A THEORETICAL EXAMINATION OF IONIC INTERACTIONS BETWEEN NEURAL AND NON-NEURAL MEMBRANES

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ABSTRACT Evidence from electron microscopy indicates that the separation between adjacent membranes of the central nervous system (CNS) is less than 500 A and perhaps as small as 100–250 A. The rapid K^+ efflux associated with the neural action potential may therefore be sufficient to affect the local extracellular potassium concentration and, via their partial dependence upon the potassium equilibrium potential, alter the electrical states of nearby neural and glial membranes. This new concept of a transient and local depolarizing "ionic interaction" between active and inactive membranes of the CNS is here examined theoretically and its magnitude calculated as a function of (*a*) the intermembrane separation, (*b*) the membranes' electrochemical characteristics, and (*c*) the rate at which K^+ can diffuse away from the vicinity of the active (neural) membrane. My results indicate that the interaction is in the millivolt range and therefore significant in the modulation of postsynaptic and presynaptic information processing; in particular configurations the postulated interaction alone may be suprathreshold. Membrane noise and local synchrony in groups of neurons may reflect these local, K^+-mediated interactions. The transient ionic interaction between active neural and nearby glial membrane is also in the millivolt range; however, the relevance of neuroglia to neuronal function is obscure. Certain pathological states, such as seizure and spreading depression, have an obvious phenomenological correspondence to the results presented here and are briefly discussed.

INTRODUCTION

Investigations of the squid giant axon clearly demonstrated the functional dependence of that bioelectric membrane upon the transmembrane sodium and potassium ionic concentration gradients (Hodgkin, Huxley, and Katz, 1952; Grundfest, Shanes, and Freygang, 1953). Even though the original quantitative formulation (Hodgkin and Huxley, 1952) adequately described the electrogenic phenomena, the physical mechanisms upon which these were based was, and remains, unclear. Fortunately, this empirical model was sufficiently explicit to have spawned over a decade

of direct and indirect validating experiments. It now appears that the sodium-potassium (or "two ion") hypothesis can account for the major bioelectric activity of vertebrate neural membrane as well; see reviews by Grundfest (1961) and Noble (1966).

Because of the transfer of sodium (inward) and potassium (outward) across the neural membrane during the course of an action potential, there must result some transient alteration in their concentrations both at the exterior and the interior faces of the active membrane. Although the resting and active states of neural membrane are dependent on the potassium and sodium transmembrane ionic gradients, it is admittedly hard to imagine that the actual variations could be significant in view of the small quantity of ion transferred during each action potential.[1] However, the postspike behavior of some invertebrate nerve fibers gave evidence of just such activity-dependent variations in the K^+ concentration gradient (Frankenhaeuser and Hodgkin, 1956). The basis for these effects seemed to be twofold: (*a*) a small extracellular space — the axon membrane had ready access to an adjacent aqueous phase only 250–300 A wide, and (*b*) a small extracellular potassium concentration that was significantly altered by the net potassium efflux during the repolarization phase of the action potential. Similar phenomena have been investigated in mammalian peripheral nerve preparations (Arnett and Ritchie, 1963; Blackman, Ginsberg, and Ray, 1963).

Turning to the mammalian CNS, the distance between membranes appears to be less than 500 A (Torack, 1965; van Harreveld, Cromwell, and Malhotra, 1965) and possibly in the range of from 100 to 250 A (Karlsson and Schultz, 1965). (The points of conflict between electron microscopy (EM) and physiological measures of the extracellular space have recently been reconsidered by Nevis and Collins, 1967). Thus, the immediately adjacent extracellular volume is again extremely small relative to the intracellular volume. Also, whereas the extracellular sodium concentration is on the order of 120–140 μeq/cm^3, the extracellular potassium concentration is in the range 2.5–3.5 μeq/cm^3 (Bito and Davson, 1966; Katzman, 1961). Perhaps, therefore, the normal activity of at least a certain class of mammalian central neural structures would be capable of producing significant alterations of their own extracellular environment, viz., transiently increased external potassium concentration. The work reported here is part of a larger study devoted to a quantitative theoretical examination of this phenomenon and its relation to neuronal and glial functioning.

The point which I examine in this paper is that there is a certain reciprocity in the

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[1] Since $\Delta V = \Delta Q/C$, the total charge transferred (ΔQ) during a 100 mv action potential (ΔV) is $(10^{-1}) \cdot (10^{-6}) = 10^{-7}$ coul/cm^2 for a membrane with capacitance $C = 1\ \mu F/cm^2$. This corresponds to $\Delta Q/F = 10^{-12}$ eq/cm^2 of monovalent ion per impulse and is a minimum value; the actual sodium and potassium currents partially overlap such that the gross transfer of a given ionic species may be 2–4 times larger (for references see Hodgkin, 1964, Table 3). Taking a factor of 3 and an extracellular space 200 A wide, the above represents a quantity of K^+ at the concentration $(3 \times 10^{-12})/(200 \times 10^{-8}) = 1.5$ μeq/cm^3.

behavior of the membranes facing one another across a narrow extracellular cleft. That is, after a spike in one of them, any transients produced in the external potassium concentration affects in parallel *all* membranes facing that immediate volume. From this follows the interesting concept of ionic interaction between the active membrane and the membranes, themselves active or passive, which share a common extracellular cleft. This phenomenon has been examined by a digital computer simulation of the system: "(active neural membrane)–(limited extracellular environment)–(adjacent neural and/or glial membranes)." The model and its analysis show that this postulated and heretofore uninvestigated ionic interaction takes the form of a transient depolarization of the neural and the glial membrane adjacent to the spike-producing membrane. The magnitude and time course of the interaction are determined as functions of the electrochemical characteristics of the membranes, the width of the intermembrane cleft, and the communication of the cleft with the bulk of the tissue (i.e., the ease with which potassium equilibrium is reestablished). For the particular case of a resting neural membrane influenced by a sufficiently close active neighbor, the ionic interaction may exceed the neural firing level and thereby communicate a spike across the intervening extracellular space. The more general case, however, appears to be such that there would exist a patchwork of local potassium-mediated depolarizations that would modulate rather than initiate the neuron's firing. Via a similar ionic depolarization of presynaptic terminals, an active membrane could exert significant control over its own input as well as the input to cells in the immediate vicinity (Lebovitz, in preparation). Glial membrane is not known to demonstrate active or spikelike depolarizations so that the significance of a potassium-mediated depolarization of glial cells is unclear. Portions of this work were submitted in partial satisfaction of requirements for the Ph.D. in Physiology at UCLA (Lebovitz, 1967 *a*); preliminary reports have appeared (Lebovitz, 1966 and 1967 *b*).

MODEL OF CLOSELY PACKED ELECTROCHEMICAL MEMBRANES

A description of the dynamics of a restricted extracellular space is facilitated by some idealization of the neuronal architecture, such as is shown in Fig. 1. At the left is represented a portion of some neural structure (say, neural soma or large process) whose membrane is here termed the "primary" neural membrane. Adjacent to this (at right) are represented portions of the arbitrary array of neighboring neural and glial processes. This array of membranes *immediately adjacent* to the primary neural membrane is correspondingly denoted as the neural and glial "boundary". The immediately adjacent extracellular space (IXS) consists of the cleft between the primary membrane and the boundary membranes. In what follows we shall assume that the primary membrane experiences an action potential, and then examine the consequent alterations in the potassium ion concentration in its

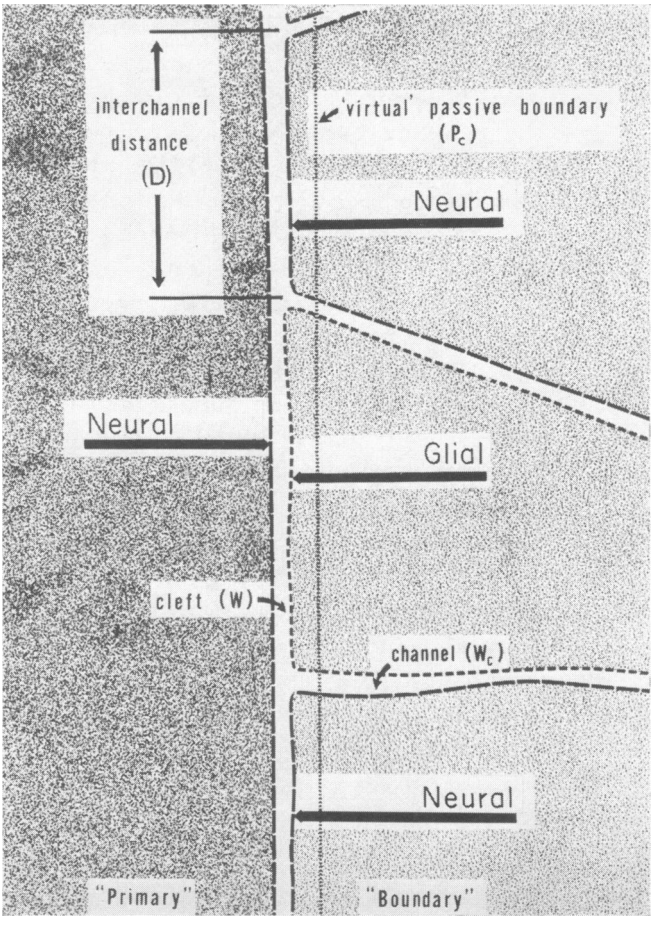

FIGURE 1 Schematic of the ultrastructure in the immediate vicinity of a portion of neural soma membrane (left "primary" membrane). The collection of channels between adjacent structures is equivalent to a "virtual" membrane that is functionally in parallel with the adjacent neural and glial "boundary" membranes (right). The width of the intermembrane cleft and of the interelement channels are grossly exaggerated for clarity.

immediate vicinity and the effect of this on the electrical behavior of the adjacent (boundary) membranes. All electrical and ionic events are assumed to take place uniformly over the primary membrane so that there are neither lateral concentration gradients nor lateral current flow (that is, we assume that the action potential is either nonpropagating or propagating slowly with respect to its time course). Furthere, I shall neglect the small diffusion time across the cleft (Eccles and Jaeger, 1958), and assume that the K^+ concentration is uniform across the width of the IXS. By these assumptions the analysis of a nonpropagating event over an arbitrary patch of primary neural membrane serves to characterize the behavior of the whole.

The model is developed in detail elsewhere (Lebovitz, 1969) and follows the outline below:

(*a*), electrical behavior of spike-producing primary membrane and attendant ejection of K^+ into the immediate extracellular space (IXS), net transmembrane potassium flux denoted by M_n ;

(*b*), K^+ flux through adjacent neural membranes (neural boundary) and their electrical behavior, potassium flux = M_{nb} ;

(*c*), as (*b*) above, for adjacent glia (glia boundary), potassium flux = M_g ;

(*d*), passive diffusion of K^+ from the IXS through the extracellular channels between boundary structures, net flux into IXS denoted by M_c ;

(*e*), metabolic flux of potassium across the primary and boundary membranes;

(*f*), variations in the extracellular potassium concentration $\Delta K_o(t)$ in the IXS as determined by the algebraic sum $M_n + M_{nb} + M_g + M_c$ and the translation of these variations into changes in the potassium equilibrium potentials ($\Delta V_k(t)$), hence, into changes in the electrical state of all the contiguous membranes via the membrane models (*a–c*, above).

The time course of the effects of potassium accumulation overlaps the second half of the spike, hence a set of relations between the electrical and ionic events of active neural membrane was required. The Hodgkin and Huxley model (1952) was not derived for mammalian neural membrane nor has it yet been extended this far. Nevertheless, it was used for that purpose here since its applicability appears, at least, qualitatively correct; for detailed references on this topic see the review by Noble, 1966. The details of a precise fit of the equations to mammalian action potential electrogenesis were not crucial to this investigation; appropriate adjustments in the time course and amplitude were included.

The electrochemical behavior of glial membrane is simply described since it is electrically passive and approximates a potassium electrode (Hild and Tasaki, 1962; Kuffler and Nicholls, 1966). Glial membrane adjacent to active neural membrane was, therefore, represented by a "leaky capacitor."

An effective tissue diffusion constant was too gross a parameter for keeping track of potassium concentration fluctuations in the IXS. The interstitium is a collection of narrow channels which are the separations between the membranes (Fig. 1) and has been likened to the water phase of a foam; K^+ movement must be followed on a similar scale. The equations for the electrochemical behavior of neural and glial membrane already included transmembrane potassium flux. Under the assumptions that extracellular K^+ moves by passive diffusion and that the width of the extracellular clefts (W_c) are much less than their spacing (D), the collection of channels abutting on the primary membrane was treated as equivalent to a "virtual membrane" with permeability

$$P_c = \frac{f_c d_{aq}}{kL} \qquad \text{cm/sec}, \tag{1}$$

where f_c is the fraction of the composite boundary which is open channel entrance,

L is the mean length of the channels, d_{aq} is the aqueous diffusion constant for K^+ (2.5×10^{-5} cm^2/sec [Washburn, 1929]), and k is a coefficient to reflect any restriction upon extracellular diffusion in the interelement channels such as might be imposed by fixed charge (Katchalsky, 1964) or gel structure (Bennett, 1963; Pease, 1966).

Viewed in three dimensions, the structurally continuous primary membrane faces a complementary surface scored by "slits" that are the open ends of the abutting interelement channels. If the average channel spacing is D (refer to Fig. 1), in each of two orthogonal directions coplanar with the membrane, then $f_c = 2W_c/D$, and hence

$$P_c = \frac{2W_c d_{aq}}{kLD} \quad \text{cm/sec.} \tag{2}$$

The geometrical parameters and any restriction upon free K^+ diffusion were in this way treated together by the simple variable, P_c (see its elaboration below and numerical examples in the Appendix).

The behavior of the system (active neural membrane)–(restricted extracellular space)–(neural and/or glial boundary membranes) was simulated by stepwise-linear solution of the differential equations on a digital computer (step size was 5 μsec or less). For each particular configuration of adjacent membranes and extracellular geometry, the procedure was to begin with the membranes and IXS at steady-state equilibrium (including leakage and metabolic ionic flux) with no net transmembrane currents. At $t = 0$, a brief (0.1 msec) current pulse was simulated to elicit a spike from the primary membrane. The subsequent electrical behavior of all membranes was then followed in time; particular examples were drawn directly via a CALCOMP plotter (California Computer Products, Inc., Anaheim, Calif.) (Figs. 3 A, 5, 7, 8). Boundary membranes had zero net transmembrane current at all times.

IONIC INTERACTIONS BETWEEN VERY CLOSE MEMBRANES

Given the electrochemical characteristics of the membranes, there are two key variables: (*a*) W, the width of the IXS and (*b*) P_c, the equivalent permeability of the collection of channels between the various structures adjacent to the primary neural membrane (Fig. 1). The physical significance of the former variable is clear and its morphological counterpart is the separation between the membranes. Although a sufficiently dense extracellular gel of mucopolysaccharide (Pease, 1966) might lead to a valid distinction between *functional* and *morphological* extracellular volume, the distinction will not be pressed here. I have considered intermembrane separations ranging from 50 to 500 A and thus covered the most likely range of values for the mammalian CNS.

In contrast, P_c is a new measure of the local extracellular milieu and one for which the numerical values are not immediately obvious. It is determined by the distribu-

tion and size of the individual neighboring structures which, relative to the primary active structure, together form the second boundary to the IXS. The numerical evaluation of this multipurpose variable and its derivation from the specific electron-micrographic architecture are taken up in the Appendix. From a less formal point of view, the value of P_c connotes the local ultrastructure in the following sense. When the structures adjacent to the primary membrane are large, such as with closely packed neural soma (Green and Maxwell, 1961) or envelopment by large glial sheets (Blackstad and Dahl, 1962; Ralston, 1968), there are *few channels per unit area*, hence P_c assumes a relatively *small* value (the minimum value considered here is (min P_c =) 5×10^{-5} cm/sec). When the boundary structures are small, such as dendrites, axons or portions of small cells and processes, there are *many channels per unit area* hence potassium can *more readily diffuse* from the IXS and P_c has a relatively *large* value, (max P_c =) 320×10^{-5} cm/sec.

If we consider the diffusion of K^+ from the IXS via the interelement channels only, then

$$[\dot{K}]_o(t) = P_c W^{-1}([K]_o(0) - [K]_o(t)); \tag{3}$$

Substituting

$$\Delta[K]_o(t) = [K]_o(t) - [K]_o(0),$$

the above equation has the simple solution

$$\Delta[K]_o(t) = \Delta[K]_o(0)\exp(-tP_c/W). \tag{4}$$

For this extracellular diffusion component we may therefore define a diffusion time constant of the IXS

$$\tau_{IXS} = W/P_c \text{ sec}, \tag{5}$$

where W is the width of the IXS in cm, and P_c is the equivalent channel permeability in cm/sec. When W = 200 A,

$$\min \tau_{IXS} = \frac{200 \times 10^{-8}}{320 \times 10^{-5}} = 0.61 \text{ msec.}$$

and

$$\max \tau_{IXS} = \frac{200 \times 10^{-8}}{5 \times 10^{-5}} = 40 \text{ msec.}$$

Neural-Neural Interactions

In a previous study it was found that the expected transient K^+ fluctuations in the narrow cleft between a spike-producing membrane and the adjacent membranes

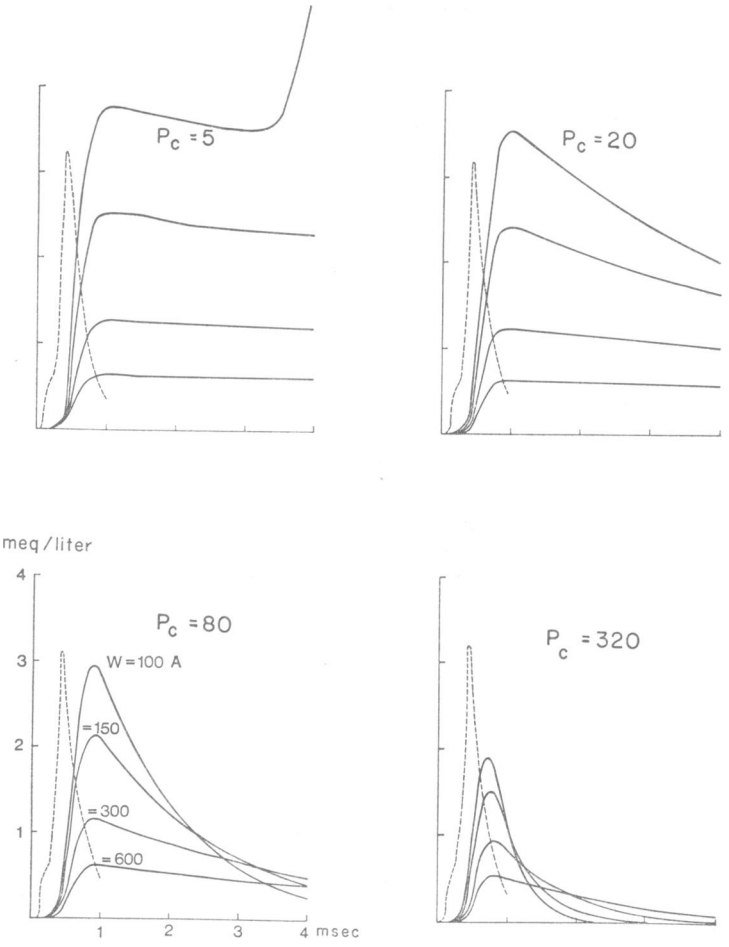

FIGURE 2 Time course of extracellular potassium transients, $\Delta[K]_o(t)$, following a single action potential in the primary neural membrane. W in angstroms and P_c in 10^{-5} cm/sec. Approximate time course of the action potential is shown by dotted profile ($C_n = 1\mu F/cm^2$). Effects of adjacent boundary membrane not included; for $P_c = 5 \times 10^{-5}$ cm/sec, $W = 100$ A, the second increase in $\Delta[K]_o(t)$ is due to a spontaneous spike in primary membrane (see "burst" response in text).

were significant over a wide physiological range (Fig. 2; also Lebovitz, 1966). The qualitative form of $\Delta[K]_o(t)$ follows from the time course of the outward potassium current. During the initial repolarization phase of the spike, $V_m - V_k \gg 0$, so that the K^+ efflux exceeded that which could be accommodated by the interelement channels ($| M_n | > | M_c |$). As the membrane potential approached its prespike level, M_n decreased until $| M_n | < | M_c |$. Correspondingly, $\Delta[K]_o(t)$ reached a peak nearly coincident with the foot of the action potential and thereafter decayed at a rate governed by the diffusion time constant, τ_{IXS}.

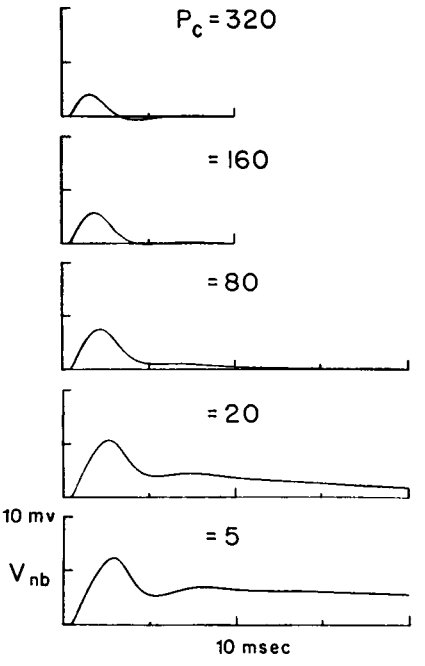

FIGURE 3A

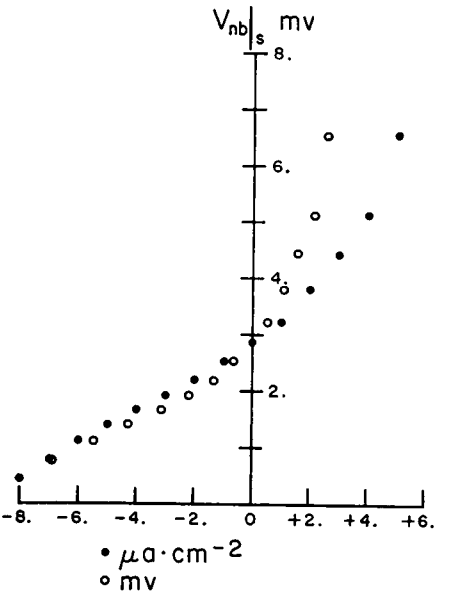

FIGURE 3B

FIGURE 3 A Transient depolarization of neural "boundary" membranes due to action potential in adjacent primary neural membrane; $f_n = 1.0$, $C_n = C_{nb} = 1.0\ \mu F/cm^2$ and time course of primary spike as in Fig. 2. $W = 300$ A and P_c in 10^{-5} cm/sec. The subthreshold neural-neural ionic interaction resembles an EPSP, except for the long "tail" at $P_c < 80 \times 10^{-5}$ cm/sec ($\tau_{IXS} > 4$ msec).

FIGURE 3 B. Effect of polarization of boundary membrane on peak magnitude of neural-neural interaction ($V_{nb}|_s$); $W = 300$ A and $P_c = 160 \times 10^{-5}$ cm/sec (see Fig. 3 A). Abscissa shows boundary membrane current in $\mu a/cm^2$ (closed circles) or boundary membrane polarization in mv (open circles).

When a more restricted space was considered, the reduced equivalent channel permeability implied that $| M_n | > | M_c |$ for a larger fraction of the repolarization phase. The peak of the transient was therefore delayed as well as larger for smaller values of P_c (Fig. 2). The upper limit on this peak was of course determined by the width of the IXS. However, it was the finding that the time constants of these extracellular potassium transients were on the same order or greater than the neural or glial membrane time constants that indicated that these membranes would therefore be responsive to them. To examine the effects of these transients on an adjacent membrane, the primary membrane was first assumed to be everywhere adjacent to other neural membranes. Adjacent glial membrane is treated below; more complicated boundary structures could be imagined, as will be discussed elsewhere.

It is well known that the resting neural membrane is sensitive to the external potassium concentration and that its increase has a depolarizing effect on the mem-

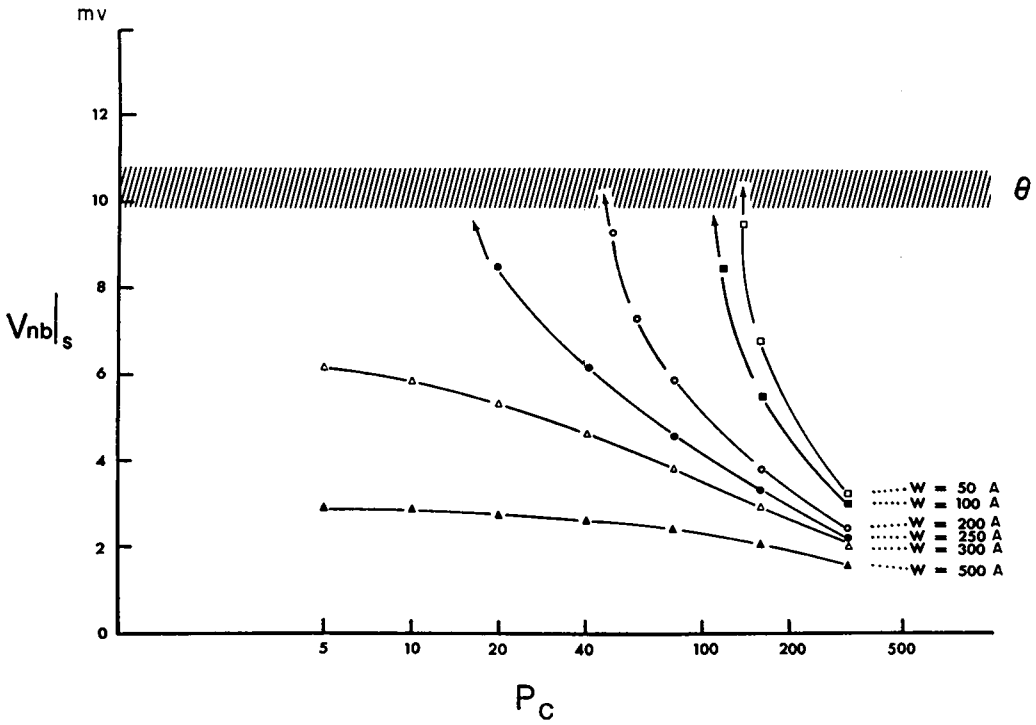

FIGURE 4 Magnitude of peak of K^+-mediated neural-neural interaction ($V_{nb}|_s$) caused by single primary membrane spike. P_c in 10^{-5} cm/sec. Arrows indicate where further reduction of P_c yielded suprathreshold interaction and therefore a spike in boundary neural membrane. $f_n = 1.0$ and $C_n = C_{nb} = 1\ \mu F/cm^2$.

brane (Noble, 1966). Here the simulated neural membranes adjacent to an active membrane showed a depolarization which roughly followed the time course of the deduced K^+ transients (Fig. 3 A). However, both in experiment and in theory the neural rest potential is only in part determined by V_k, hence the effect of the $\Delta[K]_o(t)$ on the adjacent neural membrane potential was correspondingly attenuated. In addition, the capacitance of the neural boundary membrane retards its depolarization in response to fast transients. Thus in Fig. 3 A, where the primary neural and neural boundary membranes were given $C_n = C_{nb} = 1\ \mu F/cm^2$, the peak of the depolarizing interaction occurred up to 1 msec after the peak change in the potassium equilibrium potential and was smaller by a factor of 3–6. The combined effect of significant sodium and chloride conductances (g_{na}, g_{cl}) at rest and the RC lag was to reduce the magnitude of the postulated local K^+-mediated interactions to a matter of a few millivolts over much of the physiological range. As was discussed elsewhere (Lebovitz, 1966), the repolarizing active membrane has itself an enhanced g_k and would therefore be expected to respond more directly to $[K]_o$ fluctuations.

It is important to note that the magnitude of this interaction was dependent on

the electrical state of the boundary membranes. The response of a *hyper*polarized membrane to a neighbor's action potential was reduced, while a *de*polarized neural boundary membrane showed an enhanced response (Fig. 3 B). This variability arose primarily from the shift in the ratio of g_k/g_{na} caused by the membrane polarization; that is, from the nonlinear relation between membrane and potassium equilibrium potentials. In these respects the postulated ionic interaction showed behavior opposite from the chemically transmitted excitatory synaptic potential (EPSP), which it otherwise much resembles in form and effect. This would provide a needed experimental test for establishing the identity of ionic interactions between adjacent neural membranes.

The results of simulating this neural-neural interaction at various values of W and P_c are summarized in Fig. 4. For each case the peak depolarization in response to a single primary spike is shown. For much of the physiological range, and in particular for $W > 250$ A, the K^+-mediated interaction had the general form shown in Fig. 3 A, viz., a peak nearly coincident with the terminal foot of the primary action potential, followed by a monotonic decay. When a higher membrane capacitance was assigned ($C_n = C_{nb} = 2\ \mu F/cm^2$), the peak of the depolarizing interaction was only slightly reduced. More significantly however, it then lagged the primary action potential by 2–5 msec.

These subthreshold depolarizations occur via an inward K^+ flux, hence, $M_{nb} < 0$ and the external potassium concentration transient was damped by the boundary depolarization. However, this potassium flux through the adjacent neural membranes was only a small fraction of the total flux from the IXS. At small separations this was not necessarily the case, since the peak of the neural-neural interaction increased rapidly with decreased P_c. In particular, at the more intimate geometries with $W < 250$ A, the interaction was sufficient to exceed the firing level of the adjacent neural membranes and thus communicate a spike across the intervening extracellular space. In interpreting this result, it must be kept in mind that the interactions with adjacent membranes were, strictly speaking, local. Whereas the primary membrane can be considered as a complete active surface, the model and its simulation only treats the behavior of *immediately adjacent portions* of the boundary membranes (neural or glial). However, regardless of whether or not we consider the entire adjacent unit to be discharged by such suprathreshold coupling, the induced local spikes would have as much effect on the IXS as the spikes of the primary membrane. Hence, the boundary potassium flux, M_{nb}, is no longer negligible in these cases.

Examples of such tight coupling are shown in Fig. 5. With $P_c = 80 \times 10^{-5}$ cm/sec, a spike in the primary membrane initiated a subthreshold response in the composite neural boundary membrane 200 A distant (Fig. 5 A). At decreased P_c or W (Fig. 5 B), the primary spike could induce a boundary spike which elicited in return a subthreshold response in the primary membrane. The latter looked like a pronounced depolarizing afterpotential (DAP). With P_c or W decreased still further, the DAP

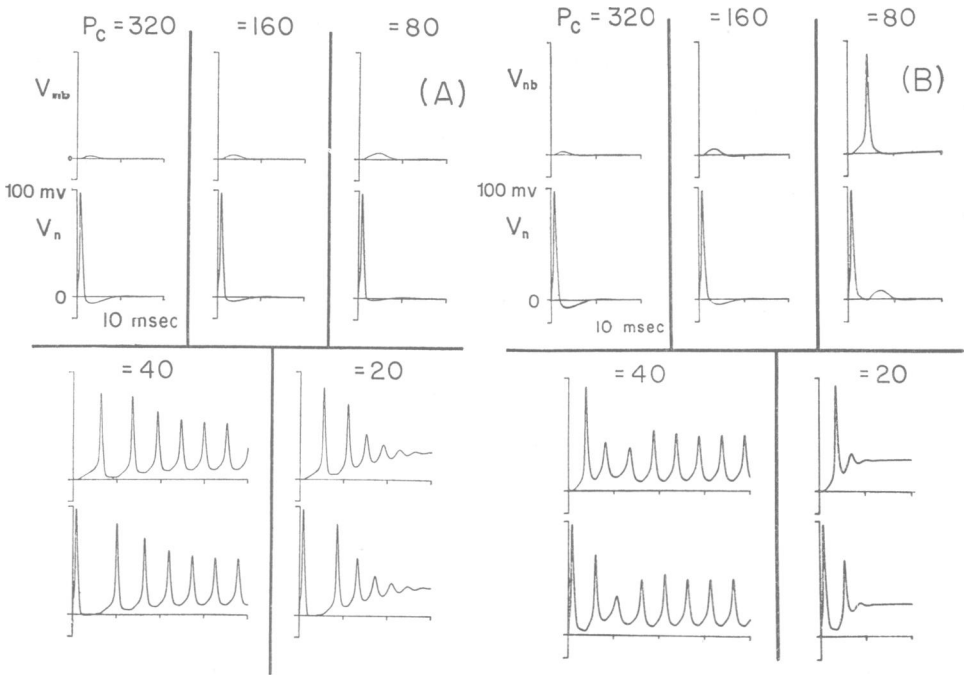

FIGURE 5 Neural-neural ionic interaction. $f_n = 1.0$. Behavior of neural boundary (upper) and primary neural (lower) membrane potentials following single triggered spike in latter. P_c in 10^{-5} cm/sec. (A), $W = 200$ A. (B), $W = 100$ A. Decreased P_c reduced and ultimately inverted the postspike hyperpolarizing overshoot in the primary membrane. Train ($P_c = 40 \times 10^{-5}$ cm/sec) and burst ($P_c = 20 \times 10^{-5}$ cm/sec) responses discussed in text. $C_n = C_{nb} = 1$ $\mu F/cm^2$.

became suprathreshold and the primary membrane thereby yielded a second and spontaneous spike.

Thus, if the width of the IXS or the rate at which K^+ can attain its extracellular equilibrium concentration were reduced sufficiently to allow suprathreshold coupling, more complicated behavior was found which took one of two forms (Fig. 5, $P_c = 40, 20 \times 10^{-5}$ cm/sec.): (*a*) a train of interdigitated spikes, or (*b*), at still smaller W or P_c, a burst of successively smaller spikes terminating in a sustained depolarization of 30–40 mv. From neither state would the membrane spontaneously recover to the initial resting state; this required the inclusion of additional dynamics. Either a simulated inhibitory synaptic potential (IPSP) or a simulated increase in the rate of movement of extracellular K^+ by metabolic uptake or phasic changes in the geometry would, for example, terminate these states (Lebovitz, 1966). It was significant that such termination was abrupt and all-or-none (i.e. had a threshold). Hyperpolarizing responses, which are found to occur in certain membranes im-

mersed in an environment of abnormally high potassium concentration (Tasaki, 1959), were not part of the membrane model assumed in this study.

The train or burst responses from ionically coupled neural membrane pairs were responses that would not have been expected from the unbounded membrane. They could be best understood as new equilibrium states in which augmented K^+ flux from the neural membranes (due to their depolarization and to increased g_k) attains dynamic but stable equilibrium with the extracellular diffusion of K^+ ($M_e = M_{nb} + M_n$). Depending on the geometry, the new, stable potassium equilibrium potential could be just below or just above the firing level for the membrane; it could not be very much below the firing level since the membrane would then simply repolarize to $V_n = V_{nb} = 0$. Consider first the case where it is a few millivolts below the firing level. Then, following an action potential, the membrane rapidly repolarizes to V_k, since g_k is high. As g_k subsequently falls towards its prespike value, the membrane potential rises. This is exactly the process of postspike overshoot and recovery, except that now the recovery potential is not zero but a potential somewhat above the firing level (remember that the membrane equilibrium potential is above the potassium equilibrium potential due to the nonzero g_{na} and g_{cl}). The membrane therefore fires another spike and the process repeats with pacemaker-like regularity (Fig. 5, $P_e = 40 \times 10^{-5}$ cm/sec).

If V_k was at or above the firing level, then the membrane began active (sodium current) depolarization before this mechanism had fully recovered (see Hodgkin and Huxley, 1952). The successive spikes were therefore reduced until finally the regenerative sodium conductance component was completely inactivated and the membrane potential stabilized near the *new* potassium equilibrium potential (Fig. 5, $P_e = 20 \times 10^{-5}$ cm/sec). A similar burst response could be induced in a primary membrane that was bounded solely by glial membrane (see below, Fig. 7 D); however, the intermembrane separations and equivalent channel permeability had to be much smaller since there was only one neural component now contributing to the extracellular K^+. On the other hand, a stable spontaneous train could be produced only if some responsive neural membrane were adjacent to the primary neural structure or if the local extracellular potassium concentrations altered via some other source. That is, to sustain a train of seemingly spontaneous action potentials, the K^+ transients have to be suprathreshold at their peak yet fall off sufficiently fast to preclude successive summation and membrane inactivation.

Neural-Glial Interaction

To examine the postulated transient glial depolarizing interaction it was assumed that the primary membrane was everywhere adjacent to glial membrane. This K^+-mediated depolarization was similar in form to the neural depolarizing interaction (Fig. 6); it was larger since the model of glial membrane was more directly responsive to transmembrane potassium gradients. No active (self-regenerative) behavior was

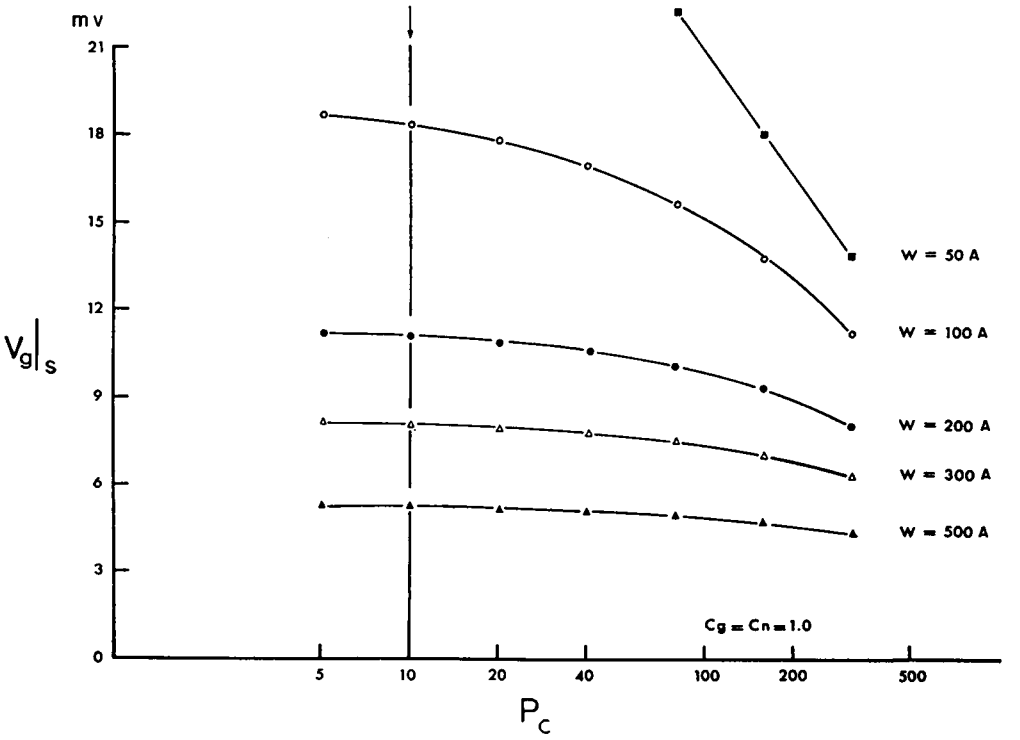

FIGURE 6 Magnitude of peak of K^+-mediated neural-glial interaction ($V_g|_s$) caused by a single primary membrane spike. P_c in 10^{-5} cm/sec. $f_g = 1.0$, $C_n = C_g = 1$ $\mu F/cm^2$ and $(\bar{g}_k)_g = 100$ mmho/cm^2.

included in the glial model and the membrane acted always as a simple potassium sink. The net result was that the glial membrane potential changes reflected the alterations in the local potassium concentration. That is, the decay constant of the transient glial depolarization is the larger of τ_{IXS} and τ_g ; the peak magnitude of the transient is sufficient to estimate the width of the extracellular space. This may prove useful in the investigation of the dynamics of the extracellular space. Unfortunately, perisomal glia is frequently in the form of broad but thin laminae, as difficult to deal with experimentally as is the IXS itself.

Some of the qualitative properties of the neural-glial interaction are shown in Fig. 7. The low resistance glial membrane had a time constant that was shorter than the spike width, hence with multiple stimuli and τ_{IXS} significantly smaller than the interstimulus interval, successive transients were independent (Fig. 7 A). When τ_{IXS} was significantly greater than the interstimulus interval, the neural-glial interaction showed the logarithmic summation characteristic of a passive, K^+-dependent membrane (Fig. 7 B and C). The successively augmented external potassium concentration was also reflected in additive neural afterpotentials (Fig. 7 C) which were sufficient, in particular cases, to trigger a burst response (Fig. 7 D).

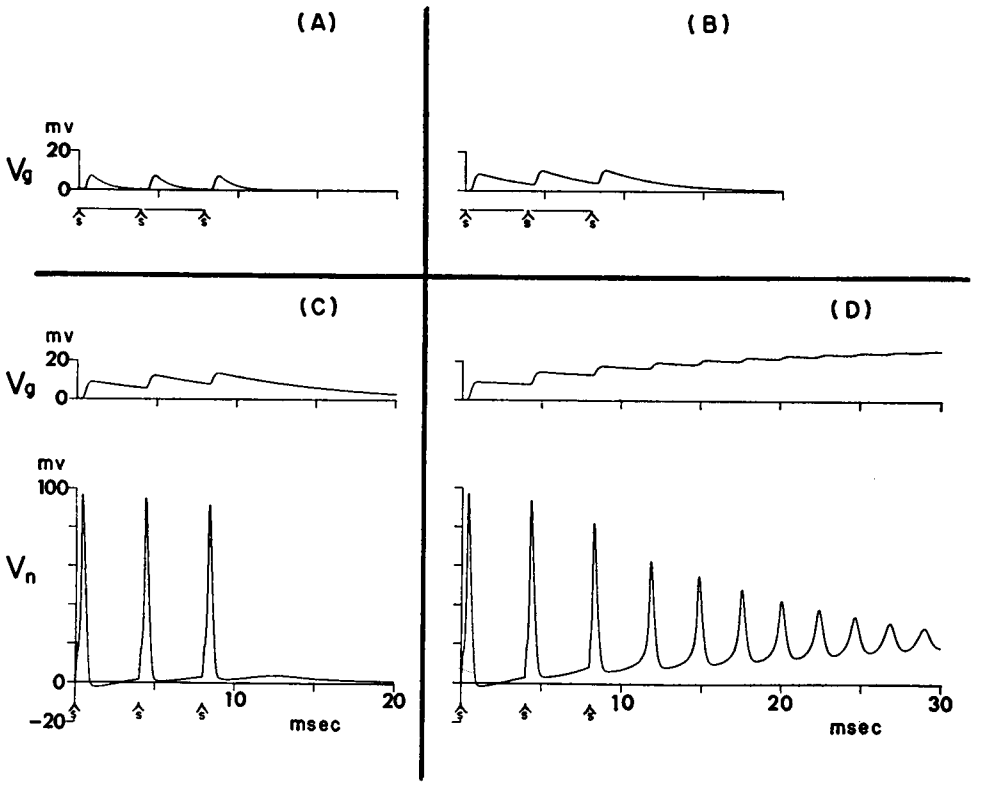

FIGURE 7 Examples of neural-glial ionic interaction. Primary membrane triggered three times (at arrows) with $f_g = 1.0$, $C_n = C_g = 1\ \mu F/cm^2$, and $(\bar{g}_k)_g = 100$ mmho/cm^2. $V_g(t)$ alone is shown in (A) and (B); in (C) and (D), $V_g(t)$ is plotted above and $V_n(t)$ below in each panel. $W = 250$ A. (A), $P_c = 320 \times 10^{-5}$ cm/sec; (B), $P_c = 80$; (C), $P_c = 40$; (D), $P_c = 10$. Note depolarizing afterpotential following last primary spike in (C) and burst response following last triggered spike in (D).

The assigned conductance of the glial membrane illustrated in Fig. 7 was 100 mmho/cm^2, approximately 100 times larger than the conductance of the resting neural membrane. The glial time constant was therefore quite small and did not contribute to the slow decay of V_g. More recent investigations of glial membrane have indicated that the specific conductance is comparable to that of neural membrane (Kuffler and Nicholls, 1966). Simulation of such a high resistance glial membrane ($g_g = 1$ mmho/cm^2) showed that with the increased membrane time constant, the peak of the K^+-mediated interaction was reduced by as much as 20% and retarded by 2–5 msec, depending on the values of W and P_c. Otherwise the local neural-glial interactions were quite similar for high and low resistance glial membrane (Fig. 8). As above, the form of the transient could be used to estimate the parameters of the extracellular space. However, because of the increased time con-

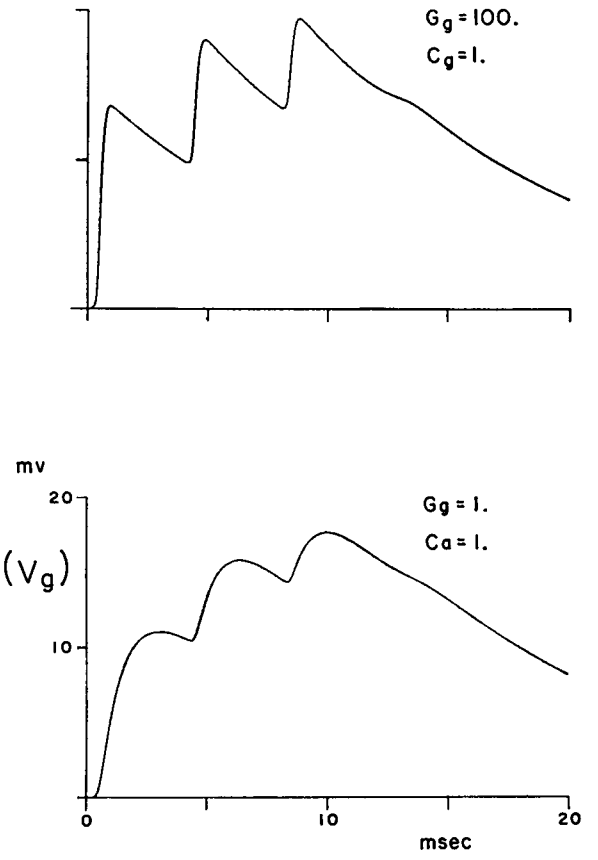

FIGURE 8 Neural-glial ionic interaction for low resistance glial membrane (above — $(\bar{g}_k)_g$ = 100 mmho/cm²) and for high resistance glial membrane (below — $(\bar{g}_k)_g$ = 1 mmho/cm²). f_g = 1.0 and $C_n = C_g = 1\ \mu F/cm^2$. W = 150 A and $P_c = 20 \times 10^{-5}$ cm/sec. Primary membrane triggered three times as in Fig. 7. Note decremental summation of the glial transients.

stant of the high resistance glial membrane (τ_g = 1 msec if $C_s = 1\ \mu F/cm^2$) the estimate of W is a function of τ_{IXS} (hence, of P_c). Current flow through glia will be taken up in another place.

DISCUSSION

It has been shown that the hypotheses of (*a*) the ionic basis of neural electrical activity, and (*b*) a restricted extracellular space, together imply the existence of transient local depolarization of neural and glial membrane by spike activity in nearby neural membrane. Since this depolarization is mediated by variation in the extracellular K^+ concentration, the phenomenon is called an ionic interaction. The available magnitudes of this new, nonsynaptic interaction are in the millivolt range and therefore sufficient to modulate neural discharge. In particular geometrical situations

they are theoretically sufficient to depolarize neighbor neural membrane beyond the firing level and thereby trigger a local spike. The proposed interactions with glial membrane are likewise in the millivolt range.

I have used the Hodgkin and Huxley equations (1952) to model the neural membranes in this analysis, even though there has been no precise experimental fit to mammalian somal electrogenesis. Some such dynamical description was a necessary part of this analysis since the time course of the K^+ efflux is a factor in accumulation effects and, further, this rapid efflux is itself altered by such accumulation. The above results are not, however, bound to the details of the Hodgkin and Huxley formulation but rather to the phenomena of potassium efflux during membrane repolarization and *partial* dependence of the membrane potential on the potassium equilibrium potential, neither of which is in serious question. Given a more precise membrane model, the above results would require quantitative but not qualitative revision.

A more serious objection is to the idealization that the primary membrane is separated from a constant interstitial compartment by a single "layer" of adjacent neural and glial structures. Characterizing this layer simply by the average diameter of its elements (Appendix) neglects the local inhomogeneity which is obvious in electron- and photomicrographs of the CNS. In addition, neuronal structures in the boundary layer or beyond it certainly may themselves be active and release potassium into the interstitium. Thus, K^+ gradients could be established in the extracellular channels which would oppose its movement from the vicinity of the active unit upon which our attention was focused. These objections are quite valid and can only be met by more complete analysis of particular cases. Such an extension is the study of ionic interactions among the fine processes of the neuropil, for which the approximations of this analysis are not well suited (see Lebovitz, 1969).

In this first consideration of the electrophysiological consequences of a restricted extracellular environment, I have chosen to consider the active surface of a single neuronal soma (the primary membrane) plus only the neural or glial membrane *immediately adjacent* to it (boundary membranes). Extracellular current flow has not been included. Hence, the postulated interactions with these neighboring units are local and patchlike depolarizations. Where the neural-neural interaction is suprathreshold and we can consider that the communicated spike propagates over the surface of the adjacent unit, we could speak in simplified terms about the stability of a population, that is, of its tendency to partial or total synchronous discharge. The general case, however, requires a discussion of the distribution of weak interactions among geometrically close neurons. These effects would be most potent near trigger zones and when the individual EPSP's are themselves weak. In sum, this appears to add another order of complexity to the analysis of neural response to dispersed, subthreshold EPSP's (see Rall, 1964).

Again like the EPSP, the proposed ionic interaction is spike dependent and transient, and if detected with intracellular micropipettes, would be quite similar to

it in form. Although their disparate responses to membrane polarization (Fig. 3 B) provide a basis for distinguishing between postsynaptic and ionic interactions, these atter phasic and nonsynaptic interactions have not yet been identified experimentally. There had been little to prompt such a close examination of the small intracellular potentials except the speculations that a build up of extracellular potassium may contribute to seizure spread (Green and Petsche, 1961) and/or to the phenomenon of spreading depression (Grafstein, 1956). Nevertheless, there are reports of neuronal interaction potentials which have the form of EPSP's but which, in response to membrane polarization, do not behave like EPSP's (Grinnell, 1966; Nelson, 1966; Stefanis and Jasper, 1964) or in fact behave opposite to them (Takahashi, Kubota, and Uno, 1967). In accordance with the above results, I propose that these occasionally reported potentials are ionic interactions and that they require careful reexamination.

It may also be significant that a component of membrane noise has been found to behave in the predicted fashion, viz., to be reduced as the membrane is hyperpolarized (Verveen and Derksen, 1965). The suggestion had previously been offered, on statistical grounds, that the discharge patterns of spontaneously active neurons may relate to a localized, nonsynaptic noise process (Enright, 1967). As that author rightly points out, the intracellular recording of neural transmembrane potentials is a spatial average of the local potentials; unless these potentials are in near synchrony over large areas of membrane, their recorded values will be much less than their "local" values, say, at a trigger zone. Finally, the relation between membrane noise and nerve impulses has been demonstrated (Hubbard, Stenhouse, and Eccles, 1967); prior to the theoretical results presented here, their results could only be interpreted in terms of synaptic events.

Of fundamental importance for the concept of ionic interaction is the fact that it would be effective only over short distances and would be readily altered by tonic or phasic variations in local structure (W or P_c) or in the metabolic activity of nearby membranes, particularly glial membrane. Considering first the localization of the interaction, the sensitivity of synaptic terminals to polarization presents a powerful extension of these effects. That is, the local potassium accumulation resulting from spike activity would depolarize nearby synaptic boutons and thus enable a neuron to exert a measure of control over its own input and, perhaps, over the input to *nearby* neurons. Depending on the ultrastructure and upon the timing of synaptic activity entering the region of influence of the active membrane, the local K^+ transients could cause the input to be depressed (via subthreshold depolarization similar to that postulated on other grounds for presynaptic inhibition (Eccles, Kostyuk, and Schmidt, 1962 *a* and *b*) or perhaps enhanced (by facilitating invasion of the terminals when the safety factor is low). In spreading depression, there is the expected cessation of spontaneous neural discharge followed by a brief flurry of spikes before the prolonged silence and unresponsiveness of cathodal block of neuron and synapse (Morlock, Mori, and Ward, 1964).

Alterations in the extracellular architecture might be related to neural function, as in the report of short latency impedance changes in the lateral geniculate in response to light (Morlock, Bak, and Marshall, 1966). More familiar examples are certain pathological states, such as anoxia and the spreading depression mentioned above, in which the marked reduction of the extracellular cleft is well known (van Harreveld and Khattab, 1967). Reduction of the extracellular cleft would of course enhance ionic coupling and, in the range of 100–200 A, promote dominating suprathreshold interactions. Such pathological states in cortex are perhaps favorable conditions under which to seek evidence for phasic, K^+-mediated interactions.

As regards metabolic variations, the work on short time course metabolic proccesses in neural and glial membrane has barely begun. To extrapolate from this work, the concept of metabolic control of extracellular fluid composition by activated transmembrane "pumps" (Skou, 1965) provides an additional mechanism for adjusting the relative effects of the rapid K^+ efflux.

The significance of the neural-glial interaction is obscure since the functional significance of glial elements is not completely resolved. The above results simply indicate that there is a direct phasic link between neural activity and whatever service the glial population provides, be it metabolic support (Giacobini, 1962), memory (Cummins and Hyden, 1962) or environmental control (Nicholls and Kuffler, 1964). At the moment, the conceptual value of the proposed neural-glial interaction lies rather in the possibility of using the glial membrane as a part of the apparatus to monitor the variations in local extracellular potassium concentration. The magnitude, decay constant, and summation properties of the intracellularly recorded glial depolarization could be used to estimate the various parameters of the local extracellular geometry (Lebovitz, 1966).

A potassium-mediated neural-glial interaction has been described (Orkand, Nicholls, and Kuffler, 1966) and appears to be the steady-state counterpart of the transients which I propose here. The published experimental reports show the summated effects of many impulses as the potassium released from the active neurons distributes through the interstitial fluid. Intracellular recordings from presumed glial cells (Atkinson and Ward, 1964; Karahashi and Goldring, 1966) verify the existence of transient depolarization — these in a class of cells which are classically devoid of synaptic input. However, their origin in a potassium-mediated transient interaction with nearby neurons has not been verified.

Note Added in Proof. Two experimental studies of K^+ accumulation have appeared since this paper was submitted for publication. They are:
Eccles, V. C., H. Korn, H. Taborikova, and N. Tsukahara. 1969. *Brain Res.* **15**:276.
Balor, D. A., and J. G. Nicholls. 1969. *J. Physiol. (London).* **203**:555.

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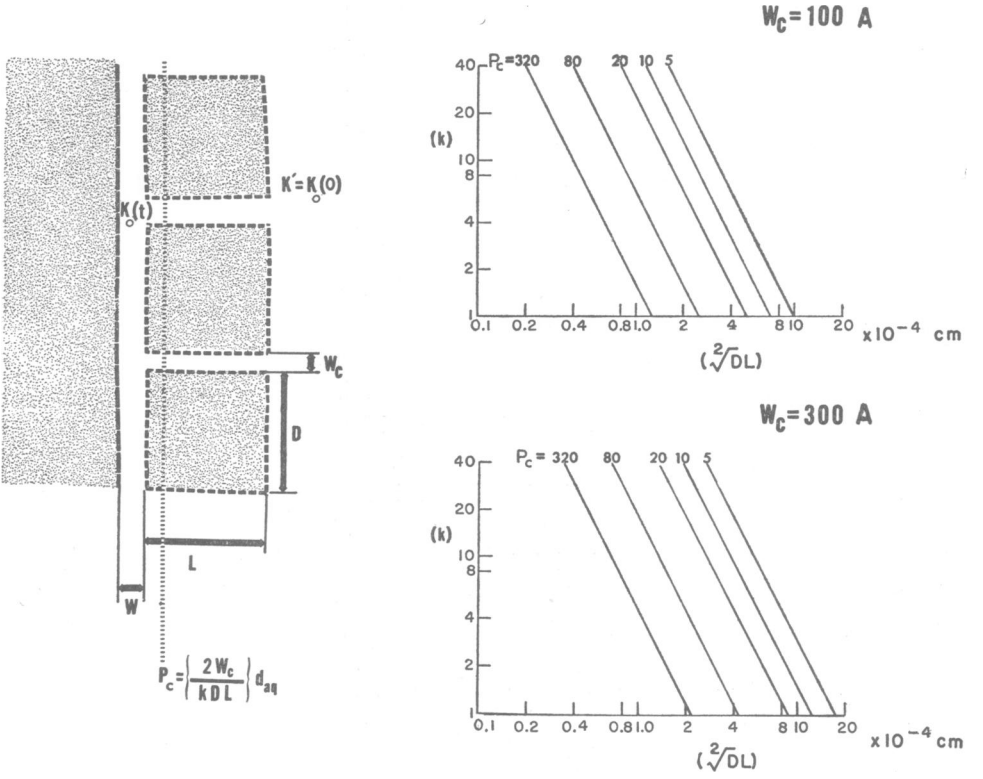

FIGURE 9 Definition of equivalent channel permeability (P_c) in terms of the channel geometry and characteristics. Schematic at left shows assumptions from which the permeability of the "virtual" membrane (finely divided vertical line) was derived. The graphs at the right show the relation between the size of the boundary elements ($\sqrt{DL}$), restriction upon extracellular diffusion (k) and P_c, for channels of width W_c = 100 and 300.

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APPENDIX

The diffusion of liberated potassium from the vicinity of an active neural membrane has been described by constructing a "virtual membrane" which represents the collection of extracellular channels between the neural and glial elements adjacent to that primary membrane. The total channel flux is, therefore,

$$M_c = P_c(\Delta[K]_o(t)) \text{ eq/cm}^2\text{sec}, \qquad \text{(A1)}$$

where $P_c = 2W_c/kLD$ is the permeability of the virtual membrane, the size of the first layer of boundary structure is given by D and L, W_c is the width of the extracellular channels, and

k is a coefficient which represents any restriction upon free extracellular diffusion of K^+ ($k \geq 1$). A schematic of this approximation to the local extracellular geometry is shown in Fig. 9. In any given region of the CNS, the dimensional terms D, L (here, best interpreted as weighted average values over the surface of a given active primary structure, viz., neural soma) are well defined, as is the aqueous diffusion constant, d_{aq}. In order to preserve the generality of the model, the extracellular diffusion is described in terms of P_c rather than by a particular extracellular geometry. The one-to-many correspondence between P_c and the extracellular geometry is, however, easily obtained from equation A 1 (see graph in Fig. 9).

If, for example, the composite boundary comprised a large number of elements having small diameters (such as a collection of small cell bodies, dendrites or glial processes), then $\sqrt{DL}$ would likely fall in the range 0.4–2.0 μ, say 1.0 μ. With W_c = 100 A, consider two cases.

(*a*), k = 1.0. If potassium diffuses as in a free, aqueous system, such a collection of small boundary elements corresponds to $P_c = 500 \times 10^{-5}$ cm/sec; any K^+ transients would be small and quite brief; τ_{IXS} = 0.2 msec.

(*b*), k = 10. In a restrictive extracellular matrix, the same extracellular geometry corresponds to $P_c = 50 \times 10^{-5}$ cm/sec, or one-tenth the previous value; τ_{IXS} = 2 msec.

Next consider that the active soma is surrounded by large structures such as other soma or extensive glial sheaths. In this case $\sqrt{LD}$ would likely fall in the range 2.0–10.0 μ, say 5 μ. With W_c = 100 A, again consider two cases.

(*a*), k = 1. Under the assumption of free diffusion, $P_c = 20 \times 10^{-5}$ cm/sec and the τ_{IXS} = 5 msec; reequilibration of the extracellular phase would be slow.

(*b*), k = 10. Now P_c is one-tenth its former value or $P_c = 2. \times 10^{-5}$ cm/sec due to the added restriction of "sticky" as well as infrequent interelement channels; τ_{IXS} = 50 msec.

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