J. Physiol. (1970), **210**, *pp.* 565–580
With 7 text-figures
Printed in Great Britain

THE CONTRIBUTION BY GLIAL CELLS TO SURFACE RECORDINGS FROM THE OPTIC NERVE OF AN AMPHIBIAN

By M. W. COHEN*

From the Department of Neurobiology, Harvard Medical School, Boston, Mass., U.S.A.

(*Received 21 January 1970*)

SUMMARY

1. The contribution by glial cells to surface recordings has been examined in the optic nerve of the amphibian *Necturus maculosus*. The method of current injection was employed selectively to alter the membrane potential of glial cells without affecting that of the axons. The resulting changes in potential were recorded simultaneously from the surface of the nerve using the sucrose gap method and intracellularly from a glial cell near the gap.

2. The sucrose gap method recorded 40 % of the changes in glial membrane potential. This percentage was not affected when the current electrode was inserted into different glial cells while maintaining the recording conditions constant.

3. Following axonal degeneration, produced by removing the eye 2–3 months earlier, the percentage contribution by glia increased to 84 %.

4. By measuring sucrose gap responses to changes in K_o it was possible to estimate that the sucrose gap method recorded 31–60 % of changes in axonal membrane potential. It was also determined that the axons, unlike glial cells, are relatively insensitive to reductions in K_o. Surface responses to decreases in external potassium thus reflect the magnitude of the glial contribution.

5. It is concluded that changes in glial membrane potential contribute about as much to surface recordings from the optic nerve of *Necturus* as do equivalent changes in axonal membrane potential. The contributions by the glial cells and axons are related to the relative volumes of tissue they respectively occupy. The significance of these findings to the analysis of surface recordings from the mammalian brain is discussed. Since mammalian glial cells, like those in Amphibia and the leech, become depolarized during neuronal activity and on the basis of electron microscopic evidence

* Fellow of the National Research Council of Canada. Present address: Department of Physiology, McGill University, Montreal, Canada.

appear to be electrically coupled, it is likely that they contribute to the electroencephalogram.

INTRODUCTION

Recent physiological studies by Kuffler and his co-workers have shown that in the central nervous systems of Amphibia and the leech glial cells become depolarized as a result of neuronal activity. The changes in glial membrane potential are relatively slow, measured in seconds, and can be quite large (up to 50 mV) in individual cells. They are brought about by an accumulation of potassium ions which are released by the active neurones into the surrounding intercellular clefts (Kuffler & Nicholls, 1966; Orkand, Nicholls & Kuffler, 1966; Kuffler, 1967; Baylor & Nicholls, 1969).

The present study was undertaken to decide whether changes in glial membrane potential contribute to potentials which one records with surface electrodes from nervous tissue. If this can be shown it would follow that portions of the conventionally recorded electroencephalogram (EEG) could be caused by glial cells. This expectation is made likely by several studies which have shown that the previously unidentified 'idle' cells in the mammalian brain are in fact glial cells and that these undergo analogous fluctuations in membrane potential as have been studied in detail in amphibians and the leech (Karahashi & Goldring, 1966; Kelly, Krnjevic & Yim, 1967; Grossman & Hampton, 1968; Dennis & Gerschenfeld, 1969).

To establish the contribution of glial cells to surface recordings in a quantitative manner the optic nerve of the amphibian *Necturus maculosus* (mud puppy) proved suitable. Being a tract of the central nervous system it is made up of axons and glial cells whose relative volume contributions to the tissue are known (Kuffler, Nicholls & Orkand, 1966). When current is injected through an intracellular electrode into one glial cell it spreads to neighbouring glial cells causing changes in their membrane potentials. The amplitudes of the potential changes and their rates of rise and fall decline with distance away from the site of current injection (Kuffler *et al.* 1966). Surface potentials resulting from these changes in glial potential were recorded in the present study using the sucrose gap method. Their magnitudes and time courses were compared with the 'true' changes in glial potential registered with a second intracellular electrode inserted in a glial cell close to the sucrose gap. It was found that changes in glial membrane potential contribute as much to surface recordings as do equivalent changes in axonal potential. Therefore it now seems established that the glial contribution to surface recordings has to be considered whenever the membrane potential of a group of glial cells is changed.

METHODS

The intracranial portion of the optic nerve of the mud puppy was dissected as previously described (Kuffler *et al.* 1966). The nerve is 80–150 μ in diameter and contains non-myelinated axons and glial cells in approximately equal volumes. It is surrounded by a stocking-like sheath containing blood vessels which do not penetrate the nerve proper. The sheath and its blood vessels were entirely removed in order to minimize shunting factors which would reduce recordings made with the sucrose gap method (see below). In some experiments nerves were used from animals in which one or both eyes were removed 2–3 months earlier. All animals were kept unfed at room temperature.

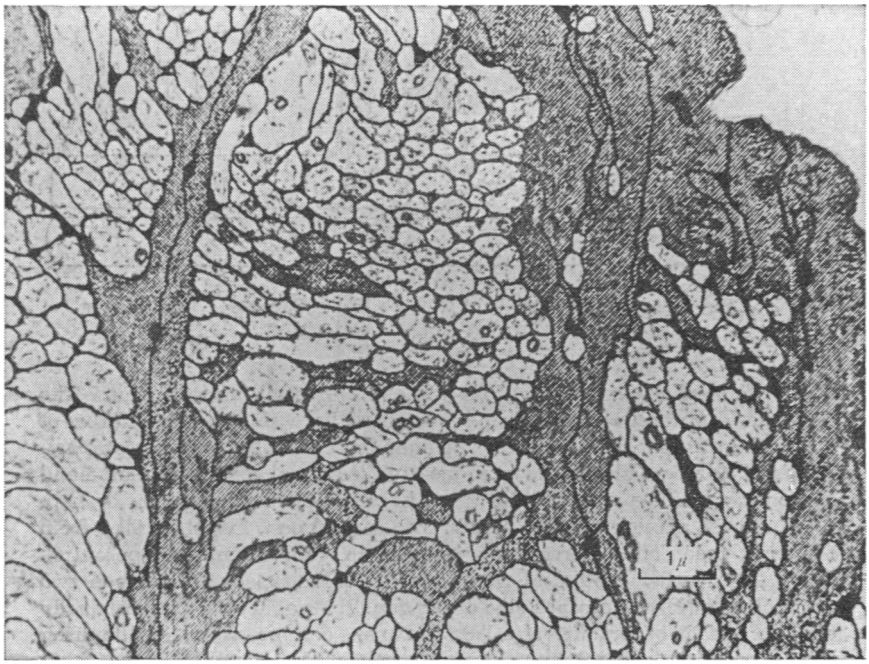

Fig. 1. Distribution of glial cytoplasm, nerve fibres, and intercellular clefts in an optic nerve of *Necturus*. The electron micrograph shows a cross-section through the outer portion of a desheathed nerve. The glial cytoplasm has been stippled in and the intercellular clefts blacked in for contrast (reprinted from Kuffler *et al.* 1966).

An electron micrograph of a transverse section through part of a desheathed nerve is shown in Fig. 1. From measurements on this and other such micrographs it was found that the glial cells occupy about 40 % of the nerve's volume. The axons occupy about 50 % of the nerve's volume and are all less than 2 μ in diameter. The remaining 10 % is taken up by the intercellular clefts.

Recording conditions. The desheathed nerve was placed in a sucrose gap chamber containing three compartments as shown diagrammatically in Fig. 2. The central

compartment, or gap, was 1 mm wide and was separated from the other two by partitions made from sheets of polyethylene, 50 μ thick. Each partition contained a single hole, about 100 μ in diameter, through which the nerve was drawn. The gap was perfused with isotonic sucrose whose rate of flow was adjusted so that, if increased, it did not produce any further increase in resistance across the gap (see below). The left-hand compartment was perfused with Ringer solution. The inflow was placed close to the partition and was connected to a 6-way tap of the Hodgkin–Horowicz type to permit rapid changes of solution (Hodgkin & Horowicz, 1959). The volume of the left-hand compartment was about 0·2 ml. and flow rates of about 2 ml./min were used. The compartment was drained via a strip of filter paper. This

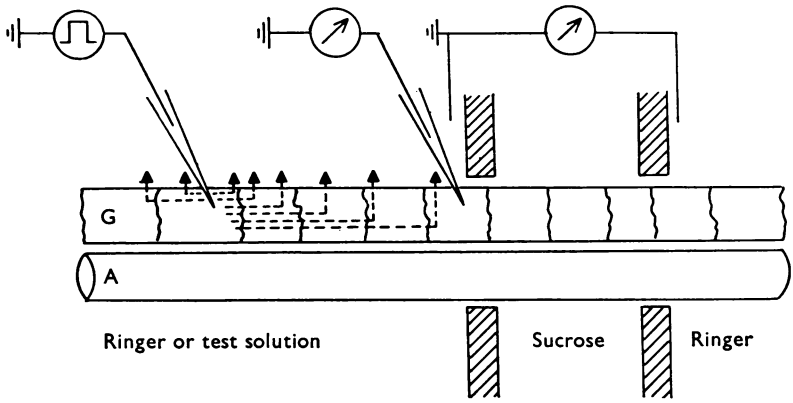

Fig. 2. Schematic representation of experimental arrangement. The desheathed optic nerve, which is represented by a single axon and row of glial cells, was placed in a sucrose gap chamber consisting of three compartments. Two micro-electrodes were inserted into glial cells in the left-hand compartment. The one distant from the central compartment, or gap, was used for passing current pulses. The second one, less than 90 μ from the gap, recorded the resulting changes in glial membrane potential. Changes in surface potential were recorded simultaneously between two other electrodes, one on each side of the gap. Since glial cells are electrically coupled to each other, current injected into one cell spreads to neighbouring cells causing their membrane potentials to change.

method was also preferred for draining the gap. The right-hand compartment was filled with Ringer solution but in most experiments was not perfused. To ensure minimal leakage of solutions between this compartment and the gap, small spaces between the nerve and the partition were plugged with silicone grease.

Sucrose gap recordings from the optic nerve were made with glass pipette electrodes, about 100 μ in diameter at the tip and filled with 3 M-KCl in 1 % agar. Chlorided silver wires served as leads. The electrode in the left-hand compartment was connected to ground and that in the right-hand compartment to a DC amplifier and an oscilloscope. The resistance across the gap was also monitored continuously and in successful experiments remained constant for several hours. To achieve this, and to prevent deterioration of the optic nerve, the sucrose flow was interrupted every 20 min or less and replaced by Ringer solution for about 10 min. The drift over a 20 min period of sucrose flow was usually less than 5 mV.

In preliminary experiments the sucrose gap apparatus was evaluated by testing its performance on desheathed frog tibial nerves. Whole nerve stimulation gave monophasic action potentials with peak amplitudes of up to 85 mV. Responses to changes in the concentration of external potassium, K_o, from 2·5 to 114 mM were as large as 60 mV. These values compare favourably with those obtained in previous studies (Stampfli, 1954; Schmidt, 1965).

To alter glial membrane potentials in a controlled fashion two glass capillary micro-electrodes filled with 3 M-KCl were inserted into glial cells in the left-hand compartment of the sucrose gap apparatus, one within 90 μ from the gap and the other 300–1200 μ from the gap (Fig. 2). The electrode near the gap served to measure the 'true' changes in glial membrane potential for comparison with the changes in surface potential recorded simultaneously with the sucrose gap method. Therefore a penetration with this electrode was accepted only if it recorded a stable membrane potential of at least 75 mV. The electrode away from the gap was used to inject current into the glial cells and membrane potentials above 60 mV were accepted. The current pulses injected through this distant micro-electrode were 1 sec in duration and of varying intensity. For some experiments a suction electrode was used to stimulate the whole nerve. Most of these methods have previously been described in detail (Kuffler *et al.* 1966).

In the course of prolonged experimentation it was found that the condition of optic nerves could be judged by the amount of depolarization caused by a standard increment of K_o. Thus in the most careful dissections a change of K_o from 3 to 18 mM gave a surface depolarization of 30 mV or more. Such a test was a useful criterion for the absence of injury to the nerve and was made at the beginning of each experiment. Only optic nerves with responses of 25 mV or more were accepted for further experimentation.

Solutions. Sulphate-Ringer of the following composition was used in all experiments: Na_2SO_4, 55·5 mM; K_2SO_4, 1·5 mM; $CaSO_4$, 8 mM; Tris maleate brought to pH 7·4 with NaOH, 5 mM; sucrose, 66 mM; glucose, 11 mM. Solutions of altered potassium concentration were made by substituting potassium for sodium in equimolar amounts. The sucrose solution for perfusing the gap contained 210 mM sucrose. All solutions were made up in glass distilled water and were evacuated before use in order to prevent any bubble formation during the experiments, which were carried out at room temperature (20–24° C).

RESULTS

The glial contribution in response to intracellular current injections

Previous studies have shown that glial cells are electrically coupled to each other by low resistance pathways (Kuffler & Potter, 1964; Kuffler *et al.* 1966). When current is injected through a micro-electrode into one glial cell the current spreads to neighbouring glial cells causing their membrane potentials to change. The size of these potential changes and their rates of rise and fall decline with distance away from the current electrode. In the optic nerve of *Necturus* such passive spread of current can be detected between glial cells separated by more than 1 mm. However, no significant current spread occurs between axons and their surrounding glial cells. For example, the current flow during an impulse volley in the optic nerve does not alter glial membrane potentials (Orkand *et al.* 1966). The lack of electrical interaction has been shown more

directly in the nervous system of the leech where neighbouring neurones and glial cells can both be impaled simultaneously (Kuffler & Potter, 1964). Glial cells also behave passively, showing no change in membrane resistance even if large depolarizing or hyperpolarizing currents are passed through them (Kuffler & Potter, 1964; Kuffler *et al.* 1966). In the following experiments the method of current injection was therefore used in order to selectively change the membrane potentials of glial cells without

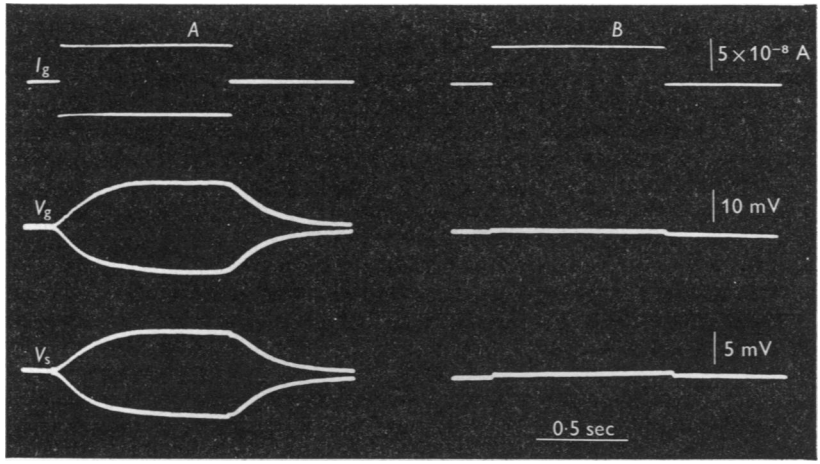

Fig. 3. *A.* Simultaneous potential changes recorded with an intracellular electrode (V_g) in a glial cell near the sucrose gap and with extracellular leads across the gap (V_s). The currents, monitored in the top trace (I_g), were injected into a glial cell 1020 μ from the gap (see scheme of Fig. 2). Note similar time courses of the potentials, but V_s is about one half of V_g (see calibration).

B. Same conditions as in *A*, but the current electrode was advanced into the intercellular space just outside the glial cell. Note the absence of significant potentials when current does not cross glial membrane.

affecting the axons. The resulting changes in potential were recorded simultaneously from the surface of the nerve using the sucrose gap method and intracellularly from a glial cell near the sucrose gap.

A typical experiment is shown in Fig. 3*A*. The top trace monitors the current injected into a glial cell 1020 μ from the sucrose gap and having a resting potential of 76 mV. The middle trace shows the resulting changes in the membrane potential of a glial cell within 90 μ from the gap, having a resting potential of 83 mV. On the bottom trace are the changes in the surface potential recorded with the sucrose gap method. During the current pulses the glial membrane potential and the surface potential changed with similar time courses, rising to 67 % of maximum in about 0·25 sec. Only their amplitudes differed, the potential changes recorded with the sucrose gap method being about one half of those recorded intra-

cellularly. A series of similar recordings, but with different current intensities, were made on the same optic nerve and are plotted in Fig. 4. As expected from previous experiments (Kuffler *et al.* 1966) the changes in glial membrane potential, ΔV_g, were linearly related to current intensity, I_g, over the entire range (Fig. 4*A*; filled circles). The changes in surface potential, ΔV_s, were also linearly related to I_g (Fig. 4*A*; open circles), as would be expected if they were caused by the changes in glial membrane potential.

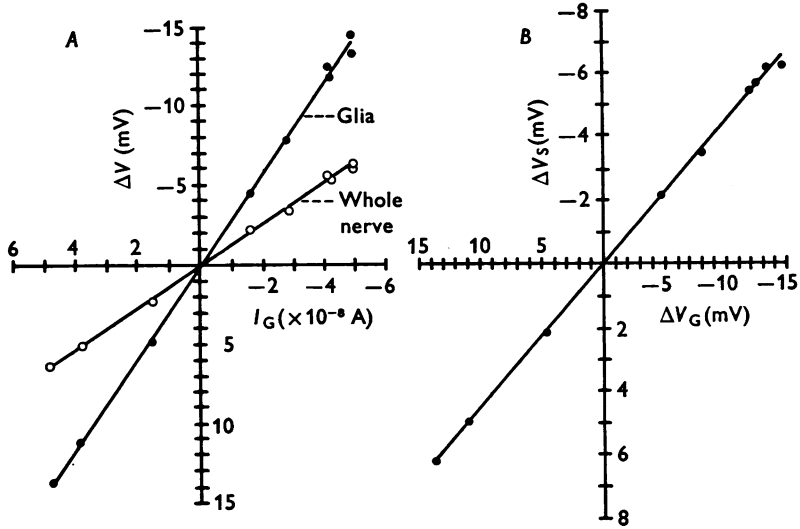

Fig. 4. *A*. Current–voltage relationships for intracellular and sucrose gap recordings. From the same experiment as in Fig. 3. The changes in glial membrane potential (filled circles) as well as in surface potential (open circles) were linearly related to current intensity over the entire range.

B. The data are replotted to show that the change in surface potential, ΔV_s, remained a constant fraction of the change in glial membrane potential, ΔV_g, for all current intensities. The fraction is given by the slope of the straight line, which in this experiment was 0·45.

In fact the surface recordings remained a constant fraction of the intracellular recordings at all current intensities used. An accurate measure of this fraction was obtained by plotting ΔV_s against ΔV_g, as shown in Fig. 4*B*. The straight line drawn through the points was calculated by the method of least squares and has a slope of 0·45, which indicates that whenever the membrane potential of the glial cell near the gap changed, 45 % of the change was recorded with the sucrose gap method. In seven experiments of this kind the ratio $\Delta V_s/\Delta V_g$ was $0{\cdot}40 \pm 0{\cdot}03$ (mean ± S.E. of mean).

The changes in potential described above proved to be entirely dependent on the impalement of a glial cell by the current electrode. If the current electrode was advanced or withdrawn so that its tip was just

outside, rather than inside, a glial cell the current pulses produced no significant changes in either surface potential or glial membrane potential (Fig. 3*B*). This result eliminates the unlikely possibility that the current pulses caused changes in axonal membrane potentials upon which the surface potentials depended. Instead it is necessary to conclude that the changes in surface potential were invariably linked with changes in glial membrane potential.

Cross-sections through the optic nerve of *Necturus* always reveal several glial nuclei (see Fig. 2 in Kuffler *et al.* 1966). It is therefore apparent that the sucrose gap method recorded the contribution of many glial cells. In order to determine whether the intracellular recordings were representative of the potential changes in all glial cells near the gap, in some experiments the current electrode was inserted into different glial cells along the nerve while maintaining the recording conditions constant. As expected, when the current electrode was moved closer to the gap the potential changes for both the surface and intracellular recordings became larger and their rise times became faster; however, the ratio $\Delta V_s/\Delta V_g$ remained constant (Table 1; expt. nos. 2, 4, 7, 8). Presumably all glial cells in the optic nerve are effectively coupled to each other by low resistance pathways, so that the current injections several hundred micra from the gap caused the membrane potential of all glial cells near the gap to change by the same amount. Otherwise one would expect to have found differences in the ratio $\Delta V_s/\Delta V_g$ upon penetration of different glial cells with the current (or recording) electrode.

Increase in glial contribution following axonal degeneration

Since glial cells occupy about 40% of the optic nerve's volume (see Methods) and the sucrose gap method recorded this same percentage of changes in glial membrane potential, it was of interest to examine further how the contribution by glial cells to surface recordings is related to the volume of tissue they occupy. Experiments were therefore made on optic nerves of animals in which one or both eyes had been removed 2–3 months earlier in order to allow the axons to degenerate. The diameters of these chronic 'eyeless' nerves were less than those of control nerves and their resistances as measured across the sucrose gap were larger, being 1·0–4·5 MΩ as compared to 0·4–1·2 MΩ (Table 1). Passing the usual stimulating pulses through them did not elicit action potentials or any type of regenerative response. On the other hand the glial cells had the usual membrane potentials and their degree of electrical coupling, as determined by the ratio $\Delta V_g/I_g$, did not appear to be markedly altered (Table 1).

The same experiments as described in connexion with Figs. 3 and 4, of injecting current into individual glial cells and recording the consequent

changes in glial membrane potential and surface potential, were repeated on 'eyeless' optic nerves. The results were strikingly different. In three experiments the changes in surface potential were 0·75, 0·88 and 0·88 of the changes in glial membrane potential. Thus by degenerating the axons, the glial contribution to the surface recording approached 100%, suggesting

TABLE 1. Intracellular and surface recordings in response to current injections into glial cells. R.P. resting potential

	I_g		V_g			V_s		
Expt. no.	Dist. from gap (μ)	R.P. (mV)	R.P. (mV)	Rise time* (sec)	$\frac{\Delta V_g}{I_g}$ (MΩ)	Resistance across gap (MΩ)	Rise time* (sec)	$\frac{\Delta V_s}{\Delta V_g}$
				'Normal' nerves				
1†	1020	76	83	0·23	0·28	0·7	0·24	0·45
2	540	67	83	0·25	0·47	0·6	0·29	0·46
	450	75	—	0·20	0·52	—	0·25	0·46
3	570	79	90	0·05	0·07	0·4	0·06	0·43
4	660	83	85	0·09	0·16	0·9	0·11	0·44
	390	78	—	0·08	0·17	—	0·10	0·44
	330	80	—	0·06	0·20	—	0·08	0·44
5	510	75	76	0·08	0·19	1·0	0·10	0·30
6	600	62	80	0·07	0·06	0·4	0·08	0·32
7	900	84	80	0·18	0·23	1·2	0·17	0·37
	810	71	—	0·18	0·24	—	0·17	0·37
	720	84	—	0·17	0·30	—	0·16	0·37
				'Eyeless' nerves				
8†	630	86	85	0·19	0·45	1·6	0·19	0·88
	600	83	—	0·18	0·56	—	0·18	0·88
	360	80	—	0·14	0·70	—	0·14	0·88
9	720	90	86	0·09	0·40	1·0	0·09	0·75
10	900	63	82	0·16	0·30	4·5	0·18	0·88

* Time to reach 67% of final amplitude.

† The nerves for expts. nos. 1 and 8 were taken from the same animal.

that the axons, which in control nerves had served as parallel shunts to the glial cells, had now virtually disappeared. Recent electron microscopic studies have shown that non-myelinated axons in the optic nerves of alligators and crocodiles entirely disappear 1–3 weeks after they are severed from their cell bodies (Kruger & Maxwell, 1969). The present findings therefore demonstrate that the contribution by glial cells to surface recordings depends, among other factors, on the relative volume of tissue they occupy.

The glial contribution in response to decreases in external potassium

Among the physiological properties which distinguish glial cells from neurones is the sensitivity of their membrane potential to decreases in the concentration of external potassium, K_o (Kuffler & Nicholls, 1966; Kuffler, 1967). In the optic nerve of *Necturus* reduction of K_o to one tenth, from the standard 3 mM to 0·3 mM, hyperpolarizes glial cells by 30–40 mV (Kuffler *et al.* 1966). In contrast, the membrane potentials of a wide variety of neurones are relatively insensitive to such changes in K_o and hyperpolarize by only a few millivolts (Curtis & Cole, 1942; Huxley & Stampfli, 1951; Julian, Moore & Goldman, 1962; Nicholls & Kuffler, 1964). This difference in sensitivity presumably arises because, in the case of neurones, ions other than potassium, such as sodium, have a significant effect in determining the membrane potential when K_o is low (Hodgkin, 1958), whereas for glial cells this does not appear to be the case. In any event the sensitivity of glial cells to reductions in K_o suggests that surface responses to such changes should be large whenever the glial contribution is significant.

To test this prediction surface responses to reductions in K_o were recorded with the sucrose gap method in the same optic nerves which were used for measuring the glial contribution by the current injection method. K_o was reduced from the standard 3 mM to 1·5 mM and to 0·3 mM. These reductions in external potassium elicited hyperpolarizations which, as seen in the example of Fig. 5, reached a steady value in about 30 sec and were completely reversible. For seven 'normal' nerves the hyperpolarizations were $7 \pm 0{\cdot}5$ mV (mean $\pm$ S.E. of mean) in response to 1·5 mM-K_o and $14 \pm 1{\cdot}2$ mV in response to 0·3 mM-K_o. These values are approximately 40% of those which have been recorded intracellularly from glial cells (Kuffler *et al.* 1966). As described above, the sucrose gap method was found, by using current injections, to record 40% of changes in glial membrane potential. Thus the surface responses to reductions in K_o appear to be entirely attributable to the changes in glial membrane potential and thereby reflect the relative magnitude of the glial contribution. The membrane potentials of the axons are apparently insensitive to reductions in K_o.

These conclusions were further reinforced by the results obtained with three 'eyeless' nerves. The responses to 1·5 mM-K_o were 10, 11 and 12 mV, and to 0·3 mM-K_o they were 24, 27 and 28 mV, approximately twice as large as those obtained with the 'normal' nerves. The glial contribution as measured by the current injection method was also twice as large in 'eyeless' nerves (84% compared to 40%). It is therefore clear that the surface responses to reductions in K_o provide an alternative and reliable measure of the relative magnitude of the glial contribution.

The axonal contribution

Since the sucrose gap method recorded 40 % of changes in glial membrane potential in 'normal' nerves the remaining 60 % must have been shunted. Assuming the shunt to be due entirely to the axonal population of the optic nerve, it follows that the sucrose gap method would record 60 % of changes in axonal membrane potential. This value represents an upper limit for the axonal contribution. It would be less if in addition to the axons some extraneous shunt existed across the gap.

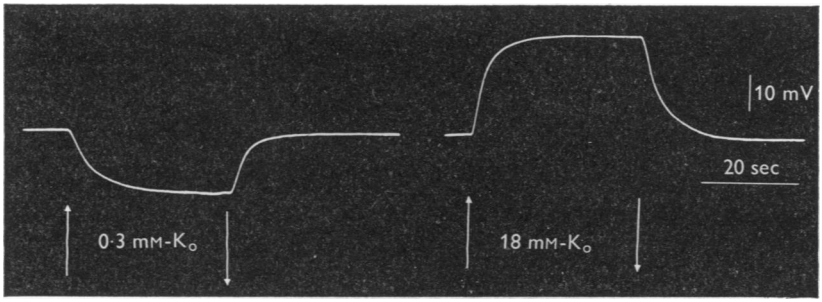

Fig. 5. Responses to changes in the potassium ion concentration of the bathing fluid recorded from an optic nerve with the sucrose gap method. Reducing the potassium ion concentration in the bath from 3 to 0·3 mM gave a reversible hyperpolarization of 16 mV. Increasing it from 3 to 18 mM gave a reversible depolarization of 27 mV. From such measurements the relationship between change in surface potential and potassium ion concentration was plotted (Fig. 6).

An estimate of the minimum contribution by the axonal population was obtained in the following way. In each of the experiments described above surface recordings were made in response to changes in K_o from the standard 3 mM to the following values: 0·3, 1·5, 9, 18, 36 and 72 mM. The responses to decreases in K_o have already been described in the previous section. Increases in K_o elicited depolarizations which, as seen in the example of Fig. 5, reached a steady value in about 30 sec and were completely reversible. The results for the entire series of potassium ion concentrations are plotted in the graph of Fig. 6 (filled circles). For K_o between 9 and 72 mM the change in surface potential was linearly related to the logarithm of K_o with a slope of 41 mV for a tenfold change. For K_o below 9 mM the slope became progressively less steep.

It is known that the glial membrane behaves like a potassium electrode for K_o above 1·5 mM; a tenfold change in K_o causes a 58 mV change in membrane potential (at 20° C) as predicted by the Nernst equation (Kuffler *et al.* 1966). In the present experiments the sucrose gap method recorded 40 % of changes in glial membrane potential. It therefore follows

that the glia contributed 23·2 mV (0·40 × 58 mV) to the 41 mV slope and that the remainder, 17·8 mV, was due to the axons. The actual slope relating membrane potential to the logarithm of K_o is not known for the axons of the optic nerve of *Necturus* because non-myelinated fibres with diameters of 1–2 μ or less cannot be impaled with micro-electrodes. It could be as large as 58 mV but might be less if the axonal membrane were relatively permeable to ions other than potassium. Assuming the slope to

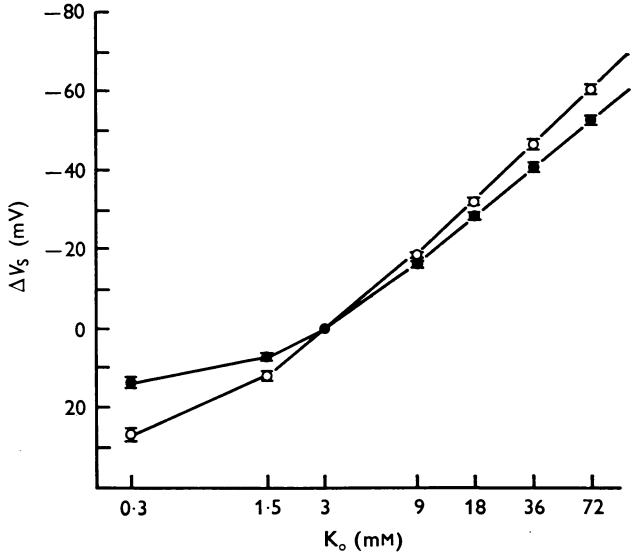

Fig. 6. Relation between change in surface potential, ΔV_s, and the external potassium ion concentration, K_o. The mean (± S.E. of mean) of the responses to each potassium ion concentration is shown for seven 'normal' nerves (filled circles) and for three 'eyeless' nerves (open circles). Potassium concentrations are plotted on a logarithmic scale. Note the large responses, especially for the 'eyeless' nerves, when K_o was reduced below 3 mM. For K_o between 9 and 72 mM the values lie on straight lines, with slopes of 41 mV for the 'normal' nerves and 46 mV for the 'eyeless' nerves. The results are from the same nerves which were used for measuring the glial contribution by the current injection method.

be 58 mV it follows that the sucrose gap method recorded 31 % (17·8/58) of the changes in axonal membrane potential. This value represents the minimum percentage contribution by the axons and would of course be larger if the axonal slope were less than 58 mV. Thus the estimated limits for the axonal contribution (31–60 %) are close to the measured value of 40 % for the glial contribution. In other words, changes in glial membrane potential contribute about as much to surface recordings from the optic nerve as do equivalent changes in axonal membrane potential.

Responses to changes in K_o were also recorded from the 'eyeless' optic

nerves. As was the case for 'normal' nerves the change in surface potential was linearly related to the logarithm of K_o for concentrations above 9 mM (Fig. 6, open circles). The slope, 46 mV for a tenfold change, was close to the value of 48·7 mV (0·84 × 58 mV) which would be expected if the nerve contained only glial cells, and was 5 mV larger than that obtained with the 'normal' nerves. Such an increase following axonal degeneration would be expected if the axonal slope were less than the glial slope. An alternative possibility is that the axons have the same slope as the glial cells but there was less extraneous shunting across the sucrose gap with the 'eyeless' nerves.

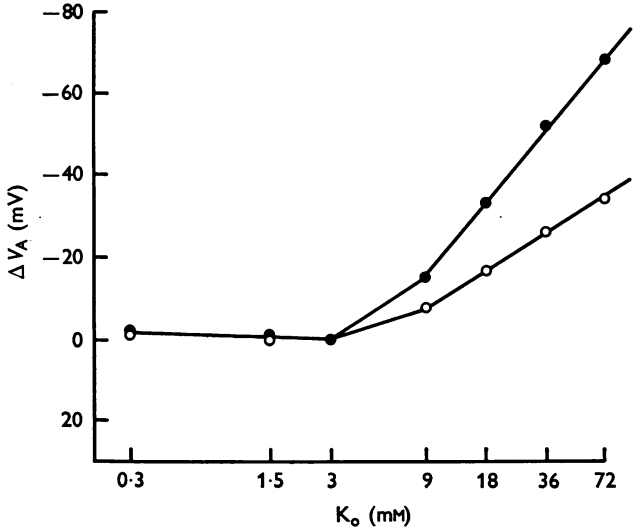

Fig. 7. Minimum (open circles) and maximum (filled circles) estimates of changes in axonal membrane potential produced by changes in the potassium ion concentration of the bathing fluid. These estimates were calculated on the basis that the sucrose gap method recorded 31–60 % of changes in axonal membrane potential. The axons, unlike glial cells, appear to be insensitive to reductions in K_o below 3 mM. Above 9 mM the change in axonal membrane potential for a tenfold change in K_o lies between 31 and 58 mV (see text for further detail).

The axonal membrane potential

Fig. 7 shows minimum and maximum estimates of the changes in axonal membrane potential in response to changes in K_o. These estimates were calculated using the following relationship:

$$\Delta V_s = 0{\cdot}40 \times \Delta V_g + 0{\cdot}31 \text{ (or } 0{\cdot}60) \times \Delta V_a.$$

The numbers represent the fractional contributions by the glial cells and axons respectively, as determined earlier. The values for ΔV_s have already been presented (Fig. 6, filled circles) and those for ΔV_g were taken from the

results of Kuffler *et al.* (1966). It is seen, as already pointed out, that reductions in K_o below the standard 3 mM had negligible effects on the axonal membrane potential. For K_o greater than 9 mM the slope relating axonal membrane potential to the logarithm of K_o lies between 31 and 58 mV. The resting potential, assuming K_i to be about 100 mM (see Kuffler *et al.* 1966), lies between 39 and 76 mV. This range compares favourably with that of 34–82 mV estimated for the non-myelinated fibres in the rabbit vagus nerve (Keynes & Ritchie, 1965). It is interesting to note that even in neurones which have relatively low resting potentials as revealed by reliable intracellular recordings, such as 48 mV for the large neurones in leech ganglia (Nicholls & Kuffler, 1964), the slope relating membrane potential to the logarithm of K_o approaches 58 mV. It may therefore be that the actual values for the optic nerve fibres are closer to the upper limits. However, any definitive conclusion must await more direct measurements.

DISCUSSION

The present findings have demonstrated that for the optic nerve of *Necturus* changes in glial membrane potential contribute about as much to surface recordings as do equivalent changes in neuronal membrane potential. This large glial contribution is readily explained by the fact that the glial cells are electrically coupled to each other by low resistance pathways and thus behave like a single long 'core' conductor. Consequently, a change in the membrane potential of a group of glial cells at one point along the nerve will generate current flow over a sufficient length of nerve, thereby creating an extracellular potential difference which can be detected by surface recording methods. In the case of a nerve having a single population of cells, all of which undergo the same change in membrane potential, the change in surface potential is directly proportional to the ratio $r_o/(r_o+r_i)$ and will be large if the intracellular resistance, r_i, is small compared to the extracellular resistance, r_o (Katz, 1966). It follows that in a nerve containing two populations of cells, axons and glia, the population with the smaller internal resistance will provide the larger contribution. The finding that the axonal and glial percentage contributions are approximately the same indicates that their internal resistances must be similar. This is not unexpected since the glial cells and axons in the optic nerve of *Necturus* occupy approximately equal volumes (see Methods).

The question arises whether glial cells in the mammalian central nervous system, where surface recording techniques have been applied most extensively, also make significant contributions. For example, the neurone–glial relationship is similar to that seen in the optic nerve of *Necturus* and rough

estimates indicate that the glial cells occupy as much as one half the volume (Kuffler, 1967). Several recent studies have further indicated that mammalian glial cells have membrane properties which are similar to those found in Amphibia and the leech. They have high resting potentials which are sensitive to changes in K_o and they cannot be made to fire action potentials (Kelly *et al.* 1967; Dennis & Gerschenfeld, 1969). They also undergo slow depolarizations in response to neuronal activity (Grossman & Hampton, 1968; see also Karahashi & Goldring, 1966). Attempts to demonstrate electrical coupling between them have, however, been unsuccessful, presumably because of technical difficulties (Dennis & Gerschenfeld, 1969). On the other hand 'tight junctions', which appear to be an anatomical correlate of electrical coupling (Furshpan, 1964), have been observed between mammalian glial cells (Peters, 1962; Brightman & Reese, 1969) thereby suggesting that they are in fact connected by low resistance pathways. This being the case they should contribute significantly to surface recordings, as in *Necturus*. Indirect evidence for a glial contribution in the cat cerebral cortex has recently been obtained by Castellucci & Goldring (1970).

Whereas glial cells become depolarized when neighbouring neurones are active, the neurones themselves may undergo potential changes of the same or opposite polarity (Baylor & Nicholls, 1969). The surface potential, being the algebraic sum of the contributions from different cell populations, may therefore have a wave form which is entirely different from those of the potential changes in the contributing cells. Examples of such differences in wave form have recently been observed for simultaneously recorded glial potentials and surface potentials in the cat cerebral cortex (Grossman, Whiteside & Hampton, 1969; Castellucci & Goldring, 1970) as well as in the optic nerve of *Necturus* (unpublished observations).

It is a pleasure to thank Professor Stephen W. Kuffler for continual advice and encouragement throughout the course of work and for reading the manuscript. Mr R. B. Bosler gave unfailing technical assistance. This work was supported by Grant No. NB-02253 from the National Institutes of Health, U.S. Public Health Service.

REFERENCES

BAYLOR, D. A. & NICHOLLS, J. G. (1969). Changes in extracellular potassium concentration produced by neuronal activity in the central nervous system of the leech. *J. Physiol.* **203**, 555–569.

BRIGHTMAN, M. W. & REESE, T. S. (1969). Junctions between intimately apposed cell membranes in the vertebrate brain. *J. cell Biol.* **40**, 648–677.

CASTELLUCCI, V. F. & GOLDRING, S. (1970). Contribution to steady potential shifts of slow depolarization in cells presumed to be glia. *Electroenceph. clin. Neurophysiol.* **28**, 109–118.

CURTIS, H. J. & COLE, K. S. (1942). Membrane resting and action potentials from the squid giant axon. *J. cell. comp. Physiol.* **19**, 135–144.

DENNIS, M. J. & GERSCHENFELD, H. M. (1969). Some physiological properties of identified mammalian neuroglial cells. *J. Physiol.* **203**, 211–222.

FURSHPAN, E. J. (1964). 'Electrical transmission' at an excitatory synapse in a vertebrate brain. *Science, N.Y.* **144**, 878–880.

GROSSMAN, R. G. & HAMPTON, T. (1968). Depolarization of cortical glial cells during electrocortical activity. *Brain Res.* **11**, 316–324.

GROSSMAN, R. G., WHITESIDE, L. & HAMPTON, T. L. (1969). The time course of evoked depolarizations of cortical glial cells. *Brain Res.* **14**, 401–415.

HODGKIN, A. L. (1958). Ionic movements and electrical activity in giant nerve fibres. *Proc. R. Soc.* B **148**, 1–37.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* **148**, 127–160.

HUXLEY, A. F. & STAMPFLI, R. (1951). Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibres. *J. Physiol.* **112**, 496–508.

JULIAN, F. J., MOORE, J. W. & GOLDMAN, D. E. (1962). Membrane potentials of the lobster giant axon obtained by use of the sucrose-gap technique. *J. gen. Physiol.* **45**, 1195–1216.

KARAHASHI, Y. & GOLDRING, SIDNEY (1966). Intracellular potentials from 'idle' cells in cerebral cortex of cat. *Electroenceph. clin. Neurophysiol.* **20**, 600–607.

KATZ, B. (1966). *Nerve, Muscle and Synapse.* New York: McGraw Hill.

KELLY, J. S., KRNJEVIC, K. & YIM, G. K. W. (1967). Unresponsive cells in cerebral cortex. *Brain Res.* **6**, 767–769.

KEYNES, R. D. & RITCHIE, J. M. (1965). The movements of labelled ions in mammalian non-myelinated nerve fibres. *J. Physiol.* **179**, 333–367.

KRUGER, L. & MAXWELL, D. S. (1969). Wallerian degeneration in the optic nerve of a reptile: an electron microscopic study. *Am. J. Anat.* **125**, 247–269.

KUFFLER, S. W. (1967). Neuroglial cells: physiological properties and a potassium mediated effect of neuronal activity on the glial membrane potential. *Proc. R. Soc.* B **168**, 1–21.

KUFFLER, S. W. & NICHOLLS, J. G. (1966). The physiology of neuroglial cells. *Ergebn. Physiol.* **57**, 1–90.

KUFFLER, S. W. & POTTER, D. D. (1964). Glia in the leech central nervous system: physiological properties and neuron-glia relationship. *J. Neurophysiol.* **27**, 290–320.

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

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.

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.

PETERS, A. (1962). Plasma membrane contacts in the central nervous system. *J. Anat.* **96**, 237–248.

SCHMIDT, H. (1965). Die Wirkung von Tetraäthylammoniumchlorid auf das Membran potential und den Membranwiderstand von Bundeln markaltiger Nervenfasern. *Pflügers Arch. ges. Physiol.* **282**, 351–361.

STAMPFLI, R. (1954). A new method for measuring membrane potentials with external electrodes. *Experientia* **10**, 508–509.