cent relative humidity. Feeding was carried out every other day with the medium described above.

The appearance of the glioblasts, which originally resembled an epithelial cell carpet, was transformed in 1 day by the factor into that of a cell net of interconnected, multipolar astrocytes. Prolonged culture of the cells in the presence of the factor led to further extension of the processes so that the picture of a crisscrossing network emerged between 4 and 7 days. Table 1 shows the concentration of adenosine 3′,5′-monophosphate (cyclic AMP) and S-100 protein in these cells. No change could be detected at the 1-day point, but considerable increases in these compounds were observed thereafter. At the 7-day point, the concentrations of cyclic AMP and S-100 in the experimental cells were three times and nine times, respectively, those in the control cells.

Cyclic AMP and the glia-specific protein S-100 (*4*) were chosen in this work as the chemical indicators of glioblast maturation. This study shows that although chemical differentiation does not precede or accompany the initial morphological change (at the 1-day point), where cell retraction is the main feature, it is correlated with the long-term morphological effect (4- and 7-day points), where process elongation is the major event. While the exact relationship between chemical and morphological differentiation in the glioblasts requires further investigation, the data reported here strongly suggest that they are parallel events triggered by the same maturation factor.

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Glial-Neural Interaction Demonstrated by the Injection of Na^+ and Li^+ into Cortical Glia

Abstract. *Injection of Na^+ or Li^+ into cortical glia evokes glial depolarization, discharge of adjacent neurons, and vascular pulsation. The effects can be explained by the extrusion of K^+ from glia after cation injection, glial swelling, and the slow removal of the cation from glia. The data suggest that the reduced rate of reuptake of K^+ into Na^+-loaded glia results in epileptiform firing of neurons, and support the hypothesis that glia function to buffer the environment of neurons.*

Neuronal discharge evokes depolarization of glia that is mediated by K^+ released from neurons (*1*). It has been suggested that during depolarization the glia take up K^+ to maintain the constancy of the neuronal environment by a process termed spatial buffering (*2*). The spatial buffering hypothesis predicts that generalized glial depolarization would interfere with glial uptake of K^+. It has also been proposed that disturbance of glial uptake of K^+ in areas of injury could be a contributing cause in the generation of epileptic neuronal discharge (*3*). These hypotheses were investigated by injecting Na^+ and Li^+ into glia with the objective of electrochemically displacing the intracellular K^+ and depolarizing the glia so that the effect on the activity of adjacent neurons could be studied.

Intracellular recordings in the motor cortex of cats anesthetized with pentobarbital were obtained with beveled micropipettes (tip resistances, 15 to 30 megohms) filled with 0.5 to 3*M* solutions of NaCl, LiCl, $LiCO_3$, or KCl. Glia were identified by a stable membrane potential of −95 to −70 mv, the presence of slow depolarizations of 2 to 4 mv during electrocortical spindle bursts, and the absence of spike and synaptic potentials on penetration (*4–6*). Iontophoretic injection of ions was carried out with current pulses of $0.1 - 1 \times 10^{-8}$ amp and 100 msec duration (*7–9*).

Single injections of Na^+ into glia with a 1×10^{-8} amp pulse (*10, 11*) evoked glial depolarizations of 7 to 30 mv in 90 percent of over 200 glia (Figs. 1 and 2). Repolarization had a duration of 3 to 20 seconds in different cells, and had an irregular course, marked by small depolarizing shifts of potential, quite different from the smoothly decaying repolarization that glia exhibit after neural activity evoked by thalamocortical stimulation (*4*). When two injections were made at intervals greater than 20 seconds, depolarizations of approximately equal amplitude were evoked. The rate of repolarization after the second injection was often more rapid than after the initial injection (Fig. 2B). However, if injections were made at shorter intervals the depolarizations were initially additive. A train of injections produced an incrementally increasing series of depolarizations for the first two to four pulses of the train, followed by a plateau of depolarization, and then repolarization despite continued injection (Fig. 1, third injection). Glial responses to Li^+ injection were similar to those evoked by Na^+. The depolarization was generally larger, and repolarization was more rapid. Repolarization was followed by hyperpolarization of 2 to 5 mv in some glia.

In Na^+- and Li^+-injected glia we recorded, through the glial membrane, the high-frequency discharge of neurons during the period of glial depolarization. Neuronal spike discharges, generally of low frequency, have occasionally been recorded in uninjected glia (*4, 12*). In contrast, an intense multiunit discharge composed of spikes of different amplitudes was detected, by means of auditory and oscilloscopic monitoring in all glia depolarized by cation injections. Subsequent injections evoked more intense and larger discharges than the initial injection (Fig. 2A). The spikes were as large as 20 mv in some cases and could be resolved by the ink-writing pen of the oscillograph. When the spikes were of large amplitude, the cessation of neural firing was often accompanied by an abrupt return of the glial membrane potential to preinjection levels (Fig. 2A, middle trace). This suggests that the glial depolarization recorded after cation injection may be in part mediated by K^+ or other substances released by active adjacent neurons.

Repetitive Na^+ and Li^+ injections also evoked a marked increase in the vascular pulsations that are recorded as small os-

cillations of the membrane potential synchronous with the arterial pulse in uninjected cells. After repetitive injection, pulsations of large amplitude were recorded in virtually all glia. The increased pulsation began during the period of glial depolarization and persisted for 1 to 2 minutes after repolarization had occurred. A similar increase in vascular pulsation can also be recorded in glia during the depolarization and hyperpolarization evoked by intense electrocortical activity. The increased pulsation is probably due to the dilation of cortical resistance vessels that occurs with neuronal activity (*13*) that is mediated by CO_2, and possibly K^+, released from neurons.

To control for changes in micropipette tip and junctional potentials that might be generated by iontophoresis, and for possible depolarizing effects of the currents used, ion injections were made in the extracellular space and into neurons. The extracellular injection of Na^+ or Li^+ either produced no change of potential across the micropipette, or a 1- to 2-second negative potential of 1 to 2 mv. The injection of Na^+ into neurons either had no effect on the membrane potential or produced a brief pause in firing and slight hyperpolarization, as previously reported by others (*11*). No effect on the discharge of neurons was detected with extracellular ion injections. To determine if the responses observed were characteristic of the ions injected, or were due to passage of current or injection of osmotically active particles, injections of K^+ and Cl^- were made into glia. Single and repetitive injections of K^+ into glia either had no effect on the glial membrane potential or produced a 2- to 4-mv hyperpolarization. No effects on adjacent neurons or vascular resistance were recorded. Single injections of Cl^- evoked depolarizations of up to 10 mv, lasting 1 to 2 seconds, followed by hyperpolarization of 2 to 4 mv in some cases, without evoking neural firing or changes in vascular pulsation.

Let us assume that the glial cell has a passive membrane that is a perfect K^+ electrode, and that no coupling occurs between glia. Then the effects of the initial Na^+ injection on the glial membrane potential and on adjacent neurons and vessels can be explained according to hypothesis that K^+ is extruded from glia in an amount equivalent to the cation injected. If we assumed that 4×10^{-14} mole of K^+ were a reasonable estimate of K^+ content and, as a first approximation that each Na^+ injection extruded an equivalent amount of K^+, then each Na^+ injection would displace 25 percent of the intracellular K^+ and reduce $[K^+]_i$ by 33 mmole per liter (*7*), provided that the membrane were highly permeable to K^+ and impermeable or only very slightly permeable to Na^+ (*14*). The successive levels to which $[K^+]_i$ and the membrane potential would be brought by three current pulses, each of which depleted $[K^+]_i$ by 33 mmole/l, for a cell with an initial $[K^+]_i$ of 131 mmole/l and a membrane potential of -100 mv would be: first injection, $[K^+]_i$ 98 mmole = -92 mv; second injection, $[K^+]_i$ 65 mmole = -82 mv; third injection, $[K^+]_i$ 32 mmole = -63 mv (*15*). The observed range of amplitude of

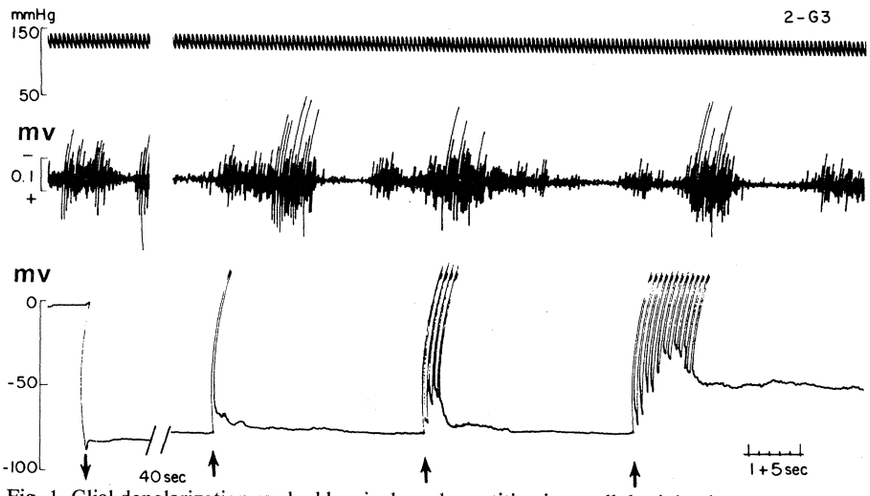

Fig. 1. Glial depolarization evoked by single and repetitive intracellular injections of Na^+. Ink-writing oscillograph recording. Upper trace, femoral blood pressure; middle trace, electrocorticogram; lower trace, intracellular recording from a glial cell with a membrane potential of -85 mv on penetration. The micropipette penetrated the cell at the point in time marked with the downward arrow. The three upward arrows mark the injection of Na^+ with 1, 4, and 12 pulses of 1×10^{-8} amp and 100-msec duration, respectively.

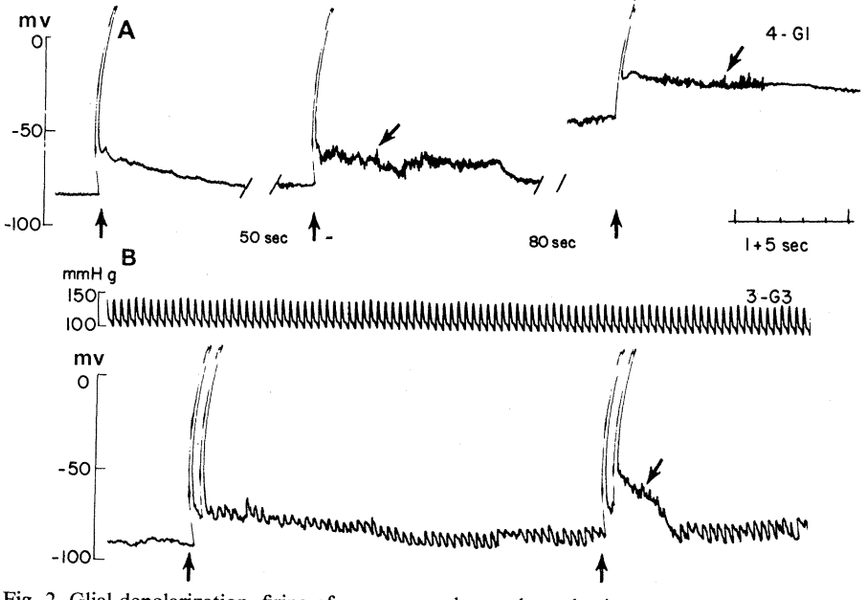

Fig. 2. Glial depolarization, firing of neurons, and vascular pulsations evoked by intracellular injection of Na^+ into glia. (A) Neuronal discharge evoked during successive Na^+-evoked depolarizations of a glial cell, at three different levels of membrane potential. Intracellular recording from a glial cell with a membrane potential of -85 mv on penetration. Upward arrows mark injection of Na^+ with a 100-msec pulse of 1×10^{-8} amp. The responses to injections 1, 6, and 13 of a series are shown. Spike discharges of neurons recorded through the glial membrane are marked with downward arrows. The recorded amplitude of spike discharge is attenuated by the frequency response characteristics of the oscillograph pen. (B) Increase in vascular pulsations produced by Na^+ injection in a glial cell. Upper trace, femoral artery pressure; lower trace, recording from a glial cell with membrane potential of -90 mv. Upward arrows mark the injection of Na^+ with two 100-msec pulses of 1×10^{-8} delivered at each arrow. Note the neuronal spike discharges marked with a downward arrow that are superimposed on the vascular pulsation-induced oscillations of glial membrane potential.

depolarization evoked by the initial Na^+ injection corresponded well with the range of depolarization of 8 to 20 mv predicted for cells with membrane potentials of −100 to −82 mv at the time of injection. The first few Na^+ injections of a train would be expected to produce depolarizations of increasing magnitude, a phenomenon that was also observed. An additional increment of depolarization after each pulse would be expected if the extruded K^+ accumulated in the extracellular clefts, and evoked firing of neurons (*1, 2, 16*).

However, the effects of repetitive Na^+ injections indicate that additional processes must occur after the initial cation injection. The fact that after four or five Na^+ injections of a train, depolarization was no longer evoked by subsequent injections suggests a change in state of the cell produced by the injections. The observation that glia were not totally depolarized by a train of injections suggests that not all of the K^+ within the glia could be exchanged by repetitive injections. These phenomena may be related to glial swelling, extrusion of Na^+ from glia and reuptake of K^+, and to the possibility that glia function as a syncytium. The physical capacity of glia to swell greatly is evident from electron micrographs of edematous cortex (*17*). The injection of Na^+ in excess of intracellular anion should be accompanied by entry of Cl^- or other anions to maintain electrical neutrality within the cell, which would result in additional swelling. Uptake of Cl^- and swelling of glia occur when extracellular K^+ levels are increased to greater than 13 mmole per liter (*18*). It is likely that the extrusion of K^+ from the glia by cation injection raised the extracellular K^+ to at least this level and that this mechanism also may have resulted in swelling. Glial swelling per se should result in depolarization due to dilution of intracellular K^+ Therefore, the repolarization observed during repetitive injections of Na^+ suggests Na^+ removal from the soma, and reuptake of K^+. Transport of Na^+ out of glia has been demonstrated in a glial cell line and may be linked to inward movement of K^+ (*19*). Also, Na^+ may move from the soma into glial processes, which can swell greatly, and Na^+ may also eventually pass into contiguous glia by way of the gap junctions that exist between opposed glial processes (*20*).

It is surprising that the disturbance of a single glial cell could result in intense discharge of neurons, considering the relative size of glia and neurons and the probable relationship of the processes of many glia to each neuron. It is possible, therefore, that the firing is evoked from axons or synaptic terminals that are wrapped, or compartmentalized, by glia (*21*). The simplest explanation of the cause of the firing is neuronal depolarization by K^+ extruded from glia. However, additional factors must be involved, as injections of K^+ into glia did not evoke discharge. Presumably, these injections did not raise extracellular K^+ sufficiently to depolarize neurons. This might be due to the normal K^+ removal mechanisms, that are believed to include uptake into neurons, reuptake into glia, diffusion and transfer into the blood (*22*). Injection of Na^+ into glia would appear to be primarily capable of blocking the glial route of K^+ removal. Therefore, it was probably the extracellular accumulation of K^+ caused by its slow reuptake into Na^+-loaded glia that was necessary for neural discharge. Glial swelling may also reduce the volume of the extracellular clefts and thereby increase the concentration of substances released into the clefts. The recording of neuronal firing within glia may also be facilitated by glial swelling and closer apposition of glial and neural membranes, and it is possible that distortion of neural membranes may evoke discharge, although single Cl^- injections that evoked depolarization did not produce discharge.

Glial depolarization and the introduction of cations may also alter the metabolism of glia, and the possible glial function of catalyzation of CO_2 to HCO_3^- (*18*), neurotransmitter uptake (*23*), and active K^+ uptake (*19*). Glial depolarization linked to neural discharge has been well documented in the invertebrate and mammalian nervous systems (*1, 4, 6, 16*). The present experiments demonstrate a reciprocal effect of glia on neurons, neuronal discharge that is linked to glial depolarization after cation loading of glia. The data support the hypothesis that glia function to accept substances released from neurons. Conditions that produce Na^+ accumulation in glia, such as cerebral edema (*18*), may be expected to interfere with the glial buffering of K^+, and perhaps other substances, released from neurons.

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