records such as those in Figs. 5, 6 and 8 in which bath $[K^+]$ was increased from 7·5 mM to 17·9 mM were analysed. The mean rate of increase of a_K^i over the 3 min of most rapid increase was obtained for each cell with $V_m \leqq -45$ mV. *A*, da_K/dt as a function of depth, z, of the cell from the surface of the slice, $\bigcirc$: glial cells; $\bullet$: photoreceptors. *B*, da_K/dt as a function of initial a_K^i for glial cells. The two glial cells that showed no uptake were from the same retina and had previously been exposed to the high K^+ solution for 31 min in the previous 90 min. These two graphs illustrate that there is no apparent correlation of da_K/dt with depth in the retina and that da_K/dt is not greater in cells with a low initial a_K^i.
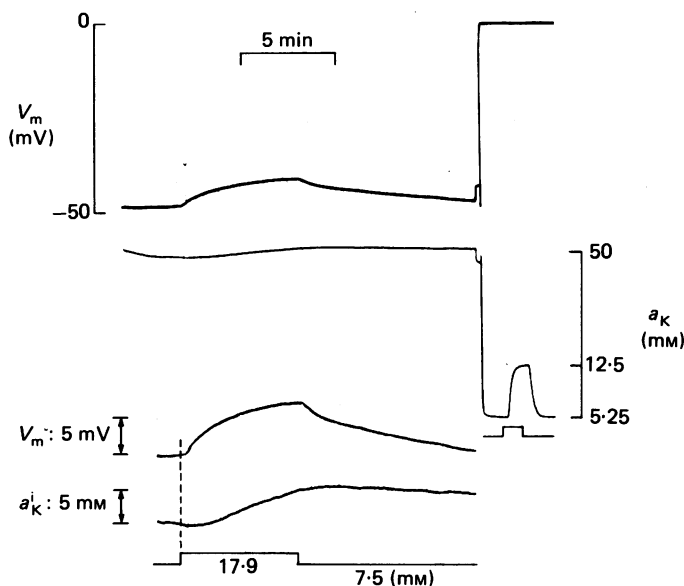
Fig. 8. Response of a deep glial cell to an increase in bath [K+]. The upper trace is the potential recorded by the reference barrel of the micro-electrode. It shows the depolarization induced by superfusion with the high K+ solution and, after the recovery, the rapid withdrawal of the electrode to the bath. The second trace is the K+ signal at a low sensitivity. The fall in K+ signal when the electrode was withdrawn to the bath gives an indication that the electrode responded sufficiently rapidly to changes in a_K. With the electrode in the bath (140 μm above the slice) the superfusate was again changed; the response of the electrode gives an indication of the delay between the operating of the valve (indicated below the trace) and the change of solution near the retina. The third and fourth traces show the potential and the K+ signal on magnified scales. This recording site was 280 μm from the surface, about 15 % of the thickness of the slice away from the centre line.

approximately the same properties in the surface layers and near the middle of the slice.

Evidence for net uptake. We now argue that the results are evidence for an uptake of K+ that does not depend on there being a gradient of $[K^+]_o$, i.e. it is space-independent (Gardner-Medwin, 1980). Since we do not know the permeability of the glial membranes to anions we consider two possible cases. Firstly, if K+ is the only ion that can cross the membranes passively, then as much must leave as enters and there can be no net increase in a_K^i by spatial buffering. Indeed, in the region where the current leaves the cells there would be a decrease. Our results showed only increases and no decreases, and so demonstrate that there must have been an entry of K+ by a mechanism that does not depend on spatial buffer currents. Secondly, if the membranes are also permeable to anions, then the K+-induced depolarization of the glial syncytium will cause an entry of anions into all parts of it; if this mechanism exists it would necessarily allow space-independent uptake. In either of these cases, our results demonstrate space-independent uptake.

As well as net uptake, spatial buffering also must change glial a_K^i, but its contribution would change in magnitude and sign with the depth from the surface. Since we did not observe differences in the rate of increase of a_K^i with depth (Fig. 7 A)
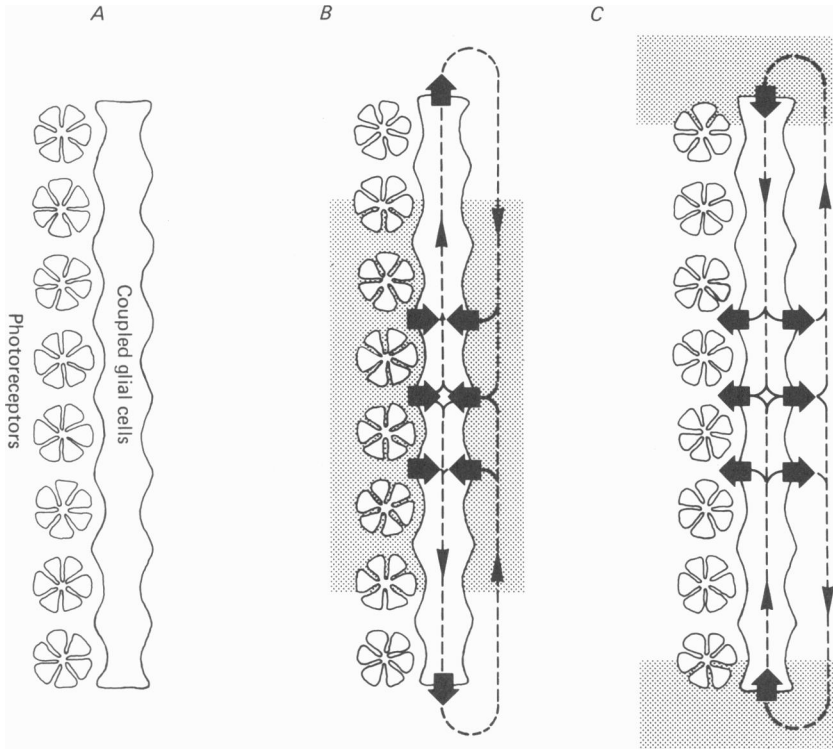
Fig. 9. Spatial buffering currents in slices of retina. *A*, representation of a section through a slice. The retinulae (not to scale) are shown in cross section. The glial cells, which in reality fill nearly all the space between the photoreceptors, are indicated by a syncytial column. In *B* and *C* arrows represent spatial buffer currents. Where current crosses membranes it is carried solely by K^+ (fat arrows). Within the glial cells and in the extracellular clefts all mobile ions carry current (thin arrows). The stippled areas show where K^+ is increased in the first few seconds following either light stimulation (*B*) or changing the bath [K^+] (*C*). *B*, light stimulation. K^+ is released into the extracellular clefts by the photoreceptors and most of this passes into the glial cells, K^+ leaves the glial cells near the surface of the slice where [K^+]$_o$ is lower. The electrical current loops are completed in the glial syncytium and in the extracellular clefts. *C*, raised bath [K^+]. The spatial buffering currents are reversed compared to *B*.

we conclude that net uptake predominates. In his experiments on the cut-head preparation, Coles (1981) observed similar increases in a_K^i in cells at different depths, and we will assume that in the cut-head preparation, as well as in slices, changes in a_K^i are due mainly to net uptake.

Possible mechanisms of K^+ uptake. In order to maintain charge neutrality, the K^+ must enter the cells either in association with an anion or in exchange for a cation. In frog skeletal muscle fibre a_{Cl}^i is approximately in Nernst equilibrium with a_{Cl}^o (Bolton & Vaughan-Jones, 1977) and the consequences of increasing [K^+]$_o$ can be successfully accounted for by supposing that Cl^- enters the cell passively with K^+ to re-establish a Donnan equilibrium (Boyle & Conway, 1941; Hodgkin & Horowicz, 1959). In our slices of drone retina, the glial cells did not depolarize as much as predicted by the Nernst equation for the change in [K^+] that was made in the bath.

This was probably due in part to the fact that since the glial cells are coupled their membrane potential depended on extracellular [K+] throughout the tissue, including the deep layers where $[K^+]_o$ was always elevated and only very slowly affected by the changes in bath [K+]. Given this uncertainty, we could not rigorously test the idea that a Donnan equilibrium was maintained, although the observed increase in a_K^i was of the predicted order of magnitude. However, in papers on those preparations, other than frog skeletal muscle, in which a_{Cl}^i has been measured it has been reported that Cl⁻ is not at electrochemical equilibrium (see Vaughan-Jones, 1979, for references, also Bührle & Sonnhof, 1981, and Aickin & Brading, 1982). Hence, in perhaps the majority of cells, Cl⁻ is actively transported across the membrane, and this may also be the case in drone glial cells.

Besides Cl⁻, the only other anion in our bathing solution was HCO_3^-, which generally also enters cells against an electrochemical gradient (Boron & De Weer, 1976; Thomas, 1977). The remaining likely candidate mechanism, Na/K exchange, would require that an increase in $[K^+]_o$ in the range 7·5–18 mM stimulate that Na–K-ATPase, as it does, for example, in squid axon (Baker, Blaustein, Keynes, Manil, Shaw & Steinhardt, 1969). The present results do not allow us to decide which of these mechanisms are responsible for the space-independent K+ uptake.

Flux of K+ through the retina. In the experiments in which $[K^+]_o$ was raised at the surface of a cut-head preparation, there were three factors that could have affected the arrival of K+ in the extracellular space in the depths of the retina. These were: (1) diffusion through extracellular clefts, (2) spatial buffering, and (3) net uptake by cells. Although the rate of arrival was roughly that expected from extracellular diffusion alone, the following arguments show that both spatial buffering and net uptake must have mediated relatively large K fluxes, although, under these experimental conditions, their effects on extracellular [K+] approximately cancelled. We observed that in glial cells a_K^i increased by about the same amount as bath a_K. Given that the volume of the glial syncytium is about 10 times the volume of the extracellular space, such uptake would be equivalent to decreasing the effective diffusion constant D (eqn. 1), by a factor of 11, and hence increasing $t_{\frac{1}{2}}$ (eqn. 2) by the same factor. To cancel this effect, about 10 times as much K+ must move as a result of spatial buffering than by diffusion through extracellular clefts. The equations for making a more sophisticated analysis of the situation have been given by Gardner-Medwin (1983*b*), but they require knowledge of electrical properties of the glial syncytium that are not available for the drone retina. Our results, which show no detectable change with depth on the time course or amplitude of the glial depolarization in response to an increase in bath [K+], only set a lower limit (of a few hundred microns) on the space constant of the syncytium (Gardner-Medwin, 1983*b*).

Gardner-Medwin (1977, 1983*a*) measured the flux of K+ resulting from an electrical field applied to rat brain. He concluded that 5 times as much K+ moved through cells as through the extracellular clefts, but he could not be certain which cell type was involved. Our results suggest a contribution of the same order of magnitude in the drone retina. In this preparation the transcellular K+ flux must be through the glia rather than the neurones, because the glial cells, as we have now shown, are electrically coupled, while the retinulae are not.

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