EFFECT OF NERVE IMPULSES ON THE MEMBRANE
POTENTIAL OF GLIAL CELLS IN THE CENTRAL
NERVOUS SYSTEM OF AMPHIBIA

R. K. ORKAND, J. G. NICHOLLS, AND S. W. KUFFLER
Neurophysiology Laboratory, Department of Pharmacology,
Harvard Medical School, Boston, Massachusetts
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Neurons and glial cells in most parts of the nervous system are intimately apposed, separated from each other by channels about 150 Å wide. It is natural to wonder whether the two types of cell influence one another either "electrically" (i.e., by current flow from one cell to the other) or by the release of a substance. It has been shown in the leech nervous system that if current is supplied to the interior of a nerve cell through an intracellular electrode, very little will enter the neighboring glial cell; most will pass through the intercellular clefts which have a relatively low resistance. Similarly, currents which flow during nerve impulses alter the membrane potential of the surrounding glia to a negligible extent (10). These results were in good agreement with subsequent findings that the clefts between neurons and glial cells served as channels for the rapid diffusion of ions and of small molecules both in the leech and in amphibia (12, 9). In the central nervous system of the leech, which contains smooth muscle cells in its connective tissue capsule, the effect of massive neuronal activity on the resting potential of glial cells could not be studied adequately because the preparation moved under these conditions and dislodged the electrode (10).

In the present study no direct electrical interaction between neurons and glial cells was found in the optic nerves of the mud puppy (Necturus) or the frog. Nerve impulses, however, caused a slow depolarization of glial cells, an effect which resulted from diffusion of materials liberated during the impulses. We have concluded that during neuronal activity K⁺ accumulates in the intercellular clefts and depolarizes the membrane of the nearby glial cells. Since, as shown in the preceding paper (9), the glial membrane potential is very sensitive to the extracellular K⁺ concentration, the effects observed might have been expected from earlier observations of Frankenhaeuser and Hodgkin (3). They showed that, following a nerve impulse in the giant axon of the squid, K⁺ accumulates in the intercellular clefts between axon and

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2 Present address: Dept. of Physiology, University of Utah College of Medicine, Salt Lake City, Utah; Special Fellow of the National Institutes of Health.
3 Present address: Dept. of Physiology, Yale University School of Medicine, New Haven, Conn.; Fellow of the National Multiple Sclerosis Society.
EFFECT OF NERVE ON GLIA

Schwann cells. This results in a fall of the membrane potential in the axon, the well-known negative afterpotential.

METHODS

Most of the experiments were made on the mud puppy (Necturus maculosus), but frogs (Rana pipiens) or the bullfrog (Rana catesbiana) were also used. The "glial depolarization" was also observed in the optic nerve of one green snake (Opheodrys aestivus). The techniques and other features of the preparations have already been described in preceding papers (9, 10, 14). For recording and stimulating, the two ends of the optic nerve were placed in suction electrodes. In order to stimulate the unmyelinated axons rectangular pulses of relatively long duration (1-3 msec.) were used. The Ringer fluid contained: NaCl, 111 mM; KCl, 3 mM; CaCl2, 1.8 mM; tris-maleate brought to pH 7.4 with NaOH, 5 mM; glucose, 11 mM. When the K+ concentration was changed, "SOJ-Ringer fluid" of the following composition was used to avoid swelling of the cells due to movements of KCl: Na2SO4, 55.5 mM; K2SO4, 1.5 mM; Na-tris-maleate, 5 mM; glucose, 11 mM; sucrose, 66 mM. To maintain the ionized Ca ++ level at about 1 mM, we added 8 mM CaSO4. K+-rich solutions were made by substituting K+ for Na+. All experiments were done at room temperature (22-24°C.).

RESULTS

Effect of nerve stimulation on the glial membrane potential in Necturus

In histological studies of Necturus optic nerve myelinated axons were not found (15; and Wolfe, unpublished). Electrical measurements in the present investigation gave conduction velocities between 0.2 and 0.5 m/sec. (0.1- to 1.5-μ, axon diameters) and a compound action potential similar to that observed in other unmyelinated nerves.

An effect of nerve impulses on glial cells was demonstrated in the following manner. When a microelectrode inserted into a glial cell gave a stable recording of the resting potential, the optic nerve fibers were stimulated at some distance from the recording site; a transient depolarization of the glial cell resulted. An example is shown in Fig. 1. The depolarization following a single maximal nerve stimulus rose to a peak of 2.4 mV. in about 0.15 sec. and declined to half in about 2 sec. (Fig. 1, top record); the potential changes summed when the nerve stimulation was repeated at relatively brief intervals (Fig. 1, lower record).

Some of the features of the glial depolarization were as follows: a) The

![Fig. 1. Depolarization of a glial cell produced by nerve impulses in the Necturus optic nerve. Glial membrane potential recorded with an intracellular electrode was 86 mV. The isolated nerve was stimulated maximally a few millimeters from the site of microelectrode penetration. Upper record: depolarization following a nerve volley rises to a peak in about 150 msec. and declines with a half-time of about 2 sec. Lower record: three stimuli at 1-sec. intervals. The glial depolarizations sum. The brief initial downward deflections result from current flow in the external bathing fluid produced by propagating nerve impulses (see Fig. 3A).](http://jn.physiology.org/cookie/10.220.32.246/1965/66/3/789/fig01)
potentials following a single maximal stimulus to the unmyelinated axons of the optic nerve had a much slower time course than that of the nerve impulses; the times to peak of the response were 50-150 msec. while the half times of decline were 1.5–3 sec. (for still slower changes with repetitive stimuli see later). b) The response could be observed only when a glial resting potential was recorded. If the glial membrane potential fell, e.g., as a result of damage produced by the electrode, the glial depolarization decreased. c) The amplitude of the response varied with the strength of stimulation within a certain range, indicating that it depended on the number of axons which had been stimulated. The largest single response was 3.1 mV. d) The maximum amplitude of the summated response depended on the frequency of nerve stimulation, as illustrated in Fig. 2. Thus, when stimulation was continued for 22 sec. at a rate of 5/sec. the final depolarization reached 17 mV., whereas at 2/sec. the value was only 8 mV. The magnitude of the maintained depolarization varied greatly in different preparations and much higher values were sometimes obtained (see Fig. 7A). The variability in the amplitude of glial depolarization apparently reflected injury of axons during the somewhat technically difficult dissection. Thus, the largest potentials occurred in vivo when dissection was kept to a minimum by exposing for recording only the central portion of the optic nerve, while stimulating it behind the eye.

The mechanism by which nerve impulses depolarize the glial cells is revealed in part by Fig. 3. In the top record of Fig. 3A the electrode tip was within the optic nerve but had not penetrated a cell because no resting
FIG. 3. Lack of effect of external current generated by nerve impulses on the glial membrane potential. A: top record; microelectrode within the optic nerve was extracellular (membrane potential, $V_m = 0$) and the isolated nerve was electrically stimulated. The triphasic potential is caused by current flow from the active axons across the resistance in the extracellular fluid between the microelectrode and the bath electrode. Bottom record; the microelectrode was advanced, recording a glial resting potential of 84 mV. Identical nerve stimulation sets up the same triphasic potential which, however, is followed by a slowly rising glial depolarization (similar response, but faster time base, as in Fig. 1). The similarity of the triphasic potential recorded outside and inside of the glial cell shows that there was no significant current flow across the glial membrane. B: same experiment as in A performed in an anesthetized circulated animal. Upper record; electrode extracellular in the intracranial portion of the optic nerve. Maximal stimulation of the eye end of nerve does not produce a measurable nerve action current. Lower record; microelectrode was slightly advanced and a 87-mV. glial resting potential was recorded. The stimulus now produced a glial depolarization which rose to a peak of 3.1 mV. in about 75 msec. 

The slow glial depolarization is not produced by current spreading from the axons or by an effect triggered by such a current spread. These possibilities are excluded for the following reasons: a) The records of Fig. 3A indicate that no potential was produced across the glial membrane by the nerve action currents because the potential field was the same when recorded outside or inside the glial membrane. Furthermore, the nerve action potentials are over before the glial potential starts, or while it is still rising. Therefore, no direct electrical connection exists between neurons and glia. This consideration
also excludes the possibility that a slow negative afterpotential in the axons could spread to the glia. b) A triggering of the glial depolarization by action currents can be ruled out because even large potential changes across the glial membrane, of more than 100 mV., produced by passing current through an intracellular electrode, do not evoke such a glial response (9). In some experiments the potential field produced by the nerve volley was not larger than the noise level of the recording, e.g., Fig. 3B. Yet, the glial depolarization could still be recorded following nerve stimulation. These results exclude a direct role of current flow in either causing or triggering the glial depolarization.

Glial depolarization in the frog

Similar observations to those in *Necturus* were made in the frog optic nerve; the results were more easily obtained because the dissections were much simpler. On the other hand, while resting potentials were high *in vivo* (more than 80 mV.), they were rarely more than 60 mV. in isolated preparations (9).

Unlike the optic nerve of *Necturus*, the frog optic nerve contains some myelinated fibers (11) which give rise to an early peak in the compound action potential (later, Fig. 9A). Large glial depolarizations were seen when the unmyelinated fibers were stimulated (Fig. 9B, lower record); however, no effect on glia was detected when the low-threshold fast-conducting myelinated fibers were stimulated on their own, even at high frequencies (30-50 /sec.); possibly because they are few in number and the “active” response is confined to the nodes of Ranvier.

Ionic basis of the glial depolarization

In vertebrate and invertebrate nerves so far examined, K+ has been found to move out during the nerve impulse (e.g., 6, 7). Since the glial membrane is remarkably sensitive to changes in the extracellular K+ concentration, we have tested the following hypothesis: during impulse activity, K+ released from the axons accumulates in the intercellular clefts surrounding glial cells and produces a glial depolarization.

It was shown in the preceding study that the membrane potential of the glial cells in the *Necturus* optic nerve is normally at the K+ equilibrium potential, $E_K$ (9). It follows the logarithmic relation predicted by the Nernst equation over a remarkably wide range of external K+ concentration (1.5–75 mEq/liter). Thus, if the depolarizing substance released by the nerve impulses is K+, its effect will vary in a predictable way when the extracellular K+ concentration ($K_0$) is altered; the effect will be larger at low $K_0$ and smaller when $K_0$ is increased.

Figure 4 is an example of one experiment testing this prediction. The resting potential of the glial cell was 89 mV., with the normal concentration of K+ (3 mEq/liter) in the Ringer fluid. A brief train of 10 nerve stimuli at 10/sec. set up a glial depolarization of 12.3 mV. Upon changing to 4.5 mEq/liter K+, the resting potential fell to 78 mV., as predicted by the Nernst equa-
When the same nerve stimulation was repeated the glial depolarization was now only 9.3 mV. When $K_0$ was subsequently reduced to 1.5 mEq/liter the new resting potential was 105 mV. and the glial depolarization 18.5 mV. On returning to 3 mEq/liter $K_0$ the glial depolarization (11.9 mV.) was close to its original size.

We now can plot our data from Fig. 4 on a graph relating $K^+$ concentration to glial membrane potential. Solid circles in Fig. 5 are experimentally determined resting potentials of the same glial cell at the three concentrations of 1.5, 3.0, and 4.5 mEq/liter $K^+$. The various glial depolarizations caused by the same train of nerve stimulation are drawn as vertical lines (and open circles) at the three $K^+$ concentrations. The average peak depolarization of the glial cell in the standard solution (3 mEq/liter) was 12.1 mV., equivalent to an increase of 1.8 mEq/liter $K^+$ in the bathing solution, i.e., one would have had to add that much $K^+$ to depolarize the glial cell by 12.1 mV. When we reduced the $K^+$ in the bath to 1.5 mEq/liter the resting potential rose to 105 mV. (lowest filled circle). If the nerve stimulation now liberated the same amount of $K^+$, increasing the concentration around the glial cell by 1.8 mEq/liter, one would have predicted a larger depolarization of 18.9 mV., indicated by the lower dotted horizontal line. In fact, the observed effect of stimulation (18.5 mV.) came to within 0.4 mV. of the predicted value and is plotted in Fig. 5 (lowest open circle). The upper horizontal dotted line of Fig. 5 gives the calculated membrane potential if we added 1.8 mEq/liter of $K^+$ to a bath which already had 4.5 mEq/liter in it. Stimulating the optic nerve once more depolarized the glial cell by 9.3 mV.
FIG. 5. Test for the liberation of $K^+$ by nerve impulses as the cause of glial depolarization. All measurements were made on one glial cell in the optic nerve of Necturus. Data obtained from experiment illustrated in Fig. 4. The solid line is the theoretical $K^+$ concentration-glial membrane potential curve predicted by the Nernst equation with a slope of 59 mV. for a 10-fold change in $K_0$. Filled circles indicate the glial resting potential at the three concentrations of external $K^+$, 1.5, 3.0, and 4.5 mEq/liter. At the normal resting potential (89 mV.) in Ringer solution (3.0 mEq/liter $K^+$) the optic nerve was stimulated by a train of impulses which caused a glial depolarization of 12.1 mV. (middle open circle). From the curve it can be seen that this depolarization is equivalent to the addition of 1.8 mEq/liter $K^+$ to the bathing fluid. If this is the amount of $K^+$ liberated by the train of nerve impulses in Ringer solution one can predict from the curve the size of glial depolarizations which will be set up by the same train of impulses when the $K^+$ content in the bath is lower or higher. With 1.5 mEq/liter of $K^+$ in the bath the same train of nerve impulses depolarized the glial cell by 18.5 mV. (lower open circle); the predicted effect of adding 1.8 mEq/liter $K^+$ to 1.5 mEq/liter was almost the same (lower dotted line). Similar good agreement between predicted and observed values is seen when the bath contained 4.5 mEq/liter $K^+$. Data from this and additional experiments are compiled in Table 1.

We conclude from such results as shown in Figs. 4 and 5 and Table 1 that the glial depolarization after nerve stimulation can be explained by the assumption that $K^+$ accumulates in the intercellular clefts following nerve
Table I. Effect of varying external potassium on glial depolarization

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>E_m, mV.</th>
<th>V_m, mV.</th>
<th>K_1, mEq/liter</th>
<th>K_0, mEq/liter</th>
<th>V_n, mV.</th>
<th>V_0, mV.</th>
<th>V_n/V_0</th>
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<td>1</td>
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<td>11.9</td>
<td>1.7</td>
<td>4.5</td>
<td>8.8</td>
<td>8.5</td>
<td>1.04</td>
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<tr>
<td>2</td>
<td>91</td>
<td>10.2</td>
<td>1.4</td>
<td>2.4</td>
<td>7.2</td>
<td>7.4</td>
<td>0.97</td>
</tr>
<tr>
<td>3*</td>
<td>89</td>
<td>12.1</td>
<td>1.8</td>
<td>1.5</td>
<td>7.1</td>
<td>7.2</td>
<td>0.90</td>
</tr>
<tr>
<td>4</td>
<td>86</td>
<td>10.0</td>
<td>1.4</td>
<td>1.5</td>
<td>7.1</td>
<td>7.2</td>
<td>1.00</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>19.3</td>
<td>3.3</td>
<td>1.5</td>
<td>7.1</td>
<td>4.6</td>
<td>1.02</td>
</tr>
<tr>
<td>6</td>
<td>94</td>
<td>6.7</td>
<td>0.9</td>
<td>0.9</td>
<td>10.3</td>
<td>9.1</td>
<td>1.13</td>
</tr>
</tbody>
</table>

E_m = resting potential in normal potassium (3 mEq/liter). V_m = average glial depolarization produced by a train of maximal stimuli to the nerve in normal potassium solution. K_1 = calculated increase in potassium concentration which would produce the same depolarization as the stimulation in the normal solution. K_0 = test concentration of external potassium. V_n = depolarization produced by nerve stimulation in the test K_0 solution. V_0 = predicted depolarization which would be produced in the test solution by the addition of K_1 (as in Fig. 5). * Result illustrated in Figs. 4 and 5.

stimulation. We tested over a limited range of 1.5 mEq/liter K^+ above and below the normal bathing solution because within these limits the liberation of K^+ per impulse is not expected to be significantly altered (see Fig. 6).

Additional evidence for K^+ as the cause of glial depolarization. The change in amplitude of summating glial depolarizations, set up by nerve volleys, adds further support to previous evidence for K^+ being the depolarizing substance. From the logarithmic relation between K_0 and the glial membrane potential one would expect that successive summating glial depolarizations, produced by a constant quantity of K^+ lost from the axons, should diminish in amplitude in a predictable manner. This prediction has been tested by measuring the amplitude of successive glial depolarizations and calculating the increase in K_0 which would produce the observed depolarization. From the previous experiments we would expect that the amount of K^+ lost from the axons as a result of impulse activity should remain constant during small changes in external potassium.

In the experiment of Fig. 6 nine successive maximal stimuli were applied to the nerve while recording the summed depolarization from a glial cell. The voltage scale permits one to measure the depolarization of the cell, contributed by each of the nine nerve stimulations. The dashed lines after volleys 1 and 7 indicate the extrapolation of the falling phase of these two glial depolarizations, to illustrate how the contribution of each nerve volley was measured. On the right ordinate the corresponding K^+ concentrations are

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Fig. 6. Record of nine successive glial depolarizations set up by maximal nerve volleys at 1-sec. intervals. Extrapolation of the falling phase of the first and seventh depolarization shown by a dashed line. The depolarization is given in millivolts on the left side of the scale. On the right side are given the values of external K⁺ concentrations in mEq/liter which would produce equivalent depolarizations (calculated from the Nernst equation). The second depolarization with an amplitude of 2.04 mV. is equivalent to the addition of 0.26 mEq/liter K⁺ (distance on scales between lower two arrows). The eighth depolarization, 1.52 mV., is equivalent to the addition of 0.25 mEq/liter K⁺ (upper two arrows, see Table 2).

shown, calculated from the relation $\Delta E = 59 \log \frac{K^+}{K_{Ringer}}$. Thus, one can read off the amount of K⁺ contributed by each of the nine nerve volleys. For example, the amplitude of the second depolarization is 2.04 mV. (the potential bracketed by the lower two white arrows), corresponding to 0.26 mEq/liter K⁺. A similar conversion was made for potential number 8 which was only 1.52 mV. (upper white arrows). When converted into K⁺ concentration it corresponded to 0.25 mEq/liter. The results obtained in the same way for the other seven glial depolarizations in Fig. 6 are included in Table 2, and they all range between 0.23 and 0.26 mEq/liter K⁺. It should be noted that the potentials presented in Fig. 6 cover a relatively small range of glial depolarization, about 8 mV. in a glial cell that had a membrane potential of 91

<table>
<thead>
<tr>
<th>Volley No.</th>
<th>Amplitude, mV.</th>
<th>Calculated K⁺ Liberated, mEq/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.10</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td>2.04</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>1.85</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>1.73</td>
<td>0.24</td>
</tr>
<tr>
<td>5</td>
<td>1.62</td>
<td>0.23</td>
</tr>
<tr>
<td>6</td>
<td>1.59</td>
<td>0.24</td>
</tr>
<tr>
<td>7</td>
<td>1.54</td>
<td>0.24</td>
</tr>
<tr>
<td>8</td>
<td>1.52</td>
<td>0.25</td>
</tr>
<tr>
<td>9</td>
<td>1.44</td>
<td>0.23</td>
</tr>
</tbody>
</table>
mV. With larger depolarizations the conversions showed a progressively decreasing liberation of K⁺ by nerve impulses, in line with experiments on squid axons where it appeared that the net efflux of K⁺ was decreased when K₀ was raised (3).

These results are therefore in good agreement with the proposal that K⁺ is the agent which causes the glial depolarization and that in the physiological range nerve impulses released nearly constant amounts of K⁺ into the clefts.

Disappearance of K⁺ from the intercellular clefts. If the glial depolarization is produced by the accumulation of K⁺, the declining phase of the potential

![Graph showing decay of depolarization produced by prolonged and brief trains of impulses](image)

Fig. 7. Decay of depolarization produced by prolonged and brief trains of impulses. Same glial cell in both A and B; isolated Necturus optic nerve, resting potential 86 mV. A: ten per second train of stimuli for 27 sec. Peak depolarization is 48 mV. and time for \( \frac{1}{2} \) decay of potential 14 sec. B: brief train of stimuli at 75/sec. Peak depolarization 18 mV. and the time for \( \frac{1}{2} \) decay 4.5 sec. Depolarizations have been displaced so that times when stimulation was stopped are lined up. Note slower decay of potential following prolonged stimulation.

is due to the disappearance of K⁺ from the spaces around the glial cells. The mechanisms involved are presumably a combination of diffusion of K⁺ from clefts and its reabsorption by cells. One way to study the problem is to make an estimate of the apparent K⁺ concentration that surrounds the glial cell membrane during the decline of the potential. Figures 7 and 8 illustrate the result of this procedure. A glial cell was impaled and the nerve was stimulated at two frequencies, each for a different duration, adequate time being left between stimulations for recovery. In Fig. 7A maximal stimulation of the nerve was continued for 28 sec. at 10/sec., the glial depolarization building up to a plateau of 48 mV. After cessation of stimulation the potential fell to \( \frac{1}{2} \) in 14 sec. In Fig. 7B a brief train of stimuli was given at 75/sec., causing a depolarization of 18 mV. with a subsequent decline to \( \frac{1}{2} \) in 4.5 sec. The two records are lined up so that the potential decline starts at the same time, for easier visual comparison. In Fig. 8 the falling phases of the potentials in Fig. 7, A and B, were converted into K⁺ concentrations and scaled appropriately.
In curve A a calculated peak concentration of 19.8 mEq/liter K⁺ was reached; the K⁺ liberated by the axons fell back to normal (i.e., 3 mEq/liter) with a half-time of 6.3 sec., while in B the smaller concentration of 6.2 mEq/liter K⁺ fell to normal more rapidly, with a half-time of 1.7 sec. (arrows).

The plot of the recovery phase in Fig. 8 represents the rate at which K⁺ disappears from the clefts around a glial cell after the spaces had been filled by K⁺ coming out of the axons. There was great variability in times of K⁺ loss from the environment of different glial cells. The result, however, was consistent, that prolongation of stimulation causing large glial depolarizations led to progressively slower restoration of the normal cleft concentrations.

![Graph of calculated fall of potassium concentration following prolonged and brief trains of impulses.](http://jn.physiology.org/)

**Fig. 8.** Calculated fall of potassium concentration following prolonged and brief trains of impulses. Using the Nernst equation the decay of potentials for the two trains of impulses, illustrated in Fig. 7, have been converted to equivalent K⁺ concentrations and drawn on comparable scales. The calculated peak concentration of K⁺ in A (prolonged train) was 19.8 mEq/liter and in B (brief train) 6.2 mEq/liter. The half-times for fall in K₀ to normal (3 mEq/liter) were 6.3 sec. in A and 1.7 sec. in B (indicated by arrows).

Repolarization of a glial cell after reducing the K⁺ concentration in the bathing fluid from 30 to 3 mEq/liter is shown in Fig. 7 of the preceding paper (9). When the rate of loss of K⁺ from the clefts is calculated in the same way as in Fig. 8 one finds that K⁺ disappeared with a half-time about 8 sec. The rate under these conditions was always slower than after nerve stimulation.

Several possible explanations may be offered for the different rates at which K⁺ is cleared from the intercellular spaces after various periods of nerve stimulation and after equilibrating the nerve in high K⁺ concentrations. For example, in the latter case the K⁺ is likely to be distributed more uniformly in the complex intercellular cleft system. Furthermore, after nerve stimulation reabsorption of K⁺ by axons must contribute to the faster disappearance of K⁺ out of the clefts.

*Maximal glial depolarization by nerve stimulation and block of conduction.*
The glial depolarization was surprisingly large in some preparations. In Fig. 7A a plateau of 48 mV was attained after 28 sec. stimulation at 10/sec. and an apparent concentration of 19.8 mEq/liter K⁺ was reached. The concentration in the immediate environment of the axons must have been still higher (see DISCUSSION). The question arises whether axons continue to conduct at such concentrations. It was found that concentrations of K⁺ in the bath up to 22.5 mEq/liter K⁺ (7.5 X normal) never blocked conduction, whereas 30 mEq/liter (10 X normal) always blocked the nerve impulses (9). This finding furnishes an indirect check for the conclusion that K⁺ accumu-

Fig. 9. Intracellular and extracellular recordings from frog optic nerve. A: extracellular “monophasic” record of the compound action potential set up by a single maximal nerve stimulus; the short-latency deflection, 1, is due to medullated axons and is followed by the more slowly conducting nonmedullated fibers, 2. Last is the small negative afterpotential, 3. B: Upper record; same recording conditions as in A, but stimulation at 10/sec. displayed at a slower sweep speed. Negative afterpotentials sum. Lower record; simultaneous recording with an intracellular electrode in a glial cell. Note the similar time course of the rising phase of the glial depolarization with the potential recorded with surface leads. Calibrations: A and B, upper trace, same amplification, different time base; B, both records, same time base, different amplification.

lates in the intercellular clefts and depolarizes the glia. If, for example, 20 mEq/liter K⁺ or a smaller concentration had blocked conduction, the K⁺ hypothesis and our calculations of intercellular K⁺ concentrations would have been invalid.

In addition to testing the K⁺ hypothesis, these experiments set an upper limit for the possible K⁺ accumulation with nerve stimulation, between 22.5 and 30 mEq/liter. In Necturus and frog optic nerves the nonmedullated axons did not follow maximal stimulation frequencies much above 10/sec. for longer than several seconds. The compound action potentials (Fig. 9) gradually declined, apparently due to a block of the conduction mechanism by K⁺.

Negative afterpotentials and glial depolarization. The compound action potential recorded with suction electrodes in the frog indicated that there
was a group of fast-conducting fibers as well as one or more groups with slower conduction velocity of 0.2-0.5 m/sec. (1). The potential peaks in Fig. 9A (labeled 1 and 2) represent the larger myelinated and the much smaller unmyelinated fibers which form the bulk of the optic nerve axons (11). A small but distinct negative afterpotential (3 in Fig. 9A) followed the nerve volley. The diphasic character of the action potential could not be abolished. In Fig. 9B simultaneous recordings were made, at a much slower sweep speed than in Fig. 9A, with a suction electrode near one end of the optic nerve (Fig. 9B, upper record) and an intracellular electrode in a glial cell in the midportion (Fig. 9B, lower record). The nerve was stimulated maximally at 10/sec.; the dense white band in Fig. 9B, upper record, is a photographic fusion of compound action potentials identical to those of Fig. 9A. The slow shift in the potential results from summation of the negative afterpotentials. Especially noteworthy is the similarity of the time course of the glial depolarization in Fig. 9B, lower record, with the slow potential change recorded with extracellular leads (Fig. 9B, upper record). The falling phase of the extracellular potential, however, is more rapid. These observations raise the possibility that the glial depolarization contributed to the negative afterpotentials recorded with surface electrodes (see DISCUSSION).

Glial depolarization by illuminating the eye

There remained the question whether glial potential changes occurred in vivo with natural stimulation of the eye. Recordings were therefore made from glial cells in the optic nerves of lightly anesthetized mud puppies. Figure 10 shows an experiment in which white light was shone into the eye. In Fig. 10A a single flash of 100 msec. duration was followed by a depolarization which rose to a peak of 4 mV. in about 0.5 sec. and declined to half in a further 4 sec. If three flashes were given (Fig. 10B) the second and third made additional, but smaller contributions. The depolarizations were graded with light intensity. No attempt was made to study the characteristics of impulse activity arising in the Necturus eye. Cursory observations indicate that adaptation of impulse discharge was rapid in our preparations. For example, if a flash was prolonged to 27 sec. (Fig. 10C) there was an initial “on” effect lasting several seconds. The depolarization, however, declined afterward toward the resting potential. When the light was turned off there was another depolarization similar to the initial one, but smaller. The effects increased in dim light. It is apparent that the glial depolarization in these examples reflects optic nerve discharges. In recent experiments Bortoff (2) found that brief flashes of light shone in the eye of Necturus cause bursts of impulses in the retinal ganglion cells which adapt during more prolonged illumination. We conclude that “natural” impulse activity in vivo has an effect on the membrane potential of glial cells.

DISCUSSION

Liberation of K+ from axons as a cause of glial depolarization. The direct action of current flow from axons to glial cells can be excluded because a)
FIG. 10. Effect of "natural" nerve stimulation on glial resting potentials. Light flashes were shone into the eye while recording intracellularly from a glial cell in a circulated optic nerve in an anesthetized Necturus. Lower beams monitor currents applied to light bulb. A: single light flash of 100 msec. duration sets up transient depolarization. B: same flash repeated three times. C: light stimulus is maintained for 27 sec. The initial glial depolarization declines due to adaptation of neuronal discharges. When the light is turned off an additional depolarization is produced.

there is a discrepancy in the time course of the currents in the nerves and the depolarization of the glial cells. The nerve action currents, lasting about 30 msec. or less, have practically disappeared when the glial depolarization is still rising, a phase which lasts from 50–150 msec. followed by a decline which is measured in seconds. Furthermore, the potential changes accompanying the axonal currents are nearly equal inside and outside the glial cells so that only a negligible potential drop is present across the glial membrane (Fig. 3). b) The glial depolarization observed in this study cannot be reproduced by currents artificially supplied through microelectrodes placed inside glial cells. The conclusion that current flow has no direct action is in agreement with previous results in the leech, that currents passed across either glial or nerve membranes did not flow appreciably into the other cell type (10).

The quantity of K⁺ liberated during activity from giant axons in the squid and from mammalian unmyelinated fibers has been measured by Keynes and Lewis (6) and Keynes and Ritchie (7). Frankenauæeuer and Hodgkin (3) have presented convincing evidence that the leakage of K⁺ leads to a transient increase in the K⁺ in the intercellular spaces between the axon and the Schwann cells and accounts for the negative afterpotential seen in squid nerves, measured with intracellular electrodes across the axonal membrane. K⁺ leakage has also been thought to account for the negative afterpotentials of vertebrate unmyelinated nerve, recorded with surface leads (4, 7, 17; see later).

The present evidence suggests that the release of K⁺ from active nerve
fibers also explains the following results: a) the variation in the amplitude of the glial depolarization with changes in external potassium concentration (see Figs. 4 and 5); b) the summed effects of nerve stimulation, i.e., successive glial depolarizations, were predictably smaller, as if each nerve volley liberated a similar quantity of K+. In squid and cockroach axons (3, 13) and in unmyelinated mammalian fibers (17) successive negative afterpotentials sum linearly, whereas in Necturus successive glial depolarizations decreased in amplitude. This dissimilarity is predicted from the different relation between K₀ and resting potential in neurons and glia. The resting potential of neurons is linearly related to K₀ in the physiological range, whereas the resting potential of glia is related to the log of K₀. 

As we have already noted, an effect of nerve impulses on glia was not found in the leech, perhaps because technical complications were introduced by movement artifacts from the contraction of smooth muscle fibers (10). It would now be of interest to re-examine the leech with better immobilization of the tissue. In recordings made from Schwann cells around giant axons of the squid during stimulation with single shocks (19) no depolarization of the Schwann cell was seen. The possibility remains that the effect is too small to be measured with single stimuli and only becomes apparent with repetitive stimulation.

Width of the intercellular clefts. If the net efflux of potassium and the increase in potassium concentration in the clefts following a nerve volley were known, the width of the intercellular clefts could be calculated (3). Thus

\[
\text{width of cleft} = \frac{\text{amount of } K^+ \text{ released from the axon (moles/cm}^2\text{)}}{\text{increase in } K^+ \text{ concentration in clefts (moles/cm}^3\text{)}}
\]

The loss of K⁺ from unmyelinated nerve fibers in the Necturus optic nerve is not known but an estimate can be made assuming it to be similar to that found by Keynes and Ritchie (7) for mammalian unmyelinated fibers, namely, 1 pmole/cm² per nerve volley. The largest glial depolarization recorded following a single maximum nerve volley was 3.1 mV, which is equivalent to an increase of 0.39 mEq/liter K⁺ in the space surrounding the glial cell. Using these values, the width of the cleft =

\[
\frac{1 \times 10^{-12} \text{ mole/cm}^2}{0.39 \times 10^{-6} \text{ mole/cm}^3} = 2.6 \times 10^{-6} \text{ cm} = 260 \text{ Å}.
\]

This value is likely to be inaccurate for the following reasons: first, the loss of K⁺ in Necturus may differ from that found by Keynes and Ritchie (7); second, the calculation assumes that the glial membrane is everywhere apposed to active axons. This assumption is not correct (see below) and leads to an overestimate of the width of the clefts. Nevertheless, 260 Å, is within the range of values determined by the same method for the width of the cleft separating the squid axon from the Schwann cell (3); it is, however, somewhat larger than usually seen in electron micrographs.

Alternative hypotheses. A number of substances are known to be released from active nerves (e.g., lactic acid, CO₂, and NIL⁺) and they may exert a depolarizing action on glial cells. Since we know that K⁺ actually is liberated by nonmedullated fibers (7) and in view of the good agreement between the observations and the K⁺ hypothesis, there seems at present no need for alternate assumptions.

Distribution of K⁺ in the extracellular spaces. Electron microscopic studies of optic nerves in the frog (11) and in Necturus (Wolfe, unpublished) show a
considerable anatomical complexity of the cleft system (Fig. 3B, ref. 9). Our calculations of the K+ concentrations in clefts assume that glial cells face a uniform concentration all along their surface, as is the case when K+ is applied through the bath. This, however, is not correct because part of each glial membrane is adjacent to other glial cells or is folded upon itself. In some cells a considerable area faces the surface of the optic nerve and the large extracellular space. The glial membrane potential during nerve stimulation, therefore, gives us an average, apparent concentration of K+, the actual concentration being greatest near the active axons. In addition, the axons themselves are arranged in bundles, for the most part facing each other (Fig. 3A, ref. 9). Whenever K+ is liberated it will be most concentrated in the interaxonal spaces with a gradient toward the surface of the bundle where it borders glia. In Fig. 7A, for instance, the apparent final concentration around the glial cell which corresponds to a depolarization of 48 mV., is close to 20 mEq/liter K+. It must have been greater by an unknown amount around some of the axons because some of the nerve fibers became blocked during the stimulation, as checked by extracellular recording of the compound action potentials. Conduction block does not occur at K0 of 23 mEq/liter but is complete at 30 mEq/liter, the next higher concentration which was tested (9). The limitation of glial depolarization by nerve impulses would be set by an accumulation of K+ which blocks conduction, i.e., 23–30 mEq/liter which would produce a 52- to 59-mV. depolarization.

The concentration of K+ required to block impulse conduction in a nerve that has been inactive is probably greater than that required to produce block in an axon after prolonged impulse activity. Active axons lose K+ and gain an equivalent amount of Na+. The effect of this exchange on the intracellular ion concentrations will be greatest in the smaller axons which have a high surface:volume ratio. For example, a single impulse in a 1-μm. axon will increase the internal Na+ concentration by about 0.2 mM, whereas in a 0.1-μm. axon the increase will be about 2 mM (4). An increase in internal Na+ and decrease in internal K+ may result in a lower safety factor for conduction and a greater susceptibility to block by an increase in K0.

Individual nonmyelinated optic nerve fibers in the frog can fire at frequencies of 50/sec. with visual stimulation, whereas many of them become blocked with sustained maximal electrical excitation of the whole nerve at much lower frequencies (11, 12). A conduction block in some fibers occasionally develops even at frequencies below 10/sec. if synchronous stimulation is maintained in the frog or Necturus optic nerves. Apparently asynchronous impulses, in small groups of axons, do not build up K+ concentrations large enough to block conduction.

The rise time of the glial depolarization is much slower than the K+ efflux from individual axons (3). The slow time course is probably due a) to the dispersion of impulses in axons of varying diameter, b) to the tortuous cleft system which introduces appreciable distances for diffusion between the axons and many portions of the glial membranes.

Recording glial potentials with surface electrodes. The question arises whether the glial depolarizations can be recorded from the surface of the nerve and contribute to the various afterpotentials which have been ex-
tensively studied. To set up an extracellular potential, current has to flow between different parts of a cell. Although glial cells in the optic nerve may be relatively short, they are electrically coupled to each other so that a potential change arising in one cell will spread, with accompanying current flow, to adjacent cells (9). Therefore, if nerve impulses liberate K+ which depolarizes some of the glial cells, current will be drawn from electrically coupled glia which are not near active axons, as in the experiment of Fig. 9 where the potentials are recorded monophasically. To estimate the separate contribution of axons and glial cells to the extracellular currents one has to know their respective membrane depolarizations, their membrane resistances, and their internal resistances. The total internal resistances are likely to be similar because the cross-sectional areas of neurons and glia are not very different. Thus, in view of the greater sensitivity of the glial membrane potential to K+ in the physiological range and the similarity of neuronal and glial membrane resistances that is found in the leech, it seems possible that glial cells, in certain situations, can contribute as much as axons, or perhaps more, to the potentials recorded with surface electrodes.

The surface potential will be the result of the relative contributions of neuronal and glial potential changes to extracellular current flow. It should be pointed out that these potential changes need not be in the same direction. Thus, when K+ increases following nerve activity the glial cells are depolarized but the axons presumably go through a sequence of depolarizing and hyperpolarizing afterpotentials. A hyperpolarization in the nerves could occur in the presence of an increased K+ as a result of potentials produced by uncoupled sodium pumping (5) or by a prolonged increase in K+ permeability (16).

Similar conditions apply elsewhere in the nervous system. In the brain a focal activation of neurons could produce glial potentials which would contribute to extracellular potentials recorded from the surface of the brain (for a discussion see ref. 8).

General physiological consequences of fluctuations of K+ concentrations. The depolarization set up in glial cells by illuminating the eye of the circulated animal shows that the effect also occurs in vivo with natural stimulation. The possibility is therefore raised that depolarization acts as a stimulus for the glial cell. We have as yet no indication of the nature of any glial activity, perhaps metabolic, that might be induced by a fall in its resting potential.

Another question concerns an effect of K+ accumulation on neuronal activity. A relatively large increase in the K+ concentration in intercellular clefts during nerve impulses is expected to have significant consequences, especially at synapses where changes in the nerve membrane potentials of even a few millivolts could alter the patterns of transmission (18). It is therefore interesting that in the leech the membrane potential of neurons is much less sensitive than that of glial cells to increasing K+ concentrations. The same seems to apply to Necturus whose axons conduct in 23 mEq/liter K+ concentrations. This insensitivity appears a useful adaptation and allows neurons to function in a high K+ environment (Fig. 25, ref. 8).

The current flow which accompanies glial depolarization when circumscribed groups of cells are activated, suggests that such currents might distribute the accumulated K+ in the clefts, without significantly altering the
internal \(K^+\) of glial cells. If a glial cell becomes depolarized by \(K^+\) which has accumulated in the clefts, the resulting current carries \(K^+\) inward, in the high \(K_0\) region, and out again, through electrically coupled glial cells in low \(K_0\) regions. Under such conditions, glial cells might serve as "spatial buffers" in the distribution of \(K^+\) in the cleft system. The quantitative aspects of such \(K^+\) movement are not known, nor is the contribution of \(Cl^-\) movement. With widespread synchronous maximal stimulation, e.g., in the present experiments in the optic nerve, the distribution of \(K^+\) by currents would be negligible because most of the glial surfaces would be depolarized practically simultaneously by \(K^+\) which leaks out of the distributed axon bundles.

Another consideration is the possible significance of the glial potentials as indicators of over-all neuronal activity. Each astrocyte is apposed to numerous axons and, depending on location, also to synapses. By sampling the \(K^+\) concentration around its surface it registers indiscriminately impulse traffic in excitatory and inhibitory axons and, to an unknown extent, the synaptic events. Thus a high membrane potential of say 90 mV. in a group of glial cells will reflect low-level neuronal activity in their environment, while a lower potential will indicate a high level of neuronal activity. The magnitude of the potential fields and their influence on neighboring neurons created by the glial potentials is not known. Available experiments do not support the idea that currents in glial cells influence neurons because the clefts greatly attenuate current spread between the two cell types. As a first step in the study of these problems, as well as the correlation of glial potentials with slow potentials recorded from the brain, a reliable method has to be developed for recording intracellularly from glial cells elsewhere in the brain.

**Summary**

The effect of nerve impulses on glial cells has been studied in isolated as well as in circulated optic nerves of two amphibia: the mud puppy and the frog.

1. A single maximal stimulus to the optic nerve sets up a slow depolarization in the glial cells which surround the nonmyelinated axons. The depolarization rises to a peak in 50–150 msec. and declines in about 2 sec. Electrical recordings made inside and outside the glial membrane show that the effect of impulses is not a direct result of action current flow.

2. The glial depolarization is graded and its magnitude depends on the number of axons activated and on the frequency of nerve stimulation. Trains of impulses at 10/sec. can reduce the normal glial resting potential by up to 48 mV. The time of decline of the glial depolarization is slower after prolonged stimulation.

3. Evidence is presented for the following mechanism of glial depolarization: during impulse activity axons release \(K^+\) which accumulates in the intercellular clefts, thereby reducing the glial membrane potential.

4. With repeated maximal stimulation the average \(K^+\) concentration in the clefts, estimated from the glial depolarization, can increase to 20 mEq
/liter from its normal value of 3 mEq/liter. Within axon bundles the K+ concentration can be even higher, leading to block of conduction.

5. Depolarization of glial cells also occurs in circulated animals when light flashes are given to the eye. The consequences of K+ accumulation in the intercellular spaces during neuronal activity and reasons for thinking that depolarization of glial cells contributes to potentials recorded with surface electrodes are discussed.

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REFERENCES