# MODELING REPETITIVE FIRING AND BURSTING IN A SMALL UNMYELINATED NERVE FIBER

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ABSTRACT The Hodgkin-Huxley equations, originally developed to describe the electrical events in the squid giant axon, have been modified to simulate the ionic and electrical events in a small unmyelinated nerve fiber. The modified equations incorporate an electrogenic sodium-potassium pump, a finite intra-axonal volume, a periaxonal space, a calcium current, and calcium-dependent potassium conductance ($G_{KCa}$). The model shows that adaptation can occur in two ways: increased Na-K pump activity because of periaxonal K accumulation or intra-axonal Na accumulation; or from an increase in ($G_{KCa}$) caused by calcium accumulating within the axon. Bursting is an extension of adaptation and occurs when the sensitivity of the Na-K pump or ($G_{KCa}$) to changes in ionic concentration is increased.

## INTRODUCTION

The Hodgkin-Huxley equations (1952) were originally developed to describe the propagation of a single impulse down a large axon 500–1,000 $\mu$m in diameter. Because the squid axon is so large there was no need to take account of any changes in ionic concentration that might be caused by ionic fluxes across the membrane. However, when modeling the repetitive firing of a small (of the order of 1 $\mu$m) unmyelinated fiber, one has to take into account, not only the changes in ionic concentration caused by the ionic fluxes, but also the ionic pumps, which maintain the differential concentrations across the membrane. I show here that it is possible to modify the Hodgkin-Huxley equations so that they describe both the electrical and ionic behavior of a small axon.

The Hodgkin-Huxley model assumes that the axon is initially at rest and in electrical balance ($dV/dt = 0$). To model the small nerve, I assume an equivalent but stronger initial condition; that there is no net ionic flux across the membrane at rest. The ionic balance condition implies electrical balance. Since ions are not interchangeable this assumption also implies that each ion must be in balance separately.

The model axon has four ion fluxes across its surface: the two major charge carriers, sodium and potassium, as well as chloride and calcium. I assume that for each of these ions there is at least one voltage at which there is no net flux across the membrane; either because the voltage is equivalent to the equilibrium potential or because the passive flux is balanced by a pump flux. If the system is to be in ionic as well as electrical balance, the voltage at which ionic balance occurs must be the same for all ions; however, it is not obvious which ions determine the membrane potential and which equilibrate passively. I assume that, as in the squid axon, it is the sodium and potassium ions that are the major determinants of the membrane potential and that the chloride and calcium ions are in balance at the voltage determined by the sodium-potassium system. The nerve model is therefore developed as three separate systems: sodium-potassium (Na-K), chloride (Cl) and calcium (Ca).

*The Na-K system*

The sodium and potassium fluxes are kept in balance by a coupled Na-K pump, the properties of which have been extensively reviewed (Thomas, 1972; De Weer, 1975). The properties that have been incorporated into this model are (*a*) the pump is electrogenic, (*b*) the pump current is independent of the membrane voltage, (*c*) the pump fluxes are dependent upon the external sodium and potassium concentrations, and (*d*) the pump ratio is dependent upon the internal sodium concentration.

When the axon is in equilibrium and at rest the voltage-dependent (Hodgkin-Huxley) fluxes are balanced exactly by the pump currents so that there is no net ionic flux across the membrane. Thus,

$$I_{Na} = -I_{Nap}; I_K = -I_{Kp}, \tag{1}$$

where the subscript p denotes pump. The sodium and potassium pump currents are related by the equation

$$I_{Nap} = -rI_{Kp}, \tag{2}$$

where the coupling ratio $r \geq 1$. Substituting Eq. 2 into 1,

$$I_{Na} + rI_K = 0, \tag{3}$$

at rest. Writing the voltage-dependent fluxes explicitly,

$$\bar{G}_{Na}m^3h(E_{Na} - V) + r\bar{G}_Kn^4(E_K - V) = 0. \tag{4}$$

Where $\bar{G}_{Na}$ and $\bar{G}_K$ are the maximum sodium and potassium conductances, respectively, $m$, $n$, and $h$ are the Hodgkin-Huxley variables, and $E_{Na}$ and $E_K$, the sodium and potassium equilibrium potentials.

The equilibrium potentials are written in terms of the Nernst equation:

$$E_j = \mathcal{R}T/zF \ln (j_0/j_{in}): j \equiv \text{Na,K} \tag{5}$$

Where $\mathcal{R}$ is the gas constant, $T$ the absolute temperature, $z$ the charge carried by the ion, $F$ is Faraday's constant, $j_o$ the ion concentration outside the excitable membrane and $j_{in}$ the ion concentration inside. In using Eq. 5, I assume that there is no significant binding of Na or K either inside or outside the membrane. The $m_\infty$, $n_\infty$, $h_\infty$ variables and their associated $\alpha$ and $\beta$ have the same form as in the original equations but are shifted along the voltage axis by various amounts ($m_s$, $n_s$, $h_s$). Thus $V$ in the equations for $\alpha_m$ and $\beta_m$ is replaced by $V + m_s$; in the equations for $\alpha_h$ and $\beta_h$ by $V + h_s$ and in the equations for $\alpha_n$ and $\beta_n$ by $V + n_s$.

The value of $V$ that satisfies Eq. 4 represents the resting membrane potential $V_{eq}$. Of the eight parameters in Eq. 4, only two, $E_{Na}$ and $E_K$, have been measured in the small unmyelinated nerve (Keynes and Ritchie, 1965; Rang and Ritchie, 1968*b*) and one other parameter, $r$, can be assumed to be the same as that found in other neurones. The five remaining parameters $\bar{G}_{Na}$, $\bar{G}_K$, $m_s$, $n_s$, $h_s$ were estimated by an iterative procedure that tested all integer values of $m_s$, $n_s$, and $h_s$ between $-40$ and $+10$mV and the integer values of the ratio $\bar{G}_{Na}/\bar{G}_K$ in the range 3–9. Those parameter sets that gave values of $V_{eq}$ within 5 mV of the desired (experimental) value were further tested to ensure that the behavior of the system is biologically reasonable. The three criteria of acceptance are (*a*) $V_{eq}$ is a unique solution of Eq.

4 for a given parameter set; (*b*) The system produces a properly shaped impulse when given a suprathreshold stimulus. (*c*) The system obeys the "all or none" law.

These criteria eliminated most of the possible parameter sets. The parameter set used for the simulation was chosen from those remaining so that the model had a nonzero threshold and the resting ionic fluxes and the net ion movement per impulse were similar to those measured experimentally.

*The Periaxonal Space*

In some unmyelinated axons the volume immediately adjacent to the excitable membrane is separated from the bulk extracellular fluid by a barrier that limits the rate of ionic movement (Frankenhaeuser and Hodgkin, 1956). During neuronal activity the movement of ions across the excitable membrane results in sodium and potassium concentrations in the space that are different from those in the extracellular fluid. With these changes in concentration there is a concomitant change in both $E_{Na}$ and $E_K$.

I assume that the diffusion barrier is of the same type that Frankenhaeuser and Hodgkin (1956) postulated for the squid axon. The equations describing the changes in potassium concentration are derived first. The volume of the periaxonal space surrounding a cylindrical axon is $\pi(2R\theta + \theta^2)l$ where $R$ represents the radius of the axon; $l$ is the length of the space-clamped or equipotential portion; and $\theta$ the thickness of the periaxonal space.

The flux of K ions into this volume is thus

$$\pi(-I_{Kp} - I_K)\, 2Rl/F \tag{6}$$

where $F$ is Faraday's constant. The flux leaving by diffusion is

$$D_K(K_{sp} - K_o)\pi 2(R + \theta)l \tag{7}$$

where $D_K$ is the permeability of the barrier to potassium and $K_{sp}$ and $K_o$ represent the potassium concentrations in the periaxonal space and the extracellular fluid, respectively.

The change in potassium concentration is therefore given by

$$dK_{sp}/dt = [(-I_{Kp} - I_K)2R/F - 2D_K(R + \theta)(K_{sp} - K_o)]/(2R\theta + \theta^2) \tag{8}$$

If $R \gg \theta$ this equation reduces to

$$dK_{sp}/dt = [(-I_{Kp} - I_K)/F - D_K(K_{sp} - K_o)]/\theta \tag{9}$$

which is the equation of Frankenhaeuser and Hodgkin(1956).

An equation similar to Eq. 9 can be derived for the change in sodium concentration:

$$dNa_{sp}/dt = [(-I_{Nap} - I_{Na})/F - D_{Na}(Na_{sp} - Na_o)]/\theta \tag{10}$$

where $D_{Na}$ is the permeability of the barrier to sodium and $Na_{sp}$ and $Na_o$ are the sodium concentrations in the periaxonal space and the extracellular fluid, respectively.

*The Axon Interior*

The concentration of ions in the interior of the axon is dependent upon the ionic currents and the Na-K pump. If it is assumed that the ions within the volume mix instantaneously, the change in potassium concentration is given by

$$dK_{in}/dt = (I_K + I_{Kp})2/FR. \tag{11}$$

The analagous equation for the change in sodium concentration is

$$d\mathrm{Na}_{in}/dt = (I_{Na} + I_{Nap})2/FR. \tag{12}$$

*The Pump Currents*

Baker et al. (1969), working on the squid axon, found that the activity of the Na-K pump was strongly dependent on the external potassium concentration and weakly dependent on internal sodium concentration. They showed that both the Na efflux and the K influx could be described by the equation

$$\frac{k}{[(1 + B^{K}/\mathrm{K}_{sp})(1 + \mathrm{Na}_{sp}/B^{Na})]^{2}} \tag{13}$$

where $B^{K}$, and $B^{Na}$ represent the dissociation constants for K and Na, respectively, and $k$ represents a proportionality constant. The Na efflux and K influx differ only in the value of this constant; the Na efflux is always greater than the K influx. Baker et al. (1969) also found that the pump fluxes are insensitive to changes in $\mathrm{Na}_{sp}$ when $\mathrm{Na}_{sp}$ is large. Since $\mathrm{Na}_{sp}$ changes little even with prolonged repetitive firing, Eq. 14 can be simplified by dropping the second term in the denominator. Noting that K influx is equivalent to $I_{Kp}$ , the potassium pump current can be written

$$I_{Kp} = \frac{A}{(1 + b_1/\mathrm{K}_{sp})^{2}} \tag{14}$$

where $A$ is a constant of proportionality and $b_1$ is the dissociation constant for $\mathrm{K}_{sp}$.

Mullins and Brinley (1969) showed that the K influx becomes highly dependent on $\mathrm{Na}_{in}$ when this concentration drops below half its normal resting value. Although Mullins and Brinley did not propose an equation to describe this dependence, a plot of the data (their Fig. 13) shows that it could be described by a first-order Michaelis-Menten equation. Adding this to the equation for $I_{Kp}$, Eq. 14 becomes

$$I_{Kp} = \frac{a}{(1 + b_1/\mathrm{K}_{sp})^{2}(1 + b_2/\mathrm{Na}_{in})} \tag{15}$$

where $a$ is constant of proportionality and $b_2$ is the dissociation constant for $\mathrm{Na}_{in}$. From Mullins and Brinley's data (1969; Fig. 13, *inset*), $b_2$ can be estimated to be ~30 mM. For a given $b_1$ and $b_2$, $a$ is calculated so that $I_K = -I_{Kp}$ when $V = V_{eq}$.

It is not known whether there is any delay in the pump response to a change in ionic concentration. Hodgkin and Keynes (1956) estimated that the delay in the squid axon, if it existed, was <1 s. Since there are no data that support the existence of a delay I have assumed that the pump responds instantaneously to a change in ion concentration.

*The Pump Ratio*

Mullins and Brinley (1969) found that the Na-K pump ratio was a quasilinear function of $\mathrm{Na}_{in}$. I approximate this relationship by the equation

$$r = c\,\mathrm{Na}_{in} + d \tag{16}$$

where $c$ and $d$ are constants; $c$ is arbitrary and $d$ is determined by the constraint that $r = 1.5$ at rest (Thomas, 1972). In the squid axon $c$ has a value of 0.05 (Mullins and Brinley, 1969, Fig. 15).

The results of Baker et al. (1969), which show that the dependence of K influx and Na efflux on external sodium and potassium differs only in a constant of proportionality, imply that $r$ must be independent of both these quantities.

### *The Cl System*

If the axon is to be in steady state the Cl system must be in equilibrium at the same voltage as the Na-K system. Chloride balance across the membrane is maintained either by passive diffusion or by a chloride pump. Each mechanism will be considered in turn.

### *Passive Diffusion*

It is obvious that for passive diffusion to maintain ionic balance the chloride equilibrium potential and the axon rest potential must be equal. The net chloride current is written in the same form as the Hodgkin-Huxley leak current:

$$G_{Cl}(V_{eq} - V). \tag{17}$$

However, it has been shown that in the squid axon $E_{Cl}$ is greater than the rest potential by ~30mV and that equilibrium is maintained by a pump (Keynes, 1963; Landowne and Scruggs, 1976; Russell, 1976).

It is interesting to note that whereas the leak current (which includes but is not necessarily equivalent to the chloride current) is not equilibrated by diffusion, the net movement of ions during an impulse is small. This arises from the near coincidence of the calculated $E_l$ (−49.402 mV for the squid axon) to the value of the voltage averaged over one impulse (~−48 mV) which implies a net efflux of the order of 0.17 · pmol/cm$^2$ impulse; about an eightieth of the Na and K influxes.

### *The Chloride Pump*

The chloride pump has been examined by Russell (1976) who found that the pump flux varied quasilinearly with voltage and was inversely dependent on $Cl_{in}$. If it is assumed that chloride conductance is not voltage dependent and that the pump current varies linearly with voltage, then

$$I_{Cltot} = I_{Cl} - I_{Clp} = G_{Cl}(E_{Cl} - V) - (k_1 V + k_2) = 0 \tag{18}$$

when $V = V_{eq}$. Thus

$$\begin{aligned} k_2 &= G_{Cl}E_{Cl} - (G_{Cl} + k_1)V_{eq} \\ I_{Cltot} &= (G_{Cl} + k_1)(V_{eq} - V) \\ &= G'_{Cl}(V_{eq} - V) \end{aligned}$$

which is equivalent to Eq. 17.

Inasmuch as the chloride efflux is small, and since the resting membrane potential is independent of $Cl_{in}$ and $I_{Clp}$ is weakly dependent upon $Cl_{in}$ (Russell, 1976), the internal and external chloride concentrations were assumed to be constant.

*The Calcium System*

A calcium current is present in most axons although its contribution to the total membrane current varies from axon to axon. In this model it is assumed that the calcium current is small compared with the Na and K currents.

Brown et al. (1978) described the calcium conductance in snail neurons in terms of the Hodgkin-Huxley variables $m$ and $h$:

$$G_{Ca} = \bar{G}_{Ca} m_c h_c \tag{20}$$

where $\bar{G}_{Ca}$ is the maximum value of the calcium conductance. The time constants for activation and inactivation are the same as $\tau_n$ and $\tau_h$, respectively. Using this formulation the calcium current is written as

$$I_{Ca} = \bar{G}_{Ca} m_c h_c (E_{Ca} - V) \tag{21}$$

where $E_{Ca}$ is the calcium equilibrium potential and is described by the Nernst equation (Eq. 5). With a value of 100 nM for the internal calcium concentration (Baker, 1978) and 2 2 mM for the external concentration ($Ca_o$) (Keynes and Ritchie, 1965), $E_{Ca}$ has a value of 126.3 mV at 20°C. This is very close to the value of 120 mV estimated by Baker et al. (1971) for the squid axon. The calcium fluxes across the membrane are assumed to be so small that the concentration of calcium in the periaxonal space is the same as $Ca_o$.

*The Calcium-dependent Potassium Conductance*

There is considerable evidence (Meech, 1978) that many neurons have a potassium conductance the magnitude of which depends upon the concentration of free calcium within the neuron. (Gorman and Thomas, 1978; Eckert and Tilotson, 1978). It is believed that the calcium binds reversibly with a receptor situated on the interior of the membrane, perhaps next to the pore through which the potassium enters. It is not yet clear whether the conductance $G_{KCa}$ represents a modulation of a voltage-dependent channel or is entirely separate. I assume the latter.

Measurements of $G_{KCa}$ show that it is activated very slowly, its value being proportional to the level of intraneural calcium (Eckert and Tilotson, 1978). It will therefore be assumed that $G_{KCa}$ is voltage independent and that the total potassium conductance is

$$\bar{G}_K n^4 + G_{KCa}. \tag{22}$$

Furthermore, if the equilibrium voltages for both conductances are assumed to be equal,

$$I_K = (\bar{G}_K n^4 + G_{KCa})(E_K - V). \tag{23}$$

As before, $I_K$ is precisely balanced by $I_{Kp}$ at rest.

Since nothing is known about the calcium-receptor interaction the scheme proposed by Plant (1978) will be followed and it will be assumed that the calcium-receptor interaction is a first-order process:

$$Ca + R \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CaR \tag{24}$$

and that

$$G_{KCa} = \frac{\bar{G}_{KCa}}{1 + K_D/Ca_{in}} \tag{25}$$

where $K_D = k_{-1}/k_1$ is the dissociation constant for the calcium-receptor complex and $\bar{G}_{KCa}$ is the maximum value of this conductance.

### *Ionized Calcium Balance*

Ionized calcium is kept in balance by a pump and by sequestration into a bound store. Since it is unclear how either of these mechanisms work, two assumptions will be made:

(*a*) The resting Ca influx is precisely balanced by an outward calcium current $I_{Cap}$. This current, unlike $I_{Nap}$ and $I_{Kp}$, is assumed to be independent of both ion concentration and voltage. (*b*) The excess free calcium within the axon is removed (either by the pump or sequestration) at a rate proportional to the excess.

Thus the change in internal calcium concentration is written

$$dCa_{in}/dt = (I_{Ca} + I_{Cap})/FR - D_{Ca}(Ca_{in} - Ca_{inr}) \tag{26}$$

where $D_{Ca}$ represents the rate of removal of calcium and $Ca_{inr}$, the resting level of $Ca_{in}$.

### *The Membrane Current*

The membrane current is the sum of the contributions of the three ionic systems.

$$C\frac{dV}{dt} = I_{Na} + I_{Nap} + I_K + I_{Kp} + I_{Cltot} + I_{Ca} + I_{Cap} + I_{inj} \tag{27}$$

where $I_{inj}$ is an experimentally injected current.

This equation together with Eqs. 2, 5, 9, 10, 11, 12, 15, 16, 19, 21, 23, 25, and 26, and the Hodgkin-Huxley equations for the rate constants form the basis of the model.

## METHOD

The equations were integrated using the International Mathematical and Statistical Libraries (IMSL)-supplied subroutine DGEAR, which uses Gear's (1971) method for stiff equations. The method is stable and with an analytic Jacobian is between six and eight times faster, and as accurate as, a sixth order Runge-Kutta or a variable order Adams Bashford method.

### *Small Fiber Parameters*

The mammalian C fiber is the only small unmyelinated nerve fiber for which there are sufficient data for parameter estimates to be made. Although I shall use this fiber as a source for some of the parameters it is intended that the model describe a wide range of small nerve behavior and there will be no attempt to make an exact simulation of C fiber behavior.

The average diameter of the C fiber is 0.75 $\mu$M (Keynes and Ritchie, 1965). The width of the periaxonal space is ~ 14.5 nm and labeled potassium leaves this space with a time constant of 150 ms, implying that $D_K$ is 0.1 $\mu$m/s (Greengard and Straub, 1958). The resting membrane potential of the C fiber is not known but an estimate can be made using Mullins and Noda's (1963) version of the

Goldman equation:

$$E_m = \mathcal{R}T/F \ln \left[ \frac{rK_o + eNa_o}{rK_{in} + eNa_{in}} \right] \tag{28}$$

where $e = P_{Na}/P_K$.

Assuming that $P_{Na}/P_K = 0.25$ (Armett and Ritchie, 1963) and $r = 1.5$: $E_m = -40.5$ mV at 20°C. If, as Keynes and Ritchie (1965) suggest, a value of 0.25 for the $P_{Na}/P_K$ ratio is too high, then $E_m$ is probably more negative than −40 mV. The model value of −46.5 mV was thought to be a reasonable (though arbitrary) estimate.

The spike width of the C fiber impulse is 1.25 ms at 37°C (Paintal, 1967), which, if a $Q_{10}$ of 3 is assumed for the rate variables, gives a spike width of 8 ms at 20°C. The spike width generated by the model with the parameters listed in Table I is 5 ms. To reconcile these two values the time constants of the $m$, $n$, and $h$ variables were multiplied by 1.6. The values of the conductances were adjusted to give a net Na and K movement of ~ 1 pmol/cm$^2$ impulse (Keynes and Ritchie, 1965).

### *Calcium Parameters*

No measurements have been made of the calcium parameters in a small unmyelinated fiber and so measurements from the squid axon were used instead. It was assumed that, as in the squid axon, the net

TABLE I
PARAMETERS OF THE MODEL AXON

| Variable | Value | | Source |
|---|---|---|---|
| $C$ | 1 | μF | squid: Hodgkin and Huxley (1952) |
| $\bar{G}_{Na}$ | 54 | mS | Present work |
| $\bar{G}_K$ | 9 | mS | Present work |
| $G_{Cl}$ | 0.07 | mS | Present work |
| $m_s$ | −9 | mV | Present work |
| $h_s$ | −10 | mV | Present work |
| $n_s$ | −20 | mV | Present work |
| $Na_{in}$ | 63.4 | mM | C fiber: Keynes and Ritchie (1965) |
| $Na_o$ | 154.0 | mM | C fiber: Keynes and Ritchie (1965) |
| $K_{in}$ | 144.9 | mM | C fiber: Keynes and Ritchie (1965) |
| $K_o$ | 5.6 | mM | C fiber: Keynes and Ritchie (1965) |
| $T$ | 20°C | | C fiber: Keynes and Ritchie (1965) |
| $R$ | 0.375 | μm | C fiber: Keynes and Ritchie (1965) |
| $\theta$ | 14.5 | nm | C fiber: Greengard and Straub (1958) |
| $D_{Na}$ | 0.1 | μm/s | C fiber: Greengard and Straub (1958) |
| $D_K$ | 0.1 | μm/s | C fiber: Greengard and Straub (1958) |
| $b_1$ | 1 | mM | C fiber: Rang and Ritchie (1968a) |
| $b_2$ | 30 | mM | squid: Mullins and Brinley (1969) |
| $c$ | 0.05 | mM$^{-1}$ | squid: Mullins and Brinley (1969) |
| $r$ | 1.5 | | Thomas (1972) |
| Calcium parameters | | | |
| $Ca_{inr}$ | 100 | nM | squid: Baker (1978) |
| $Ca_o$ | 2.2 | mM | C fiber: Keynes and Ritchie (1965) |
| $G_{Ca}$ | 0.01 | mS | Present work |
| $D_{Ca}$ | 0.001 | μm/s | Present work |
| $K_D$ | 0.01 | μM | Present work |
| $m_{ca}$ | −20 | mV | Present work |
| $h_{ca}$ | 0 | mV | Present work |

calcium movement was about a hundredth of the sodium and potassium movements. The parameters (Table I) were therefore adjusted to give an inflow of ~ 10 fmol/cm$^2$ impulse.

In order to separate the multiple effects of the various pumps and conductances the modeling was done in two stages: first with the pumps active and the calcium-related parameters set to zero, and then with no periaxonal space, the Na-K pumps insensitive to changes in concentration, and the calcium parameters nonzero.

## RESULTS

### *Single Impulse*

Variations in the shape of the single impulse are mainly due to periaxonal potassium accumulation. The decrease in $E_K$ that accompanies the increase in periaxonal potassium slows the repolarization phase and decreases the depth of the hyperpoliarization. If the Na-K pump is sensitive to the increase in $K_{sp}$, the hyperpolarization phase is slightly flatter and prolonged (Fig. 1). The increase in $Na_{in}$ over a single impulse was found to be too small to cause any significant alteration to the pump ratio and to the shape of the spike.

With the parameters as listed in Table I the accumulation of $Ca_{in}$ over a single spike has little effect: the concomitant changes in $G_{KCa}$ are too small to significantly alter the shape of the spike.

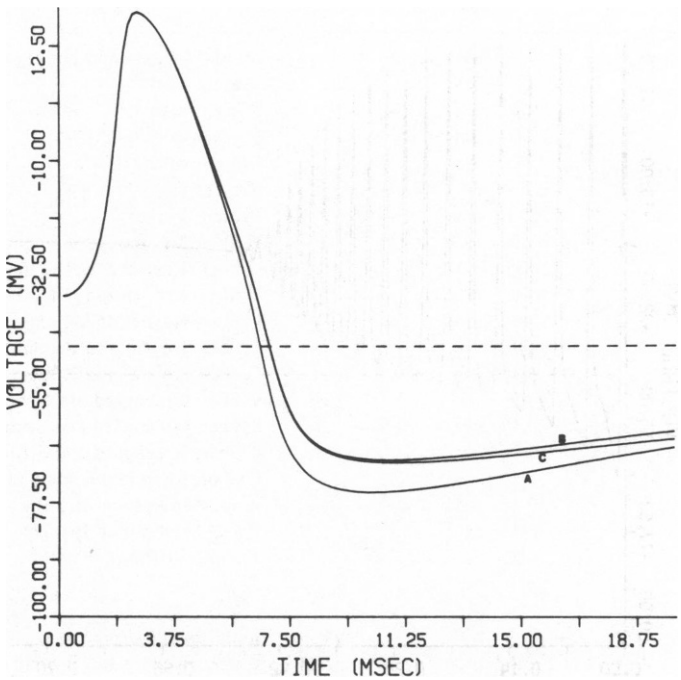


FIGURE 1 Effect of periaxonal potassium accumulation and Na-K pump activity on impulse shape. Curve *A* shows the impulse shape without either periaxonal K accumulation or K-stimulated pump activity. In curves *B* and *C*, ion diffusion is limited by periaxonal space 14.5 nm wide. In curve *C* the pump is sensitive to changes in periaxonal K whereas in curve *B* it is not. The dotted line represents the resting membrane potential.

*Repetitive Firing*

Repetitive firing can be induced with an injected current of 100 nA. With the appropriate choice of $G_{KCa}$ or pump parameters, two phenomena, adaptation and bursting, can be simulated. As before, the Na-K pump and the $G_{KCa}$ are treated separately.

*Adaptation Due to the Na-K Pump*

Adaptation resulting from an increased Na-K pump activity was found to have two causal factors: K accumulation in the periaxonal space and intra-axonal Na accumulation. The effects of these were examined separately.

Periaxonal K accumulation has two opposing effects; decreasing $E_K$, which increases the firing rate, or stimulating the Na-K pump, which decreases the firing rate. The one of these that predominates will depend upon the sensitivity of the pump and the relative size of the potassium and pump currents during the hyperpolarization phase. With the listed parameters, $b \geq 4$ mM always caused adaptation irrespective of the value of $D_K$. For values of $b_1 < 4$mM the firing rate increased with time, an equilibrium being reached if $D_K$ was sufficiently large. If, however, both $b_1$ and $D_K$ are small the nerve fiber fires decremental pulses ever more rapidly until there is a maintained depolarization (Fig. 2). The parameter $b_2$ has little effect on the behavior of this axon because the value of $Na_{in}$ during repetitive firing is much greater than $b_2$ and the receptor is saturated.

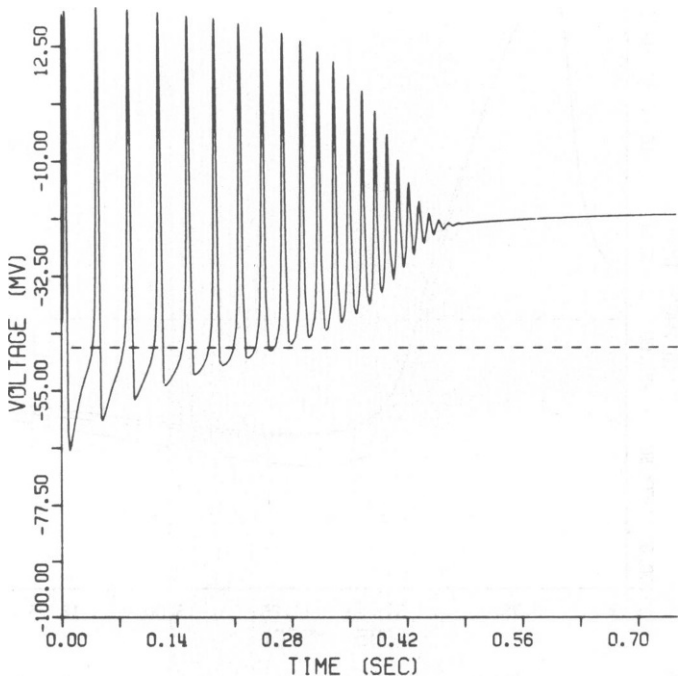


FIGURE 2 Repetitively firing axon ($I_{inj}$ = 100 nA) with a very low rate of diffusion from the periaxonal space. Massive accumulation of potassium within the space results in decremental impulses followed by a maintained depolarization.

Intra-axonal Na accumulation will always cause adaptation if the pump ratio is sensitive to changes in $Na_{in}$($c > 0$). If $c = 0$, however, the axon can fire for long periods with little change in the firing rate. In one simulation, a doubling of $Na_{in}$ occurred with only a 4% increase in the firing rate.

Adaptation in a nerve fiber with both mechanisms working ($c > 0$ $b_1 > 0$) is shown in Fig. 3.

*Adaptation Due to $G_{KCa}$*

Provided that the axon is firing rapidly enough and $D_{Ca}$ is sufficiently small, both $Ca_{in}$ and $G_{KCa}$ will increase with each impulse. However adaptation does not necessarily occur, because the frequency of repetitive firing is dependent upon the rate at which the hyperpolarization phase of the impulse decays, which in turn is dependent upon the values of the currents and their associated conductances. Thus, even though $G_{KCa}$ may be a large proportion of the potassium conductance at rest, increasing $G_{KCa}$ will have no effect on the firing rate if the ratio of $G_{KCa}$ to $\overline{G}_K n^4$ is small when the nerve fiber is hyperpolarized. For increases in $G_{KCa}$ to cause adaptation it is necessary that the resting value of the voltage-dependent K conductance is not small compared with the maximum value of this conductance. The value of the voltage shifts as well as the ratio of $\overline{G}_{Na}$ to $\overline{G}_K$ will therefore determine the sensitivity of the axon to changes in $G_{KCa}$. The parameters in Table I satisfy the above condition and adaptation occurs. Equilibrium is reached when the net movement of calcium per impulse is zero.

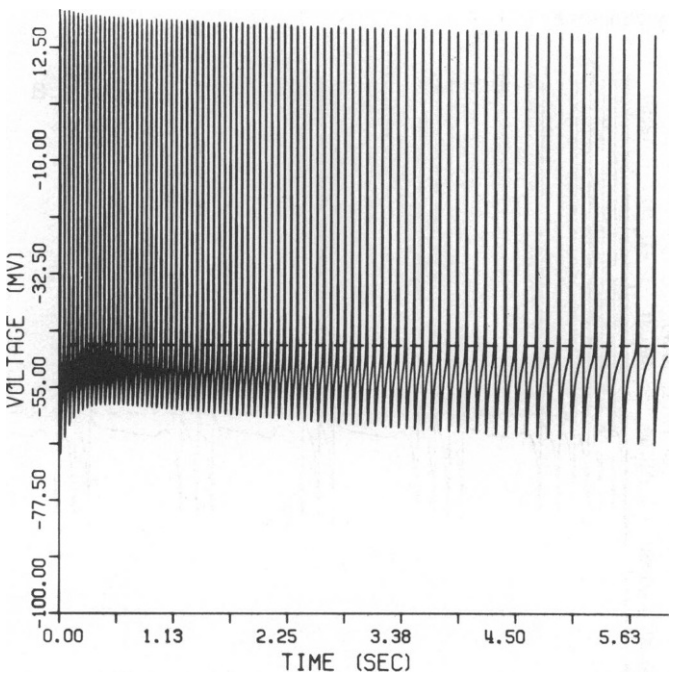


FIGURE 3 Adaptation of a repetitively firing axon due to Na-K pump activity which is stimulated by intra-axonal sodium accumulation and periaxonal potassium accumulation ($I_{inj}$ = 250 nA; pump parameters: $b_1$ = 1 mM, $b_2$ = 30 mM, $c$ = 0.05 $mM^{-1}$).

*Bursting*

Bursting (periods of firing separated by periods of silence) is, in this model, a natural extension of adaptation. If, for instance, the Na-K pump is sufficiently strong to stop the fiber firing, diffusion from the periaxonal space and activity of the pump restores the ionic concentrations to equilibrium. This, in turn, reduces the activity of the pump and allows the nerve fiber to recommence firing. The ion concentrations in the interior and the periaxonal space increase and the cycle is repeated.

Calcium build-up within the nerve fiber causes bursting by a similar mechanism. In this case when $G_{KCa}$ becomes large enough to stop the nerve fiber firing the calcium inflow is decreased and the pump and sequestration mechanisms return $Ca_{in}$ to its base level. This, in turn, reduces $G_{KCa}$ and the nerve fiber starts firing again. Such a mechanism has been proposed to explain bursting in the R15 cell of *Aplysia* (Junge and Stephens, 1973; Gorman and Thomas, 1978) and has been modeled, in a manner different than that described above, by Plant (1978). The behavior of a nerve fiber in which both the Na-K pump and $G_{KCa}$ are active is shown in Fig. 4*a*. The changes in internal Na and K and calcium levels during the burst cycle are shown in Figs. 4*b* and *c*, respectively.

The relationship between burst frequency and spike frequency within the burst was investigated by doing a series of simulations in which the sensitivity of the pump ratio to variations in $Na_{in}$ (*c*) was varied. Since the nerve fiber is switched on and off by the same mechanism, it was no surprise to find that there is a linear relationship between the burst frequency and the spike frequency within the burst (Fig. 5). The mean frequency was, therefore, virtually constant.

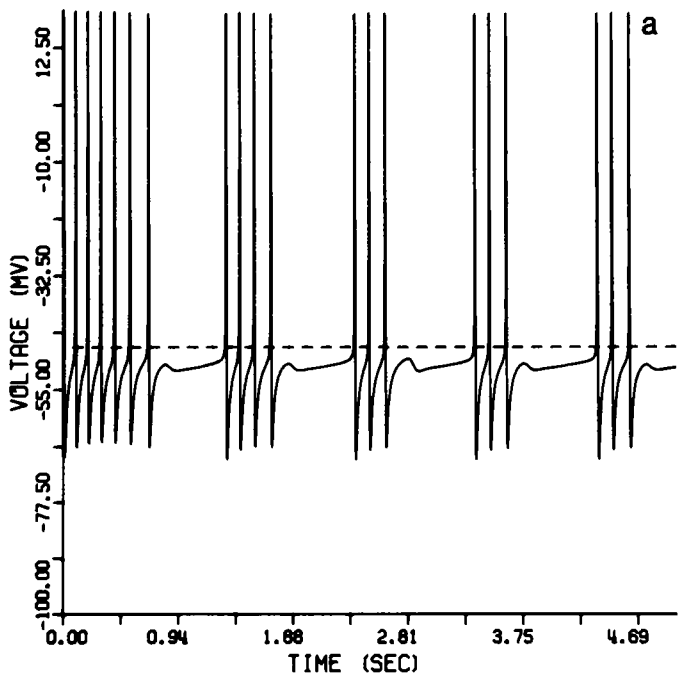


FIGURE 4 Continued on next page.

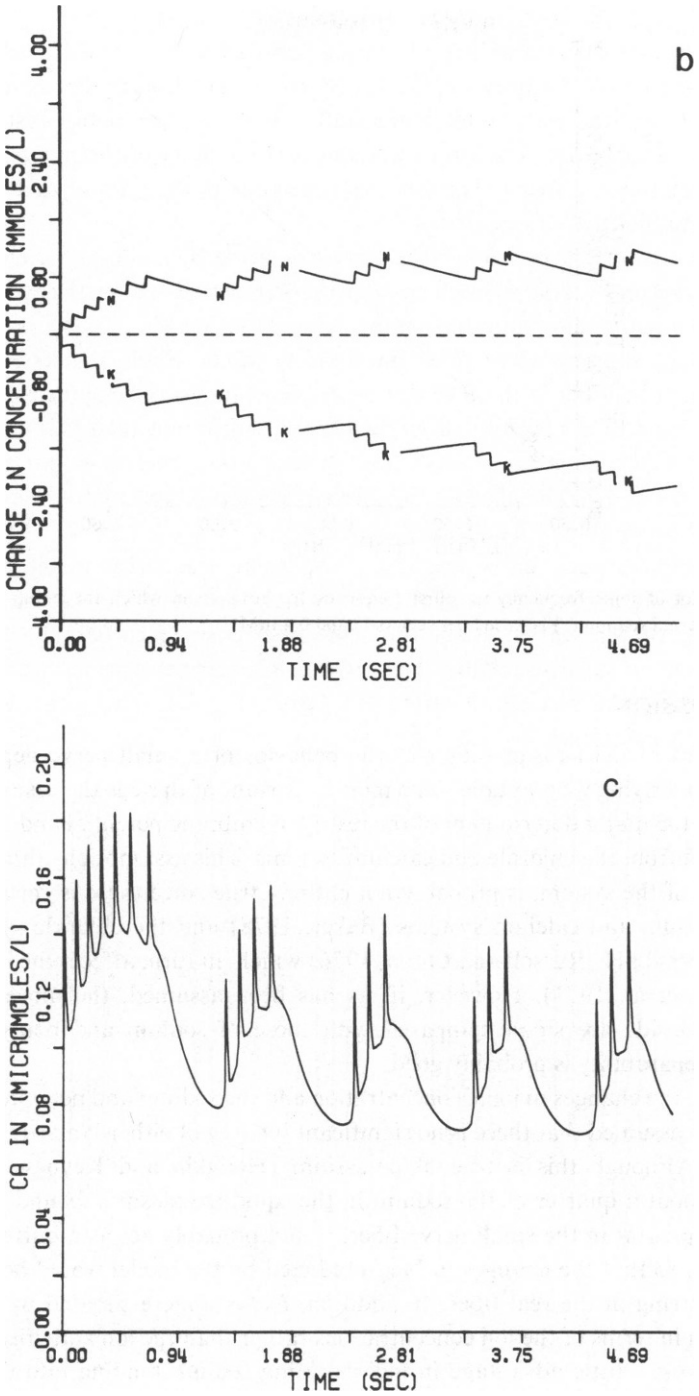


FIGURE 4 (*a*) Bursting due to combined Na-K activity and cyclic changes in $G_{KCa}$. ($I_{inj}$ = 100 nA; $b_1$ = 1 mM, $b_2$ = 30 mM, $c$ = 0.05 $mM^{-1}$, proportion of $G_K$ that is calcium dependent: 25%, $D_{Ca}$ = 0.001 $\mu m/s$.) (*b*) Changes in the internal Na and K concentrations of the axon shown in Fig. 4*a*. (*c*) Changes in the internal calcium concentration of the axon shown in Fig. 4*a*.

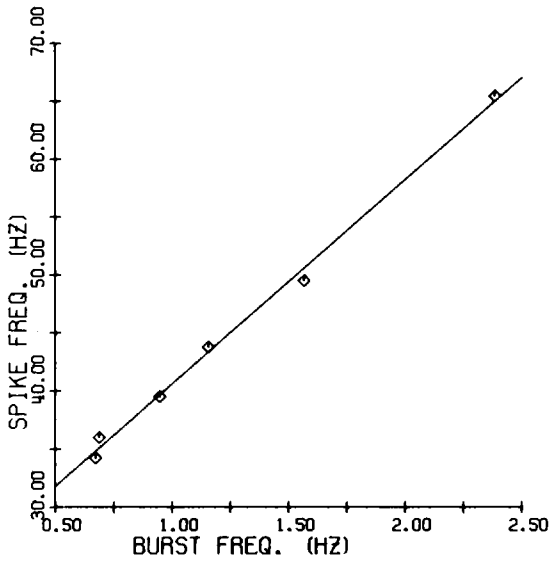


FIGURE 5 Plot of spike frequency vs. burst frequency for an axon in which the pump sensitivity to changes in internal sodium ($c$) is varied between 0.05 and 0.4 $mM^{-1}$.

## DISCUSSION

The value of this model as a predictor of the behavior of a small nerve depends upon the validity of its underlying assumptions. The most important of these is the assumption that the Na-K system is the major determinant of the resting membrane potential and that this system can be separated from the chloride and calcium systems. This assumption, although necessary for the analysis of the system, is probably not entirely true since there is certainly some link between the sodium and calcium systems (Baker, 1978) and the chloride system probably controls the internal pH (Russell and Boron, 1976) which, in turn, affects the levels of ionized calcium (Baker et al. 1971). However, if, as has been assumed, the movements of ionic calcium and chloride are small compared with those of sodium and potassium then the assumption of separability is probably good.

In calculating the changes in ionic concentration and the sodium and potassium equilibrium potentials it was assumed that there is no significant binding of either Na or K to molecules in the axoplasm. Although this is true of potassium (Hodgkin and Keynes, 1953) there is evidence that about a quarter of the sodium in the squid axoplasm is bound (Hinke, 1969). This system, if present in the small nerve fiber, would probably act as a buffer against large changes in $Na_{in}$, so that the changes in $Na_{in}$ predicted by the model would be more extreme than those occurring in the real fiber. In addition, $E_{Na}$ is underestimated by expressing the Nernst equation in terms of the ion concentrations rather than the ion activities.

There is, however, little advantage in incorporating sodium binding into the model since nothing is known of the binding kinetics of Na to its carrier molecule and any equations describing this are entirely speculative and have little predictive value. Also, as has been shown, the behavior of the model is only weakly dependent on $E_{Na}$ so small errors in this parameter should have little effect.

The formulation of the chloride system is incomplete. Eq. 19 implies that if the system is at rest, any change in $G'_{Cl}$ or $E_{Cl}$, no matter how large, would not affect the equilibrium of the system; a prediction which is almost certainly false. The problem can be solved by altering two of the assumptions made in deriving Eq. 19. If $G_{Cl}$ *is* dependent on voltage and $E_{Cl}$ is *not* constant then Eq. 19 becomes

$$I_{cltot} = (G_{Cl} + k_1)(V_{eq} - V) + G_{Cl}E_{Cl} - G_{Cleq}E_{Cleq} \tag{19a}$$

where $G_{Cleq}$ and $E_{Cleq}$ are the values of $G_{Cl}$ and $E_{Cl}$ when the system is at rest and $V = V_{eq}$. Eq. 19a is nonzero if either $E_{Cl}$ or $G_{Cl}$ are changed when $V = V_{eq}$. Although Eq. 19a is probably a better description of the chloride system than Eq.19 it requires an accurate knowledge of the voltage dependence of $G_{Cl}$ and the way in which $I_{Clp}$ depends upon $E_{Cl}$. Since this information is either unknown or not accurately determined, any increase in accuracy obtained by using Eq. 19a is lost in the arbitrary choice of the unknown parameters.

The lack of available data makes the section of the calcium system highly speculative. However, since its effects are mediated solely through $G_{KCa}$, any system that gives calcium accumulation with an exponential decay (Gorman and Thomas, 1978) would work equally well (see Plant, 1978, for example).

This model is thus an approximation to a highly complex system. Despite its limitations, the results show that it is possible to simulate a wide range of nerve behavior with a few simple modifications to the Hodgkin-Huxley model. Although this model is designed as a general description of the small unmyelinated nerve fiber it is not implied that all of the effects, especially the calcium-related behavior, are present in all small unmyelinated nerve fibers.

The model is currently being used to investigate a number of phenomena, among which are; the postactivity hyperpolarization, the effect of temperature changes on the resting membrane potential, and the effect of temperature change on repetitive firing.

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