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ION AND TRANSMITTER MOVEMENTS DURING SPREADING CORTICAL DEPRESSION

HENRY C. TUCKWELL and CHRISTOPHER L. HERMANSEN

Department of Mathematics, Monash University, Clayton, Victoria, Australia 3168

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A reaction–diffusion system of equations whose components are the extracellular concentrations of K^+, Ca^{++}, Na^+, Cl^-, an excitatory neurotransmitter and an inhibitory neurotransmitter, is developed in order to model the movements of these substances through various kinds of membrane in brain structures. Expressions are derived from probabilistic arguments for the conductances induced in subsynaptic membrane by transmitter substances at various concentrations, for one-way active transport rates and for an exchange pump rate. These expressions are employed in the reaction terms of the system. The meaning of the many constants is explained and, with appropriate choices for their values, the model predicts subthreshold responses to small enough local elevations of KCl or glutamate and stable propagating SD waves if the local elevations of these chemicals is sufficient. The SD waves consist of elevated K^+ and transmitter substances and diminished Ca^{++}, Na^+ and Cl^-, the velocity of propagation in cortex being about 0.6 mm/minute. This is in the experimental range, the K^+-amplitude being 17 mM relative to a baseline of 3 mM, as the model developed ignores the effects of action potentials. There is no SD response to either NaCl or GABA. The effects of no K^+ and no glutamate diffusion are investigated, both being manifest as a failure in propagation of the stable SD waves. The wave solutions are analysed in terms of phase portraits. The roles of various amino acid uptake and release processes by neurons and glia are discussed, as are the complications with regard to their incorporation in a model for SD. The roles of neurons and glia are analysed and the six basic fluxes of K^+ are outlined. It is postulated that under some circumstances, in cortex treated with TTX, there may be practically no transmitter release, but SD may propagate if TTX does not completely abolish g_{Na} for nonsynaptic membrane, a corresponding system of model equations being developed. The data and ideas of K^+-based and glutamate-based SD of Van Harreveld are discussed and interpreted in terms of which reaction terms are operative in the K^+ equation. An appendix contains the values of the parameters used in the numerical calculations.

Spreading cortical depression (SD), discovered first in the rabbit brain by Leao (1944), is a complicated wave phenomenon that moves slowly (typically about 3 mm/minute) across brain structures endowed with a large proportion of gray matter. The many concomitant phenomena are well described in the reviews of Marshall (1959) and Bures, Buresova, & Krivanek (1974). Recently, ion selective electrodes have enabled measurements of ion concentrations in the extracellular compartment during spreading depression (Vyskocil, Kriz, & Bures, 1972; Kraig & Nicholson, 1976; Nicholson, Ten Bruggencate, Steinberg, & Stockle, 1977; Kraig & Nicholson, 1978; Nicholson & Kraig, 1979; Nicholson, Ten Bruggencate, Stockle & Steinberg, 1978). These experiments have revealed an increase in potassium ion concentration, confirming earlier investigations (Brinley, Kandel, & Marshall, 1960; Krivanek & Bures, 1960), large decreases in sodium and chloride ion concentrations and a drop in calcium ion concentration to about a tenth of its normal value.

Twelve years after SD was discovered, a theory of the phenomenon was advanced by Grafstein (1956; see also, 1963, and the book by Bures *et al.* (1974)). In that theory, potassium played a central role. It was suspected that the intense neuronal activity that accompanied the SD wave released potassium into the extracellular compartment. This accumulated K^+ diffused to regions yet uninvaded by the SD wave, depolarizing nerve cells and causing them to fire action potentials and release more K^+ etc. A reaction–diffusion equation for the concentration of extracellular K^+,

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$$K_t^0 = D_K K^0_{xx} + f(K^0), \qquad (1)$$

where $K^0(x,t)$ is the ion concentration at x at time t, D_K is the diffusion coefficient, subscripts x and t represent partial differentiation and $f(K^\circ)$ is a cubic. This form of the equation was apparently suggested by Hodgkin and later solved for travelling wave solutions by Huxley (see Grafstein, 1963; Bures *et al.*, 1974). This equation could predict the rise of the K^+ concentration during SD but not the return to resting values. It is known now that one needs a coupled system of reaction diffusion equations with at least two components before one may obtain wave solutions with a departure and return to resting conditions.

An alternative theory of SD was proposed by Van Harreveld and Fifkova (1970) in which glutamate, the putative excitatory neurotransmitter substance in many nervous structures (see Krnjevic, 1974, for a review; Sandoval & Cotman, 1978), plays the "primary" role. This idea was based on the earlier observation (Van Harreveld, 1959) that the threshold concentration for elicitation of SD in rabbit cortex was much lower for glutamate than for KCl when topically applied. To quote from Van Harreveld & Fifkova (1970): "If glutamate would be released in the way proposed by Grafstein for K^+, all the known concomitants of SD would follow."

There have been a number of subsequent developments in the controversy as to which chemical (K^+ or glutamate) is the "primary" one in SD. Do Carmo & Leao (1972) found that topical application of glutamate could only cause SD in rabbits and rats when accompanied by mechanical stimulation. This led them to believe, along with other authors (Bures *et al.*, 1974), that glutamate was not the primary chemical in SD and that this role belonged to K^+. A follow up study by Fifkova & Van Harreveld (1974) seemed to account very well for the findings of Do Carmo and Leao. Age was found significantly to affect the ability of glutamate to cause SD. The final conclusion was that when glutamate fails to elicit SD by topical application, whereas KCl does elicit SD, the reason is that glutamate is not as successful in penetrating the upper layers of the cortex, hence not reaching nerve cells in sufficient concentration to give rise to an SD wave.

More recently, Van Harreveld (1977) has reported the existence of two kinds of SD. One of these is called "glutamate based," the other "K based." It had been shown previously (Van Harreveld & Fifkova, 1970) that glutamate was released from the isolated chicken retina during SD elicited either by KCl, glutamate or direct current stimulation, though it could not be demonstrated to be released in spontaneous SD. In the more recent communication it was stated that SD in the retina is blocked by Mg^{++}, but only at temperatures greater than 23°C. At lower temperatures it was claimed that metabolism is slowed and that then K ions are involved in SD. Also, ouabain causes SD at elevated temperature in the presence of Mg^{++}. A difference in speeds of the two kinds of SD was also noted. At 22°C the "glutamate based" SD had a velocity of 2.3 mm/minute, on average, whereas the "K based" SD had a velocity of only 1.3 mm/minute.

These recent findings make the picture of what happens during SD more complicated. In this paper, a model is developed for the movements of the four basic ions (K^+, Ca^{++}, Na^+ and Cl^-) and two neurotransmitter substances, one excitatory and the other inhibitory, which one could tentatively take to be glutamate and GABA (gamma-aminobutyric acid), respectively. In distinction to a previously analysed model (Tuckwell & Miura, 1978; Tuckwell, 1979a) in the model equations developed here the transmitter substances have their own reaction–diffusion equations and thus appear explicitly. This enables the response of the system to applied doses of either ions or transmitters to be ascertained, and thus puts us in a better position to ask which chemicals are "primary" in SD. Expressions are derived below for transmitter induced conductance changes as functions of transmitter concentrations too are expressions for the pump rates including those expected for an exchange pump as functions of ion concentrations. The equations to be employed in the numerical calculations are described below with a description of the meaning of the several parameters.

The next section contains the results of the numerical calculations. In response to elevated local concentrations of either KCl or glutamate stable solitary SD waves form consisting of elevated K^+ and transmitter concentrations and depressed Ca^{++}, Na^+ and Cl^-. With a K^+ amplitude of 17 mM relative to a resting level of 3 mM, the velocity of propagation is estimated at about 0.6 mm/minute. No SD waves form with local application of either NaCl or GABA.

The final section contains a discussion of the various fluxes during SD, including the uptake and release of amino acids from neurons and glia. The

roles of neurons and glia are also discussed and seemingly crucial matters such as whether the K^+ levels are sufficiently high in TTX treated cortex to induce transmitter release are considered. An alternative model which relies on the incomplete abolition of sodium conductance by TTX is outlined.

DEVELOPMENT OF THE MODEL EQUATIONS

We will restrict our attention here to the movements of K^+, Ca^{++}, Na^+, Cl^- and an excitatory transmitter which we shall denote by T_E and an inhibitory transmitter which we will denote by T_I. The ion concentrations will be represented by the symbols K, Ca, Na, Cl and the transmitter concentrations will be represented by simply their labels T_E and T_I.

We will need to distinguish between the extracellular compartment and several kinds of intracellular compartment. Concentrations in the extracellular compartment will be designated by the superscript ^{0} (e.g., Na^0). We will focus attention on two kinds of intracellular compartment. The first is what may be called the postsynaptic intracellular compartment and the second we will refer to as the presynaptic intracellular compartment. To distinguish concentrations in these compartments, we will use the superscripts $i,1$ and $i,2$ respectively. This $K^{i,1}$ is the concentration of potassium ions in the postsynaptic intracellular compartment.

The reason for considering two intracellular neuronal compartments is that the preterminal synaptic endings can probably be viewed as localized volumes which contain high concentrations of transmitter substances (McLennan, Note 1). It has been also shown that synaptasomes are viable compartments with their own membrane potential and ion concentrations (Campbell, 1976). Other direct evidence exists that the cell body (and presumably at least the proximal parts and possibly distal parts of the dendrites) is distinct from the presynaptic terminal endings (of the same cell). Whittaker (1965) found that about two-thirds of total acetylcholine in cortex was localized in the synaptosomal fraction. This matter is discussed by Eisenstadt & Schwartz (1975) who point out that in another study (Potter, 1970) uptake, turnover and release of acetylcholine can occur at neuromuscular junctions independently of the nerve cell body for short periods of time. Transport of transmitter synthesized in the cell body of the cholinergic neuron L10 of aplysia to nerve terminals was found to take a few hours (Koike, Kandel, & Schwartz, 1974) providing further supporting evidence that in modelling populations of nerve cells account should be taken of the synaptic terminals by regarding them as a different intracellular compartment which is not in rapid contact (except when invaded by an action potential) with the somatic regions of the cell. There is another noteworthy intracellular compartment, namely that of glial cells but this will not appear explicitly in the model equations. Glial cells reputedly behave like K^+ electrodes, do not have synapses, and are thus believed to play a "passive" role in SD.

We will consider a spatially homogeneous sheet of cortical tissue and though SD is a phenomenon in three space dimensions, we will restrict our attention here to one space dimension. This simplification is made for two reasons: the essential phenomenon of wave propagation can be studied in one space dimension and there is an enormous increase in the computational task of solving the reaction–diffusion equations when we go from one space dimension to two. The question of phenomena related to two or more space dimensions will have to be addressed at a later date. The assumptions of the model are as follows and are mainly similar (with the exception of (i) and (vi)) to those previously employed (Tuckwell & Miura, 1978):

i) Ions and transmitter substances are free to diffuse in the extra-cellular compartment.

ii) The equilibrium membrane potentials are given by the Goldman–Hodgkin–Katz formula (Goldman, 1943; Hodgkin & Katz, 1949). Thus for the postsynaptic membrane the equilibrium potential is

$$V_1 = \frac{RT}{F} \ln \left[\frac{K^0 + p_{Na}\, Na^0 + p_{Cl} Cl^{i,1}}{K^{i,1} + p_{Na}\, Na^{i,1} + p_{Cl} Cl^0} \right], \qquad (2)$$

where R is the gas constant, T is the absolute temperature, F is Faraday's constant, and p_{Na}, p_{Cl} are the permeabilities of the membrane to sodium and chloride ions relative to that of potassium ions. A similar equation holds for V_2, the equilibrium membrane potential of the presynaptic membrane.

iii) The ions K^+, Ca^{++}, Na^+ and Cl^- have individual equilibrium potentials given by the

Nernst formula. Thus, for example, the post-synaptic potassium equilibrium potential is

$$V_{K,1} = \frac{RT}{F} \ln \left[\frac{K^0}{K^{i,1}}\right], \tag{3}$$

with similar expressions for the sodium and chloride equilibrium potentials. The presynaptic calcium equilibrium potential is,

$$V_{Ca,2} = \frac{RT}{2F} \ln \left[\frac{Ca^0}{Ca^{i,2}}\right]. \tag{4}$$

iv) The postsynaptic and presynaptic membranes have certain conductances per unit area to the various ions. These are denoted by $g_{K,1}$, $g_{K,2}$, etc., where again the additional subscripts 1, 2 refer to postsynaptic and presynaptic membrane respectively.

v) The flux (current) of an ion species through the membrane is proportional to the product of the conductance for that ion and the difference between the membrane potential and the equilibrium potential of that ion. Thus, for example, the rate at which potassium ions flow through postsynaptic membrane is proportional to $g_{K,1}(V_1 - V_{K,1})$.

vi) The postsynaptic membrane is endowed with receptors for the various neurotransmitters. The conductance of the postsynaptic membrane to various ions depends on the number of non-refractory receptors which are bound to transmitter molecules.

vii) The presynaptic membrane calcium conductance is a function of the presynaptic membrane potential. Thus, $g_{Ca,2} = g_{Ca,2}(V_2)$.

viii) The outward flux of neurotransmitters from presynaptic terminals is proportional to the inward presynaptic calcium current.

ix) Over the various kinds of membrane there are sites at which active transport of both ions and transmitter occurs. The action of these transport mechanisms is such that it tends to restore the resting distribution of concentrations in the extracellular and intracellular compartments. The active transport processes are referred to by the popular name, "pumps."

Each of the equations, which take the form of reaction–diffusion equations, for the extra-cellular concentrations thus contains, apart from the diffusion term, a sink term and a source term. Let $\mathbf{C}^0$ be the vector whose components are the ion and transmitter concentrations in the extracellular space and let $\mathbf{C}^{i,1}$ and $\mathbf{C}^{i,2}$ be the vectors whose components are the concentrations of ions and transmitter substances in the postsynaptic and presynaptic intracellular compartments, respecively. It is also convenient to let $\mathbf{C}$ be the vector whose components are all the extracellular and intracellular concentrations so that $\mathbf{C} = (\mathbf{C}^0, \mathbf{C}^{i,1}, \mathbf{C}^{i,2})$. Then the general model equations take the form

$$\frac{\partial \mathbf{C}^0}{\partial t} = D \frac{\partial^2 \mathbf{C}^0}{\partial x^2} + \mathbf{F}_1(\mathbf{C}) + \mathbf{F}_2(\mathbf{C}), \tag{5A}$$

$$\frac{\partial \mathbf{C}^{i,1}}{\partial t} = -\alpha_1 \mathbf{F}_1(\mathbf{C}), \tag{5B}$$

$$\frac{\partial \mathbf{C}^{i,2}}{\partial t} = -\alpha_2 \mathbf{F}_2(\mathbf{C}), \tag{5C}$$

where D is the (diagonal) matrix of diffusion coefficients and α_1 and α_2 are the ratios of the extracellular volume to the postsynaptic and presynaptic intracellular volumes. Here $\mathbf{F}_1$ and $\mathbf{F}_2$ refer to fluxes of chemicals between the extracellular compartment and the post- and presynaptic intracellular compartments. Before giving details of the equations which are to be employed in the calculations, we devote some time to the derivation of expressions for the pump rates (including the case of an exchange pump) and the dependence of postsynaptic membrane conductance on transmitter concentrations.

EXPRESSIONS FOR CONDUCTANCES AND PUMP RATES

Conductances Depending on Transmitter Concentration

We shall derive some simple expressions for the dependence of post-synaptic membrane conductance on transmitter concentrations. There do not appear to be models for the attachment of glutamate or GABA to receptors as have been proposed for the way in which acetylcholine attaches to receptors (Katz & Thesleff, 1957; Katz & Miledi, 1973). We will have to assume that the manner in which these amino acid molecules cause conductance changes to various ions is similar to that proposed (though tentatively) for acetylcholine.

We begin by assuming that the mode of action of the transmitter is as follows. A transmitter molecule in the synaptic cleft may form a complex with a receptor site (molecule). We will ignore the possibility of an intermediate state and assume that when this complex is formed, the conductance of the postsynaptic membrane to certain ions is increased. The transmitter–receptor complex has a certain lifetime after which it breaks up into a transmitter molecule and a receptor which is refractory for a certain length of time. This can be represented as

$$T+R \rightarrow (TR) \rightarrow T+R' \tag{6}$$

where T denotes transmitter, R is the receptor and R' is the receptor in the refractory state. The simplifying assumption has been made (cf. Katz & Thesleff, 1957) that the reactions in (6) are not reversible, except R' can later go to R.

Now consider a patch of postsynaptic membrane on which are N receptor sites per unit area. We will assume that the number of transmitter molecules in the synaptic cleft is large in comparison with the number of receptor sites. Note that at neuromuscular junction there are normally only about 6 receptor sites per square micron (Fambrough, 1974) so that the estimate we will make of the conductance will be slightly inaccurate when the transmitter concentration is small. When the transmitter concentration has reached values typical of those expected in spreading depression (greater than 0.1 mM) the number of transmitter molecules in the synaptic cleft (of assumed "thickness" 200 Å) over an area of 1 square micron is approximately 1200 or greater, so this approximation should have only a small effect on the resultant expressions.

At time t we assume that the transmitter concentration is $T(t)$, that of the N total receptor sites, $N_1(t)$ are in the state (TR) and $N_2(t)$ are in the state R'. The conductance is $g(t) = kN_1(t)$ where k is the conductance due to a single receptor–transmitter complex. We now assume that the probability per unit time that a receptor site is hit by a transmitter molecule is p; that the probability per unit time that a receptor in the state (TR) becomes refractory is p_1; and that the probability per unit time that a refractory receptor becomes nonrefractory is p_2. It is further assumed (though this could easily be relaxed) that any collision between a transmitter molecule and a non-refractory receptor results in the formation of the active receptor–transmitter complex (TR).

Under these assumptions the expected change in the number of active receptor sites (TR) in time Δt is

$$E[\Delta N_1] = (p[N-\{N_1+N_2\}]-N_1p_1)\Delta t, \tag{7}$$

whereas the expected change in the number of refractory sites is

$$E[\Delta N_2] = (p_1N_1-p_2N_2)\Delta t. \tag{8}$$

In the limit as $\Delta t \rightarrow 0$ we obtain differential equations for the expected number of active and refractory sites:

$$\frac{dN_1}{dt} = p[N-\{N_1+N_2\}]-N_1p_1 \tag{9A}$$

$$\frac{dN_2}{dt} = p_1N_1-p_2N_2. \tag{9B}$$

Given initial conditions for this second order linear system, we can easily find N_1 and N_2 as functions of time. However, the time scale for the reactions in (6) is expected to be faster than the typical time scale of spreading depression (milliseconds versus seconds) so that we can regard the system (9A) and (9B) as being in a state of equilibrium. To find the nontrivial equilibrium state, we set $dN_1/dt = dN_2/dt = 0$ and solve for N_1 to obtain

$$N_1 = \frac{pN}{p_1+p(1+p_1/p_2)}. \tag{10}$$

It seems reasonable to assume that the number of collisions of transmitter molecules with receptor sites is proportional to the concentration T of transmitter. This means that p, the probability of such a collision can be written

$$p = k_1T, \tag{11}$$

where k_1 is a constant. Hence, our expression for the conductance of post-synaptic membrane as a function of transmitter concentration is

$$g(T) = \frac{kk_1NT}{k_1T(1+p_1/p_2)+p_1}. \tag{12}$$

At zero transmitter concentration, we have $g = 0$ and as the transmitter concentration becomes very large, the value of g approaches an upper limit. The dependence of g on transmitter concentration is not unlike the dependence of reaction rate on

substrate concentration in the Michaelis–Menten chemical kinetic picture. We can, of course, simplify Eq. (12) by setting

$$c_1 = \frac{kk_1N}{k_1(1+p_1/p_2)} \tag{13}$$

$$c_2 = \frac{p_1}{k_1(1+p_1/p_2)}, \tag{14}$$

whereupon we obtain

$$g(T) = \frac{c_1T}{T+c_2}. \tag{15}$$

At $T = c_2$ the value of $g(T)$ achieves its half maximal value. A typical plot of conductance versus transmitter concentration is shown in Figure 1, where the values $c_1 = 1$ and $c_2 = 1$ have been employed.

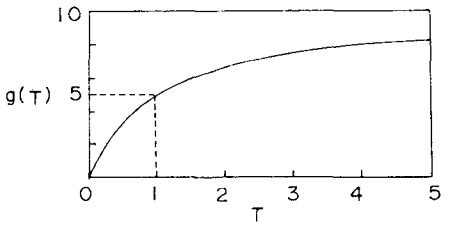

FIGURE 1 The conductance $g(T)$ in subsynaptic membrane induced by transmitter of concentration T in the synaptic cleft. The dashed line represents the half maximal value. The function $g(\cdot)$ corresponds to Eq. (15) with $c_1 = c_2 = 1$.

Pump Rate as a Function of Ion or Transmitter Concentration

Here we derive some simple expressions for the rate of active transport of either an ion or transmitter substance across the neuronal (or possibly glial) membrane. Consider a patch of membrane of unit area on which there are N pump sites. At time t we assume that N_1 sites are engaged in active transport and the remaining $N_2 = N - N_1$ sites are available. (We could incorporate a refractory period for pump sites but it will be seen that the functional form of the resultant expression is the same without this added complication). The derivation is along the same lines as that for the conductance as a function of transmitter concentration.

We assume that there is a probability p per unit time that an available site becomes busy and a probability p_1 per unit time that a busy site becomes available. The actual pump rate is taken to be proportional to the number of busy pump sites. The expected change, in time Δt, of the number of active sites is

$$E[\Delta N_1] = (pN_2 - p_1N_1)\Delta t. \tag{16}$$

In the limit as $\Delta t \to 0$ we get the following differential equation

$$\frac{dN_1}{dt} = pN_2 - p_1N_1. \tag{17}$$

Again we assume that the time scale in this equation is rapid relative to that of spreading depression and the system of pump sites quickly equilibrates. This equilibrium will occur when $dN_1/dt = 0$ which gives, on solving (17),

$$N_1 = \frac{pN}{p+p_1}. \tag{18}$$

Suppose now that the concentration of the substance being actively transported is S and that the transport rate is kN. Then the pumping rate per unit area is

$$P(S) = \frac{kk_1NS}{k_1S+p_1}, \tag{19}$$

where we have assumed that the probability of a collision between molecules of S and pump sites is k_1S. Introducing new constants we can write

$$P(S) = \frac{c_1S}{S+c_2}, \tag{20}$$

which has the same form as the dependence of conductance on transmitter concentration.

An Exchange Pump

For an exchange pump (for example, that picks up sodium ions on the inside of cells, deposits sodium ions on the outside, picks up potassium ions on the outside and deposits them on the inside, etc.) we suppose the situation is as follows. Suppose the two substances being transported are S_1 and S_2 which symbols we will also employ for their concentrations. Again we suppose that in a unit area of membrane, there are a total number N of pump sites. There are now four states that a pump

site can be in. These are "busy transporting S_1," "available for transporting S_1," "busy transporting S_2" and "available for transporting S_2." We let the numbers of sites in these states at time t be N_1, N_3, N_2 and N_4 respectively.

A site which is busy transporting an S_1 molecule can in the next short time interval either remain in that state or become available for transporting an S_2 molecule. A site which is available for transporting an S_1 molecule can in the next short time interval either remain in that state or make a transition to being busy transporting an S_1 molecule. Similar remarks apply with S_1 replaced by S_2 and vice versa.

We now invoke a set of transition probabilities per unit time for transitions amongst the various states. Let p_1 be the probability that site available for transporting S_1 becomes busy transporting S_1; let p_2 be the probability that a site available for transporting S_2 becomes busy transporting S_2; let p_3 be the probability that a site busy transporting S_1 becomes available for transporting S_2; and let p_4 be the probability that a site busy transporting S_2 becomes available for transporting S_1.

Taking into account the expected changes in a small time interval Δt we obtain the following differential equations:

$$\frac{dN_1}{dt} = p_1N_3 - p_3N_1 \tag{21A}$$

$$\frac{dN_2}{dt} = p_2N_4 - p_4N_2 \tag{21B}$$

$$\frac{dN_3}{dt} = p_4N_2 - p_1N_3 \tag{21C}$$

$$\frac{dN_4}{dt} = p_3N_1 - p_2N_4. \tag{21D}$$

Again we seek the equilibrium point of the system. Utilizing the relation $N_4 = N - N_1 - N_2 - N_3$ and setting these derivatives equal to zero gives

$$N_1 = \frac{p_1p_2N}{p_1p_2(1+p_3/p_4)+p_3(p_1+p_2)}, \tag{22}$$

and

$$N_2 = \frac{p_1p_2N}{p_1p_2(1+p_4/p_3)+p_4(p_1+p_2)}. \tag{23}$$

We now assume that p_1 is proportional to the concentration of S_1 and that p_2 is proportional to the concentration of S_2. Defining constants c_1, c_2 and c_3 we find that the pump rate for S_1 is

$$P_1(S_1, S_2) = \frac{c_1S_1S_2}{S_1S_2 + c_2S_1 + c_3S_2}, \tag{24}$$

with a similar expression for the pump rate of S_2. Note that the pump rate is zero when either S_1 or S_2 are zero. Furthermore, at a fixed S_2 as S_1 gets large the pump rate saturates at a particular value which is maximal for $S_2 = \infty$. We have derived this expression because it has been pointed out that the pump for Na^+ and K^+ is an exchange pump. However, it is not known what the nature is of the active transport mechanisms of the other ions and transmitter substances, though models of Na-dependent active transport of glutamate and GABA have been devised (Wheeler & Hollingworth, 1978, 1979). We will employ the exchange pump expression for Na^+ and K^+ and the simpler versions as in Eq. (20) for the remaining ions and transmitter substances.

EQUATIONS EMPLOYED IN THE NUMERICAL CALCULATIONS

Since we are focusing on the concentrations of the four ions and two transmitter substances in three separate compartments, the model Eqs. (5A)–(5C) constitute a set of eighteen partial differential equations. The only recourse is to solve such complicated systems by numerical methods, so it is very desirable in order to reduce the computational task, to reduce the number of equations but still retain the most important features of the model.

We make the following simplifications:

i) There is only one membrane potential V_M, given by the Goldman–Hodgkin–Katz formula (2), evaluated using the extracellular and postsynaptic intracellular concentrations of K^+, Na^+ and Cl^-.

ii) The only fluxes of the ions K^+, Na^+ and Cl^- taken into account are through postsynaptic membrane, whereas the only fluxes of Ca^{++}, T_E and T_I taken into account are through presynaptic membrane.

iii) There is approximately a local conservation of the ion concentrations. For example,

$$K^{i,1}(x,t) = K_R^{i,1} - \alpha_1(K^0(x,t) - K_R^0), \tag{25}$$

where the subscript R denotes resting value. This equation states that the value of the internal postsynaptic concentration of K^+ is its initial value minus the amount by which the extracellular concentration has increased, which allowance for the fact that the ratio of the extracellular to postsynaptic intracellular volumes is α_1.

To avoid excessive superscripts and subscripts we will change the notation. The symbols U, V, W, X, Y, Z will denote the extracellular concentrations of the ions K^+, Ca^{++}, Na^+, Cl^- and the transmitters T_E, T_I respectively. The corresponding internal concentrations alluded to above will be denoted U_i, V_i, W_i, X_i, Y_i, Z_i and the resting values of these twelve concentrations will be designated by the superscript R. The collection of the set of concentrations will be designated by the old symbol $\mathbf{C}$. The model equations now become

$$\frac{\partial U}{\partial t} = D_1 \frac{\partial^2 U}{\partial x^2} + F_1(\mathbf{C}) \tag{26A}$$

$$\frac{\partial V}{\partial t} = D_2 \frac{\partial^2 V}{\partial x^2} + F_2(\mathbf{C}) \tag{26B}$$

$$\frac{\partial W}{\partial t} = D_3 \frac{\partial^2 W}{\partial x^2} + F_3(\mathbf{C}) \tag{26C}$$

$$\frac{\partial X}{\partial t} = D_4 \frac{\partial^2 X}{\partial x^2} + F_4(\mathbf{C}) \tag{26D}$$

$$\frac{\partial Y}{\partial t} = D_5 \frac{\partial^2 Y}{\partial x^2} + F_5(\mathbf{C}) \tag{26E}$$

$$\frac{\partial Z}{\partial t} = D_6 \frac{\partial^2 Z}{\partial x^2} + F_6(\mathbf{C}) \tag{26F}$$

The reaction terms are as follows:

$$F_1(\mathbf{C}) = k_1(V_M - V_K)\left[\frac{YH(Y)}{Y+k_2} + \frac{k_3 ZH(Z)}{Z+k_4}\right] - P_K(U,W) + k_5 + k_6 H(V_M - V_M^R)(V_M - V_M^R)(V_M - V_K), \tag{27A}$$

$$F_2(\mathbf{C}) = k_7(V_M - V_{Ca})\, g_{Ca}(U, W, X) + P_{Ca}(V) - k_8 \tag{27B}$$

$$F_3(\mathbf{C}) = k_9(V_M - V_{Na})\left[\frac{YH(Y)}{Y+k_2} + \frac{k_{10} ZH(Z)}{Z+k_4}\right] + P_{Na}(U,W) - k_{11} \tag{27C}$$

$$F_4(\mathbf{C}) = k_{12}(V_M - V_{Cl})\left[\frac{ZH(Z)}{Z+k_4} + \frac{k_{13} YH(Y)}{Y+k_2}\right] + P_{Cl}(X) - k_{14} \tag{27D}$$

$$F_5(\mathbf{C}) = k_{15}(V_M - V_{Ca})\, g_{Ca}(U, W, X) - P_E(Y) \tag{27E}$$

$$F_6(\mathbf{C}) = k_{16}(V_M - V_{Ca})\, g_{Ca}(U, W, X) - P_I(Z). \tag{27F}$$

In these equations $H(\cdot)$ is the Heaviside unit step function defined by

$$H(x) = \begin{cases} 1, & x > 0, \\ 0, & x \leqslant 0. \end{cases} \tag{28}$$

It is incorporated in the postsynaptic conductance expressions (and the pump expressions below) because negative transmitter concentrations do not make sense. In the numerical calculations it is necessary to take this into account, because in generating numerical solutions, when a variable gets very close to zero the finite differencing may at some subsequent time step make it go slightly negative, whereas in the exact model or in reality this could not occur.

The local conservation conditions lead to the following relations:

$$U_i = U_i^R - \alpha_1(U - U^R) \tag{29A}$$

$$V_i = V_i^R + \alpha_2(V^R - V) \tag{29B}$$

$$W_i = W_i^R - \alpha_1(W - W^R) \tag{29C}$$

$$X_i = X_i^R - \alpha_1(X - X^R). \tag{29D}$$

The membrane and ion equilibrium potentials become:

$$V_M = \frac{RT}{F} \ln\left[\frac{U + p_{Na} W + p_{Cl} X_i}{V_i + p_{Na} W_i + p_{Cl} X}\right], \tag{30A}$$

$$V_K = \frac{RT}{F} \ln\left[\frac{U}{U_i}\right], \tag{30B}$$

$$V_{Ca} = \frac{RT}{2F} \ln\left[\frac{V}{V_i}\right], \tag{30C}$$

$$V_{Na} = \frac{RT}{F} \ln\left[\frac{W}{W_i}\right], \tag{30D}$$

$$V_{Cl} = \frac{RT}{F} \ln\left[\frac{X_i}{X}\right]. \tag{30E}$$

The pump rate expressions in (27A)–(27F) are, on utilizing the exchange pump expression for Na^+ and K^+:

$$P_K(U,W) = \frac{k_{17}\, UW_iH(U)H(W_i)}{UW_i + k_{18}U + k_{19}\, W_i}, \tag{31A}$$

$$P_{Ca}(V) = \frac{k_{20}V_iH(V_i)}{V_i + k_{21}}, \tag{31B}$$

$$P_{Na}(U,W) = \frac{k_{22}\, UW_iH(U)H(W_i)}{UW_i + k_{23}\, U + k_{24}\, W_i}, \tag{31C}$$

$$P_{Cl}(X) = \frac{k_{25}\, X_iH(X_i)}{X_i + k_{26}}, \tag{31D}$$

$$P_E(Y) = \frac{k_{27}\, Y\, H(Y)}{Y + k_{28}}, \tag{31E}$$

$$P_I(Z) = \frac{k_{29}\, Z\, H(Z)}{Z + k_{30}}. \tag{31F}$$

Note that there are additional constants k_1, k_8, k_{11}, k_{14}, in the "pump rates" for the four ions because the pump rates vanish at zero concentrations, whereas in practice the source terms must become zero at resting levels.

To complete the description of the reaction terms, we need only one more quantity which is the dependence of presynaptic membrane conductance on membrane potential. This is given approximately by the following expression for depolarizations encountered during SD:

$$g_{Ca}(U, W, X) = [1 + \tanh\{k_{31}(V_M + V_M^*)\} - k_{32}] \times H(V_M - V_M^T),$$

where k_{32} is the value of $1 + \tanh[k_{31}(V_M + V_M^*)]$ at the membrane potential $V_M = V_M^T$. Thus, the presynaptic calcium conductance is zero for potentials below V_M^T and thereupon rises smoothly to approach an upper limit. This is, in fact, the empirically obtained qualitative behavior of this quantity (see Eccles (1969) and Llinas, Steinberg & Walton (1976) and the functional form given by (32) can provide a reasonable quantitative description as well. Note that in the second of these last two mentioned references a more involved expression for g_{Ca} is derived, but for computational purposes we will use the comparatively simple expression (32). The functional form of g_{Ca} is shown in Figure 2 where g_{Ca} is plotted against V_M, the membrane potential.

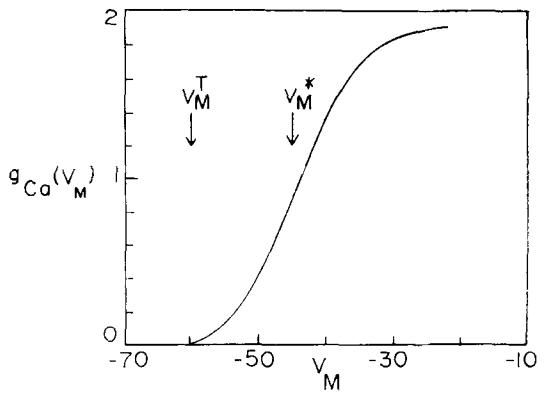

FIGURE 2 The dependence of presynaptic terminal calcium conductance g_{Ca} on membrane potential V_M according to Eq. (32). The resting membrane potential is $V_M{}^R$ and the conductance is zero at values of V_M less than $V_M{}^T$.

It will be noted that in addition to the transmitter induced potassium conductance term in Eq. (27A) there is an additional flux with coefficient k_1. This represents the "passive" flux of K^+ through nonsynaptic membrane as occurs in the Hodgkin–Huxley equations. The dependence of this conductance on membrane potential has been assumed to be a linear function of the difference between the membrane potential and the resting membrane potential $V_M{}^R$. This is expected to be a reasonable assumption for small depolarizations as will occur during spreading depression in TTX treated cortex. Furthermore, the role of this extra term is not vital; it is inserted in the equations for physiological completeness. There is no such term for the Na^+ flux (because TTX is supposed to block the corresponding ion channels) and it has been ignored for Cl^- due to lack of information.

It is worth mentioning that in the above Eqs. (27), the transmitter induced conductance changes in postsynaptic membrane for each of the ions K^+, Na^+ and Cl^- has been attributed to both the extracellular excitatory transmitter concentration (Y) and the inhibitory transmitter concentration (Z), with, of course, adjustable weights. It is known, for example, that the putative excitatory

transmitter glutamate increases both K^+ and Na^+ conductances in a certain ratio and that GABA causes large increases in chloride conductance (Krnjevic, 1974). However, the possibility has to be allowed for in the model equations that glutamate and GABA (for example) may both contribute to an increase in, say, K^+ conductance.

It can be seen that there is a large number of parameters and physical constants in the above set of reaction–diffusion equations, but all have well-defined meanings and are, in principle, measurable in some preparations. Firstly, there are the six diffusion coefficients, those of the four ions and of the two transmitter substances. There are the two relative permeabilities, p_{Na} and p_{Cl}, and there are eight resting ionic concentrations (4 extra- and 4 intracellular) which determine the resting equilibrium potential $V_M{}^R$. The external resting transmitter concentrations are assumed to be zero and the internal transmitter concentrations do not enter the model equations. With the functional form of the presynaptic calcium conductance as given by Eq. (32) there are three parameters: $V_M{}^T$ determines the value of the depolarization at which this conductance becomes non-negligible; k_{31} determines how rapidly the conductance rises with further depolarization and $V_M{}^*$ is approximately the value of the membrane potential at which the conductance is about half maximal. The additional parameter in (32) is k_{32} which is determined by k_{31}, $V_M{}^*$ and $V_M{}^T$. There are also the ratios α_1 and α_2 of the extracellular volume to the volumes of the post- and presynaptic compartments and the absolute temperature T. The remaining constants, $k_1, \ldots, k_{30}$ all have well-defined meanings as follows. The quantities k_2 and k_4 are the values of the extracellular excitatory and inhibitory transmitter concentrations at which the postsynaptic conductances due to attachment of transmitter molecules to receptors achieve their half-maximal values. The parameters k_{21}, k_{26}, k_{28} and k_{30} are the values of the intracellular calcium concentration, the intracellular chloride ion concentration, the extracellular excitatory transmitter concentration and the extracellular inhibitory transmitter concentration at which the corresponding pump rates (and possibly other clearance mechanisms) achieve their half-maximal values.

The exchange pump parameters are a little more involved. To see the meaning of k_{19}, for example, divide expression (31A) by UW_i and then take the limit as W_i (the internal Na^+ concentration) goes to infinity. It can then be seen that k_{19} is the value of the external K^+ concentration at which the potassium pumping rate achieves its half-maximal value at the theoretical upper limit of (infinite) internal Na^+ concentration. Similarly, k_{18} is the value of the internal Na^+ concentration at which the potassium pump rate achieves its half-maximal value at the theoretical upper limit of (infinite) external K^+ concentration. The parameters k_{23} and k_{24} have definitions as for k_{18} and k_{19} except with reference to the rate of pumping of sodium ions.

Consider each of the four pump rate expressions P_K, P_{Ca}, P_{Na} and P_{Cl}. These expressions achieve the value zero when the ions being pumped reach zero (internal or external as the case may be) concentrations. Since the corresponding resting ionic concentrations are not zero, we have to insert constant terms k_5, k_8, k_{11}, and k_{14} which give rise to fluxes in the opposite direction to the pumps to maintain the resting conditions in the absence of other source and sink terms. Thus the values of these constants represent the strength of the "leakage" currents of the ions which are counterbalanced by the pumps. Such constants do not enter the equations for the transmitter concentrations because it is assumed that in resting conditions the transmitter is sequestered in presynaptic terminals. This assumption could, of course, be easily relaxed. Note that the four constants k_5, k_8, k_{11} and k_{14} are determined completely by the specified resting ionic concentrations and the pump strength parameters.

The constants k_{17}, k_{20}, k_{22}, k_{25}, k_{27} and k_{29} have the following definitions. We will assume that the active transport processes do, in fact, occur at definite "sites." Then k_{17} must be the number of (K^+–Na^+ exchange) pump sites per unit volume of nervous tissue multiplied by the maximum pumping capacity of a single site in terms of the amount of decrease in external K^+ concentration effected by that site per unit time. The other five pump strength parameters have the same meaning for the other five substances being actively transported.

The constants k_1, k_3, k_9, k_{10}, k_{12} and k_{13} reflect the conductance changes at the various synapses. For potassium we have that k_1 is the number of excitatory synapses per unit volume multiplied by the K^+ conductance of a single such synapse when it is in an equilibrium situation with a very large (theoretically infinite) excitatory transmitter concentration in the extracellular compartment. This

definition can be refined to allow for the part that different synapses may have different numbers of receptors, in which case, it would be better to define k_1 as the total area of excitatory subsynaptic membrane per unit volume of cortical tissue multiplied by the K^+ conductance per unit area of the postsynaptic membrane when in the presence of a very large concentration of excitatory transmitter substance. Furthermore, k_1 will be roughly the number of excitatory receptors per unit volume multiplied by the conductance for K^+ induced by the attachment of transmitter to the receptor which takes it from state R to (TR) in accordance with the scheme of Eq. (6). This assumes that the time spent on the state R' is negligible. Taking only the first definition into account, we see that $k_1 k_3$ is the number of inhibitory synapses per unit volume multiplied by the K^+ conductance induced at a single inhibitory synapse when it is in an equilibrium situation with a very large external concentration of inhibitory transmitter substance. Also, k_9 and k_{10} play the corresponding roles for Na^+ that k_1 and k_3 do for K^+. For Cl^- k_{12} plays the role that $k_1 k_3$ did for K^+ and $k_{12} k_{13}$ plays the role that k_1 did for K^+. The reason for this is that emphasis for chloride fluxes switches to those induced by inhibitory rather than excitatory transmitter.

To define k_7 we have to note that $k_7 g_{Ca}(V_M)$ is the calcium conductance of all the presynaptic membrane in a unit volume of cortex when that membrane is at the potential V_M. We may decompose this further as the number of synapses per unit volume multiplied by the conductance per synapse or as the total presynaptic area per unit volume multiplied by the conductance per unit area.

The quantity $k_6(V_M - V_M{}^R)$ is the K^+ conductance at membrane potential V_M per unit area of cell membrane, not connected with synaptic membrane or active transport, multiplied by the total area of such membrane per unit volume of cortical tissue. Recall that the dependence of this conductance on membrane potential is here being approximated by a linear function of the depolarization from resting potential $V_M{}^R$. According to the equilibrium ($t = \infty$) data of Hodgkin & Huxley (1952), this is not quite true because $g_K{}^\infty$ is small but finite at zero depolarization, the passive leak of K^+ in such a state being presumably countered by the active transport processes. This leak has in the above model equations been taken care of by ensuring that the pump rate is countered at resting conditions by the insertion of the extra constant k_1.

The remaining two parameters are k_{15} and k_{16}. These contain the rates of efflux of excitatory and inhibitory transmitter per unit area of presynaptic membrane per unit inward flux of calcium ions multiplied by the total area of excitatory and inhibitory presynaptic membrane per unit volume of the cortical tissue.

RESULTS

The system of Eqs. (26A)–(26F), with the reaction terms as defined in Eqs. (27A)–(27F), is integrated numerically for given initial and boundary conditions. The method of integration is due to Lees (1969), and is an adaptation of the Crank–Nicolson procedure. To illustrate the details, consider the scalar reaction–diffusion equation

$$\frac{\partial U}{\partial t} = D\frac{\partial^2 U}{\partial x^2} + F(U). \tag{33}$$

One sets, in the finite-difference approximation, $U_m^n = U(m\Delta x, n\Delta t)$, and replaces the differential equation by the scheme

$$\begin{aligned}\frac{1}{\Delta t}\left(U_m^{n+1} - U_m^n\right) = {} & \frac{D}{2(\Delta x)^2} \\ & \times\left[\left(U_{m+1}^{n+1} - 2U_m^{n+1} + U_{m-1}^{n+1}\right)\right. \\ & \left.+\left(U_{m+1}^{n} - 2U_m^{n} + U_{m-1}^{n}\right)\right] \\ & + F\left(\frac{3}{2}U_m^n - \frac{1}{2}U_m^{n-1}\right).\end{aligned} \tag{34}$$

This scheme is stable and involves the solution of a tridiagonal system of equations at each time step.

The model equations described above have been obtained based on several generally accepted principles of the factors governing fluxes of ions and transmitter substances in several experimental studies. The reaction terms are considered to contain the essential factors which will be operative when action potentials are blocked by the application of TTX. To establish that the system of equations could provide a plausible basis for variations in ionic and transmitter concentrations such as are encountered in SD it is necessary to show that when the conditions which are known to

lead to waves of SD in cortical structures are applied as initial conditions for the equations, the qualitatively correct behavior of the various concentrations is predicted.

With the very large number of parameters in the model equations, many of which, as is clear from their physiological and anatomical meanings as outlined in the last section, are unknown, it may seem a formidable task to ascertain whether the system does in fact support solitary wave solutions of increased extracellular K^+ and neurotransmitter concentrations and diminished extracellular Ca^{++}, Na^+ and Cl^- concentrations. To make the task less formidable, there was some preliminary knowledge obtained from experience in computing solutions of the system of equations involving only the concentrations of K^+ and Ca^{++} (Tuckwell & Miura, 1978; Tuckwell, 1979a, b, c). Experience was then gained on a system of equations involving the concentrations of K^+, Ca^{++} and an excitatory transmitter substance; then on a system with the four ions K^+, Ca^{++}, Na^+, Cl^- and an excitatory transmitter substance. As a final preliminary step a system was studied in which the movements of K^+, Ca^{++}, Na^+ and Cl^- together with an excitatory and an inhibitory transmitter substance were taken into account, but in which there was no exchange pump for Na^+ and K^+. The results to be presented here are for the system of equations described in the last section where the four ions, two transmitter substances and the exchange Na^+–K^+ pump are taken into account. The parameter values used in the results to be presented here are as outlined in the Appendix.

Response to Potassium Chloride Application

In sufficient concentrations, potassium chloride is known to instigate propagating waves of SD. Recalling that the symbols U and X indicate the external concentrations of K^+ and Cl^-, the system of model equations were integrated numerically on the space interval $0 \leqslant x \leqslant 1$ with the initial conditions

$$U(x,0) = U^R + U_A \exp\left[-\left(\frac{x-0.3}{0.05}\right)^2\right], \quad (35A)$$

$$V(x,0) = V^R, \quad (35B)$$

$$W(x,0) = W^R, \quad (35C)$$

$$X(x,0) = X^R + X_A \exp\left[-\left(\frac{x-0.3}{0.05}\right)^2\right], \quad (35D)$$

$$Y(x,0) = Y^R, \quad (35E)$$

$$Z(x,0) = Z^R, \quad (35F)$$

the resting external concentrations being 3 mM, 1 mM, 120 mM, 136 mM for K^+, Ca^{++}, Na^+ and Cl^- respectively, and zero for the excitatory and inhibitory transmitter substances. The boundary conditions at $x = 0$ and $x = 1$ were that each of the external concentrations took their resting values. In these numerical calculations the diffusion coefficients were scaled up by a factor of 100 to ensure that the wave phenomena were amply accommodated on the unit space interval $0 \leqslant x \leqslant 1$. Based on the available figures for the diffusion coefficients, these scaled values were 2.4×10^{-3}, 1.0×10^{-3}, 1.7×10^{-3} and 2.5×10^{-3}, for the ions K^+, Ca^{++}, Na^+ and Cl^-, respectively based on the American Institute of Physics Handbook (1963), the actual values being these figures multiplied by 10^{-2} in which case their units are cm^2/sec. For the inhibitory transmitter substance, the scaled diffusion coefficient was taken as 1.3×10^{-3} which is the value given for GABA, a putative transmitter in various structures. Since glutamate is commonly postulated to be an (or the) excitatory transmitter in many neocortical structures and the cerebellum (see recent reviews by Orrego (1979) and Fagg & Lane (1979)) it was desired to make the diffusion coefficient of the excitatory transmitter that of glutamate. A search of the literature did not reveal this diffusion coefficient: hence the diffusion coefficient of glycine was used with a scaled value of 1.3×10^{-3}, since this amino acid has a similar molecular weight to that of glutamic acid. Note that the effective diffusion coefficient of glutamic acid in brain tissue (Herz, Zieglgansberger & Farber, 1969) is not appropriate here.

Subthreshold response. The values of U_A and X_A were set at 5 mM. This is equivalent to adding 5 mM KCl at the center of the stimulus so that at $x = 0.3$ the K^+ concentration in the extracellular space has a maximum value of 8 mM whereas that of Cl^- is 141 mM. At $t = 0.5$ we find that the K^+ and Cl^- concentrations have almost returned to their resting values and the concentrations of the transmitter substances are still at their resting values.

The sodium ion concentration in the extracellular space undergoes a very small increase and the calcium ion concentration is unchanged.

Let us now consider these properties of the model in light of the experimental data of Kraig and Nicholson (1978). There the preparation was the cerebellum of the catfish *Corydoras aneus* whose temperature is in fact 25°C for which the diffusion coefficients employed in the calculations and the value of $(RT/F)\ln(\cdot)$ are appropriate. In one experiment in which KCl was applied, the extracellular K^+ concentration rose to almost about 10 mM from its resting value of about 2 mM, whereas the extracellular Ca^{++} concentration did not change at all. This was called a "nonpropagating event" which can be identified with a subthreshold response.

There are a few points worth raising in this connection. In the model system the extracellular Na^+ concentration rose by about 1 mM, whereas in the above experiment, the Na^+ concentration actually fell by about 8 mM from its resting value of about 150 mM. This apparent anomaly is readily explainable, however. In the physiological preparation considered, TTX was not applied, which means that in certain regions where the applied K^+ concentration was high enough to locally depolarize nerve cells past their thresholds for firing action potentials, some Na^+ would be transported to the (postsynaptic) intracellular compartment. The concomitant ion exchanges of K^+, Na^+ and probably Cl^- were not sufficient to overcome diffusive dissipation and removal of abnormal ion distributions by active transport and other clearance mechanisms. In the model the only Na^+ transport effected was induced by the Na^+–K^+ exchange pump which in order to remove some of the extracellular K^+ had to transport some Na^+ to outside the cells where it remained transiently before resting conditions were restored.

Consider now the fact that in this experimentably reported nonpropagating event the external Ca^{++} concentration did not drop at all even though the K^+ concentration had risen to about 10 mM (which implies a local depolarization of synaptic terminal membrane and other neuronal membrane by about 10 mV). This seems to imply, firstly, that at such depolarizations the calcium conductance of presynaptic terminals had not reached a sufficient level to cause an inward calcium current with its consequent release of transmitter substances. It also implies that if calcium spikes were occurring in the dendrites of the Purkinje cells (Llinas & Hess, 1976) that the loss of extracellular Ca^{++} due to such events was negligible in the abovementioned reported experiment.

Propagating SD wave. With the same initial conditions as in Eqs. (35A)–(35F), except now with $U_A = X_A = 17$ mM, representing a local Gaussian elevation of KCl of concentration 17 mM at its center (see Figure 3), the system of equations was again integrated numerically with the same boundary conditions as before. The initial data are shown at $t = 0$ in the figure and the spatial profiles of the four extracellular ion concentrations and two transmitter concentrations are shown at $t = 2.5$.

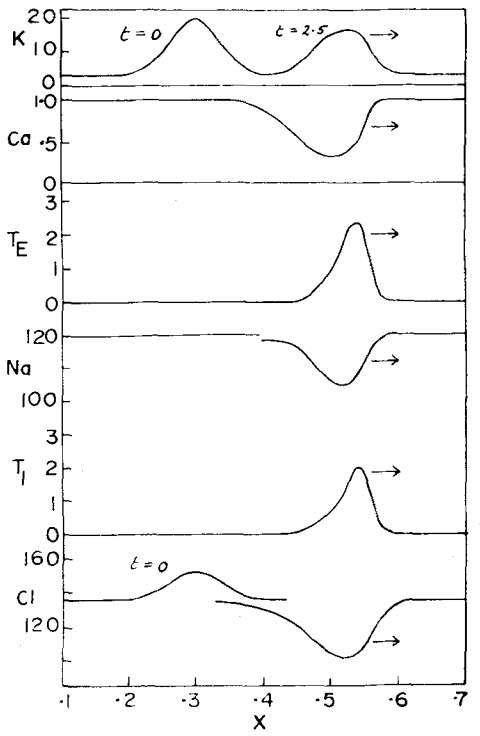

FIGURE 3 Solitary SD waves. Numerically computed solutions of the model equations in response to a suprathreshold dose of KCl (17 mM added at center of stimulus). By $t = 2.5$ well formed stable propagating SD waves have formed. The units of the ion concentrations are mM.

It can be seen that the model equations therefore do, in fact, have stable propagating SD wave solutions consisting of elevated extracellular K^+ and neurotransmitter concentrations with concomitant depressions of extracellular Ca^{++}, Na^+ and Cl^- concentrations. In these computed solutions the K^+ concentration rises from 3 mM

to 17 mM at the peak of the K^+-wave, the Ca^{++} concentration drops from 1 mM to 0.3 mM, the Na^+ concentration falls from 120 mM to 105 mM and the Cl^- concentration drops from 135 mM to about 100 mM. The excitatory transmitter concentration rises from its assumed normal resting extracellular level of zero to about 2.4 mM, while that of the inhibitory transmitter rises from zero to about 2 mM. Each of the four extracellular ion concentrations and two transmitter concentrations then return to their resting values.

There are several points to be raised here in anticipation of the possible objections to the facts that the model solutions do not replicate with precision any known set of experimental data on the variations of the extracellular ionic and transmitter concentrations during SD. Firstly, the only set of data on extracellular concentrations during SD in TTX treated cortex to date is for cat neocortex in which the K^+ concentration only was measured (Sugaya *et al.*, 1975). In this regard the agreement between computed variation in K^+ and the experimental result is reasonable. In the non-TTX experiments the decreases in Na^+ and Cl^- concentrations have been reported to be about equal (Kraig & Nicholson, 1978), whereas in the computed solutions they differ by about 15 mM. This is considered not to be a serious shortcoming of the model however because: (a) the model results are for TTX treated cortex where experimental data are not yet known and (b) with different choices of the relevant parameters in the model equations the decreases in Na^+ and Cl^- concentrations could be made closer to each other. The main point is that it is fairly certain, by changing the overall strengths of the various source and sink terms for the various ion and transmitter concentrations, with appropriate changes in the parameters that control the functional form of the pump rates and conductances, that waves of various amplitudes and shapes can be obtained. It is clear that with so many such parameters to vary a systematic appraisal of the effects of varying each one is not feasible as it was with the simpler model involving only K^+ and Ca^{++} (Tuckwell, 1979a).

Response to Other Chemical Stimuli

Having established that SD waves could be elicited by the application of a sufficient amount of potassium chloride in the model system, the known responses to the applications of other chemicals in experimental situations had to be tested in the model system to lend support or otherwise to the general validity and usefulness of the model.

Glutamate. Recall that the model system involves the concentration of an excitatory transmitter substance which we will assume is glutamic acid. It has been established that glutamate in quite small concentrations (of the order of a few mM) in some preparations can elicit spreading depression (Van Harreveld, 1959; Ochs, 1962; Van Harrevld & Fifkova, 1970; Fifkova & Van Harreveld, 1974; Van Harreveld, 1977).

In order to establish that the above model equations are capable of predicting the phenomena associated with the application of glutamate, the initial data was chosen such that the four ionic concentrations U, V, W, X (K^+, Ca^{++}, Na^+, Cl^-) and inhibitory transmitter concentration in the extracellular compartment were at their resting levels, but the external glutamate concentration was set at

$$Y(x,0) = Y^R + Y_A \exp\left[-\left(\frac{x-0.3}{0.05}\right)^2\right], \qquad (36)$$

representing a local Gaussian elevation of glutamate centered at $x = 0.3$ and of maximum value Y_A (since $Y^R = 0$).

With $Y_A = 0.5$ mM a slight transient increase in the concentrations of K^+ and Na^+ occurred but insufficient depolarization of nerve terminals occurred in order for the significant entry of Ca^{++} with its concomitant release of transmitters. This, then, corresponded to a subthreshold application of glutamate as resting conditions were soon restored, the excess glutamate being removed from the extracellular space by the clearance mechanisms.

When Y_A was set at 5 mM or higher, solitary SD waves emerged from the stimulus with the same amplitudes and shapes as had occurred with suprathreshold applications of KCl. The system thus predicted threshold phenomena for both glutamate or KCl application with solitary SD waves whose form was (eventually) independent of the method of elicitation in the case of a suprathreshold application of either KCl or glutamate. The threshold glutamate level is much lower than that for K^+.

In connection with the question as to which chemical is "primary" in SD, it seemed important to establish the time sequence events occurring

around the stimulus when SD is elicited either by KCl or glutamate application. The details are shown in Figures 4 and 5 for the applications of suprathreshold applications of KCl and glutamate respectively. Only the extracellular concentrations of K^+ and glutamate are shown in these figures.

In Figure 4 at $t = 0$ the K^+ level is elevated locally around the center of the applied stimulus whereas that of glutamate is everywhere at its resting level. By $t = 0.5$ the level of external K^+ has risen everywhere so that the profile does not yet have the pronounced peaks associated with the eventual solitary waves. The glutamate level has risen around the stimulus and well formed peaks have appeared. By $t = 1.0$ the glutamate solitary waves have almost completely formed whereas the tails of the K^+ waves are nowhere near completely formed. The wavefront of the K^+ wave, however, is slightly ahead of that of the glutamate wave.

In the case of the application of a suprathreshold amount of glutamate (Figure 5), at $t = 0$ the K^+ is at resting level everywhere and the glutamate concentration is peaked at the center of the stimulus. The potassium level has risen considerably by $t = 0.5$ in the vicinity of the stimulus and its profile is very similar to that at the same time after KCl application. At the same time the glutamate level has dropped at the center of the stimulus and peaks have started to form just as with KCl application. By $t = 1.0$ the profiles of both K^+ and glutamate are also very similar to those in Figure 4, the wavefront of the K^+ wave again being ahead of that of the glutamate wave. In both cases the formation of the glutamate wave precedes the formation of the K^+ wave, in the sense that the tail of the glutamate wave forms first. It is likely, however, that with different strength for the clearance mechanisms, the order of appearance of the "complete" solitary waves could be reversed.

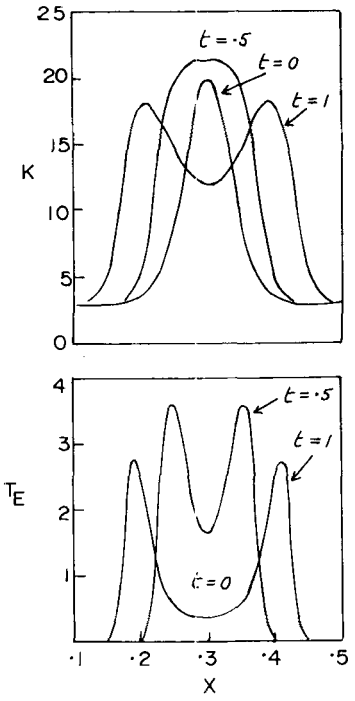

FIGURE 4 The time course of the development of the K^+ and glutamate waves when the initial stimulus is KCl.

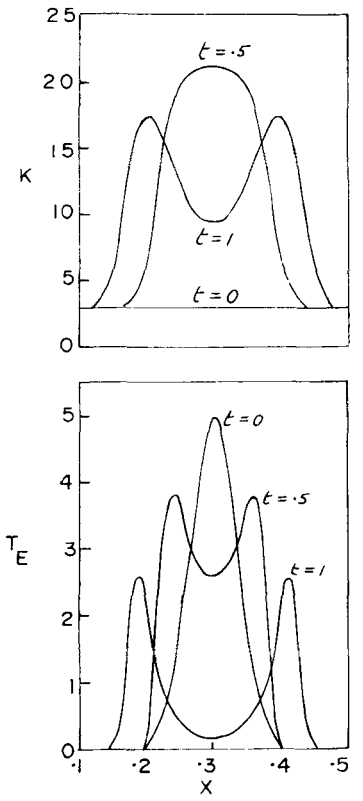

FIGURE 5 Time course of development of the K^+ and glutamate waves when SD is instigated by glutamate.

Sodium chloride. It is known experimentally that whereas potassium chloride in sufficient concentrations may elicit SD waves, the application of sodium chloride does not (Bures *et al.*, 1974). To

test the model response to NaCl application the initial values of the external concentrations of K^+ and Ca^{++} and the excitatory and inhibitory transmitter concentrations were set at their resting levels for $0 \leqslant x \leqslant 1$ whereas the Na^+ and Cl^- concentrations were raised locally in accordance with the relations,

$$W(x,0) = W^R + W_A \exp\left[-\left(\frac{x-0.3}{0.05}\right)^2\right] \quad (37A)$$

$$X(x,0) = X^R + X_A \exp\left[-\left(\frac{x-0.3}{0.05}\right)^2\right] \quad (37B)$$

Only one value for both W_A and X_A was employed and that was 17 mM as this additional concentration of KCl had elicited SD in the model system. With this amount of added NaCl no SD wave appeared. There was a small subsequent but transient local elevation of K^+ around the stimulus. The excess concentration of Na^+ above resting level around the stimulus dropped exceedingly slowly because of the exchange nature of the $Na^+ - K^+$ pump (i.e., if the K^+ level is at or near resting level then Na^+ is not actively transported to within the cells). Because in the model system the Cl^- pump was not coupled to the transport of other substances, the external Cl^- concentration quickly returned to resting levels.

GABA. It is assumed that the inhibitory transmitter is GABA in the following discussion. With all ion concentrations (K^+, Ca^{++}, Na^+, Cl^-) and the excitatory transmitter concentration initially at their resting levels for $0 \leqslant x \leqslant 1$, a local application of GABA was applied in the model system according to

$$Z(x,0) = Z^R + Z_A \exp\left[-\left(\frac{x-0.3}{0.05}\right)^2\right] \quad (38)$$

Two values of Z_A were used; 5 mM and 17 mM. In neither case did solitary waves of SD form, whereas the same added concentrations of glutamate had both elicited SD. A slight *decrease* in external chloride around the stimulus occurred but this was only transient and resting concentrations of all substances soon appeared. The fact that GABA does not elicit SD and in fact has a blocking influence on SD (Van Harreveld, 1959; Bures, Buresova & Kvivarek, 1960) is thus also predicted by the model equations.

Role of K^+ and Glutamate Diffusion

It has been much discussed whether K^+ or glutamate is the "primary" chemical in SD (Grafstein, 1963; Do Carmo & Leao, 1972; Bures *et al.*, 1974; Van Harreveld, 1978). In order possibly to aid in resolving this matter it was decided to ascertain in the model system whether SD waves could form and propagate in the absence of diffusion of either K^+ or glutamate.

The initial conditions used in these runs were resting levels of Na^+, Ca^{++} and the two transmitter substances and a Gaussian elevation of KCl as in (33A)–(33F), with $U_A = X_A = 17$ mM. Recall that this stumilus had, with the appropriate values for the diffusion coefficients of K^+ and glutamate, given rise to stable propagating SD waves. When the diffusion coefficient of glutamate was set at zero and that of K^+ was its actual value, a wave began to form in the vicinity of the center of the stimulus but it did not form properly and did not propagate. In fact, resting conditions soon prevailed uniformly in space. When the diffusion coefficient of K^+ was set at zero and that of glutamate set at its actual value, an SD wave formed but was not stable. Another "SD wave" began to form in the tail of the first SD wave whose tail was thenceforth ill-formed. Thus, in the model system stable solitary SD waves do not form in the absence of either K^+ or glutamate diffusion. It must be pointed out that of course in reality neither the diffusion coefficient of K^+ nor glutamate is zero. The idea of setting the diffusion coefficients zero was to test the truth of such statements as "stable solitary SD waves can form without K^+ diffusion but not without glutamate diffusion." Had this statement, for example, been rendered true by the model results, then one may have been tempted to call glutamate the "primary" chemical because diffusion is (ignoring electrotonic spread of depolarization) presumably the triggering event.

Velocity and Phase Portraits

Recall that the computer generated solutions are with scaled distance and time variables which we shall designate x' and t' with $x' = ax$ and $t' = bt$, there x and t are in cm and sec respectively. The velocity of the waves in the model system was determined by averaging the results for the peak K^+ level, and the values $K^+ = 17$ mM on the wavefront and the waveback. This gave a final result of 0.0848 units of x' in a unit of t'. To fix

the time scale we use the time from resting K^+ level to peak in a closely related set of experimental data on TTX treated cat cortex (Sugaya *et al.*, 1975). The time from rest to peak in the model takes 1.136 units of t' which therefore corresponds to about 30 sec. This determines the scale factor b. If the original equation was the scalar equation (33), then in the new space and time variables we have

$$\frac{\partial U}{\partial t'} = \frac{Da^2}{b}\frac{\partial^2 U}{\partial x'^2} + \frac{F}{b}, \tag{39}$$

and since $D = 2.4\times10^{-5}$ cm^2/sec for K^+ and we employed $Da^2/b = 2.4\times10^{-3}$ in the numerical calculation, the value of a is determined. It then turns out that the unit interval $0 \leqslant x' \leqslant 1$ on which the numerical calculations were performed corresponds to about 5.2 mm of distance in the actual brain structure. The velocity of the waves in the brain structure is then about 0.99 mm/min, or, if we divide by $\pi/2$ to allow roughly for the tortuosity of paths around cells as was done previously (Tuckwell & Miura, 1978; Tuckwell, 1979a) we obtain the value 0.63 mm/min. This velocity is in the range of experimental ones (Kraig & Nicholson, 1978) and is in accordance with the relationship between wave speed and the amplitude of the K^+-wave (Tuckwell, 1979a).

In the previously studied model system in which only the movements of K^+ and Ca^{++} were explicitly taken into account, it was helpful in understanding the behavior of the solutions to plot the solitary waveorbits in the "phase plane." It was found that there were three "critical" points including the rest state which were important in determining the nature of the orbits (Tuckwell, 1979a). In the presently described model we began with 18 components in the reaction–diffusion system and have been able to reduce the number of components to six consisting of the four ion concentrations and two transmitter concentrations in the extracellular compartment. It is clear that the wave orbits cannot be plotted in this six-dimensional space but we can characterize the wave orbits as shown in Figure 6.

In Figure 6A, for example, the pairs of (K^0, Ca^0) values which arise in the six component solitary sketched in Figure 4, are plotted against each other. The orbit commences at the rest point (K^0 = 3 mM, Ca^0 = 1 mM) and does a single closed loop in the (K^0, Ca^0) plane to return to the rest point. The take-off from the rest point is along the

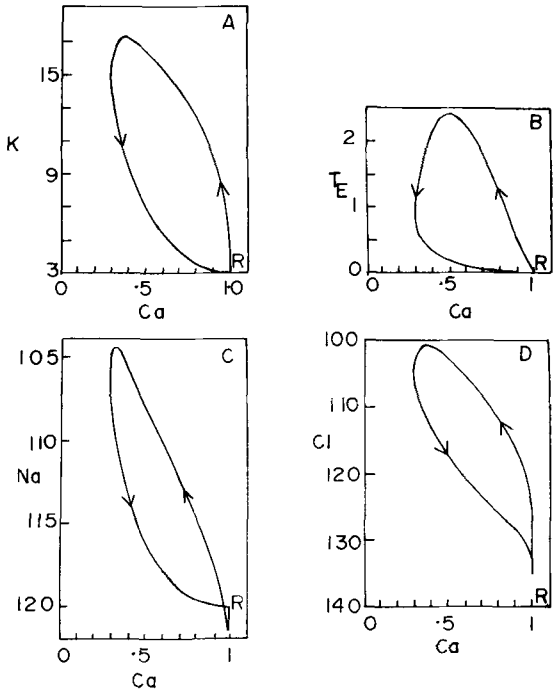

FIGURE 6 Phase portraits of the solitary SD waves shown in Figure 4. Each orbit begins and ends at R, the rest point, the arrows indicating the direction of increasing time. Note that the axes for Na^+ and Cl^- are positive downwards. The concentrations of K^+, Na^+, Cl^- and glutamate (T_E) are plotted against that of Ca^{++}. The portrait for T_I is omitted as it is very similar to that for T_E.

Ca^0 = 1 mM line, indicating that the K^0 level is rising ahead of the drop in the Ca^0 level. In contradistinction to the previously ovtained phase portraits the return to the rest point is not so much along the line K^0 = 3 mM which probably reflects the different functional forms of the source and sink terms employed here.

It was decided to characterize the solitary wave by plotting each component against Ca^0. Figure 6B shows the (T_E, Ca^0) pairs that occur on the solitary wave orbit. Here the take off from rest is not along $Ca_R{}^0$ as the glutamate (T_E) and Ca^{++} movements commence at about the same time at a given space point. This is reasonable because firstly the diffusion coefficients of T_E and Ca^{++} are quite similar in the model calculations, and secondly because it is the depletion of external Ca^{++} that leads to the build up of transmitter in the extracellular space. Thus diffusion of K^+ precedes the movements of Ca^{++} and glutamate.

The (T_E, Ca^0) solitary wave orbit has quite a different shape than the (K^0, Ca^0) wave orbit. The approach to the rest point is along the line $T_E = 0$, indicating that the excitatory transmitter concentration returns to resting level ahead of Ca^{++}. The orbit for (T_I, Ca^0) is quite similar to that of (T_E, Ca^0) and hence has been omitted from the figures.

The orbit obtained with (Na^0, Ca^0) pairs on the solitary wave has some novel features. The Na^0 level begins to rise a little before the Ca^0 begins to drop which gives the small straight line segment downward from the rest point in Figure 6C. Then the Na^+ and Ca^{++} concentrations both fall together to start the loop which eventually ends up at the rest point. The reason for the preliminary rise in Na^+ concentration is presumably the activation of the Na^+–K^+ exchange pump by the excess K^+ diffusing ahead of the wave proper. It was extremely difficult to find correct values of the parameters which led to five closed loop solitary wave orbits for (K^0, Ca^0), (T_E, Ca^0), (Na^0, Ca^0), (Cl^-, Ca^0) and (T_I, Ca^0). One of the main difficulties was the timing of the return of the K^+, Na^+ and Cl^- concentrations to resting levels. Some parameter choices would lead to five orbits which did not all end up at the rest points. It is noted that in experimental results, return to resting concentrations, for K^+ in particular, does not always occur in the course of an experiment (e.g., in Sugaya *et al.*, 1975). Hence the parameter values chosen represent the more or less "ideal" solitary wave case where all concentrations end up at resting levels.

The last phase portrait is that for (Cl^0, Ca^0) pairs on the solitary wave orbit shown in Figure 6D. The take off from rest is along $Ca^0 = 1$ showing that the chloride level drops ahead of the decline in external Ca^{++} concentration. The loop does not cross itself as the (Na^0, Ca^0) orbit does, and returns to the rest point along the line $Ca^0 = Ca_R{}^0$. That is, the chloride lags Ca^{++} in the return to rest.

DISCUSSION

The model which has been proposed herein for ion and transmitter fluxes during SD has resulted in a reaction-diffusion system with 18 components. If the glial intracellular concentrations of these substances (K^+, Ca^{++}, Na^+, Cl^-, T_E (glutamate) and T_I (GABA)) were included the system would have 24 components. This system has been reduced to one with 6 components by assuming that one could focus on the movement of Ca^{++} and the transmitters through presynaptic terminals and of K^+, Cl^- and Na^+ through postsynaptic neuronal membrane, and by assuming that the membrane potential of terminals and neighbouring postsynaptic cells were approximately equal. The glial concentrations have been omitted and the effects of action potentials have been neglected. This latter simplification fortunately does not remove all the physiological reality because SD does propagate in TTX treated cortex (Sugaya *et al.*, 1975) and in the chicken retina (Kow & Van Harreveld, 1972).

The model equations when solved with the appropriate initial data have faithfully duplicated many known properties of SD waves. These include the observed transient rise in extracellular K^+ and accompanying transient decreases in extracellular Ca^{++}, Na^+ and Cl^- as measured with ion-selective micropipettes (Vyskocil *et al.*, 1972; Nicholson & Kraig, 1975; Sugaya *et al.*, 1975; Nicholson *et al.*, 1977; Kraig & Nicholson, 1978; Nicholson *et al.*, 1978; Phillips & Nicholson, 1979). The velocity of propagation in the model system was about 0.63 mm/min which is in the range of experimental velocities.

The model solutions also predict solitary waves of increased excitatory transmitter (glutamate) and inhibitory transmitter (GABA). It is noted that in one experiment (Kraig & Nicholson, 1978) a possible reported (unknown) anion accumulates in the extracellular space and it is quite feasible that this is in fact partly an accumulation of glutamate which carries a net negative charge at pH 7.4 (Wheeler, 1979). No contribution is expected from GABA which carries no net electric charge at such a pH. Furthermore, glutamate has been shown to be released from the chicken retina during SD (Van Harreveld & Fifkova, 1970).

The model equations indicated that, in accordance with experiment, application of either KCl or glutamate could elicit SD, with a lower threshold for glutamate. Application of NaCl or GABA on the other hand, did not elicit SD. Naturally since the neuronal membrane potential is given by the Goldman–Hodgkin–Katz formula (2) and the glial membrane potential is approximately that of V_K given by formula (3), the model predicts depolarization of both neurons and glia during SD even though explicit account of the glial ion transport has not been taken into account.

There are a large number of matters to discuss both with regard to the validity or otherwise of the model equations employed here and the factors in general which will be operating during SD.

Amino Acid Uptake and Release

One of the difficult aspects of developing a mathematical model for spreading depression is that important fluxes of chemicals at synapses (including spines), are not yet well understood. There is a perplexing variety of such fluxes with an almost overwhelming set of dependencies on other substances. In the model equations glutamate and GABA uptake have been lumped into single terms whereas there may be high affinity and low affinity uptake of both of these amino acids. The high affinity uptake of GABA by cortical synaptosomes, for example, is supposed to be via a sodium-dependent, energy-dependent saturable process. A mathematical description based on a scheme of reactions involving GABA, Na^+ and carrier molecules led to reasonable agreement with experiment (Wheeler & Hollingworth, 1979). Should this more realistic model of the events at the synaptic level be incorporated into a model of GABA uptake in SD, then about another nine constants would be introduced. However, since these constants have been mostly determined with regard to best fit to experimental data, the inclusion of such a term for the GABA "pump" is something which should be considered in future modelling of SD. A model for synaptosomal high affinity uptake of glutamate has been developed by the same authors (Wheeler & Hollingworth, 1978). To complicate matters, however, some authors have found that the high affinity GABA uptake is inhibited by elevated external K^+ concentrations (Blaustein & King, 1976), in particular at such levels as arise during SD. In other studies, for example on glutamate uptake in rat dorsal sensory ganglion where there are no nerve terminals, high ($K_m = 2.06 \times 10^{-5}$M) and low ($K_m = 1.13 \times 10^{-3}$M) affinity uptake systems have been found, the former being ascribed the role of clearance of transmitter after synaptic function whereas the latter was implicated as having a purely metabolic function (Roberts & Keen, 1974). Glial cells have also been implicated in these amino acid uptake processes and are believed to be the site of conversion of glutamate to glutamine and also transamination of glutamate to aspartate (Benjamin & Quastel, 1974). It is probable that in SD both high and low affinity uptake systems of amino acid uptake are operating due to the excessive overloading of the extracellular space that occurs during this pathological condition. In the model equations it could be said that only the low affinity uptake mechanisms have been taken into account

It is noted that of the three postulated possible clearance mechanisms for amino acids, namely: (a) chemical degradation, (b) uptake and (c) diffusion, the first is reported as being not highly significant (Curtis, Duggan, & Johnston, 1970). Of the remaining clearance mechanisms, both have been accounted for in the model equations. There is also evidence that most of the high affinity uptake is into synaptosomes. This has also been taken into account in the model in the sense that only the presynaptic pool of transmitter substances is considered. It must be pointed out, however, that most of the matters pertaining to uptake of amino acids are still controversial (Fagg & Lane, 1979; Orrego, 1979).

When we turn to the quantitative investigations of the release of putative transmitter substances like glutamate, aspartate and GABA, the present picture is just as unclear. Most authors cling to the idea that one of the identifying characteristics of transmitter release is its Ca^{++}-dependence. Here the Ca^{++}-dependence of the release of glutamate from synaptosomes independently of whether induced by electrical stimulation or by elevation of external K^+ concentration, seems to have been established, whereas only under certain conditions does (for example) GABA release seem to be Ca^{++}-dependent. Again there are many complicating factors. For example, GABA release from glia, neuronal perikarya and synaptosomes from rabbit cerebellum increases as external K^+ rises to 15 mM at which level the release rate seems to saturate (Sellstrom & Hamberger, 1977). Also, GABA release from rat cortical synaptosomes has been found to depend on external GABA and external NaCl (Simon, Martin, & Kroll, 1974).

This latter study, incidentally, revealed some other interesting information. Quantitative estimeates put the intrasynaptosomal concentration of GABA at about 40 mM and the total fraction of cerebral cortical GABA within synaptosomes at about 5%. Since normal brain (human) has a gross concentration of GABA of about 2 mM (Van Gelder, Sherwin, & Rasmussen, 1972) we can estimate an approximate lower bound for the ratio of the volume of the extracellular space to the

presynaptic volume which was designated by α_2 in the above equations. Let v be the volume of synaptic terminals in one liter of tissue with an assumed GABA concentration of 40 mM/liter. Let the average concentration of GABA in the remaining $(1-v)$ liters be x. Then if the gross GABA concentration is 2 mM per liter we must have $40v+x(1-v) = 2$. Solving for x we obtain

$$x = (2-40v)/(1-v). \tag{40}$$

Since $0<v<1$ we have that $1-v>0$. Also, since x must be positive we must have $40v<2$ or $v<0.05$. Since the total extracellular space is generally accepted as being about 0.2 of the total tissue (Pope, 1978) then we see that α_2 must be greater than 4 with higher values more likely. This supports the value employed in the numerical calculations (see the Appendix) and an even higher lower bound is possible for α_2 if we use the figure of between 50 and 150 mM GABA in inhibitory synaptic terminals on cat Purkinje cells (Fonnum & Walberg, 1973).

In the model equations integrated in the present study it was assumed that the transmitter release rates (glutamate and GABA) were proportional to the presynaptic calcium current which was based on observations at the squid giant synapse. It is clear that these assumptions may, in light of the many complications touched upon here in regard to amino acid release, have to be modified when the final details of these release processes are eventually worked out.

Glial Cell Versus Neuronal Involvement in SD

In the model equations the intracellular compartment due to glial cells has not been included explicitly. This has been a matter of computational convenience and is also based on the fact that the synapses are known to contain large concentrations of putative transmitters and in normal circumstances synapses are found on neuronal rather than glial processes. Furthermore, the K^+ concentration inside Muller cells in the frog retina was found to increase during SD (Mori, Miller, & Tomita, 1976) and SD was not blocked by TTX in this preparation. These authors concluded that glial cell function in SD was to clear the extracellular space of excess K^+ which had resulted from synaptic release.

In the light of the uptake and release of amino acids by glia as well as by synapses (synaptosomes) and neuronal perikarya it is probable that a complete model of SD should include the glial intracellular compartment. The idea that glia form a syncytium (Prince, Pedley, & Ransom, 1978; Latzkovits, 1978) and convey intercellular K^+ currents (Nicholson & Kraig, 1979) is a matter which would deserve special consideration and not enough is yet known about this proposed mechanism to incorporate it into the model equations.

It has been noted that in SD the glial depolarization corresponds more closely in its time course which the negative surface potential than does the neuronal depolarization which is often hailed by a burst of activity (Sugaya *et al.*, 1975). These authors observed that glial depolarization preceded bursting activity of neighbouring nerve cells and noted that this more or less contradicted Grafstein's (1956, 1963) theory that it was K^+ released from neurons that was the primary event in SD. It is pointed out however that: (a) unmyelinated axons running along the cortex in the direction of the SD wave will be emitting K^+ ahead of the site at which the microelectrodes are placed (presumably in cell bodies), (b) diffusion of K^+ (as noted by Sugaya *et al.*) will enable the advancing SD wave to be accompanied by external K^+ levels up to about 8 mM before spiking occurs (assuming a threshold depolarization of about 10 mv). Examination of Figure 13A of Sugaya *et al.* (1975) shows that in this recording the neuronal spiking had terminated before the external K^+ level rose above 4 mM in comparison with the resting value of 3 mM. This could be due to the fact that the cause of the neuronal firing is the depolarization of synapses occurring on dendrites which extend ("backwards") in the opposite direction of travel of the SD wave. This does not seem to weaken or strengthen Grafstein's original hypothesis.

In Sugaya, Takato, Noda, & Sekiya (1978) it was claimed that treating the cortex with TTX should help delineate the contributions of neurons and glia. However this is only partially true because it has been established that TTX does not interfere with glutamate induced conductance charges (Zieglgansberger & Puil, 1972), a fact that has been noted by several authors with regard to SD propagation (Sugaya *et al.*, 1975; Mori *et al.*, 1976; Kraig & Nicholson, 1978; Tuckwell & Miura, 1978). The most pertinent question here is perhaps whether the external K^+ levels encountered in SD in TTX treated cortex are sufficient to cause

transmitter release (in particular, glutamate) from nerve terminals. It is noted that in the measurement of K^+ in TTX treated cat cortex the concentration of this ion rose to only 10 mM but it is important that this may not be the maximal level as the response amplitude in SD has a strong depth dependence (Ichijo & Ochs, 1970; Vyskocil *et al.*, 1972; Nicholson *et al.*, 1978). In experimental studies on the release of amino acids from slices of rat neocortex, the efflux of glutamate did not exceed "basal" levels significantly until the external K^+ concentration was over 20 mM (Mulder & Snyder, 1974), and the K^+-stimulated efflux was Ca^{++}-dependent. Suppose this result is extrapolated to excitatory transmitter (assumed glutamate or aspartate) release by elevated potassium concentration in cat neocortex. Then if the maximum level of K^+ during SD in this structure when treated with TTX is in fact of order 10 mM, then the postulated mechanisms underlying SD in TTX treated cortex both in this article and earlier papers (Tuckwell & Miura, 1978; Tuckwell, 1979a) may be strongly doubted. For cortex not treated with TTX the theory would necessarily be partially correct as the levels of K^+ then rise well above 20 mM and this event by itself will lead to transmitter release in addition to that caused by action potentials invading synapses. However, as pointed out by Fagg and Lane (1979) results such as those of Mulder & Snyder (1974) have to be interpreted with caution with respect to *in vivo* release of amino acids. Also, transmitter release has been reported at "small" increases in levels of external K^+ in some preparations (Gage & Quastel, 1965; cited by Orkand, 1969).

If K^+ levels occurring in SD in TTX treated cortex are not sufficient to cause significant transmitter release, what then would be an alternative theory? If there are no action potentials and no K^+-stimulated transmitter release the possibility exists that a passive efflux of K^+ occurs through non-synaptic membrane (e.g., that of unmyelinated axons, soma-dendritic membrane between synapses and possibly glial cells). There is a difficulty now in restoring the K^+ to its initial level if that restoration is by an exchange pump because there supposedly has been little if any inward Na^+ flux. (It is not known, in particular for cortical cells, just how much sodium conductance is blocked by TTX). If we assume no inward Na^+ flux whatsoever then the equations for the external concentrations of K^+(K) and Na^+(Na) would be

$$\frac{\partial K}{\partial t} = D_K \frac{\partial^2 K}{\partial x^2} + g_K(V)(V - V_K) - P_K(K,Na) \quad (41A)$$

$$\frac{\partial Na}{\partial t} = D_{Na} \frac{\partial^2 Na}{\partial x^2} + P_{Na}(K,Na), \quad (41B)$$

where V is the membrane potential, $g_K(V)$ the potassium conductance of nonsynaptic membrane (as taken account in an approximate way by the last term in Eq. (27A)) and P_K and P_{Na} are the exchange pump rates. If we consider these equations qualitatively we see that elevating K^+ will lead to K^+ eflux through depolarization. The exchange pump will be stimulated (even though there has been no influx of Na^+ and assuming that the application of TTX has not depleted the internal Na^+ concentration) and K^+ will be returned to the inside of cells and Na^+ will be transported to the outside. Accordingly if the only processes involved in SD in TTX treated cortex are flux of K^+ through nonsynaptic membrane and the operation of the Na^+–K^+ exchange pump, then the external Na^+ level should rise slightly under these conditions.

The experimental studies of the effects of TTX on squid giant axon reveal that in some cases "some 20% of the inward current persisted even after applying 5×10^{-7} gm/ml" (Nakamura, Nakajima, & Grundfest, 1965). In a study of the effects of TTX on cat spinal motoneurons (Blankenship, 1968), it was found that impulse conduction was "effectively blocked" and that resting membrane potential, resting membrane resistance and time constant were not significantly altered. The possibility exists that in some cortical cells under TTX there is a small residual, g_{Na}*. Under this hypothesis Eq. (41B) would become

$$\frac{\partial Na}{\partial t} = D_{Na} \frac{\partial^2 Na}{\partial x^2} + g_{Na}^*(V)(V - V_{Na}) + P_{Na}(K,Na) \quad (41C)$$

whereby transient increase in K^+ would be accompanied by a decrease in Na^+ with eventual return to resting levels. Chloride movement would follow that dictated by the membrane potential. It is thus with great interest that measurements are awaited of all the relevant ion concentrations (in particular at various depths) in a TTX treated structure such as rat cerebellum.

To ascertain the role of neurons rather than glia in SD, Sugaya *et al.* (1978) applied KCl to cortex in a state of edema at a stage when neurons were

viable and glia were not. No SD was observed under these conditions. It is difficult to interpret this result in terms of which mechanisms are operating during SD, because of complications caused by the swelling of tissue. For example, the usually available diffusion routes could have been blocked and the extracellular space so small that the extracellular concentrations of all substances become very large. If neuronal integrity was preserved then it is possible to declare that neither synaptic transmission ahead of the locally depolarized region nor electrotonic spread of depolarization along fibers running parallel to the cortical surface are sufficient to give rise to SD propagation.

"*Glutamate and K⁺-based*" *SD*

Van Harreveld (1978) has studied SD in the isolated chicken retina under a variety of conditions. It was found that at higher temperatures, 10 mM $MgCl_2$ prevented SD, but not at lower temperatures. At a temperature (21°C) where SD in the absence and presence of 10 mM $MgCl_2$ were both possible the mean velocities were 2.3 mm/min and 1.3 mm/min respectively. The addition of metabolic inhibitors also had a facilitatory effect as the addition of 5 mM iodoacetate enabled SD to develop in the $MgCl_2$ treated retina at 30°–31°C. Raising the external K^+ concentration or that of glutamate also had facilitatory effects.

From the data on SD, Van Harreveld deduced that there were two different mechanisms underlying SD. One component is called K^+-based and the other glutamate-based. When the retina is treated with $MgCl_2$ and SD is elicited at lowered temperatures (for example) the SD is supposed to be K^+-based. In the absence of $MgCl_2$ the "SD is probably in general glutamate based," which is taken to mean that "normal" SD consists of both components with the emphasis on the glutamate based SD. It was further hypothesized that SD in cerebral cortex (and presumably the cerebellum) is glutamate based as it is blocked by topically applied Mg^{++} (Bures, 1960).

It seems that Van Harreveld's ideas are basically correct but that they need elaboration to explain variety of phenomena connected with SD in various structures. This can be done quite transparently with mathematical formalism in the following way. Attention will be focused on the movements of K^+.

As far as is discernible the known fluxes of K^+ through neuronal elements in cortical structures are due to the following:

1) Membrane potential-dependent g_K in nonsynaptic membrane, (possibly including presynaptic terminal membrane but not "subsynaptic" membrane) activated by elevated K^+.

2) Membrane potential-dependent g_K in nonsynaptic membrane activated by action potentials.

3) Internal Ca^{++} and Mg^{++}-dependent g_K thought to be responsible for the postspike afterhyperpolarization in some cells (Krnjevic, Puil, & Werman, 1975; 1976).

4) Transmitter induced g_K in subsynaptic membrane due to release of transmitter from terminals depolarized by elevated K^+ levels.

5) Transmitter induced g_K in subsynaptic membrane due to release of transmitter from terminals depolarized by action potential invasion.

6) Active transport.

The first of these five factors tends to increase the external K^+ concentration whereas the last tends to decrease it. A possible subsidiary to (6) would be glial uptake.

We now designate the terms in the reaction-diffusion equation for K^+ corresponding to (1)–(6) by f_i, $i = 1, \ldots, 6$, with each $f_i \geqslant 0$. Letting K denote the extracellular K^+ concentration we now have

$$\frac{\partial K}{\partial t} = \nabla^2 \mathrm{K} + f_1 + f_2 + f_3 + f_4 + f_5 - f_6, \qquad (42)$$

where ∇^2 is the Laplacian (diffusion) operator. Each of the functions $f_i(\cdot)$ depends on several variables.

The following notation will be employed. The symbol "$>^*$" will mean sufficiently greater than in the sense that an SD wave may form and propagate. By an "SD wave" we will mean a solitary wave of elevated external K^+ moving across a brain structure with a velocity of order mm/minute.

In "normal" cortex (cf. "conditioning." discussed by Nicholson and Kraig (1979)) if an SD wave forms then we must have

$$f_1 + f_2 + f_3 + f_4 + f_5 >^* f_6 . \quad \text{(NORMAL)} \qquad (43)$$

TTX either reduces or abolishes f_2 and f_5 (and we

will assume f_3 then becomes unimportant) so then

$$f_1+f_5>^* f_6 . \quad \text{(TTX)} \qquad (44)$$

When divalent cations (Mg^{++}, Mn^{++}) are applied presumably f_4 and f_5 are abolished or reduced. Since SD does not then propagate we must have

$$f_1+f_2+f_3+^*< f_6 . \quad \text{(DIVALENT CATIONS)} \qquad (45)$$

If metabolic inhibitors or lowered temperatures reduce f_6 to a sufficiently small level f_6' then we can have

$$f_1+f_2+f_3 >^* f_6' . \quad \text{(DIVALENT CATIONS + METABOLIC INHIBITION)} \qquad (46)$$

Metabolic deprivation will of course affect the uptake of transmitters as well, but we have focused on the simplest system that shows the observed effects. It is, thus, possible to interpret the effects of TTX, divalent cations and metabolic inhibitors in terms of the underlying reaction terms in the appropriate reaction–diffusion equations. In the mathematical model developed in this paper, only the terms f_1, f_4 and f_6 were taken into account. In an earlier paper (Tuckwell, 1979a) a very approximate treatment of f_5 was made. Van Harreveld's K^+ based SD must be of the type indicated by the relation (46) whereas his glutamate based SD is the "normal" one as in (43).

Factors Not Yet Considered in the Theoretical Treatment

It can be seen from the matters discussed in this section that the modelling of SD is still in its very early stages. The chief difficulty is the bewildering complexity of the chemical factors affecting fluxes of various substances through various kinds of membrane. Each chemical and each compartment adds an extra component in the reaction–diffusion systems. The question arises as to whether the number of ions considered should be extended from four to five to include Mg^{++}; and there is the matter of whether an energy variable (e.g., ATP) should also be incorporated in the model equations.

One of the greatest difficulties is the inclusion of action potentials, the proper treatment necessitating another potential apart from equilibrium potential and another time scale and the variables corresponding to sodium conductance activation and inactivation as well as potassium conductance activation. A fast time scale (of order milliseconds) in addition to the slow time scale so far employed (seconds) is an additional difficulty in numerically computing solutions, together with the fact that action potentials generated at one space point may propagate to remote space points.

The "conditioning" process necessary to enable SD instigation is not yet understood. It is possible that in untreated cortex a local depolarization leads to activation of inhibitory synapses around the depolarized region (in layer II of cat motor cortex for example (Asanuma & Rosen, 1973) thus causing, say, a GABA build up which blocks SD propagation. The slow surface potential, the EEG activity, the compartmental volume changes (swelling of apical dendrites, Van Harreveld & Schade, 1959) the water movements, the increase in tissue impedance are all matters which as yet have not been approached in a quantitative theoretical fashion.

CONCLUSIONS

The mathematical model of SD which consists of a system of reaction–diffusion equations whose chief components are the extracellular concentrations of the ions K^+, Ca^{++}, Na^+, Cl^-, an excitatory transmitter (tentatively identified with glutamate) and an inhibatory transmitter (GABA), has been demonstrated with reasonable choices of the various parameters to passess solitary wave solutions consisting of elevations in K^+ and transmitters and decreases in Na^+, Cl^- and Ca^{++}. The velocity of propagation of the waves is approximately 0.6 mm/min, the amplitude of the K^+-wave being about 17 mM (resting value 3 mM). The model has subthreshold (nonpropagating) responses is the amount of K^+ or glutamate added as a stimulus is not great enough. Sufficiently large local applications of either KCl or glutamate gave rise to propagating SD waves in the model system. Applications of NaCl or GABA did not give rise to SD waves.

The concentrations of K^+, Ca^{++}, Na^+ and Cl^- have been measured during SD and the predicted changes in ion concentrations are reasonable when the experimental values are considered, though the effects of action potentials have not been included in the model in a quantitative way. The model is supposed, therefore to apply in the case of a

structure treated with TTX. A term has been included in the K^+ equation corresponding to g_K associated with nonsynaptic membrane.

Two pieces of information are necessary to test the model and an alternative model (Eqs. (41A) and (41C)). These are: (a) whether the K^+ levels encountered in the TTX treated structure are high enough to induce transmitter release, and (b) whether a reasonable amount of g_{Na} is available through nonsynaptic membrane when TTX is applied. If (a) is not the case and (b) is the case then the alternative model may be more appropriate for some structures.

There are five basic ways that K^+ can accumulate in the extracellular space and one (possibly two if glial uptake is considered) way in which it is removed apart from diffusion. Various chemicals (TTX, divalent cations) eliminate some of the five source terms and one may still obtain SD through the application of metabolic inhibitors may be then necessary to reduce the sink strength (exchange pump). The observations of Van Harreveld (1978) and his so-called K^+-based and glutamate-nased SD can be explained in terms of these various source and sink terms.

It is suggested that in order to delineate the importance of the various source and sink terms, measurements be made of the four ion concentrations at various depths during SD when the structure is treated with: (a) TTX, (b) divalent cations, and (c) both TTX and divalent cations. These results should assist in the development of a mathematical model for SD. At present we can only claim to have a rudimentary model whose development is hampered by the complexities of the neurochemical environment of brain cells, the details of the processes therein involved not yet being completely known.

APPENDIX

Many of the parameters employed in the numerical solution of the model equations were chosen in accordance with existing information from experiments on various structures, whereas others had to be guessed at reasonably until the desired solitary were solutions were obtainable.

The space and time variables were scaled in the numerical calculations. The space grid consisted of 100 subintervals on (0, 1) and the (scaled) diffusion coefficients were $D_1 = 2.4\times10^{-3}$, $D_2 = 1.0\times10^{-3}$, $D_3 = 1.7\times10^{-3}$, $D_4 = 2.5\times10^{-3}$, $D_5 = 1.3\times10^{-3}$ and $D_6 = 1.3\times10^{-3}$. These correspond to the values given by the American Institute of Physics Handbook for the various ions and GABA in aqueous solution (multiplied by 10^{-2} the D_i are in cm^2/sec), the value for glutamate being approximated by that of glycine. The values of the resting ion concentration (in mM) were: $K_R{}^0 = 3$, $K_R{}^i = 140$, $Ca_R{}^0 = 1$, $Ca_R{}^i = 0.001$, $Na_R{}^0 = 120$, $Na_R{}^i = 15$, $Cl_R{}^0 = 136.25$, $Cl_R{}^i = 6$, these being reasonable values based on observations during SD in various structures and other sources of data mentioned in previous articles (Tuckwell & Miura, 1978; Tuckwell, 1979a). The values of p_{Na} and p_{Cl} (0.05, 0.4) were also based on previously given references and the value of RT/F ln(·) was set at 58 $\log_{10}(\cdot)$. The values of α_1 and α_2 were chosen as 0.25 and 10, the first being the generally accepted value and the second being explained in the text. The parameters $k_{31} = 0.11$ and $V_M{}^* = 45$ mV were based on experimental data (Llinas *et al.*, 1976) and the value of $k_{32} = 0.071142$ follows by the definition of $V_M{}^T$ which was set at -60 mV (the voltage at which g_{Ca} becomes non zero). The values of k_3 and k_{10} were set at zero as it was assumed that the inhibitory transmitter did not effect increases in either sodium or potassium conductance; similarly $k_{13} = 0$ reflects the fact that the excitatory transmitter does not effect large changes in g_{Cl}. The pump saturation rate parameters were chosen so that the half maximal rates were achieved at values which were high relative to concentration levels expected in normal activity. Thus, $k_{18} = 15$, $k_{19} = 4$, $k_{23} = 15$, $k_{24} = 4$, $k_{21} = 0.2$, $k_{26} = 9$, $k_{28} = k_{30} = 1$. It is pointed out that the parameters for glutamate and GABA are the least certain as the levels to which these rise in SD, in particular, are not yet known. The rates at which the synaptically induced conductance changes saturate were also not known with any degree of accuracy but were set at $k_2 = k_4 = 1.5$. The value of the maximal pump rates and strengths of the source and sink terms corresponding to transmitter induced conductance changes were obtained by trial and error until solitary waves of the desired characteristics were obtained. The values used were $k_1 = 30.035$, $k_7 = 0.2$, $k_{15} = -3.47$, $k_9 = 2$, $k_{12} = -104.05$, $k_{16} = -3.15$, $k_{20} = 0.8$, $k_{27} = 47.124$, $k_{29} = 47.124$, $k_{25} = 260.16$,

$k_{17} = 429.75$ and $k_{22} = 362.25$. The four pump rates at resting levels are then determined and become $k_5 = 128.93$, $k_8 = 0.0039801$, $k_{11} = 108.68$ and $k_{14} = 104.05$. The remaining parameter is k_6 which determines the contribution from g_K at nonsynaptic membrane and was set at the small value of 0.00015. The time step employed in the numerical results presented was $\Delta t = 0.0025$.

Reference Note

1. McLennan, personal communication, 1980.

REFERENCES

Asanuma, H. & Rosen, I. Spread of mono- and polysynaptic connections within cat motor cortex. *Experimental Brain Research*, 1973, **16**, 507–520.

Benjamin, A. M. & Quastel, J. H. Fate of L-glutamate in the brain. *Journal of Neurochemistry*, 1974, **23**, 457–464.

Blankenship, J. E. Action of tetrodotoxin on spinal motoneurons of the cat. *Journal of Neurophysiology*, 1968, **31**, 186–194.

Blaustein, M. P. & King, A. C. Influence of membrane potential on the sodium-dependent uptake of gamma-aminobutyric acid by presynaptic nerve terminals; experimental observations and theoretical considerations. *Journal of Membrane Biology*, 1976, **30**, 153–173.

Brinley, F. J., Kandel, E. R., & Marshall, W. H. Potassium outflux from rabbit cortex during spreading depression. *Journal of Neurophysiology*, 1960, **23**, 246–256.

Bures, J. Block of Leao's spreading cortical depression by bivalent cations. *Physiology Bohemoslov.*, 1960, **9**, 202–209.

Bures, J., Buresova, O., & Krivanek, J. Some metabolic aspects of Leao's spreading cortical depression. In: *Structure and Function of the Cerebral Cortex*, Tower, D. B. and Schade, J. P. (Eds.), Amsterdam: Elsevier, 1960.

Bures, J., Buresova, O., & Krivanek, J. *The Mechanism and Applications of Leao's Spreading Depression of Electroencephalographic Activity*. New York: Academic Press, 1974.

Campbell, C. W. B. The Na^+, K^+, Cl^- contents and derived membrane potentials of presynaptic nerve endings *in vitro*. *Brain Research*, 1976, **101**, 594–599.

Curtis, D. R., Duggan, A. W., & Johnston, G. A. R. The specificity of strychnine as a glycine antagonist in the mammalian spinal cord. *Experimental Brain Research*, 1970, **10**, 447–462.

Do Carmo, R. J. & Leao, A. A. P. On the relation of glutamic acid and some allied compounds to cortical spreading depression. *Brain Research*, 1972, **39**, 515–518.

Eccles, J. C. Excitatory and inhibitory mechanisms in brain. In: *Basic Mechanisms of the Epilepsies*, Jasper, H. H., Ward, A. A., & Pope, A. (Eds.), Boston: Little, Brown and Co., 1969.

Eisenstadt, M. L. & Schwartz, J. H. Metabolism of acetylcholine in the nervous system of *Aplysia californica* III. Studies of an identified cholinergic neuron. *Journal of General Physiology*, 1975, **65**, 293–313.

Fagg, G. E. & Lane, J. D. The uptake and release of putative amino acid neurotransmitters. *Neuroscience*, 1979, **4**, 1015–1036.

Fambrough, D. M. Acetylcholine receptors, Revised estimates of extrajunctional receptor density in denervated rat diaphragm. *Journal of General Physiology*, 1974, **64**, 468–472.

Fifkova, E. & Van Harreveld, A. Glutamate and spreading depression. *Journal of Neurobiology*, 1974, **5**, 469–473.

Fonnum, F. & Walberg, F. The concentration of GABA within inhibitory nerve terminals. *Brain Research*, 1973, **62**, 577–579.

Gage, P. W. & Quastel, D. M. J. Dual effect of potassium on transmitter release. *Nature*, 1965, **206**, 625–626.

Goldman, D. E. Potential, impedance, and rectification in membranes. *Journal of General Physiology*, 1943, **27**, 37–60.

Grafstein, B. Mechanism of spreading cortical depression. *Journal of Neurophysiology*, 1956, **19**, 154–171.

Grafstein, B. Neuronal release of potassium during spreading depression. In: *Brain Function*, Vol. 7, Brazier, M. A. B. (Ed.), Berkeley: University of California Press, 1963.

Herz, A., Zieglgansberger, W., & Farber, G. Microelectrophoretic studies concerning the spread of glutamic acid and GABA in brain tissue. *Experimental Brain Research*, 1969, **9**, 221–235.

Hodgkin, A. L. & Huxley, A. F. A quantitative description of membrane current and its applicaion to conduction and excitation in nerve. *Journal of Physiology (Lond.)* 1952, **117**, 500–544.

Hodgkin, A. L. & Katz, B. The effect of sodium ions on the electrical activity of the giant axon of the squid. *Journal of Physiology (Lond.)*, 1949, **108**, 37–77.

Ichijo, M. & Ochs, S. Spreading depression of negative wave of direct cortical response and pyramidal tract responses. *Brain Research*, 1970, **23**, 41–56.

Katz, B. & Miledi, R. The binding of acetylcholine to receptors and its removal from the synaptic cleft. *Journal of Physiology (Lond.)* 1973, **231**, 549–574.

Katz, B. & Thesleff, S. A study of the "desensitization" produced by acetylcholine at the motor end-plate. *Journal of Physiology (Lond.)*, 1957, **138**, 63–80.

Koike, H., Kandel, E. R., & Schwartz, J. H. Synaptic release of radioactivity after intrasomatic injection of ^{3}H-choline into an identified cholinergic interneuron in abdominal ganglion of Aplysia californica, *Journal of Neurophysiology*, 1974, **37**, 815–827.

Kow, L-M. & Van Harreveld, A. Ion and water movements in isolated chicken retinas during spreading depression. *Neurobiology*, 1972, **2**, 61–69.

Kraig, R. P. & Nicholson, C. Sodium liquid ion exchange microelectrode used to measure large extracellular sodium transients. *Science*, 1976, **194**, 725–726.

Kraig, R. P. & Nicholson, C. Extracellular ionic variations during spreading depression. *Neuroscience*, 1978, **3**, 1045–1059.

Krivanek, J. & Bures, J. Ion shifts during Leao's spreading cortical depression. *Physiology Bohemoslov.*, 1960, **9**, 494–503.

Krnjevic, K. Chemical nature of synaptic transmission in vertebrates. *Physiological Revue*, 1974, **54**, 418–540.

Krnjevic, K., Puil, E., & Werman, R. Evidence for Ca^{2+}-activated K^+ conductance in cat spinal motoneurons from intracellular EGTA injections. *Canadian Journal of Physiology and Pharmacology*, 1975, **53**, 1214–1218.

Krnjevic, K., Puil, E., & Werman, R. Intracellular Mg^{2+} increases neuronal excitability. *Canadian Journal of Physiology and Pharmacology*, 1976, **54**, 73–77.

Latzkovits, L. Neuronal-glial interactions in potassium transport. In: *Dynamic properties of glia cells*, Schoffeniels, E., Franck, G., Hertz, L. & Tower, D. B. (Eds.), New York: Pergamon, 1978.

Leao, A. A. P. Spreading depression of activity in the cerebral cortex. *Journal of Neurophysiology*, 1944, **7**, 359–390.

Leao, A. A. P. & Ferreira, H. M. Altracao da impedancia electrica no decurso da depressao alastrante de atividade do cortex cerebral. *Anais. Acad. bras. Cienc.*, 1953, **25**, 259–266.

Lees, M. An extrapolated Crank–Nicolson difference scheme for quasilinear parabolic equations. In: *Numerical Solution of Partial Differential Equations*, Ames, W. F. (Ed.), New York: Barnes and Noble, 1969.

Llinas, R., Steinberg, I. Z., & Walton, K. Presynaptic calcium currents and their relation to synaptic transmission; voltage clamp study in squid giant synapse and theoretical model of the calcium gate. *Proceedings of the National Academy of Science (U.S.A.)*, 1976, **73**, 2918–2922.

Llinas, R. & Hess, R. Tetrodotoxin-resistant dendritic spikes in avian Purkinje cells. *Proceedings of the National Academy of Science, (U.S.A.)* 1976, **73**, 2520–2523.

Marshall, W. H. Spreading cortical depression of Leao. *Physiological Revue*, 1959, **39**, 239–279.

Mori, S., Miller, W. H., & Tomita, T. Muller cell function during spreading depression in frog retina. *Proceedings of the National Academy of Science (U.S.A.)*, 1976, **73**, 1351–1354.

Mulder, A. H. & Snyder, S. H. Potassium-induced release of amino acids from cerebral cortex and spinal cord slices of the rat. *Brain Research*, 1974, **76**, 297–308.

Nakamura, Y., Nakajima, S., & Grundfest, H. The action of tetrodotoxin on electrogenic components of squid giant axons. *Journal of General Physiology*, 1965, **48**, 985–996.

Nicholson, C. & Kraig, R. P. Chloride and potassium changes measured during spreading depression in catfish cerebellum. *Brain Research*, 1975, **96**, 384–389.

Nicholson, C. & Kraig, R. P. The behavior of extracellular ions during spreading depression. In: *The Application of Ion-Selective Electrodes*, Zeuthen, T. (Ed.), Amsterdam: Elsevier, in press, 1979.

Nicholson, C., Phillips, J. M., & Gardner-Medwin, R. Diffusion from an iontophoretic point source in the brain: role of tortuosity and volume fraction. *Brain Research*, 1979, **169**, 580–584.

Nicholson, C., Bruggencate, G. ten, Steinberg, R., & Stockle, H. Calcium modulation in brain extracellular microenvironment demonstrated with ion-selective micropipette. *Proceedings of the National Academy of Science (U.S.A.)*, 1977, **74**, 1287–1290.

Nicholson, C., Bruggencate, G. ten, Stockle, H., & Steinberg, R. Calcium and potassium changes in extracellular microenvironment of cat cerebellar cortex. *Journal of Neurophysiology*, 1978, **41**, 1026–1039.

Ochs, S. The nature of spreading depression in neural networks. *International Revue of Neurobiology*, 1962, **4**, 1–69.

Orkand, R. K. Neuroglial–Neuronal interactions. In: *Basic Mechanisms of the Epilepsies*, Jasper, H. H., Ward, A. A. & Pope, A. (Eds.), Boston: Little, Brown & Co., 1969.

Orrego, F. Criteria for the identification of central neurotransmitters, and their application to studies with some nerve tissue preparations *in vitro*. *Neuroscience*, 1979, **4**, 1037–1057.

Phillips, J. M. & Nicholson, C. Anion permeability in spreading depression investigated with ion-sensitive microelectrodes. *Brain Research*, 1979, **173**, 567–571.

Pope, A. Neuroglia: quantitative aspects. In: *Dynamic Properties of Glia Cells*, Schoffeniels, E., Franck, G., Hertz, L., & Tower, D. B. (Eds.), New York: Pergamon, 1978.

Potter, L. T. Synthesis, storage and release of (^{14}C) acetylcholine in isolated rat diaphragm muscles. *Journal of Physiology (Lond.)*, 1970, **206**, 145–166.

Prince, D. A., Pedley, T. A., & Ransom, B. R. Fluctuations in ion concentrations during excitation and seizures. In: *Dynamic Properties of Glia Cells*, Schoffeniels, E., Franck, G., Hertz, L., & Tower, D. B. (Eds.), New York: Pergamon, 1978.

Roberts, P. J. & Keen, P. [^{14}C] glutamate uptake and compartmentation in glia of rat dorsal sensory ganglion. *Journal of Neurochemistry*, 1974, **23**, 201–209.

Sandoval, M. E. & Cotman, C. W. Evaluation of glutamate as a neurotransmitter of cerebellar parallel fibers. *Neuroscience*, 1978, **3**, 199–206.

Sellstrom, A. & Hamberger, A. Potassium-stimulated gamma-aminobutyric acid release from neurons and glia. *Brain Research*, 1977, **119**, 189–198.

Simon, J. R., Martin, D. L., & Kroll, M. Sodium-dependent efflux and ecchange of GABA in synaptosomes. *Journal of Neurochemistry*, 1974, **23**, 981–991.

Sugaya, E., Takato, M., & Noda, Y. Neuronal and glial activity during spreading depression in the cerebral cortex of cat. *Journal of Neurophysiology*, 1975, **38**, 822–841.

Sugaya, E., Takato, M., Noda, Y., & Sekiya, Y. Glial cells and spreading depression. In: *Dynamic Properties of Glia Cells*, Schoffeniels, E., Franck, G., Hertz, L., & Tower, D. B. (Eds.), New York: Pergamon, 1978.

Tuckwell, H. C. Predictions and properties of a model of potassium and calcium ion movements during spreading cortical depression. *International Journal of Neuroscience*, 1980, **10**, 145–164.

Tuckwell, H. C. Solitons in a reaction–diffusion system. *Science*, 1979, **205**, 493–495. (b)

Tuckwell, H. C. Evidence of soliton-like behavior of solitary waves in a nonlinear reaction–diffusion system. *S.I.A.M.J. Appl. Math.* (in press). (c)

Tuckwell, H. C. & Miura, R. M. A mathematical model for spreading cortical depression. *Biophysical Journal*, 1978, **23**, 257–276.

Van Gelder, N. M., Sherwin, A. L., & Rasmussen, T. Amino acid content of epileptogenic human brain: focal versus surrounding regions. *Brain Research*, 1972, **40,** 385–393.

Van Harreveld, A. Compounds in brain extracts causing spreading depression of cerebral cortical activity and contraction of crustacean muscle. *Journal of Neurochemistry*, 1959, **3,** 300–315.

Van Harreveld, A. Two mechanisms of spreading depression in the chicken retina. *Society of Neuroscience*, 1977, **3,** 325 (Abstr. #1047).

Van Harreveld, A. Two mechanisms of spreading depression in the chicken retina. *Journal of Neurobiology*, 1978, **9,** 419–431.

Van Harreveld, A. & Fifkova, E. Glutamate release from the retina during spreading depression. *Journal of Neurobiology*, 1970, **2,** 13–29.

Van Harreveld, A. & Schade, J. P. Chloride movements in cerebral cortex after circulatory arrest and during spreading depression. *Journal of Cellular Comparative Physiology*, 1959, **54,** 65–84.

Vyskocil, F., Kriz, N., & Bures, J. Potassium-selective microelectrodes used for measuring the extracellular brain potassium during spreading depression and anoxic depolarization in rats. *Brain Research*, 1972, **39,** 255–259.

Wheeler, D. D. Effects of polarizing currents and repetitive stimulation on the uptake of amino acids by peripheral nerve. *Journal of Neuroscience Research*, 1979, **4,** 123–131.

Wheeler, D. D. & Hollingworth, R. G. A model of high affinity glutamic acid transport by cortical synaptosomes from the Long-Evans rat. *Journal of Neuro-Chemistry*, 1978, **30,** 1311–1319.

Wheeler, D. D. & Hollingworth, R. G. A model of GABA transport by cortical synaptosomes from the Long-Evans rat. *Journal of Neuroscience Research*, 1979, **4,** 265–289.

Whittaker, V. P. The application of subcellular fractionation technique to the study of brain function. *Progress in Biophysics and Molecular Biology*, 1965, **15,** 39–

Zieglgansberger, W. & Puil, E. A. Tetrodotoxin interference pf CNS excitation by glutamic acid. *Nature*, 1972, **239,** 204–205.