

THE EFFECTS OF IONIC CHANNEL DENSITY ON NEURONAL FUNCTION

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Abstract

The effect of channel density on the stability of the membrane potential and the response to applied currents is examined using numerical solutions of the Hodgkin–Huxley equations. Autorhythmicity can be induced by a reduction in \bar{g}_K or an increase in \bar{g}_{Na} : as \bar{g}_K is reduced there is a subcritical Hopf bifurcation, and solutions jump from a stable fixed point to large amplitude periodic solutions. In response to strong depolarizing currents there is a supercritical Hopf bifurcation: as the current density is increased the amplitude of stable periodic responses decreases until they vanish into a stable fixed point. The current density at which this occurs is determined by the Na^+ -channel density.

1. Introduction

The electrical activity of a neurone is a consequence of its intrinsic properties—membrane conductances that may be spatially nonuniform and the electrotonic geometry of the cell—and extrinsic synaptic and ephaptic inputs on to the cell. In this paper we will only consider the intrinsic electrical properties of neurones and their response to applied currents.

The integrative functions of a neurone result from its electrotonic geometry: the theory of electric current flow in the extensively branched structures that characterise neurones is well developed and is reviewed in Jack *et al.* (1975). Whatever the geometry, the system of partial differential equations for the spread of voltage with distance and time will be linear if the membrane is passive or has a linear current–voltage relation. Thus the interesting, information processing functions of a neurone are determined by the nonlinear current–voltage relationships that define excitable membranes.

Although there are ionic fluxes across the membrane that are coupled to metabolism, these active pumps have a low maximal transport rate, and so cannot

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generate the large or fast ionic currents that produce excitation. The only roles of the active pumps in the process of excitation are to maintain and regulate the intra- and extracellular ionic activities, and perhaps to contribute to small and slow deviations in the membrane potential from its value determined by electrodiffusion. Membrane excitation requires the presence of at least two types of ion-selective membrane conductance, with different reversal potentials, and at least two types of voltage-controlled gating mechanism. A transient change in membrane potential, such as that of the action potential, is produced by changes in the specific membrane conductances, some of which are controlled by potential dependent gating mechanisms.

A number of different membrane conductances have been described in axonal and neuronal membranes: each conductance can be identified by its ion-selectivity, voltage-dependence, kinetics and pharmacology. Given maintained intra- and extracellular activities the ion-selectivity of a conductance determines its reversal potential. Different membranes contain different conductances: the squid giant axon contains voltage-dependent, Na^+ - and K^+ -selective conductances g_{Na} , g_K , and a voltage-independent leakage conductance g_L ; the nodal membrane of mammalian myelinated axons lacks a g_K (Chiu *et al.*, 1979), and six different cation-selective conductances have been described in the somatic membrane of molluscan giant neurones (Adams *et al.*, 1980). Many somatic and dendritic conductances are poorly defined; however, all specific conductances that have been examined in detail appear to be systems of discrete macromolecular specialisations, or channels, that occur with a density that can be spatially nonuniform.

A membrane conductance is a process, defined by the relation between ionic currents and electrochemical gradients—for example, the K^+ -current I_K in squid axon is given by $I_K = g_K(V - V_K)$: the K^+ -selective conductance g_K is defined by measurements of potential V , current that is normally carried by K^+ ions, I_K , and the Nernst potential for K^+ , V_K . The membrane offers a formidable barrier to diffusion of ions: the $10 \mu m$ lipid membrane has a resting resistance of $\sim 10^3 \Omega cm^2$, while a $10 nm$ thick layer of extracellular fluid with a resistivity of $\sim 100 \Omega cm$ would offer a resistance of $10^{-4} \Omega cm^2$. However, the temperature-dependence of the maximal K^+ and Na^+ conductances is low, with a Q_{10} of 1.5 for the squid giant axon (Moore, 1958): this is only slightly higher than the Q_{10} of 1.3 for aqueous diffusion. Thus the major barrier to ions crossing a membrane is an entropy, rather than enthalpy barrier: the simplest explanation is that ions cross the membrane at sparsely distributed ‘pore-like’ channels. Conti and Wanke (1975) have reviewed the evidence for the presence of different populations of sparsely distributed ionic channels in excitable membranes, with single channel conductances of the order of pS and densities ranging from < 5 to $10^4/\mu m^2$. Signals that may be identified with the current flowing through single ionic channels have been recorded (Conti and Neher, 1980), and structures that might correspond to single channels have been observed in the membrane of myelinated axons using freeze fracture methods (Rosenbluth, 1976). Stevens and Tsien (1979) have reviewed current concepts of single channel function.

Each channel may exist in two distinct macrostates—open, and fully conducting, with a single channel conductance $\gamma(V)$, and closed, with a resting conductance γ_0 that may be set to zero without any loss of generality. The transition between the open and closed macrostates is controlled by one or more variables that may or may not be independent: for example, the Hodgkin–Huxley (H–H) model (Hodgkin and Huxley, 1952) for the sodium conductance is controlled by three independent activation variables (m) and an independent inactivation variable (h). The activation and inactivation variables have voltage-dependent kinetics.

These activation and inactivation variables may be considered to represent gating processes within the membrane that control the channel conductance: for the gating processes to be voltage-dependent the voltage sensors must be charged or have a dipole moment: thus there should be small ‘gating currents’ or charge displacement currents associated with the opening and closing of membrane channels. Asymmetric displacement currents with some of the characteristics expected for ‘gating currents’ have been measured, and their relation to gating currents reviewed in Almers (1978).

The simplest channel would have no gating variables: an example is the constant, leakage conductance g_L of the H–H equations. The H–H K^+ -conductance is controlled by four activation variables (n): conductances with only one activation variable/channel are found in cardiac muscle.

In this paper we will consider the effects of channel density when there are no interactions between neighbouring membrane channels: such interactions have been postulated (e.g. Holden and Rubio, 1978) and will be considered elsewhere (Holden, 1981).

2. Estimates of channel densities

For a specific membrane conductance that is produced by a population of identical, independent channels, each of which can be open, with a single channel conductance γ pS, and closed, with a zero conductance, the maximal conductance \bar{g} mS cm^{-2} is the product of the single channel conductance γ and the channel density ρ cm^{-2} :

$$(1) \quad \bar{g} = \gamma\rho$$

If channel density or conductance is estimated using equation (1) the estimated \bar{g} is assumed to occur when all the membrane channels are fully open: the possibility of ‘silent channels’ (Romey *et al.*, 1980) or incomplete activation is ignored. The specific channel density can be estimated directly, or via estimates of single channel conductance.

The neurotoxins tetrodotoxin (TTX) and saxitoxin (STX) block Na^+ -channels at nM concentrations: the kinetics of TTX binding to nodal membrane are consistent with one molecule of TTX blocking each channel, and so TTX binding (Moore *et al.*, 1967; Keynes *et al.*, 1971) and ^3H -TTX and ^3H -STX binding (Colquhoun *et al.*, 1972) can be used to estimate an upper limit for Na^+ -channel

density. Assuming no non-specific binding, Na^+ -channel density is of the order $1\text{--}10^4$: see Table 1. Among nonmyelinated axons, giant axons have an Na^+ -channel density 10 times that of smaller axons. Mammalian myelinated axons

Table 1
 Na^+ -channel density in μm^{-2} estimated by toxin binding

Preparation		Reference
Lobster leg nerve	<13	Moore <i>et al.</i> (1967)
Crab leg nerve	49	Keynes <i>et al.</i> (1971)
Lobster leg nerve	75	
Rabbit vagal nonmyelinated	36	Colquhoun <i>et al.</i> (1972)
Rabbit vagal nonmyelinated	27	
Garfish olfactory	2.5	Ritchie <i>et al.</i> (1976)
Lobster leg nerve	16	
Rabbit vagal nonmyelinated	100	Keynes <i>et al.</i> (1975)
Lobster axon	90	
Garfish olfactory nerve	35	Strichartz <i>et al.</i> (1979)
Squid giant axon	200	
Squid giant axon	170	Ritchie and Rogart (1977)
Mammalian myelinated (at node)	12000	
Mammalian myelinated (internode)	28	

illustrate what might be an upper limit to Na^+ -channel density, produced by restraints on the packing of channels, and extreme spatial nonuniformity of Na^+ -channel density, ranging from $25\mu\text{m}^{-2}$ for internodal membrane to $10^4\mu\text{m}^{-2}$ for nodal membrane. In myelinated axons, counts of intramembraneous particles seen using freeze fracture techniques and electron microscopy might provide direct measures of Na^+ -channel density (Rosenbluth, 1976).

K^+ -channel density cannot be directly estimated: however, from the kinetics of tetraethylammonium (TEA) block of K^+ channels Armstrong (1974) estimated γ_K as 2–3 pS; this gives a density of $\sim 100\mu\text{m}^{-2}$ for squid axonal membrane.

The ionic channels of excitable membranes are voltage-dependent, and so are controlled by ‘gating processes’ that have voltage sensors: in response to a change in potential the action of the sensor must entail charge movement or dipole rotation, both of which would generate a strongly voltage-dependent displacement current. If the g_{Na} is controlled by charged particles with a total charge q , then the slope of $g_{\text{Na}}-V$ will depend on q via Boltzmann’s principle:

$$(2) \quad \frac{d}{dV} \ln\{g_{\text{Na}}/(\bar{g}_{\text{Na}} - g_{\text{Na}})\} = q/kT,$$

where k is Boltzmann’s constant and T the absolute temperature. For $g_{\text{Na}} \ll \bar{g}_{\text{Na}}$, g_{Na} grows exponentially with V , with an e -fold change in 4 mV: thus $q \sim 6$. Measurements of asymmetric displacement current (or gating current), together with estimates of q , provide a measure of channel density: the total charge moved

(the integral of the gating current) should be $q\rho$. However, not all asymmetric displacement charge need be Na^+ -channel specific and the value taken for q assumes that the sensor experiences the full membrane potential, and so is likely to be an underestimate. Bezanilla and Armstrong (1974) and Keynes and Rojas (1976) used this method to estimate the Na^+ -channel density for squid axons to be 2–300 and $400 \mu\text{m}^{-2}$. Gilly and Armstrong (1980) have described a slow component of asymmetric displacement current that appears to be a K^+ -gating current: the total charge ranged from $118\text{--}412 e^- \mu\text{m}^{-2}$ —with a channel valency of 5–6 or $13 e^-/\text{channel}$ this gives K^+ -channel densities for squid axon from $9\text{--}82 \mu\text{m}^{-2}$. Thus, in spite of the uncertainty inherent in their assumptions, estimates of channel density obtained from measurements of asymmetric displacement currents are of the same order of magnitude as estimates obtained from channel blockers.

The analysis of fluctuations in membrane current provides an indirect route of estimating channel density, via an estimate of single channel conductance: see Conti and Wanke (1975). Although the equations for the spectral densities of fluctuations that are generated by systems of gated conductances are complicated (see for example, Hill and Chen, 1972) the estimation of γ is straightforward and model-independent. For a system of M independent and identical channels, each of which has a conductance γ or 0 and a probability p of being open, the average membrane conductance

$$E(g) = Mp\gamma$$

and the variance of the conductance

$$\sigma_g^2 = \gamma^2 Mp(1 - p).$$

Thus, irrespective of the details of the kinetic model for the channel, the single channel conductance can be estimated from

$$(3) \quad \begin{aligned} \sigma_g^2/E(g) &= \gamma(1 - p) \\ &\cong \gamma, p \ll 1. \end{aligned}$$

Estimates of γ_{K} and γ_{Na} obtained by fluctuation analysis are given in Table 2, together with the resultant densities.

The patch clamp method permits the measurement of single-channel currents (Conti and Neher, 1980) and hence the direct calculation of γ : single channel K^+ conductances estimated by this method are 9–11 pS, and give K^+ -channel densities for the squid giant axon of $36 \mu\text{m}^{-2}$.

Although there are quantitative differences in the different estimates of channel densities for the same tissue, it is clear that specific ion channel density can vary over four decades, and that there can be large spatial nonuniformities in ion channel density over the surface of a neurone and its process. It can be anticipated that descriptions of the electrical properties of somatic and dendritic membranes, with their wealth of conductance systems, will lead to estimates of channel densities.

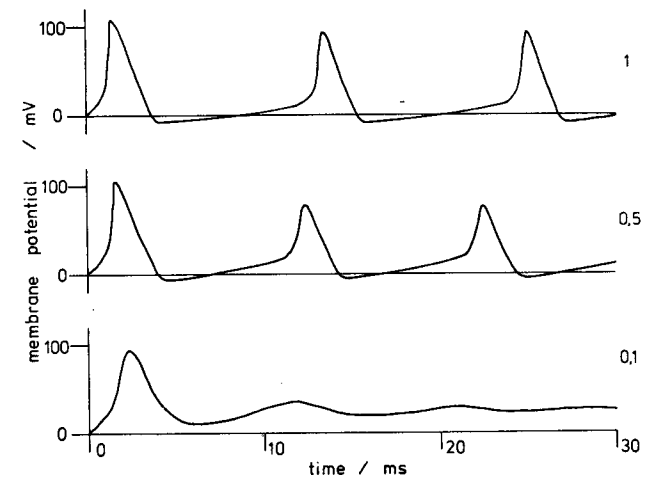


Fig. 1. Solutions of the Hodgkin–Huxley membrane equations at 6.3°C to a maintained current density of $20 \mu\text{A cm}^{-2}$ when the total membrane conductance is scaled by $\eta = 1, 0.5$ and 0.1 .

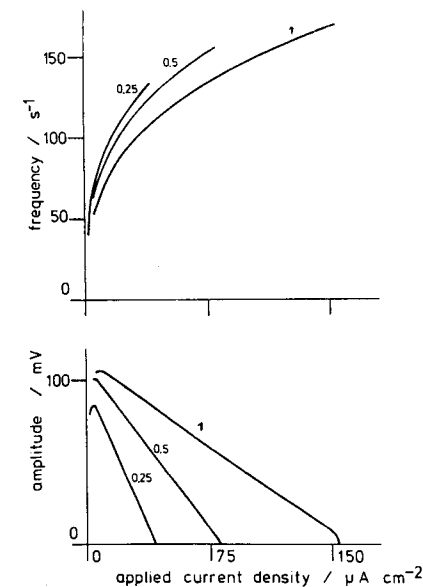


Fig. 2. Frequency and peak-to-peak amplitude of stable periodic solutions of the Hodgkin–Huxley membrane equations at 6.3°C to an applied current density, when the total membrane conductance is scaled by $\eta = 1, 0.5$ and 0.25 .

In this paper we consider some of the consequences of change in the densities of independent channels and suggest that ion channel density may be used to control the excitability and behaviour of excitable cells during development, growth and maturity.

3. Nonspecific changes in channel density and membrane responses

The maximal conductances of the H-H equations \bar{g}_K , \bar{g}_{Na} and \bar{g}_L can be considered as the products of single channel conductances and specific channel densities: thus an effect of nonspecific change in channel density would be to multiply all the conductances by a factor η : since $C_m dV/dt = -I_i(V)$, scaling the conductances and hence current would simply scale dV/dt . Membranes that generate fast action potentials have high channel densities: action potential duration decreases as channel density increases.

However, a change in channel density would also change any gating currents associated with the conductances: the effect of an increase in the density of 'gates' is a voltage-dependent increase in C_m . Adrian and Almers (1976) have described such a voltage-dependent membrane capacitance for skeletal muscle fibres, with the capacitance increasing with depolarization to a maximum. As an approximation, the voltage-dependent portion of membrane capacitance may be treated as an additional constant gating capacitance, so that a change in channel density changes the membrane capacitance by a factor Γ . Thus the general equations for an isopotential membrane may be modified to

$$(4) \quad \Gamma C_m \frac{dV}{dt} = -\eta I_i(V),$$

where both Γ and η increase with increasing channel density. If the Na^+ activation gating current is the major asymmetric displacement current, then for the squid axon with a 'resting' capacitance of $1 \mu F cm^{-2}$, Na^+ -channel density of $160 \mu m^{-2}$ (Strichartz *et al.*, 1979), and gating charge of $6 e^-/channel$, the maximal additional capacitance associated with Na^+ channel gating would be $0.15 \mu F cm^{-2}$. Thus doubling the density could be mimicked by a small increase in conductances alone.

Fitzhugh (1973) has considered the application of Huxley's (1959) dimensional analysis to excitation of a uniform membrane patch: although dimensional analysis simplifies the analysis of the effects of channel density on the H-H cable equations, effects on the membrane equations are best investigated by straight forward numerical solutions.

Solutions of the H-H equations to a maintained current density of $20 \mu A cm^{-2}$ at $6.3^\circ C$, for $\eta = 1, 0.5$ and 0.1 are shown in Fig. 1. With $\eta = 1$, the standard H-H membrane equations exhibit periodic solutions to a maintained current density J , with $6.2 < J < 154 \mu A cm^{-2}$ (see Fig. 2). Hassard (1978) has applied Hopf bifurcation theory to examine the behaviour at the bifurcation point J_2 just greater than $154 \mu A cm^{-2}$; a Hopf bifurcation occurs from a steady-state to small amplitude periodic solutions. Periodic solutions of the H-H membrane equations are investigated in Troy (1976, 1978*a,b*) and Rinzel (1978).

At $20 \mu A cm^{-2}$ and $\eta = 1$ (standard H-H membrane) there is a periodic solution of action potentials at a rate of $87 s^{-1}$: reducing η to 0.5 gives a higher rate of smaller amplitude action potentials, and at $\eta = 0.1$ the maintained response is a steady state. The effect of a reduction in η from 1 to 0.5 and 0.1 on the amplitude and frequency of periodic solutions to maintained current densities is shown in Fig. 2: as η is reduced, the membrane is more excitable, in that the threshold current for a maintained response falls slightly and the frequency is higher at any current density that supports periodic solutions. However, as η is reduced, the amplitude of the periodic solutions decreases faster with current density and the upper Hopf bifurcation occurs at a lower current density. Holden and Ramadan (1981) interpret the membrane current density, J_2 , at which the upper Hopf bifurcation from a depolarized steady state into small amplitude periodic solutions occurs, as an index of the membrane channel density: in the giant soma of a molluscan neurone this bifurcation occurs at $J_2 = 10 \mu A cm^{-2}$, suggesting a low channel density consistent with the high membrane resistance of $2.4 \times 10^4 \Omega cm^2$ and the slow ($< 50 V s^{-1}$) rate of rise of the action potential.

The block of repetitive firing seen at a current density near J_2 may be relevant to the functional behaviour of neurones. Granit and Philips (1956) noted a depolarizing block of repetitive activity in Purkinje cells of the cat cerebellum, and suggested that it is a normal mechanism for blocking excessive excitation produced by powerful climbing fibre inputs. However, in the α -motoneurone such a depolarizing block is pathological, and in normal behaviour is avoided by the adaptation of discharge rate to maintained inputs, the prolonged after hyperpolarization seen in the motoneurone and the recurrent inhibitory feedback via Renshaw cells. This is consistent with the motoneurone discharge rate controlling motor unit tension.

The amplitude and maximum rate of rise of the action potential produced by brief current pulses decrease as η is decreased—see Fig. 1 of Sabah and Leibovic (1972), when $\Gamma = \phi = 1$ and $\beta = \Gamma/\phi$, with ϕ scaling the rate coefficients. The reduction in the regenerative ability of the membrane that is produced by a decrease in η blurs the distinction between sub- and supra threshold responses: the amplitude of the response grades with stimulus intensity. Such a loss of all-or-none behaviour is also seen if $\eta = \Gamma = 1$ and ϕ is increased: this mimics the effect of temperature on solutions of the H-H equations. Such 'partial action potentials' have been obtained from squid axons at high temperature (Cole *et al.*, 1970). Sabah and Leibovic (1972) comment that a reduction in channel density, as illustrated in Fig. 1, could permit 'partially regenerative' signals that are neither simply electrotonic nor genuine all-or-none action potentials: such signals might be of importance in dendritic functioning. Direct voltage-clamp analyses of dendritic and somatic membrane are beginning to show that, although channel densities may be reduced, dendritic membranes also often have a richer variety of conductance mechanisms than axonal membrane.

4. Specific changes in channel density: autorhythmicity and membrane response

An excitable membrane contains at least two, and often several, ion-selective conductance pathways. Each conductance pathway is characterized by its selectivity, reversal potential, pharmacology and voltage-dependent kinetics, and often corresponds to the activity of a homogeneous population of gated membrane channels. Each channel is some kind of macromolecular structure, perhaps an aggregate of subunits, and is synthesized (and inserted) into the membrane matrix by specific mechanisms. Nonspecific changes in channel density could be produced by changes in the rate of synthesis of membrane matrix: changes in channel synthesis, where each type of channel is produced by separate mechanisms, would be likely to result in differential or specific changes in channel density.

The detailed properties of an excitable membrane result from the membrane conductances: the wide range of action potential shapes that are seen in different tissues reflects the variety of membrane conductances. Zeeman (1972) has emphasized that the minimal requirements for excitability are a fast excitation or depolarizing process and a slower recovery process: thus the H-H equations may be separated into fast (V, m) and slow (n, h) processes. Although specific changes in channel densities could be used to account for exotic changes in action potential shapes (for example, the different degrees of rate-independent broadening of molluscan potentials could be the result of different K^+ - or Ca^{2+} -channel densities; see Aldrich *et al.* (1979)), the simple effects of specific channel density on excitability may be explored by changing the densities for the depolarizing and repolarizing processes separately. In the H-H equations this means changing \bar{g}_K and \bar{g}_{Na} independently.

DeHaan and DeFelice (1978) have computed the effect of lowering \bar{g}_K on the small-signal membrane impedance and the response to a brief depolarizing stimulus. As \bar{g}_K is reduced the resonant peak of the impedance is increased and the membrane responds with a repetitive discharge to a brief shock. Thus a reduction in \bar{g}_K has increased the membrane excitability and can lead to autorhythmicity. Holden and Yoda (1981) have shown that the K^+ -channel density can act as a bifurcation parameter and control membrane autorhythmicity: as \bar{g}_K is reduced, the resting membrane potential becomes unstable and there is a range of \bar{g}_K ($3.84 < \bar{g}_K < 19.76 \text{ mS cm}^{-2}$) within which the modified H-H equations at 6.3°C have only periodic solutions. The frequency and amplitude of these periodic solutions are shown in Fig. 3 as a function of \bar{g}_K : if γ_K is taken to be 2 pS the standard H-H \bar{g}_K of 36 mS cm^{-2} corresponds to a channel density of $180 \mu\text{m}^{-2}$. For \bar{g}_K just below the lower, and just above the upper bifurcation points it is possible to obtain both large amplitude periodic solutions or a stable steady-state solution: such behaviour is illustrated in Fig. 4 at $\bar{g}_K = 19 \text{ mS cm}^{-2}$ at 18.2°C . These different maintained solutions are reached from different initial conditions.

The upper bifurcation of the H-H equations to a maintained current density ($J \approx 154 \mu\text{A cm}^{-2}$ in Fig. 2 with $\eta = 1$) is a supercritical bifurcation—the stable fixed point, corresponding to a depolarized membrane potential, loses its stability at a density J_2 as the current density is decreased, and bifurcates into stable small

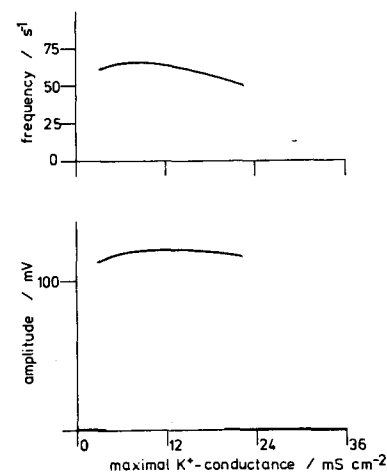


Fig. 3. Frequency and peak-to-peak amplitude of stable periodic solutions of the Hodgkin-Huxley membrane equations at 6.3°C as \bar{g}_K is changed from 0 to its standard value of 36 mS cm^{-2} .

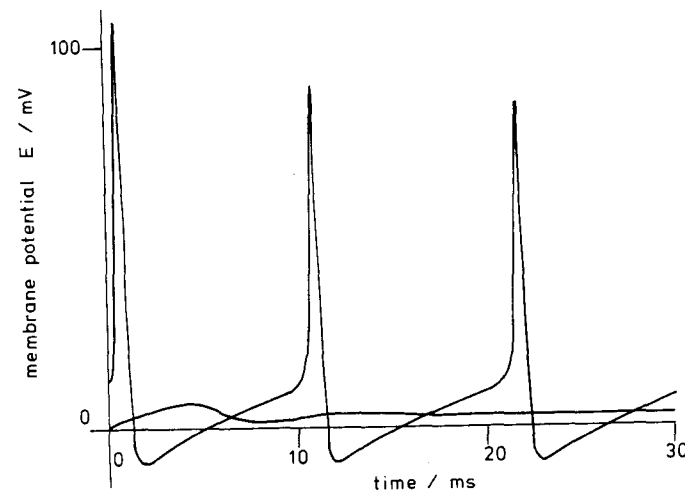


Fig. 4. Solutions of the Hodgkin-Huxley membrane equations at 18.2°C with $\bar{g}_K = 19 \text{ mS cm}^{-2}$ with initial conditions of $E = 0$ and 12 mV .

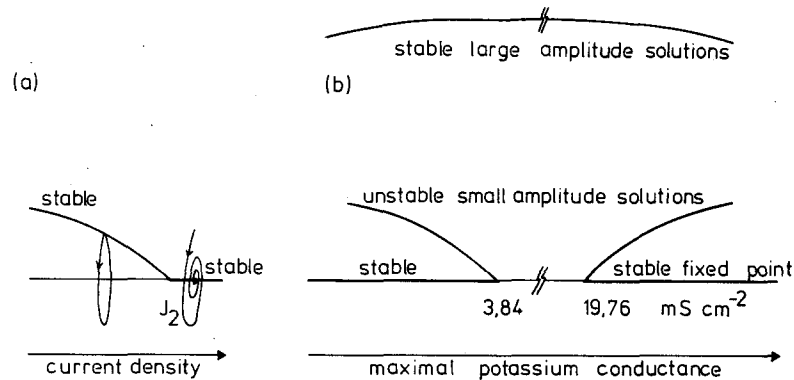


Fig. 5. (a) A supercritical bifurcation occurs at J_2 as the membrane current density is increased. For current densities $< J_2$ there are stable, small amplitude periodic orbits around unstable fixed point solutions; for current densities $> J_2$ trajectories spiral into stable fixed point solutions. (b) Subcritical bifurcations occur at \bar{g}_K near 3.84 and 19.76 mS cm^{-2} for the Hodgkin-Huxley membrane equations at 6.3°C . Between these critical conductances the only stable solutions are large amplitude periodic solutions corresponding to an autorhythmic discharge of action potentials. For \bar{g}_K just < 3.84 and just > 19.76 stable fixed point and large amplitude periodic solutions can be found, as in Fig. 4.

amplitude orbits around an unstable fixed point. As the current density is reduced further ($6.2 < J < 154 \mu\text{A cm}^{-2}$) the small stable periodic solutions grow into large amplitude solutions that correspond to a repetitive discharge of action potentials.

Evaluation of the eigenvalues and characteristic μ_2 (see Marsden and McCracken, 1976) show that, as \bar{g}_K is reduced, a subcritical Hopf bifurcation occurs: the stable fixed point bifurcates into small amplitude unstable orbits. Numerical solutions, obtained using the Euler method of integration, jump to large amplitude, periodic solutions.

The effects of \bar{g}_K on the amplitude and frequency of periodic solutions of the H-H equations to a maintained current density are illustrated in Fig. 6. As \bar{g}_K is reduced, the frequency of periodic solutions is increased and the current density at the upper bifurcation at J_2 is reduced. A reduction in \bar{g}_K permits the peak of the action potential to approach closer to V_{Na} but reduces the afterhyperpolarization: thus at low current densities the peak-to-peak amplitude of periodic solutions is increased, while it is decreased at higher currents.

A reduction in K^+ -channel density can lead to autorhythmicity: excitable membranes that lack K^+ -channels, such as the nodal membrane of mammalian myelinated fibres, are stabilized by a high leakage conductance. A reduction in the channel density for conductances with reversal potentials close to the resting membrane potential, and with the slow kinetics characteristic of recovery processes, will produce similar effects. A reduction in \bar{g}_K has little effect on the bifurcation point J_2 for maintained responses to applied currents: the large change in J_2

produced by a nonspecific decrease in membrane conductance seen in Fig. 2 is mainly due to the reduction in \bar{g}_{Na} .

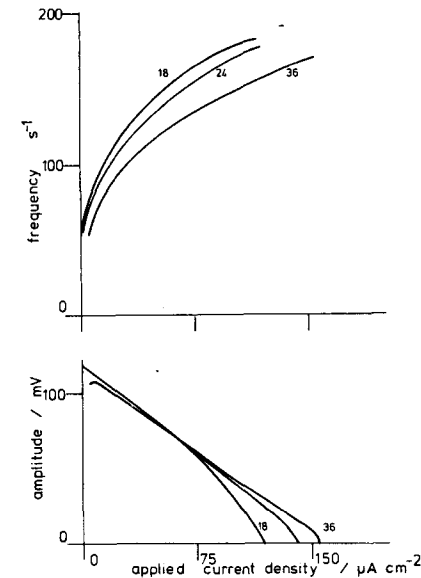


Fig. 6. Frequency and peak-to-peak amplitude of periodic solutions of the Hodgkin-Huxley membrane equations at 6.3°C to an applied current density, with $\bar{g}_K = 18, 24$ and 36 mS cm^{-2} .

The effects of \bar{g}_{Na} on repetitive activity are illustrated in Fig. 7: doubling the sodium channel density gives an autorhythmic discharge, but there is only a small increase in the discharge rates produced by maintained currents. The most pronounced effect is on the value of the membrane current density, J_2 , at which the upper Hopf bifurcation occurs, from a steady-state to small amplitude stable oscillations.

The effects of changes in Na^+ - and K^+ -channel density are antagonistic: however, they should be considered separately rather than jointly, as in Pickard's (1977) use of $\mu = g_{\text{Na}}/g_K$, as the effects on membrane excitability are partly nonspecific and synergistic, by a change in r_m , and partly specific and antagonistic.

In an isopotential membrane, the rate of rise of the action potential is $dV/dt = -I_i/C_m$: it is proportional to the total inward ionic current. At the maximal rate of rise dV/dt_{max} , the predominant current in the H-H equations is I_{Na} , so dV/dt_{max} can be used as an index of I_{Na} , and hence \bar{g}_{Na} :

$$(5) \quad \frac{dV}{dt}_{\text{max}} = (I_L + I_K)/C_m - \{m_1^3 h_1 (V_1 - V_{\text{Na}})/C_m\} \bar{g}_{\text{Na}},$$

where m_1, h_1 are estimated at V_1 where dV/dt is maximal. Even if $(I_L + I_K) = 0$, dV/dt_{max} is not proportional to \bar{g}_{Na} and channel density (Strichartz and Cohen, 1978), and so cannot be used as a measure of sodium channel density. However,

during the rise of the action potential, the membrane potential trajectory is dominated by the sodium current and so the threshold behaviour of the H-H system may be examined by considering a reduced (V , Na^+ -activation) system as in Fitzhugh (1961).

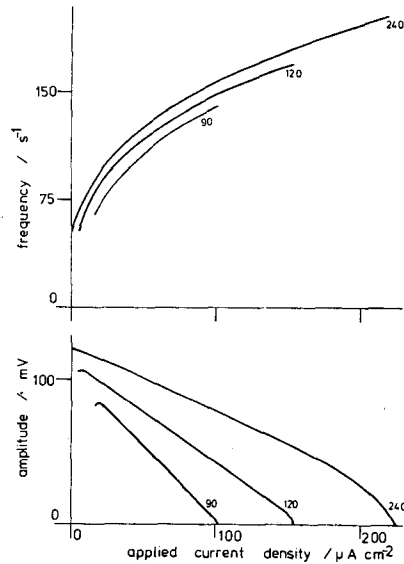


Fig. 7. Frequency and peak-to-peak amplitude of periodic solutions of the Hodgkin-Huxley membrane equations at 6.3°C to an applied current density, with $\bar{g}_{\text{Na}} = 90, 120$ (standard) and 240 mS cm^{-2} .

Lecar and Nossal (1971a,b) have developed an analysis to relate threshold properties of an excitable membrane (the probability of response to a brief stimulating current, the probability of response as a function of stimulus duration and the latency distribution) to membrane parameters including the maximal Na^+ -conductance \bar{g}_{Na} . The reduced system (V , σ), with $\sigma = m^3$ acting as a conductance control function, has three singular points: two stable points corresponding to the resting potential and the peak of the action potential, separated by a saddle point that gives rise to a threshold separatrix. The full H-H system has only a single stable singular point. The threshold separatrix clearly divides trajectories in the (V , σ)-plane into those corresponding to sub- and suprathreshold responses. The probability of a suprathreshold response, given a brief current density I and conductance and voltage fluctuations, is, for a current threshold I_{th} , an integrated Gaussian:

$$(6) \quad \text{Prob}(\text{response}|I) = 0.5\{1 + \text{erf}\{(I - I_{\text{th}})/RI_{\text{th}}\}\}$$

where R is the spread of I/I_{th} within which the probability of firing increases from 0 to 1. Ten Hoopen and Verveen (1963) fitted their experimental results with such a distribution. The relative spread R is a complicated function of the character-

istics of the membrane and the noise sources, and numerical evaluation shows the relative spread to decrease almost linearly as \bar{g}_{Na} (or sodium channel density) is decreased (see Fig. 5a of Lecar and Nossal, 1971b). Thus the effect of a decrease in Na^+ -channel density is to reduce the relative spread, or variability, of the probability of firing curves. This is a consequence of the dynamics in the (V , σ)-plane: for a finite area of membrane where a reduction in channel density gives a reduction in N_{Na} , the total number of Na -channels, the relative spread would also increase as $N_{\text{Na}}^{-1/2}$ as N_{Na} decreased with density, and so R would be proportional to the square root of the Na^+ -channel density.

5. Conduction velocity in non-myelinated fibres

The key role of the Na^+ -channel density on the membrane threshold behaviour is reflected in its influence on the conduction velocity of nonmyelinated axons: the conduction velocity of propagated responses is determined by the cable properties of the axon—how far local circuit currents will spread ahead of the propagating action potential, and the membrane excitability. An increase in \bar{g}_{K} will decrease the resting membrane resistance and the excitability of the membrane; an increase in \bar{g}_{Na} will have little effect on the ‘linear’ cable properties of the axon but will increase the excess inward current that spreads to depolarize the membrane ahead of the action potential, and will increase the excitability of the resting membrane.

The partial differential system for an H-H axon of radius a and resistivity r_a is:

$$(7) \quad \frac{a}{2r_a} \frac{\partial^2 V}{\partial x^2} = C_m \frac{\partial V}{\partial t} + I_i(V, m, n, h).$$

The assumption of a travelling wave solution with a conduction velocity θ allows equation (7) to be reduced to the differential system:

$$(8) \quad \frac{d^2 V}{dt^2} = \frac{2r_a \theta^2 C_m}{a} \left(\frac{dV}{dt} + \frac{I_i(V, m, n, h)}{C_m} \right).$$

The problem is to find a value for the conduction velocity θ that generates a homoclinic orbit as the solution for (7): Hodgkin and Huxley (1952) found $\theta = 18.8 \text{ ms}^{-1}$, and Huxley (1959) found two values of θ that generated a stable, large, fast solution and an unstable, slow, small amplitude solution. Cooley and Dodge (1966) examined the effects of scaling \bar{g}_{K} and \bar{g}_{Na} together by a factor of $0 < \eta_1 < 1$, while changing g_L and V_L to maintain the resting membrane resistance and potential. As η_1 is reduced, both the conduction velocity and peak of the fast solution decrease, until at $\eta_1 < 0.261$ the peak potentials and conduction velocities of the fast and slow solutions meet. For $\eta_1 < 0.261$ no travelling wave (non-decremental) solutions are possible. Sabah and Leibovic (1971) have analysed the effects of η scaling \bar{g}_{K} , \bar{g}_{Na} and g_L : as η is reduced small amplitude travelling waves merge into decremental propagative responses.

The wide range of Na⁺-channel densities illustrated in Tables 1 and 2 suggests that the Na⁺-channel density might be related to the diameter of nonmyelinated axons, and that the high Na⁺-channel density of invertebrate giant axons might be related to their role as rapid pathways mediating escape reflexes. Hodgkin (1975) has considered the effect of Na⁺-channel density on conduction velocity, and shown that there is a value of \bar{g}_{Na} (and hence Na⁺-channel density) at which the conduction velocity is maximal. If equation (8) is scaled by η

$$\frac{d^2V}{dt^2} = \frac{2r_a\theta^2 C_m}{a} \left\{ \frac{dV}{dt} + \frac{\eta I_i(V, m, n, h)}{C_m} \right\}$$

then for any η that supports travelling wave solutions there is a finite θ , with

$$(9) \quad \theta^2 = a/2r_a C_m f(\eta).$$

$f(\eta)$ may be evaluated numerically from travelling wave solutions. Taking the logarithms of (9) and differentiating with respect to $\ln \eta$:

$$\frac{\partial \ln \theta}{\partial \ln C_m} = -\frac{1}{2} - \left(\frac{\partial \ln \theta}{\partial \ln \eta} \right).$$

But $\bar{g}_{\text{Na}} \propto \eta$ and so

$$(10) \quad \frac{\partial \ln \theta}{\partial \ln C_m} = -\frac{1}{2} - \left(\frac{\partial \ln \theta}{\partial \ln \bar{g}_{\text{Na}}} \right)$$

which is Hodgkin's (1975) equation (9) or Huxley's (1959) equation (22). If the effect of increasing the sodium channel density is to increase \bar{g}_{Na} and to increment the capacitance by C_{Na}/m -gate, so that $C_m = C_o + 3\rho_{\text{Na}}C_{\text{Na}}$ for a channel density ρ_{Na} and 3 m-gates/channel, then

$$(11) \quad \frac{\partial \ln C_m}{\partial \ln \rho_{\text{Na}}} = \frac{C_{\text{Na}}\rho_{\text{Na}}}{(C_o + \rho_{\text{Na}}C_{\text{Na}})}.$$

At a density of ρ_{Na}^* that gives the maximum conduction velocity, $d \ln \theta / d \ln \rho_{\text{Na}} = 0$ and

$$(12) \quad \begin{aligned} C_{\text{Na}}\rho_{\text{Na}}^* &= 2C_o \frac{\partial \ln \theta}{\partial \ln \bar{g}_{\text{Na}}} \\ &= 2C_o \frac{\partial \ln \theta}{\partial \ln \eta}. \end{aligned}$$

$\partial \ln \theta / \partial \ln \eta$ is available from Huxley (1959), and so with appropriate numerical values the conduction velocity can be computed as a function of Na⁺-channel density. The conduction velocity is maximal when $\rho_{\text{Na}}C_{\text{Na}} \simeq 0.5C_o$.

These approximate calculations were confirmed by Adrian (1975), who calculated travelling wave solutions with the membrane current I_m given by

$$I_m = C_m \frac{dV}{dt} + 3\rho_{\text{Na}}z'e \frac{dm}{dt} + I_i(V, m, n, h),$$

where z' is the effective valency of the sodium activation gate. The conduction velocity, in the absence of any gating charge displacement, increased roughly as $\bar{g}_{\text{Na}}^{0.2}$. When the gating current was included in I_m there was a maximum conduction velocity for $\rho_{\text{Na}} \sim 500\text{--}1000 \mu\text{m}^{-2}$: the relationship between θ and ρ_{Na} is relatively flat, and so although the order of magnitude of the sodium channel density of giant axons is such that conduction velocity is maximized, it is not reasonable to assume that the channel density of an axon is such to maximize its conduction velocity given its diameter. The low channel densities of garfish axons would not maintain propagation in a giant axon system.

Pickard (1977) has obtained approximate expressions for the sodium channel density that maximizes the conduction velocity: the propagating action potential is treated as a sharp transition between resting ($g_{\text{K}} \gg g_{\text{Na}}$) and active ($g_{\text{Na}} \gg g_{\text{K}}$) membrane, and with these simplifications there is a broad maximum in the conduction velocity-channel density relation.

6. Spatial nonuniformity of membrane channel densities

The electrical behaviour of nerve cells is not simply the behaviour of isopotential areas of excitable membrane, but is determined by the electrotonic geometry of the neurone and its processes. A further complication is that the membrane properties can be spatially nonuniform: voltage clamp analysis shows that the somatic and dendritic membranes can have a richer variety of conductances than axonal membrane, and the initial segment of neurones with myelinated axons acts as the spike initiating locus, as if it had a lower threshold. Thus there can be both qualitative and quantitative nonuniformities in the distribution of ionic channels over the surface of the neurone. The effects of such spatial nonuniformities can be illustrated by considering the behaviour of myelinated axons.

The myelinated axon provides already isolated isopotential patches of membrane at the nodes of Ranvier: Frankenhaeuser and Huxley (1964) provide a system of differential equations that describe the ionic currents at a single node in *Xenopus*, Dodge (1963) provides a similar analysis for a node in *Rana*. Voltage clamp studies of amphibian nodal membrane are reviewed in Stampfli and Hille (1976) and Cahalan (1978). Although the area of membrane that is exposed at a node is less than $50 \mu\text{m}^2$, the nodal currents seen under voltage-clamp are up to about 10 nA: this high nodal current density suggests a high nodal channel density. The ratio of the permeability constants $\bar{P}_{\text{Na}}:P'_{\text{K}}$ is about twice that for $g_{\text{Na}}:g_{\text{K}}$ in the H-H equations. The stability of the nodal resting potential is maintained by a high nonspecific permeability and conductance.

There are qualitative differences between motor and sensory myelinated axons in the amphibian: only sensory nodes are capable of maintained, adapting responses to a maintained current. Krylov and Makovsky (1978) have accounted for these differences by numerical simulations in which the conductances are changed: the nodal membrane is repolarized by a leakage conductance g_{L} that stabilizes the resting potential, g_{K1} , a K⁺-conductance similar to g_{K} of

the H-H equations, and a slower g_{KII} that is responsible for adaptation. An increase in the density of slow K^+ channels changes the response to maintained currents from the abbreviated response characteristic of motor axons to the adapting, maintained response characteristic of sensory axons. Thus qualitative differences in the behaviour of myelinated axons can be explained simply by quantitative differences in channel density.

Voltage clamp analyses of mammalian myelinated axons (Chiu *et al.*, 1979; Brismar, 1980) show a maximum leak conductance about 10–25% the maximal sodium conductance, and the absence of a K^+ -selective conductance. The stability of the resting membrane potential is due to the large g_L .

Table 2

Single channel conductances and channel densities estimated by fluctuation analysis

Preparation	γ/pS	$\rho/\mu m^{-2}$	Reference
Squid giant axon			
Na ⁺ -channel	4	90*	Conti <i>et al.</i> (1975)
K ⁺ -channel	12	100*	
Frog node of Ranvier			
Na ⁺ -channel	8	10*	Conti <i>et al.</i> (1976)
	7		Sigworth (1977)
K ⁺ -channel	4		Begenisich and Stevens (1975)
	2.9		van den Berg (1978)

* Assuming H-H standard \bar{g}_K , \bar{g}_{Na} .

In a normal myelinated axon the axial current ahead of a propagating action potential leaves the axon through nodal membrane: the high impedance of the myelin layer around the internodal membrane prevents current crossing the internodal membrane. Thus conduction is saltatory, with the action potential jumping from node to node: only in demyelinated or dysmyelinated fibres can the conductance properties of the internodal membrane affect conduction.

Rasminsky (1978) has reviewed conduction in demyelinated fibres: in focally demyelinated axons conduction is still saltatory. Thus excitability (or a high sodium channel density) is confined to nodal areas of membrane. Ritchie and Rogart (1977) estimated saxitoxin-binding sites of $10^4 \mu m^{-2}$ for nodal and $25 \mu m^{-2}$ for internodal membranes: such a low ρ_{Na} for internodal membrane would be insufficient to support excitability if the repolarization conductances of the internodal membrane were of the same order as at the node.

Although nodal membrane lacks a K^+ -selective conductance, demyelinated and damaged myelinated axons can generate a TEA and 4-aminopyridine sensitive late outward current (Sherratt *et al.*, 1980; Chiu and Ritchie, 1980). Thus the internodal membrane might contain K^+ -selective channels.

Nodal membrane where axonal branching occurs has an ultrastructure similar to that of nonmyelinated axons (Waxman and Foster, 1980), and the first

node of Ranvier in axons innervating Pacinian corpuscles in the cat is sensitive to TEA (Akoef *et al.*, 1980): thus there might be differences in channel densities between different types of nodes. Myelinated axons represent a system where extreme spatial nonuniformity of Na^+ - and K^+ -selective channels has been demonstrated: the existence of this spatial nonuniformity favours speculations that specific channel densities may change over somatic and dendritic membranes.

The diameters of some neuronal processes are less than $1 \mu m$, and so within a given functional zone of a neurone, a restricted surface area of membrane, with a channel density that can be as low as $1 \mu m^{-2}$, implies a restricted and perhaps even small number of channels. Thus in addition to effects due to channel density there might be effects due to there being a limited number of channels: such effects are discussed in Rayner *et al.* (1976), Skaugen and Walløe (1979), Skaugen (1980) and Holden (1981).

7. Conclusions

The H-H equations provide a fairly accurate description of the electrical behaviour of the membrane of the squid giant axon: this is not a typical excitable membrane, but is specialized for rapid conduction of solitary action potentials. However, numerical studies on the H-H equations illustrate the ways in which changes in channel density can influence the electrical behaviour of excitable membranes.

Autorhythmicity can be induced by a decrease in the density of recovery-process channels, or an increase in the density of excitation-process channels. Thus the role of \bar{g}_K in stabilizing the resting membrane potential of the squid axon is taken by g_L in mammalian myelinated nerve: both maintain the stability of the resting potential, and a decrease in \bar{g}_K in the H-H membrane leads to a Hopf bifurcation, from a stable membrane potential to unstable, small amplitude oscillations. Since the bifurcation is subcritical, solutions jump to the limb of large amplitude stable periodic solutions that correspond to an autorhythmic discharge of action potentials. In the H-H equations, a modest 40% reduction in K^+ -channel density is sufficient to induce autorhythmicity. A reduction in K^+ -channel density increases the membrane excitability: the maintained discharge rate produced by an applied current density is increased.

The electrical activity of neurones is apparently stochastic—models for the stochastic activity of neurones are reviewed in Holden (1976), where it is argued that the variability of neuronal activity is a reflection of the stochastic activity of membrane conductance mechanisms. If the specific channel densities are such that the membrane is close to a bifurcation point, then the effects of the random open-close kinetics will be explosively enhanced. Thus the variability of the activity of a neurone will be strongly influenced by areas of membrane where the channel density is close to a bifurcation point.

Changes in channel density can occur during the development of the nervous system and might occur in the mature neurone. Small, quantitative changes in

channel density provide a means of producing both qualitative changes in neurone behaviour and small quantitative changes in excitability. The effectiveness of channel density in controlling neuronal behaviour will be enhanced by spatial nonuniformities, perhaps less drastic than those demonstrated in myelinated axons. Thus numerical solutions of the H-H equations provide a wealth of possible ways in which channel density can influence neuronal function: modern experimental methods are just beginning to provide the estimates of specific dendritic and somatic channel densities that are necessary for a quantitative development of theories for the ways in which channel densities do influence neuronal function.

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