

Derivation of Hodgkin-Huxley equations for a Na⁺ channel from a master equation for coupled activation and inactivation

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The Na⁺ current in nerve and muscle membranes may be described in terms of the activation variable $m(t)$ and the inactivation variable $h(t)$, which are dependent on the transitions of S4 sensors of each of the Na⁺ channel domains DI to DIV. The time-dependence of the Na⁺ current and the rate equations satisfied by $m(t)$ and $h(t)$ may be derived from the solution to a master equation that describes the coupling between two or three activation sensors regulating the Na⁺ channel conductance and a two-stage inactivation process. If the inactivation rate from the closed or open states increases as the S4 sensors activate, a more general form of the Hodgkin-Huxley expression for the open-state probability may be derived where $m(t)$ is dependent on both activation and inactivation processes. The voltage dependence of the rate functions for inactivation and recovery from inactivation are consistent with the empirically determined expressions and exhibit saturation for both depolarized and hyperpolarized clamp potentials.

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I. INTRODUCTION

The opening and subsequent inactivation of Na⁺ channels and the activation of K⁺ channels generate the action potential in nerve and muscle membranes [1]. The time-dependence of the Na⁺ current in the squid axon may be described in terms of the expression $m(t)^3h(t)$, where the activation variable $m(t)$ and inactivation variable $h(t)$ satisfy the rate equations

$$\frac{dm}{dt} = \alpha_m - (\alpha_m + \beta_m)m, \quad (1)$$

$$\frac{dh}{dt} = \alpha_h - (\alpha_h + \beta_h)h, \quad (2)$$

and α_m , β_m , α_h , and β_h are voltage-dependent rate functions for activation and inactivation transitions within the membrane.

The Hodgkin-Huxley (HH) description of the Na⁺ current is equivalent to an eight-state master equation where three independent voltage sensors may activate and open the channel, and independent inactivation may occur from each of the closed or open states [2–4]. Although this master equation is not consistent with the measurement of an almost zero Na⁺ current during repolarization of an inactivated channel, by assuming that the backward inactivation rate to the open state is zero but the recovery rate to closed states increases as the S4 sensors deactivate [5], and that inactivation is a two-stage process where the rate functions satisfy microscopic reversibility, the model provides a good description of the recovery from inactivation, and the Na⁺ current during a depolarizing clamp [6].

In this paper, it is shown that the Hodgkin-Huxley expression for the Na⁺ current and the rate equations for activation and inactivation may be derived from a master equation, which describes the coupling between two or three activation sensors regulating the Na⁺ channel conductance and a two-stage inactivation process. For a Na⁺ channel with two

independent activation sensors, where the deactivation rate during repolarization is slower between inactivated states than between closed or open states, only four of the terms of the solution to a six-state master equation for a depolarizing clamp contribute to the open state $O(t)$, which may be expressed as $m(t)^2h(t)$. Assuming that the inactivation rate from the open state is larger than the rate from closed states [7], the time-course of entry into the inactivated state and the recovery from inactivation may be approximated by biexponential functions, in agreement with kinetic data from Na⁺ channels [6]. The voltage dependence of the rate functions for inactivation and recovery from inactivation have a similar form to empirical expressions for Na⁺ channels [1,5], and in particular, the exponential variation exhibits saturation for both depolarized and hyperpolarized clamp potentials.

II. VOLTAGE CLAMP OF A Na⁺ CHANNEL WITH TWO ACTIVATION SENSORS

The Na⁺ channel protein is composed of four domains DI to DIV, each containing an S4 segment with positively charged residues at every third position [3]. Based on voltage clamp fluorometry, it has been shown that, in response to membrane depolarization, the transverse motion of the charged S4 segments of the Na⁺ channel domains DI to DIII is associated with activation, whereas the slower movement of DIV S4 is correlated with the binding of an intracellular hydrophobic motif that blocks the flow of ions through the inner mouth of the pore [8]. This may occur for small depolarizations when the ion channel is usually closed (closed-state inactivation) or for larger depolarizations when the S4 segments of the domains DI to D3 are activated (open-state inactivation).

However, during repolarization of an inactivated Na⁺ channel, the OFF gating charge has a fast component which may be attributed to the motion of the DI and DII S4 segments, and a slow component, the “immobilized” portion, that is generated by the conformational changes of the DIII and DIV S4 segments [9,10]. For an inactivation modified mutant of the human heart Na⁺ channel, it has been estimated that the

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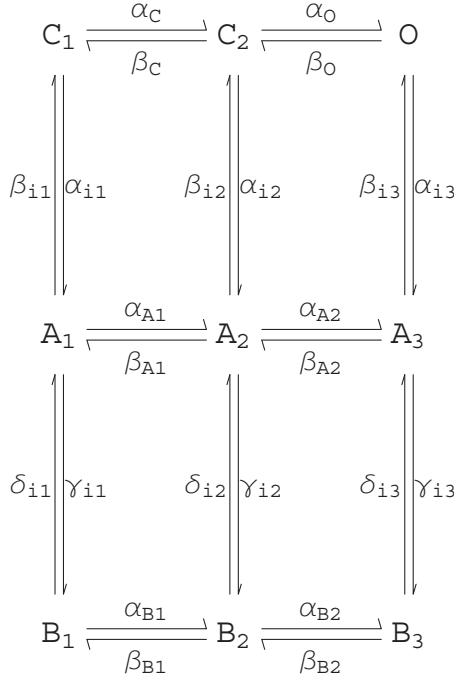


FIG. 1. State diagram for Na^+ channel gating where horizontal transitions represent the activation of two voltage sensors (DIII and either DI or DII) that open the pore, and vertical transitions represent the two-stage inactivation process of the DIV voltage sensor and the inactivation motif.

DIV S4 sensor contributes approximately 30% to the OFF charge, and approximately 20% may be attributed to the DIII S4 sensor, which is only immobilized when the inactivation gate is intact. The slow component of the OFF gating charge has the same time course as the Na^+ channel recovery from inactivation, and therefore, the rate-limiting step is the motion of the DIV S4 sensor and not the unbinding of the inactivation gate [11].

In order to account for the effect of double-cysteine mutants of S4 gating charges on the ionic current of the bacterial Na^+ channel NaChBac, it has been proposed that at least two transitions are required during the activation of each voltage sensor [12]. This conclusion is consistent with an earlier result that cross-linking a DIV S4 segment from the extracellular surface inhibits inactivation during membrane depolarization, whereas cross-linking the same segment from the inside inhibits activation of the Na^+ channel, and therefore, the DIV S4 sensor translocates across the membrane in two stages [13,14]. The measurement of currents for wild-type and charge-neutralized mutant Na^+ channels gives additional support to the conclusion that the activation of the DIV S4 sensor is correlated with ion channel inactivation [6].

In this section, we assume that the activation of two voltage sensors regulating the Na^+ channel conductance (DIII S4 and the S4 segment of either the DI or DII domains) is coupled to a two-stage inactivation process (see Fig. 1), and therefore, the kinetics may be described by a master equation where the occupation probabilities of the closed states C_1 , C_2 , A_1 , and A_2 , the open states O and A_3 , and the inactivated (or blocked)

states B_1 , B_2 , and B_3 are determined by

$$\frac{dC_1}{dt} = -(\alpha_{i1} + \alpha_c)C_1(t) + \beta_c C_2(t) + \beta_{i1} A_1(t), \quad (3)$$

$$\frac{dC_2}{dt} = -(\alpha_{i2} + \alpha_o + \beta_c)C_2(t) + \alpha_c C_1(t) + \beta_o O(t) + \beta_{i2} A_2(t), \quad (4)$$

$$\frac{dO}{dt} = \alpha_o C_2(t) - (\beta_o + \alpha_{i3})O(t) + \beta_{i3} A_3(t), \quad (5)$$

$$\frac{dA_1}{dt} = \alpha_{i1} C_1(t) - (\alpha_{A1} + \beta_{i1} + \gamma_{i1})A_1(t) + \delta_{i1} B_1(t) + \beta_{A1} A_2(t), \quad (6)$$

$$\frac{dA_2}{dt} = \alpha_{i2} C_2(t) - (\alpha_{A2} + \beta_{A1} + \beta_{i2} + \gamma_{i2})A_2(t) + \delta_{i2} B_2(t) + \alpha_{A1} A_1(t) + \beta_{A2} A_3(t), \quad (7)$$

$$\frac{dA_3}{dt} = \alpha_{i3} O(t) - (\beta_{A2} + \beta_{i3} + \gamma_{i3})A_3(t) + \delta_{i3} B_3(t) + \alpha_{A2} A_2(t), \quad (8)$$

$$\frac{dB_1}{dt} = \gamma_{i1} A_1(t) - (\alpha_{B1} + \delta_{i1})B_1(t) + \beta_{B1} B_2(t), \quad (9)$$

$$\frac{dB_2}{dt} = \gamma_{i2} A_2(t) + \alpha_{B1} B_1(t) + \beta_{B2} B_3(t) - (\alpha_{B2} + \beta_{B1} + \delta_{i2})B_2(t), \quad (10)$$

$$\frac{dB_3}{dt} = \gamma_{i3} A_3(t) + \alpha_{B2} B_2(t) - (\beta_{B2} + \delta_{i3})B_3(t). \quad (11)$$

The master equation may be derived from a Smoluchowski equation applied to the resting and barrier regions of an energy landscape for each of the S4 sensors in the domains DI to DIV [15,16]. The translocation of the S4 segment through the gating pore for Na^+ (or K^+) channels requires sufficient energy to overcome several barriers that are dependent on the Coulomb force between positively charged residues on the S4 sensor and negatively charged residues on neighboring helices, the dielectric boundary force, the electric field between internal and external aqueous crevices, and hydrophobic forces [17]. It is assumed that the transition rates for each stage of inactivation are dependent on single-barrier activation, and therefore, are proportional to $\exp(-U)$ where U is the voltage-dependent height of the barrier [18]. However, if the Na^+ channel S4 sensors of the DI, DII, or DIII domains are activated in two stages, the HH rate functions α_m and β_m may be approximated by two-state expressions [19].

In order to simplify the solution of Eqs. (3) to (11), it is initially assumed that

$$\alpha_{ik} = \alpha_{i1}, \quad \beta_{ik} = \beta_{i1}, \quad \gamma_{ik} = \gamma_{i1}, \quad (12)$$

for each k , and to ensure that the Na^+ current recovers from inactivation when the S4 sensors that regulate Na^+ conductance deactivate, it is further assumed that

$$\delta_{i1} > \delta_{i2} > \delta_{i3} \approx 0. \quad (13)$$

From microscopic reversibility or the principle of detailed balance, the product of the transition rates in the clockwise

and anticlockwise directions are equal [3], and we may write

$$\begin{aligned}\delta_{21} \frac{\alpha_{B1}}{\beta_{B1}} &= \frac{\alpha_{A1}}{\beta_{A1}} = \frac{\alpha_C}{\beta_C}, \\ \delta_{32} \frac{\alpha_{B2}}{\beta_{B2}} &= \frac{\alpha_{A2}}{\beta_{A2}} = \frac{\alpha_O}{\beta_O},\end{aligned}\quad (14)$$

where $\delta_{21} = \delta_{i2}/\delta_{i1} < 1$ and $\delta_{32} = \delta_{i3}/\delta_{i2} < 1$.

Assuming that the first forward and backward transitions for inactivation are rate limiting ($\beta_{ik} \gg \delta_{ik}$ and $\gamma_{ik} \gg \alpha_{ik}$, for $k = 1$ to 3) [19,20], and that $\beta_{ik} + \gamma_{ik}$, for each k , is greater than the activation-deactivation rate functions $\alpha_{A1}, \beta_{A1}, \alpha_{A2}, \beta_{A2}$ [6], the occupation probabilities A_1, A_2 , and A_3 rapidly attain a quasi steady state before the relaxation of the closed, open, and inactivated states (see Fig. 2):

$$A_1 \approx \frac{\alpha_{i1}C_1 + \delta_{i1}B_1}{\beta_{i1} + \gamma_{i1}}, \quad (15)$$

$$A_2 \approx \frac{\alpha_{i2}C_2 + \delta_{i2}B_2}{\beta_{i2} + \gamma_{i2}}, \quad (16)$$

$$A_3 \approx \frac{\alpha_{i3}O + \delta_{i3}B_3}{\beta_{i3} + \gamma_{i3}}, \quad (17)$$

and therefore, Eqs. (3) to (11) may be reduced to a six-state master equation (see Figs. 3 and 4):

$$\frac{dC_1}{dt} = -(\rho_1 + \alpha_C)C_1(t) + \beta_C C_2(t) + \sigma_1 B_1(t), \quad (18)$$

$$\frac{dC_2}{dt} = \alpha_C C_1(t) - (\alpha_O + \beta_C + \rho_2)C_2(t) + \beta_O O(t) + \sigma_2 B_2(t), \quad (19)$$

$$\frac{dO}{dt} = \alpha_O C_2(t) - (\beta_O + \rho_3)O(t) + \sigma_3 B_3(t), \quad (20)$$

$$\frac{dB_1}{dt} = \rho_1 C_1(t) - (\alpha_{B1} + \sigma_1)B_1(t) + \beta_{B1} B_2(t), \quad (21)$$

$$\begin{aligned}\frac{dB_2}{dt} &= \rho_2 C_2(t) + \alpha_{B1} B_1(t) - (\alpha_{B2} + \beta_{B1} + \sigma_2)B_2(t) \\ &\quad + \beta_{B2} B_3(t),\end{aligned}\quad (22)$$

$$\frac{dB_3}{dt} = \rho_3 O(t) + \alpha_{B2} B_2(t) - (\beta_{B2} + \sigma_3)B_3(t), \quad (23)$$

where the derived forward and backward rate functions for inactivation ρ_k and σ_k are, in general, voltage dependent [21–23]:

$$\rho_k \approx \frac{\alpha_{ik}\gamma_{ik}}{\beta_{ik} + \gamma_{ik}}, \quad (24)$$

$$\sigma_k \approx \frac{\delta_{ik}\beta_{ik}}{\beta_{ik} + \gamma_{ik}}. \quad (25)$$

The rates ρ_k and σ_k are dependent on the ratio β_{ik}/γ_{ik} and either α_{ik} or δ_{ik} , and therefore, are generally small relative to the activation-deactivation rate functions despite the large value of $\beta_{ik} + \gamma_{ik}$. For a 15-state master equation for a Na⁺ channel [6], $\beta_{ik} + \gamma_{ik}$, for each k , is greater than the activation-deactivation rate functions α_{Aj}, β_{Aj} for each j , and therefore, may be approximated by a ten-state gating model. If the conditions $\beta_{ik} \gg \delta_{ik}$ and $\gamma_{ik} \gg \alpha_{ik}$ for each k are not satisfied, the inactivation of the Na⁺ current during

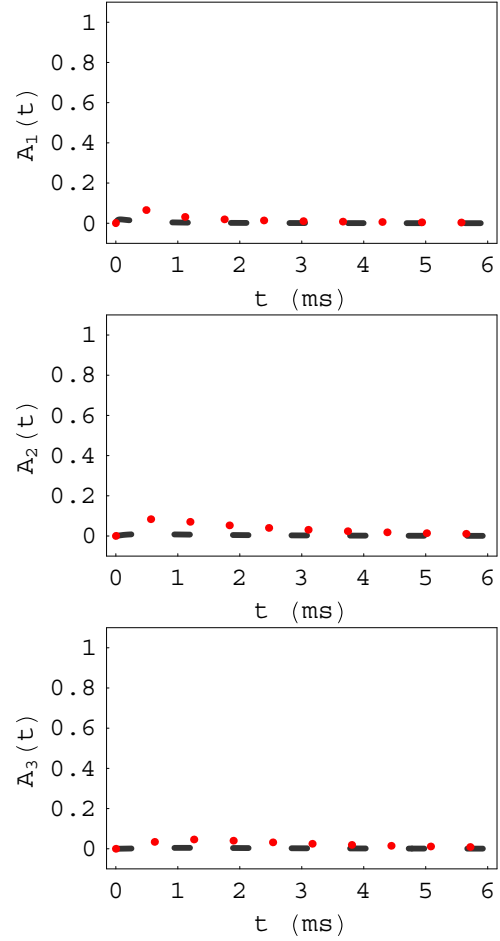


FIG. 2. During a depolarizing clamp potential for a Na⁺ channel, the occupation probabilities A_1, A_2 , and A_3 (see Fig. 1) determined from the numerical solution of a nine-state master equation, rapidly attain a quasi-steady state (dashed line) when $\gamma_{ik} = 20.1$ and $\beta_{ik}(V) = 3.4 \exp(-2.3V/25)$ are sufficiently large, and the rate functions are $\alpha_{ik}(V) = 1$ for each k , $\delta_{i1}(V) = 2.5$, $\delta_{i2}(V) = 0.0045\delta_{i1}(V)$, $\delta_{i3}(V) = 0.05\delta_{i2}(V)$, $\alpha_C = 2\alpha_m$, $\beta_C = \beta_m$, $\alpha_O = \alpha_m$, $\beta_O = 2\beta_m$, $\alpha_{A1} = \alpha_C$, $\beta_{A1} = \beta_C$, $\alpha_{A2} = \alpha_O$, $\beta_{A2} = \beta_O$, $\alpha_{B1} = 3\alpha_C$, $\beta_{B1} = 0.0135\beta_C$, $\alpha_{B2} = 3\alpha_O$, $\beta_{B2} = 0.15\beta_O$ (ms^{-1}), and $\alpha_m = 0.1(V + 25)/(1 - \exp[-(V + 25)/10])$, $\beta_m = 4 \exp[-(V + 50)/18]$ are the HH rate functions for Na⁺ channel activation, where $V = -10$ mV and the resting potential is -50 mV. However, if the inactivation rates are reduced by an order of magnitude to $\gamma_{ik} = 2.0$ and $\beta_{ik}(V) = 0.34 \exp(-2.3V/25)$, the occupation probabilities $A_1(t), A_2(t)$, and $A_3(t)$ (dotted red line) are not constant during the relaxation of the closed, open, and inactivated states.

a depolarizing clamp potential may be biexponential. From the assumptions of Eqs. (12) and (13), the inactivation rate is not state dependent ($\rho_k = \rho_1$ for each k), the recovery rates $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$, and therefore, from the microscopic reversibility conditions in Eq. (14),

$$\beta_{B1}\alpha_C < \beta_C\alpha_{B1}, \quad (26)$$

$$\beta_{B2}\alpha_O < \beta_O\alpha_{B2}. \quad (27)$$

The nonzero eigenvalues of the characteristic equation for Eqs. (18) to (23) are $\lambda_j = -\omega_j$ for $j = 1$ to 5, where ω_j

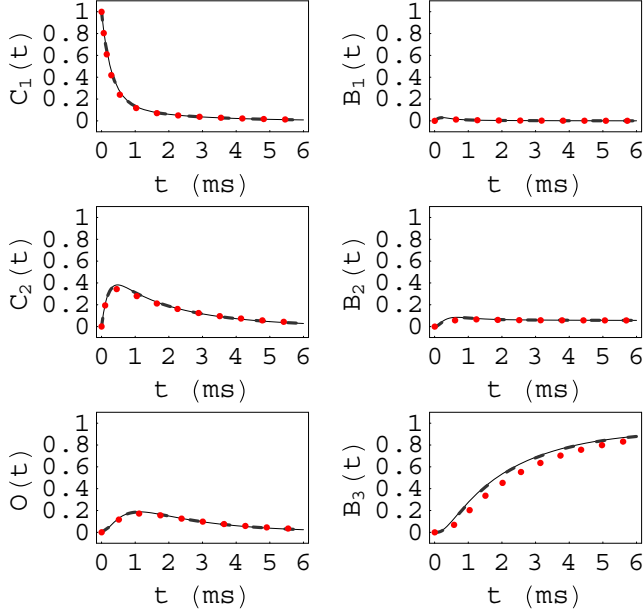


FIG. 3. During a depolarizing clamp potential for a Na⁺ channel, the solution of a nine-state master equation for $\gamma_{ik} = 20.1$, $\beta_{ik}(V) = 3.4 \exp(-2.3V/25)$ for each k (dashed line), and $\gamma_{ik} = 2.0$, $\beta_{ik}(V) = 0.34 \exp(-2.3V/25)$ (dotted red line), may be approximated by the solution of the corresponding six-state master equation (solid line) when γ_{ik} and $\beta_{ik}(V)$ are sufficiently large, and A_1 , A_2 , and A_3 are constant during the relaxation of the closed, open, and inactivated states (see Fig. 2).

may be approximated by the roots ω_{kF} and ω_{kG} of the cubic polynomials $F(\omega)$ and $G(\omega)$, defined such that $\omega_{1F} \leq \omega_{2F} \leq \omega_{3F}$, and $\omega_{1G} \leq \omega_{2G} \leq \omega_{3G}$ (see Fig. 5 and Eq. (A1) of the Appendix) and where

$$F(\omega) = \omega^3 - f_1\omega^2 + f_2\omega - f_3, \quad (28)$$

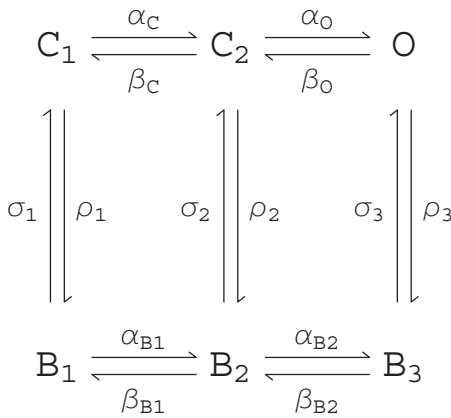


FIG. 4. The nine-state system for Na⁺ channel gating in Fig. 1 may be approximated by a six-state system when $\beta_{ik} \gg \delta_{ik}$ and $\gamma_{ik} \gg \alpha_{ik}$, for $k = 1$ to 3 , and $\beta_{ik} + \gamma_{ik}$ is greater than the activation-deactivation rate functions α_{A1} , β_{A1} , α_{A2} , and β_{A2} where ρ_k and σ_k are derived rate functions for a two-stage Na⁺ inactivation process, defined in Eqs. (24) and (25).

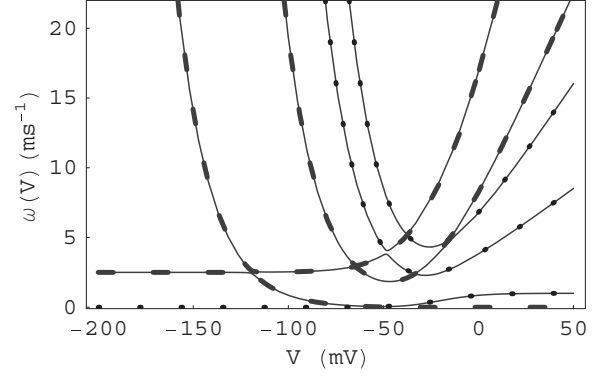


FIG. 5. The voltage dependence of $\omega_j = -\lambda_j$, $j = 1$ to 5 (solid lines), where λ_j is a nonzero eigenvalue of the characteristic equation of the six-state master equation for Na⁺ channel gating, may be approximated by $\omega_{1F} \leq \omega_{2F} \leq \omega_{3F}$ (dotted lines), and $\omega_{1G} \leq \omega_{2G} \leq \omega_{3G}$ (dashed lines), the roots of the cubic polynomials $F(\omega)$ and $G(\omega)$, and $\omega_1 = \alpha_h + \beta_h \approx \omega_{1G} + \omega_{1F}$, where the rate functions are $\alpha_{ik}(V) = 1$, $\gamma_{ik}(V) = 20.1$, $\beta_{ik}(V) = 3.4 \exp(-2.3V/25)$, for each k , $\delta_{i1}(V) = 2.5$, $\delta_{i2}(V) = \delta_{i3}(V) = 0$, $\alpha_C = 2\alpha_m$, $\beta_C = \beta_m$, $\alpha_O = \alpha_m$, $\beta_O = 2\beta_m$, $\alpha_{B1} = 3\alpha_C$, $\beta_{B1} = 0.0135\beta_C$, $\alpha_{B2} = 3\alpha_O$, $\beta_{B2} = 0.15\beta_O$ (ms^{-1}).

$$\begin{aligned} f_1 &= \alpha_O + \beta_O + \alpha_C + \beta_C + \rho_1 + \rho_2 + \rho_3, \\ f_2 &= (\beta_C + \rho_2)(\beta_O + \rho_3) + \rho_3\alpha_O \\ &\quad + (\alpha_C + \rho_1)(\alpha_O + \beta_O + \beta_C + \rho_2 + \rho_3) - \alpha_C\beta_C, \\ f_3 &= \alpha_O\rho_3(\alpha_C + \rho_1) + (\beta_O + \rho_3)(\rho_2\alpha_C + \rho_1\beta_C + \rho_1\rho_2), \end{aligned} \quad (29)$$

and

$$G(\omega) = \omega^3 - g_1\omega^2 + g_2\omega - g_3, \quad (30)$$

$$\begin{aligned} g_1 &= \alpha_{B1} + \beta_{B1} + \alpha_{B2} + \beta_{B2} + \sigma_1, \\ g_2 &= \alpha_{B1}(\alpha_{B2} + \beta_{B2}) + \beta_{B1}\beta_{B2} + \sigma_1(\beta_{B1} + \alpha_{B2} + \beta_{B2}), \\ g_3 &= \sigma_1\beta_{B1}\beta_{B2}. \end{aligned} \quad (31)$$

For a depolarizing potential, we may define $\omega_k \approx \omega_{kF}$, $\omega_{k+2} \approx \omega_{kG}$, for $k = 2, 3$, whereas for a hyperpolarizing potential, $\omega_k \approx \omega_{kG}$, $\omega_{k+2} \approx \omega_{kF}$. If β_h is the rate of inactivation and α_h is the rate of recovery from inactivation, it may be shown from the characteristic equation (see Appendix) that $\omega_1 = \alpha_h + \beta_h \approx \omega_{1G} + \omega_{1F}$, where

$$\omega_{1G} = \frac{g_3}{\omega_{2G}\omega_{3G}}, \quad (32)$$

$$\omega_{1F} = \frac{f_3}{\omega_{2F}\omega_{3F}}. \quad (33)$$

If α_{i1} , β_{i1} , γ_{i1} are, in general, exponential functions of V , the rate of inactivation,

$$\beta_h \approx \omega_{1F} = \rho_1 = \frac{\alpha_{i1}}{1 + \beta_{i1}/\gamma_{i1}}, \quad (34)$$

has an exponential voltage dependence for small clamp potentials but saturates for a larger depolarization when α_{i1} is weakly dependent on voltage (see Fig. 6 and the Appendix) [1]. In this section, it is assumed that the activation sensors

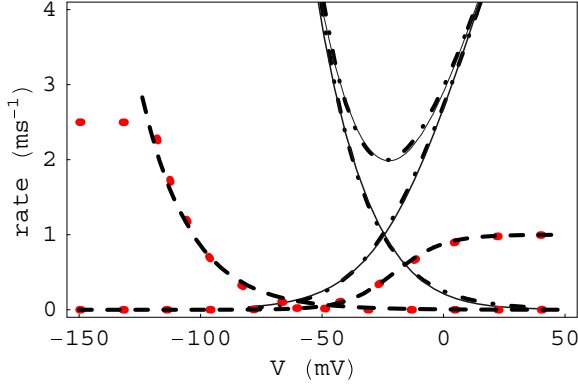


FIG. 6. Voltage dependence of the HH Na⁺ channel inactivation rate functions $\beta_h = 1/\{1 + \exp[-(V + 20)/10]\}$ and $\alpha_h = 0.07 \exp[-(V + 50)/20]$ (dashed lines) may be approximated by the expression in Eq. (34) and by the smaller root of Eq. (35) (dotted red lines), derived from a master equation for a six-state system where activation and two-stage inactivation are interdependent, and the rate functions are defined in Fig. 5. The HH Na⁺ channel activation rate functions $\alpha_m = 0.1(V + 25)/\{1 - \exp[-(V + 25)/10]\}$, $\beta_m = 4 \exp[-(V + 50)/18]$ and $\alpha_m + \beta_m$ (dot dashed lines) may also be approximated by two stage expressions $\alpha_{m,2} = 2.3 \exp[0.32(V + 50)/25]/\{1 + 8.37 \exp[-1.3(V + 50)/25]\}$, $\beta_{m,2} = 4.2 \exp[-0.77(V + 50)/18]\{0.077 + 8.37 \exp[-1.3(V + 50)/25]\}/\{1 + 8.37 \exp[-1.3(V + 50)/25]\}$, and $\alpha_{m,2} + \beta_{m,2}$ (solid lines) [19].

are independent and $\alpha_C = 2\alpha_m$, $\alpha_O = \alpha_m$, $\beta_C = \beta_m$, $\beta_O = 2\beta_m$ [2], where α_m and β_m are HH rate functions for Na⁺ channel activation, and may be approximated by two-stage expressions (see Fig. 6) [19]. If the DIII S4 sensor is the slowest to deactivate ($\beta_{B1} \ll \beta_{B2}$) [10,11], ω_{1G} and ω_{2G} are solutions of the equation (see Appendix)

$$\omega^2 - \omega(\alpha_{B1} + \beta_{B1} + \sigma_1) + \sigma_1\beta_{B1} = 0, \quad (35)$$

and the rate of recovery from inactivation $\alpha_h \approx \omega_{1G}$. For the rate functions of Fig. 5, $\alpha_h \approx \sigma_1$ when $\beta_{B1} \gg \sigma_1$, whereas for $\beta_{B1} \ll \sigma_1$ the rate of recovery for inactivation $\alpha_h \approx \beta_{B1}$. From the microscopic reversibility conditions of Eq. (14), we may assume that $\beta_{B1} \propto \beta_C$ and $\alpha_{B1} \propto \alpha_C$ and therefore, $\alpha_h(V)$ and $\beta_m(V)$ have a similar voltage dependence for small hyperpolarizing potentials, which is consistent with the HH determination of the rate functions [$\beta_m(V) \approx 57\alpha_h(V)$] [1].

If the Na⁺ channel is depolarized to a clamp potential V from a large hyperpolarizing holding potential, the solution of Eqs. (18) to (23) for $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$, when σ_2 is small, may be approximated by the solution of the corresponding master equation, for which $\sigma_1 > \sigma_2, \sigma_3 = 0$,

$$C_1(t) = k_1 C_{1s} + \sum_{j=1}^5 k_{j+1} C_{1j} \exp(-\omega_j t), \quad (36)$$

$$C_2(t) = k_1 C_{2s} + \sum_{j=1}^5 k_{j+1} C_{2j} \exp(-\omega_j t), \quad (37)$$

$$O(t) = k_1 O_s + \sum_{j=1}^5 k_{j+1} O_j \exp(-\omega_j t), \quad (38)$$

$$B_1(t) = k_1 B_{1s} + \sum_{j=1}^5 k_{j+1} B_{1j} \exp(-\omega_j t), \quad (39)$$

$$B_2(t) = k_1 B_{2s} + \sum_{j=1}^5 k_{j+1} B_{2j} \exp(-\omega_j t), \quad (40)$$

$$B_3(t) = k_1 B_{3s} + \sum_{j=1}^5 k_{j+1} B_{3j} \exp(-\omega_j t), \quad (41)$$

where

$$k_1^{-1} = \sum_{j=1}^2 (C_{js} + B_{js}) + O_s + B_{3s}, \quad (42)$$

$$C_{1s} = \sigma_1 \beta_{B1} \beta_{B2} E_0,$$

$$C_{2s} = \sigma_1 \beta_{B1} \beta_{B2} \alpha_C (\beta_O + \rho_1),$$

$$O_s = \sigma_1 \beta_{B1} \beta_{B2} \alpha_C \alpha_O,$$

$$B_{1s} = \beta_{B1} \beta_{B2} f_3,$$

$$B_{2s} = \beta_{B2} (\alpha_{B1} + \sigma_1) f_3 - \rho_1 \sigma_1 \beta_{B2} E_0,$$

$$B_{3s} = \alpha_{B2} (\alpha_{B1} + \sigma_1) f_3 - \rho_1 \sigma_1 \alpha_{B2} E_0 + \rho_1 \sigma_1 \alpha_C \alpha_O \beta_{B1}, \quad (43)$$

$E_0 = \rho_1 \alpha_O + (\beta_O + \rho_1)(\beta_C + \rho_1)$, f_3 is defined in Eq. (29), the amplitudes of the terms for each state are dependent on

$$C_{1j} = E(\omega_j), \quad (44)$$

$$C_{2j} = -\alpha_C (\omega_j - \beta_O - \rho_1), \quad (45)$$

$$O_j = \alpha_C \alpha_O, \quad (46)$$

$$B_{1j} = -\frac{F(\omega_j)}{\sigma_1}, \quad (47)$$

$$B_{2j} = \frac{1}{\sigma_1 \beta_{B1}} [-\rho_1 \sigma_1 E(\omega_j) + (\omega_j - \alpha_{B1} - \sigma_1) F(\omega_j)], \quad (48)$$

$$B_{3j} = -\frac{\rho_1 \alpha_C \alpha_O + \alpha_{B2} B_{2j}}{\omega_j - \beta_{B2}}, \quad (49)$$

and, as $\rho_k = \rho_1$ for each k (see Appendix),

$$E(\omega) = \omega^2 - \omega(\alpha_O + \beta_O + \beta_C + 2\rho_1) + \rho_1 \alpha_O + (\beta_C + \rho_1)(\beta_O + \rho_1). \quad (50)$$

Applying the initial conditions [$C_1(0) = 1$ and $C_2(0) = O(0) = B_1(0) = B_2(0) = B_3(0) = 0$], the parameters $k_j, j = 2$ to 6 may be determined from the solution in Eqs. (36) to (41). For a depolarizing potential, $\omega_4 \approx \omega_{2G}$, $\omega_5 \approx \omega_{3G}$ and therefore, from Eqs. (28) and (47), assuming that the difference between the roots of $F(\omega_j)$ and $G(\omega_j)$ is sufficiently large, $|F(\omega_2)|, |F(\omega_3)| \ll |F(\omega_4)|, |F(\omega_5)|$ and $|B_{k2}|, |B_{k3}| \ll |B_{k4}|, |B_{k5}|$ for each k . Therefore, to satisfy the initial conditions, $k_5, k_6 \approx 0$ and

$$k_2 = \frac{1 - k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_2 \omega_3}{(\omega_2 - \omega_1)(\omega_3 - \omega_1)}, \quad (51)$$

$$k_3 = -\frac{1 - k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_1 \omega_3}{(\omega_2 - \omega_1)(\omega_3 - \omega_2)}, \quad (52)$$

$$k_4 = \frac{1 - k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_1 \omega_2}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)}. \quad (53)$$

That is, each term of the open state probability in Eq. (38) with eigenvalue $\lambda = -\omega_4 \approx -\omega_{2G}$ or $-\omega_5 \approx -\omega_{3G}$ has an amplitude close to zero. If it is assumed that $\rho_1 = \rho_2 = \rho_3$ and the two activation sensors are independent, the roots of Eq. (28) are $\omega_{1F} = \rho_1$, $\omega_{2F} = \alpha_m + \beta_m + \rho_1$, $\omega_{3F} = 2(\alpha_m + \beta_m) + \rho_1$

(see Appendix) and

$$O(t) \approx m(t)^2 h(t), \quad (54)$$

$$m(t) = \frac{\alpha_m}{\alpha_m + \beta_m} (1 - \exp[-(\alpha_m + \beta_m)t]), \quad (55)$$

$$h(t) = \frac{\alpha_h + \beta_h \exp[-(\alpha_h + \beta_h)t]}{\alpha_h + \beta_h}. \quad (56)$$

When the recovery rates $\sigma_1 > \sigma_2 > \sigma_3$ are chosen to satisfy microscopic reversibility, and σ_2, σ_3 are sufficiently small, it may be shown from the numerical solution of the six-state master equation that $O(t) = m(t)^2 h(t)$ is still a good approximation, where $m(t)$ and $h(t)$ are defined in Eqs. (55) and (56), and are derived from the analytical solution of the corresponding master equation with $\sigma_2 = \sigma_3 = 0$ (see Fig. 7). It may also be shown from Eqs. (36) and (37) that $C_1(t) \approx [1 - m(t)]^2 h(t)$ and $C_2(t) \approx 2m(t)[1 - m(t)]h(t)$, as the amplitude $k_{j+1}C_{1j}$ and $k_{j+1}C_{2j}$ for $j = 4, 5$ are close to zero, even though the magnitude of C_{1j} and C_{2j} increase with membrane depolarization.

The inactivated state probability $B_3(t)$ in Eq. (41) has the largest contribution from the term with the slowest relaxation rate ω_1 , with smaller contributions from the other terms, including those with eigenvalues $-\omega_4$ and $-\omega_5$ because, although $k_5, k_6 \approx 0$, $|B_{34}|$ and $|B_{35}|$ in Eq. (49) are correspondingly large. However, following the rapid decay of the terms with the largest relaxation rates, the probability for the inactivated state $B_3(t)$ may be approximated by a biexponential function, and therefore, consistent with the experimental determination of the time-course of the development of fast inactivation in Na^+ channels [6,24] (see Fig. 8).

Assuming that the time-dependence of the Na^+ channel open state probability is described by the solution of a phenomenological master equation, as well as the HH expression $m(t)^2 h(t)$, the conditions for model reduction, $|F(\omega_2)|, |F(\omega_3)| \ll |F(\omega_4)|, |F(\omega_5)|$ for depolarizing potentials, provide constraints upon the choice of empirical activation-deactivation rate functions. If $\omega_2 \approx \omega_4$ and $\omega_3 \approx \omega_5$ for a weakly coupled model of Na^+ channel activation and inactivation, these conditions are not satisfied and therefore, the terms with eigenvalues $-\omega_4$ and $-\omega_5$ have a nonzero amplitude and also contribute to the time-dependence of $O(t)$.

When the Na^+ channel is hyperpolarized to a clamp potential V from a large depolarizing holding potential, the solution of Eqs. (18) to (23) for $\sigma_1 > \sigma_2, \sigma_3 = 0$ is given by Eqs. (36) to (41), where k_1 and the stationary solution are defined in Eqs. (42) and (43), and for $j = 1$ to 5

$$C_{1j} = \frac{\sigma_1 \beta_{B1} \beta_{B2} E(\omega_j)}{F(\omega_j)}, \quad (57)$$

$$C_{2j} = -\frac{\sigma_1 \beta_{B1} \beta_{B2} \alpha_C (\omega_j - \beta_O - \rho_1)}{F(\omega_j)}, \quad (58)$$

$$O_j = \frac{\sigma_1 \beta_{B1} \beta_{B2} \alpha_O \alpha_C}{F(\omega_j)}, \quad (59)$$

$$B_{1j} = -\beta_{B1} \beta_{B2}, \quad (60)$$

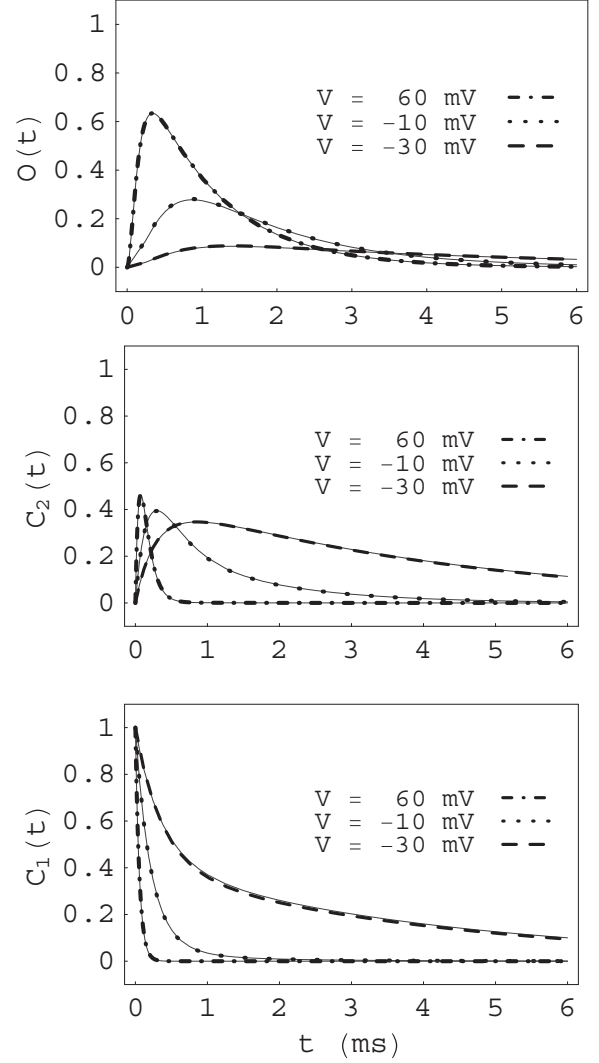


FIG. 7. During a depolarizing clamp potential for a Na^+ channel, from the numerical solution of a six-state master equation that satisfies microscopic reversibility (see Fig. 4), the open-state probability $O(t)$ (solid line) $\approx m(t)^2 h(t)$ (dashed, dotted, or dot-dashed), $C_1(t)$ (solid line) $\approx [1 - m(t)]^2 h(t)$ (dashed, dotted, or dot-dashed), and $C_2(t)$ (solid line) $\approx 2m(t)[1 - m(t)]h(t)$ (dashed, dotted, or dot-dashed), where $m(t)$ and $h(t)$ are solutions of rate equations for activation and inactivation in Eqs. (55) and (56), derived from the analytical solution of the master equation with $\sigma_2 = \sigma_3 = 0$, and the rate functions are $\alpha_{ik}(V) = 1$, $\gamma_{ik}(V) = 20.1$, $\beta_{ik}(V) = 3.4 \exp(-2.3V/25)$ for $k = 1$ to 3, $\delta_{i1}(V) = 2.5$, $\delta_{i2}(V) = 0.0045\delta_{i1}(V)$, $\delta_{i3}(V) = 0.05\delta_{i2}(V)$, $\alpha_C = 2\alpha_m$, $\beta_C = \beta_m$, $\alpha_O = \alpha_m$, $\beta_O = 2\beta_m$, $\alpha_{B1} = 3\alpha_C$, $\beta_{B1} = 0.0135\beta_C$, $\alpha_{B2} = 3\alpha_O$, $\beta_{B2} = 0.15\beta_O$, $\alpha_m = 0.1(V + 25)/\{1 - \exp[-(V + 25)/10]\}$, $\beta_m = 4 \exp[-(V + 50)/18]$ (ms^{-1}).

$$B_{2j} = \beta_{B2} \left(-\frac{\rho_1 \sigma_1 E(\omega_j)}{F(\omega_j)} + \omega_j - \alpha_{B1} - \sigma_1 \right), \quad (61)$$

$$B_{3j} = -\frac{\rho_1 \sigma_1 \alpha_C \alpha_O \beta_{B1} \beta_{B2} + \alpha_{B2} F(\omega_j) B_{2j}}{F(\omega_j)(\omega_j - \beta_{B2})}, \quad (62)$$

and $E(\omega)$ is defined in Eq. (50).

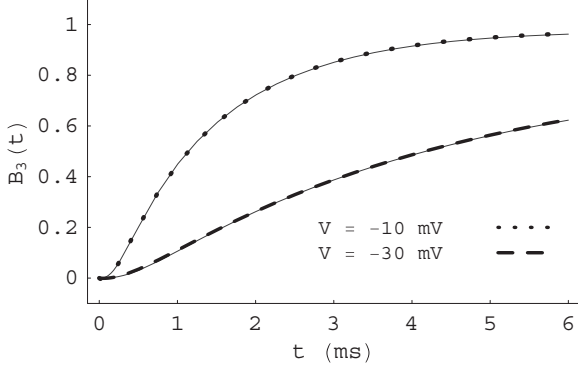


FIG. 8. During a depolarizing clamp potential for a six-state master equation for Na^+ channel gating, the probability of the inactivated state $B_3(t)$ (solid line) determined from the numerical solution of a master equation (see Fig. 7), may be approximated by the biexponential function $0.85 - 0.95 \exp(-0.24t) + 0.1 \exp(-2.6t)$ for $V = -30$ mV, and by the triexponential $0.98 - 1.04 \exp[-0.7t] - 0.36 \exp(-3.0t) + 0.42 \exp(-4.9t)$ for $V = -10$ mV, derived from the analytical solution of the corresponding master equation with $\sigma_2 = \sigma_3 = 0$.

For the nonzero eigenvalues, $\lambda_j = -\omega_j$ for $j = 1$ to 5, of the characteristic equation [see Eq. (A1) of the Appendix], $\omega_1 = \alpha_h + \beta_h \approx \omega_{1G} + \omega_{1F}$ and

$$\omega_k \approx \omega_{kG}, \quad \omega_{k+2} \approx \omega_{kF} \quad (63)$$

for $k = 2, 3$ (see Fig. 5). Applying the initial conditions [$C_1(0) = C_2(0) = O(0) = B_1(0) = B_2(0) = 0$ and $B_3(0) = 1$], and assuming that the difference between the roots of $F(\omega_j)$ and $G(\omega_j)$ is sufficiently large, from Eqs. (28) and (59), $|F(\omega_4)|, |F(\omega_5)| \ll |F(\omega_2)|, |F(\omega_3)|$, and hence $|C_{24}| \gg |C_{22}|, |C_{23}|, |C_{25}|$ and $|O_4|, |O_5| \gg |O_2|, |O_3|$. Therefore, to satisfy the initial conditions, $k_5, k_6 \approx 0$ and

$$k_2 = -\frac{1}{(\omega_2 - \omega_1)(\omega_3 - \omega_1)}, \quad (64)$$

$$k_3 = \frac{1}{(\omega_2 - \omega_1)(\omega_3 - \omega_2)}, \quad (65)$$

$$k_4 = -\frac{1}{(\omega_3 - \omega_1)(\omega_3 - \omega_2)}. \quad (66)$$

Assuming that $\alpha_C = 2\alpha_m$, $\alpha_O = \alpha_m$, $\beta_C = \beta_m$, $\beta_O = 2\beta_m$, and that the DIII S4 sensor is the slowest to deactivate ($\beta_{B1} \ll \beta_{B2}$) [10,11], from Eq. (36), we may write

$$C_1(t) \approx \left(\frac{\beta_m}{\alpha_m + \beta_m} \right)^2 \left[1 - \exp(-\omega_1 t) \times \left(1 + \frac{\omega_1 \{1 - \exp[-(\omega_2 - \omega_1)t]\}}{\omega_2 - \omega_1} \right) \right], \quad (67)$$

where the relaxation rates for biexponential recovery ω_1 and ω_2 are solutions of Eq. (35) (see Fig. 5), and the relative amplitude of the ω_1 and ω_2 components is ω_2/ω_1 .

When the recovery rates $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$ are chosen to satisfy microscopic reversibility, it may be shown from the numerical solution of the six-state master equation that the time course of the first closed-state probability during

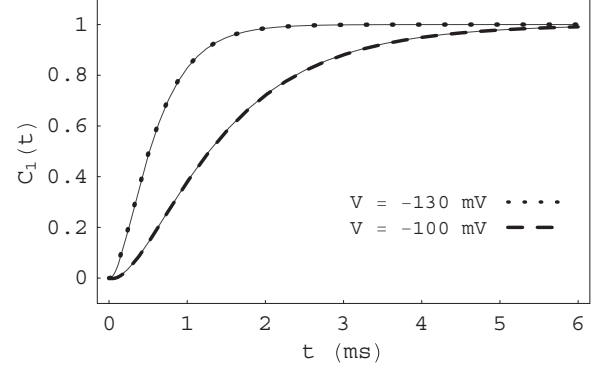


FIG. 9. During a hyperpolarizing clamp potential for a six-state master equation for Na^+ channel gating, the first closed-state probability $C_1(t)$ (solid line) determined from the numerical solution of a master equation (see Fig. 7), may be approximated by the biexponential function $1.0 - 1.58 \exp(-0.86t) + 0.58 \exp(-2.5t)$ for $V = -100$ mV, and by $1.0 - 2.2 \exp(-2.5t) + 1.2 \exp(-4.6t)$ for $V = -130$ mV [see Eq. (67)], derived from the analytical solution of the corresponding master equation ($\sigma_2 = \sigma_3 = 0$).

the recovery from inactivation may be approximated by the biexponential expression in Eq. (67), where the relative amplitude of the ω_1 and ω_2 terms is in agreement with the kinetics determined from Na^+ channels [6,24] (see Fig. 9). However, for large negative potentials, $\omega_2 \approx \beta_{B1} \gg \omega_1 \approx \sigma_1$, and Eq. (67) reduces to the HH expression $C_1(t) = [\beta_m/(\alpha_m + \beta_m)]^2 [1 - \exp(-\omega_1 t)]$. For a weakly coupled master equation, the conditions $|F(\omega_4)|, |F(\omega_5)| \ll |F(\omega_2)|, |F(\omega_3)|$ are not satisfied and therefore, the terms with eigenvalues $-\omega_4$ and $-\omega_5$ also contribute to $C_1(t)$.

When the Na^+ channel conductance is regulated by the activation of three voltage sensors in the DI, DII, and DIII domains, and coupled to a two-stage inactivation process where $\sigma_1 > \sigma_2 > \sigma_3 > \sigma_4 \approx 0$ are chosen to satisfy microscopic reversibility (see Fig. 10), it may be shown from the numerical solution of the master equation during a depolarizing clamp potential, that $O(t) \approx m(t)^3 h(t)$ where $m(t)$ and $h(t)$ are defined in Eqs. (55) and (56), and α_h and β_h are approximated by the smallest roots of two

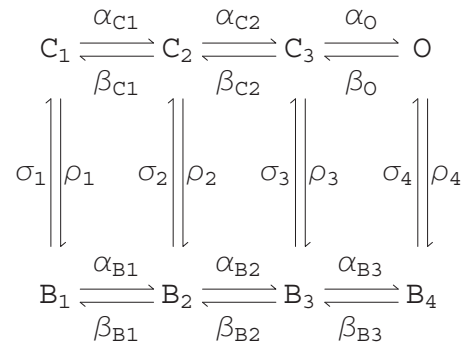


FIG. 10. State diagram for Na^+ channel gating where horizontal transitions represent the activation of DI, DII, and DIII voltage sensors that open the pore, and vertical transitions represent a two-stage Na^+ channel inactivation process, with derived rate functions ρ_k and α_k defined in Eqs. (24) and (25).

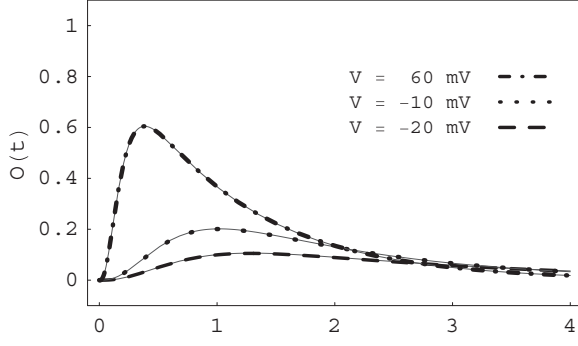


FIG. 11. During a depolarizing clamp potential for an eight-state system for Na^+ channel gating, from the numerical solution of a master equation that satisfies microscopic reversibility (see Fig. 10), the open-state probability $O(t)$ (solid line) $\approx m(t)^3 h(t)$ (dashed, dotted, or dot-dashed), where $m(t)$ and $h(t)$ are solutions of rate equations for activation and inactivation in Eqs. (55) and (56), derived from the analytical solution of the six state master equation, and the rate functions are $\alpha_{ik}(V) = 1$, $\gamma_{ik}(V) = 20.1$, $\beta_{ik}(V) = 3.4 \exp(-2.3V/25)$ for $k = 1$ to 4, $\delta_{i1}(V) = 2.5$, $\delta_{i2}(V) = 0.0045\delta_{i1}(V)$, $\delta_{i3}(V) = 0.05\delta_{i2}(V)$, $\delta_{i4}(V) = 0.05\delta_{i3}(V)$, $\alpha_m = 0.1(V + 25)/\{1 - \exp[-(V + 25)/10]\}$, $\beta_m = 4 \exp[-(V + 50)/18]$, $\alpha_{C1} = 3\alpha_m$, $\beta_{C1} = \beta_m$, $\alpha_{C2} = 2\alpha_m$, $\beta_{C2} = 2\beta_m$, $\alpha_O = \alpha_m$, $\beta_O = 3\beta_m$, $\alpha_{B1} = 3\alpha_{C1}$, $\beta_{B1} = 0.0135\beta_{C1}$, $\alpha_{B2} = 3\alpha_{C2}$, $\beta_{B2} = 0.15\beta_{C2}$, $\alpha_{B3} = 3\alpha_O$, $\beta_{B3} = 0.15\beta_O$ (ms^{-1}).

quartic polynomials and may be determined from Eqs. (34) and (35) (see Fig. 11). Similarly, during a hyperpolarizing clamp potential, if $\beta_{B1} \ll \beta_{B2}, \beta_{B3}$ [10,11], the numerical determination of the time-dependence of the first closed-state probability may be approximated by a more general form of Eq. (67) (see Fig. 12)

$$C_1(t) = \left(\frac{\beta_m}{\alpha_m + \beta_m} \right)^3 \left[1 - \exp(-\omega_1 t) \times \left(1 + \frac{\omega_1 \{1 - \exp[-(\omega_2 - \omega_1)t]\}}{\omega_2 - \omega_1} \right) \right], \quad (68)$$

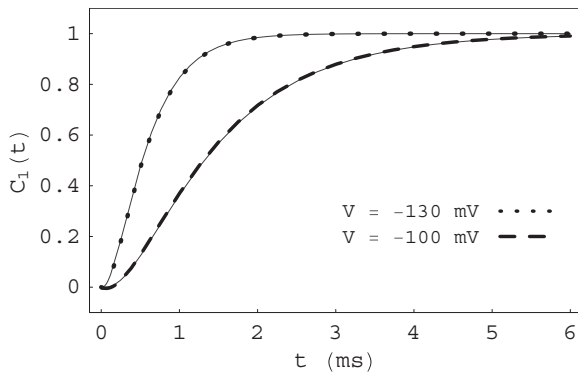


FIG. 12. During a hyperpolarizing clamp potential for an eight-state system for Na^+ channel gating, from the numerical solution of a master equation (see Fig. 11), the first closed-state probability $C_1(t)$ (solid line) may be approximated by the biexponential function $1.0 - 1.6 \exp(-0.86t) + 0.6 \exp(-2.55t)$ for $V = -100$ mV, and by $1.0 - 2.3 \exp(-2.5t) + 1.3 \exp(-4.6t)$ for $V = -130$ mV, based on Eq. (68) and the numerical determination of ω_1 and ω_2 from the matrix of coefficients of the master equation.

where ω_1 and ω_2 are the relaxation rates for biexponential recovery from inactivation, and the relative amplitude of the terms is consistent with experimental values [6,24].

III. MASTER EQUATION MODEL OF A Na^+ CHANNEL WITH A STATE-DEPENDENT INACTIVATION RATE

In this section, we consider the effect of an increase in the inactivation rate as the S4 sensors activate ($\rho_1 < \rho_2 < \rho_3$) [5,6,9], on the time-dependence of $m(t)$ and $h(t)$. If it is assumed that the DIV S4 rate functions satisfy $\alpha_{ik} = \alpha_{i1}$, $\gamma_{ik} = \gamma_{i1}$ for each k , and

$$\beta_{i1} > \beta_{i2} > \beta_{i3}, \quad (69)$$

the derived inactivation rate functions ρ_k and σ_k in Eqs. (24) and (25) are dependent on the closed, open, or inactivated state. In order to satisfy microscopic reversibility, we may write

$$\delta_{21} \frac{\alpha_{B1}}{\beta_{B1}} = \frac{\alpha_{A1}}{\beta_{A1}} = \frac{\alpha_C \beta_{i1}}{\beta_C \beta_{i2}}, \quad (70)$$

$$\delta_{32} \frac{\alpha_{B2}}{\beta_{B2}} = \frac{\alpha_{A2}}{\beta_{A2}} = \frac{\alpha_O \beta_{i2}}{\beta_O \beta_{i3}}, \quad (71)$$

and therefore, from Eq. (13), the rate functions satisfy the inequalities Eqs. (26) and (27).

The nonzero eigenvalues of the characteristic equation, Eq. (A1), are $\lambda_j = -\omega_j$ for $j = 1$ to 5, where ω_j may be approximated by the roots ω_{kF} and ω_{kG} of the cubic polynomials $F(\omega)$ and $G(\omega)$ (see Fig. 13 and the Appendix), and assuming that the activation sensors are independent ($\alpha_C = 2\alpha_O$ and $\beta_O = 2\beta_C$) and $\omega_{1F} = \Delta_{1F}$, $\omega_{2F} = \alpha_O + \beta_C + \Delta_{2F}$, $\omega_{3F} = 2(\alpha_O + \beta_C) + \Delta_{3F}$ are the roots of $F(\omega)$

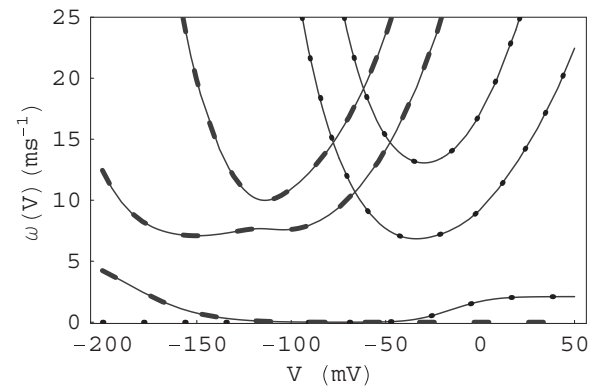


FIG. 13. The voltage dependence of $\omega_j = -\lambda_j$, $j = 1$ to 5 (solid lines), where λ_j is a nonzero eigenvalue of the characteristic equation of a master equation for a six-state system with state-dependent Na^+ inactivation, may be approximated by $\omega_{1F} \leq \omega_{2F} \leq \omega_{3F}$ (dotted lines), and $\omega_{1G} \leq \omega_{2G} \leq \omega_{3G}$ (dashed lines), the roots of the cubic polynomials $F(\omega)$ and $G(\omega)$ computed numerically, $\omega_1 = \alpha_h + \beta_h \approx \omega_{1G} + \omega_{1F}$ and the rate functions are based on those determined for Nav1.4 channels [6], $\alpha_{ik}(V) = 2.1$, $\gamma_{ik}(V) = 24.9$, for $k = 1$ to 3, $\beta_{i1}(V) = 80 \exp(-2.4V/25)$, $\beta_{i2}(V) = 40 \exp(-2.4V/25)$, $\beta_{i3}(V) = 2 \exp(-2.4V/25)$, $\delta_{i1}(V) = \exp(-0.2V/25)$, $\delta_{i2}(V) = \delta_{i3}(V) = 0$, $\alpha_C = 14.9 \exp(0.5V/25)$, $\alpha_O = 7.45 \exp(0.5V/25)$, $\beta_C = 0.8 \exp(-0.9V/25)$, $\beta_O = 1.6 \exp(-0.9V/25)$, $\alpha_{B1} = 4\alpha_C$, $\beta_{B1} = 0.01\beta_C$, $\alpha_{B2} = 5\alpha_O$, $\beta_{B2} = 0.05\beta_O$ (ms^{-1}).

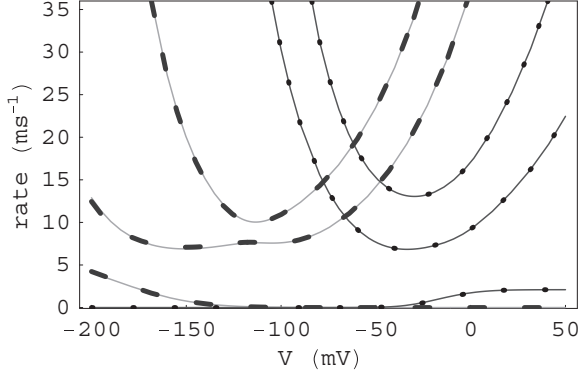


FIG. 14. The voltage-dependence of the roots $\omega_{1F} \leq \omega_{2F} \leq \omega_{3F}$ of $F(\omega)$ determined numerically (dotted lines) may be approximated by the expressions Δ_{1F} , $\alpha_O + \beta_C + \Delta_{2F}$, and $2(\alpha_O + \beta_C) + \Delta_{3F}$ (dark gray lines), where Δ_{1F} , Δ_{2F} , and Δ_{3F} are given in Eqs. (A9), (A12), and (A13), and the voltage dependence of the roots $\omega_{1G} \leq \omega_{2G} \leq \omega_{3G}$ of $G(\omega)$ (dashed lines) may be approximated by the expressions Δ_{1G} , $\alpha_{B2} + \beta_{B1} + \Delta_{2G}$, and $\alpha_{B1} + \beta_{B2} + \Delta_{3G}$ (light gray lines) where Δ_{1G} , Δ_{2G} , and Δ_{3G} are given in Eqs. (73), (74), and (75), $\Delta_G = 2$ and the rate functions are defined in Fig. 13. The inactivation rate $\beta_h \approx \Delta_{1F} \approx 2.1/[1 + \exp[-(V + 15)/10]]$, and therefore, has a similar voltage-dependence to the HH Na^+ channel-inactivation rate function for the squid axon [1].

in Eq. (28), the rate of inactivation for a depolarizing potential is $\beta_h \approx \Delta_{1F}$ (see Fig. 14), where

$$\Delta_{1F} \approx \frac{\alpha_O^2 \rho_3 + 2\alpha_O \beta_C \rho_2 + \beta_C^2 \rho_1}{(\alpha_O + \beta_C)^2}, \quad (72)$$

which reduces to Eq. (34) when the inactivation rate is not dependent on the closed or open state. Therefore, the voltage dependence of β_h has contributions from the inactivation rate ρ_k for each k , as well as the activation-deactivation functions α_O and β_C . However, most of the voltage dependence derives from ρ_k , and this is supported by the reduced voltage dependence of the time constant for inactivation in the charge-neutralized mutant Na^+ channel DIV-CN [6].

For a hyperpolarizing potential, assuming that $\omega_{1G} = \Delta_{1G} < \omega_{2G} = \alpha_{B2} + \beta_{B1} + \Delta_{2G} \ll \omega_{3G} = \alpha_{B1} + \beta_{B2} + \Delta_{3G}$ are the roots of the polynomial $G(\omega)$ in Eq. (30), it may be shown that the rate of recovery from inactivation $\alpha_h \approx \Delta_{1G}$ (see Appendix), where

$$\Delta_{1G} \approx \frac{\sigma_1 \beta_{B1} \beta_{B2}}{(\alpha_{B2} + \beta_{B1} + \Delta_{2G})(\alpha_{B1} + \beta_{B2} + \Delta_{3G})}, \quad (73)$$

$$\Delta_{2G} \approx \frac{D_1 - \sqrt{D_1^2 - 4D_2}}{2}, \quad (74)$$

$$\Delta_{3G} \approx \sigma_1 - \Delta_{1G} - \Delta_{2G}, \quad (75)$$

$$\bar{\Delta}_{1G} = \frac{\sigma_1 \beta_{B1} \beta_{B2}}{(\alpha_{B2} + \beta_{B1} + \Delta_G)(\alpha_{B1} + \beta_{B2} + \sigma_1 - \Delta_G)}, \quad (76)$$

$D_1 = \alpha_{B1} + \beta_{B2} - \alpha_{B2} - \beta_{B1} + \sigma_1$, $D_2 = \alpha_{B1} \beta_{B2} - \alpha_{B1} \beta_{B1} - \alpha_{B2} \beta_{B2} - \Delta_{1G}(\alpha_{B1} + \beta_{B2}) + \sigma_1 \beta_{B2}$, and the value of the

parameter Δ_G is chosen so that $\bar{\Delta}_{1G} \approx \Delta_{1G}$. If $\alpha_{B1}, \Delta_{2G} \ll \beta_{B2}$, Eq. (73) reduces to

$$\alpha_h \approx \frac{\sigma_1 \beta_{B1}}{\alpha_{B2} + \beta_{B1} + \Delta_{2G}}, \quad (77)$$

and may be approximated by an exponential function of V when $\beta_{B1} \ll \alpha_{B2} + \Delta_{2G}$ [1], whereas for more negative potentials, there is a gradual increase of α_h toward the saturation value σ_1 , in accord with the rate of recovery for inactivated Na^+ channels in hippocampal neurons (see Fig. 14) [5]. The recovery rate σ_1 is only weakly voltage dependent for hyperpolarizing potentials as $\beta_{i1} \gg \gamma_{i1}$, and therefore, most of the voltage dependence of α_h derives from the activation and deactivation functions between inactivated states. For the charge-neutralized mutant Na^+ channel DIV-CN, the voltage dependence of $\beta_{i1}(V)$ is reduced so that $\beta_{i1}(V) \ll \gamma_{i1}$ and $\sigma_1 \ll \delta_{i1}$, but the voltage dependence of α_{B2} and β_{B1} are not affected, and therefore, the expression for α_h in Eq. (77) is in accord with the DIV-CN data describing a slow recovery from inactivation [6].

The solution of the master equation, Eqs. (18) to (23), for $\sigma_1 > \sigma_2, \sigma_3 = 0$ and $\rho_1 < \rho_2 < \rho_3$, is given by Eqs. (36) to (41), where the stationary solution is

$$C_{1s} = \sigma_1 \beta_{B1} \beta_{B2} E_0,$$

$$C_{2s} = \sigma_1 \beta_{B1} \beta_{B2} \alpha_C (\beta_O + \rho_3),$$

$$O_s = \sigma_1 \beta_{B1} \beta_{B2} \alpha_C \alpha_O,$$

$$B_{1s} = \beta_{B1} \beta_{B2} f_3,$$

$$B_{2s} = \beta_{B2} (\alpha_{B1} + \sigma_1) f_3 - \rho_1 \sigma_1 \beta_{B2} E_0,$$

$$B_{3s} = \alpha_{B2} (\alpha_{B1} + \sigma_1) f_3 - \rho_1 \sigma_1 \alpha_{B2} E_0 + \rho_3 \sigma_1 \beta_{B1} \alpha_C \alpha_O, \quad (78)$$

$E_0 = E(0) = \rho_3 \alpha_O + (\beta_C + \rho_2)(\beta_O + \rho_3)$, f_3 is defined in Eq. (29), and the amplitudes of the terms of each state are dependent on

$$C_{1j} = E(\omega_j), \quad (79)$$

$$C_{2j} = -\alpha_C (\omega_j - \beta_O - \rho_3), \quad (80)$$

$$O_j = \alpha_C \alpha_O, \quad (81)$$

$$B_{1j} = -\frac{F(\omega_j)}{\sigma_1}, \quad (82)$$

$$B_{2j} = \frac{1}{\sigma_1 \beta_{B1}} [-\rho_1 \sigma_1 E(\omega_j) + (\omega_j - \alpha_{B1} - \sigma_1) F(\omega_j)], \quad (83)$$

$$B_{3j} = -\frac{\rho_3 \alpha_C \alpha_O + \alpha_{B2} B_{2j}}{\omega_j - \beta_{B2}}, \quad (84)$$

where $E(\omega) = \omega^2 - (\alpha_O + \beta_O + \beta_C + \rho_2 + \rho_3)\omega + (\beta_C + \rho_2)(\beta_O + \rho_3) + \alpha_O \rho_3$ (see Appendix).

If the Na^+ channel is depolarized to a clamp potential V from a large hyperpolarizing holding potential, the parameters $k_j, j = 2$ to 6 may be determined from the solution in Eqs. (36) to (41), and applying the initial conditions [$C_1(0) = 1$ and $C_2(0) = O(0) = B_1(0) = B_2(0) = B_3(0) = 0$], it may be shown that $k_5, k_6 \approx 0$ and k_2, k_3, k_4 are given by Eqs. (51) to (53). If $\rho_1 < \rho_2 < \rho_3$, and the recovery rates $\sigma_1 > \sigma_2 > \sigma_3$

are chosen to satisfy microscopic reversibility, where σ_2, σ_3 are small, it may be shown from the numerical solution of the master equation that $O(t) \approx m(t)^2 h(t)$, where $h(t)$ is defined in Eq. (56), and the activation variable is dependent on both

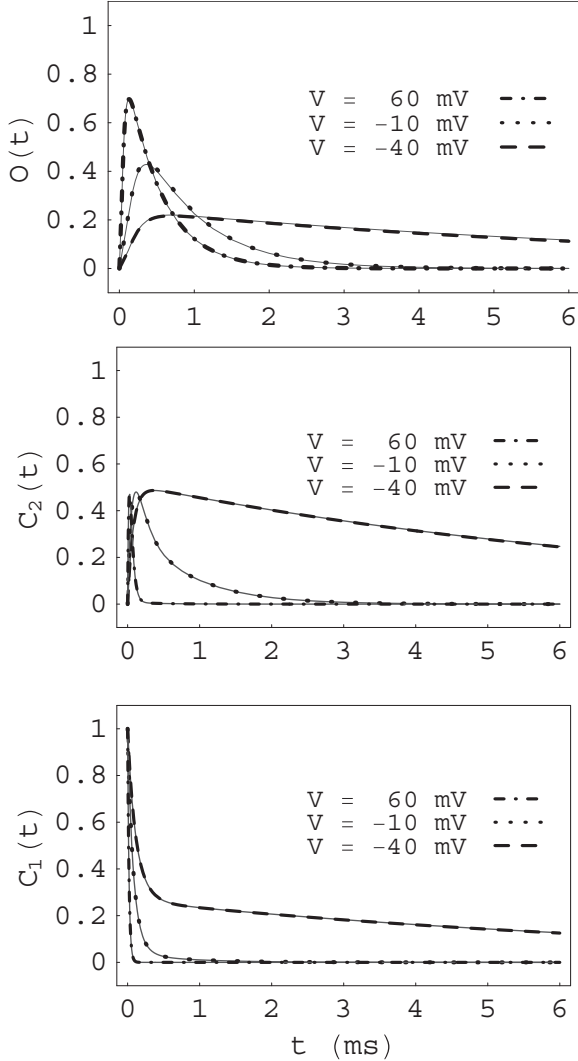


FIG. 15. During a depolarizing clamp potential for a six-state system with state-dependent inactivation, from the numerical solution of a master equation that satisfies microscopic reversibility, the open-state probability $O(t)$ (solid line) $\approx k_1 O_s + \sum_{j=1}^3 k_{j+1} O_j \exp(-\omega_j t) \approx m(t)^2 h(t)$ (dashed, dotted, or dot-dashed), $C_1(t)$ (solid line) $\approx k_1 C_{1s} + \sum_{j=1}^3 k_{j+1} C_{1j} \exp(-\omega_j t)$ (dashed, dotted, or dot-dashed), and $C_2(t)$ (solid line) $\approx k_1 C_{2s} + \sum_{j=1}^3 k_{j+1} C_{2j} \exp(-\omega_j t)$ (dashed, dotted, or dot-dashed), where $m(t)$ and $h(t)$ are solutions of rate equations for activation and inactivation in Eqs. (A8) and (A14), derived from the analytical solution of the corresponding master equation with $\sigma_2 = \sigma_3 = 0$, and the rate functions are based on those determined for Nav1.4 channels [6], $\alpha_{ik}(V) = 2.1$, $\gamma_{ik}(V) = 24.9$, for $k = 1$ to 3, $\beta_{i1}(V) = 80 \exp[-2.4V/25]$, $\beta_{i2}(V) = 40 \exp[-2.4V/25]$, $\beta_{i3}(V) = 2 \exp[-2.4V/25]$, $\delta_{i1}(V) = \exp(-0.2V/25)$, $\delta_{i2}(V) = 0.005\delta_{i1}(V)$, $\delta_{i3}(V) = 0.2\delta_{i2}(V)$, $\alpha_C = 14.9 \exp[0.5V/25]$, $\alpha_O = 7.45 \exp[0.5V/25]$, $\beta_C = 0.8 \exp[-0.9V/25]$, $\beta_O = 1.6 \exp[-0.9V/25]$, $\alpha_{B1} = 4\alpha_C$, $\beta_{B1} = 0.01\beta_C$, $\alpha_{B2} = 5\alpha_O$, $\beta_{B2} = 0.05\beta_O$ (ms^{-1}).

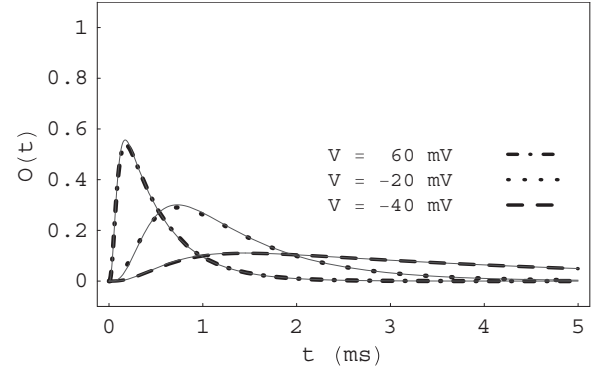


FIG. 16. During a depolarizing clamp potential for a 15-state master equation model of a Na^+ channel with three activation sensors and an opening step [6], the open-state probability $O(t)$ (solid line) $\approx a \exp(-\omega_1 t) [1 - \exp(-\omega_a t)]^3$ (dashed, dotted, or dot-dashed), where $\omega_1 = 0.26 \text{ ms}^{-1}$, $\omega_2 = 2.5 \text{ ms}^{-1}$, $\omega_a = \omega_2 - \omega_1$, $a = 0.18$ for $V = -40 \text{ mV}$, $\omega_1 = 1.13 \text{ ms}^{-1}$, $\omega_2 = 4.34 \text{ ms}^{-1}$, $\omega_a = 0.93(\omega_2 - \omega_1)$, $a = 0.95$ for $V = -20 \text{ mV}$, and $\omega_1 = 2.26 \text{ ms}^{-1}$, $\omega_2 = 22.5 \text{ ms}^{-1}$, $\omega_a = 0.89(\omega_2 - \omega_1)$, $a = 0.91$, for $V = 60 \text{ mV}$, where ω_1 , ω_2 , and a are determined from a numerical solution of the master equation, and $\omega_a/(\omega_2 - \omega_1)$ is a parameter.

activation and inactivation rate functions (see Fig. 15 and the Appendix)

$$m(t) = \frac{\alpha_O}{\alpha_O + \beta_C + \Delta} \{1 - \exp[-(\alpha_O + \beta_C + \Delta)t]\}, \quad (85)$$

$$\Delta = \frac{\alpha_O(\rho_2 + 2\rho_3) + \beta_C(2\rho_1 + \rho_2)}{\alpha_O + \beta_C} - 3\beta_h. \quad (86)$$

The HH expression for the open state during membrane depolarization also applies to strong coupling models where the probability of inactivation from a closed state is small, and inactivation generally occurs after activation and opening of the channel [3]. In the case of a Na^+ channel with three cooperative activation sensors, the HH description of the time-dependence of the open state $O(t) = m(t)^3 h(t)$ is still a good approximation if there is a separate opening step that follows the activation of the voltage sensors [6] (see Fig. 16).

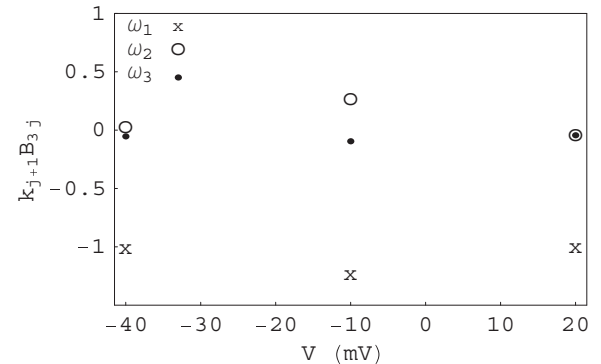


FIG. 17. During a depolarizing clamp potential, the probability of the Na^+ channel inactivated state $B_3(t)$ of a six-state master equation [see Eq. (41)] has the largest contribution from the terms with relaxation rates ω_1 and ω_2 , and the amplitude $k_{j+1} B_{3j} \approx 0$ for $j > 3$ (see Fig. 15).

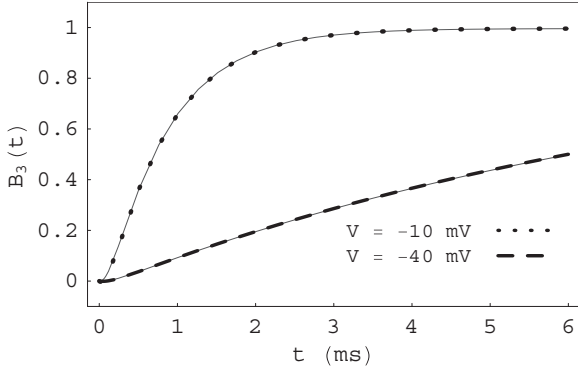


FIG. 18. During a depolarizing clamp potential for a six-state system for Na^+ channel gating, the probability of the inactivated state $B_3(t)$ (solid line) determined from the numerical solution of a master equation that satisfies microscopic reversibility, may be approximated by the biexponential function $0.98 - 1.0 \exp(-0.12t) + 0.02 \exp(-7.0t)$ for $V = -40$ mV, and by $0.996 - 1.23 \exp(-1.3t) + 0.23 \exp(-8.0t)$ for $V = -10$ mV, derived from the analytical solution of the corresponding master equation (see Fig. 15).

Assuming that the inactivation rate ρ_3 from the open state is larger than the rates ρ_1 and ρ_2 from closed states [7], from Eq. (41) the amplitude $k_{j+1} B_{3j} \approx 0$ for $j = 4, 5$ and

$$B_3(t) \approx \frac{\alpha_s}{\alpha_s + \beta_s} + \frac{B_{31} \exp(-\omega_1 t)}{(\omega_2 - \omega_1)(\omega_3 - \omega_1)} - \frac{B_{32} \exp(-\omega_2 t)}{(\omega_2 - \omega_1)(\omega_3 - \omega_2)} + a_3 \exp(-\omega_3 t),$$

where B_{31} and B_{32} are defined in Eq. (84), and the amplitude a_3 of the ω_3 term is small, and therefore $B_3(t)$ may be approximated by a biexponential function (see Figs. 17 and 18), where the ratio of the amplitude of the ω_1 and ω_2 terms is similar to experimental values for Nav1.4 channels [6]. From the numerical solution of a 15-state model of Na^+ channel gating during a depolarizing clamp potential, the inactivated

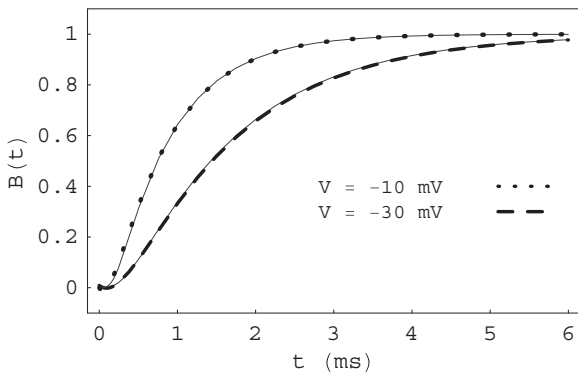


FIG. 19. During a depolarizing clamp potential, the probability of the (open) inactivated state $B(t)$ (solid line) determined from the numerical solution of a fifteen state master equation for Na^+ channel gating [6], may be approximated by the biexponential function $0.999 - 1.36 \exp(-0.69t) + 0.37 \exp(-3.2t)$ for $V = -30$ mV, and by $1 - 1.32 \exp(-1.31t) + 0.32 \exp(-7.2t)$ for $V = -10$ mV.

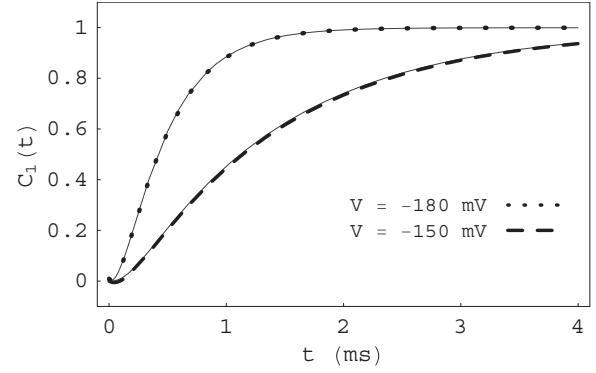


FIG. 20. During a hyperpolarizing clamp potential for a six-state system with state dependent Na^+ inactivation, from the numerical solution of the master equation that satisfies microscopic reversibility, the first closed-state probability $C_1(t)$ (solid line) may be approximated by the biexponential function $0.996 - 1.16 \exp(-0.74t) + 0.16 \exp(-7.1t)$ for $V = -150$ mV, and by $1.0 - 1.51 \exp(-2.6t) + 0.51 \exp(-8.4t)$ for $V = -180$ mV [see Eq. (67)], derived from the analytical solution of the corresponding master equation (see Fig. 15).

state probability is a sum of exponential terms but may be approximated by a biexponential function for physiological times (see Fig. 19).

For the six-state model of Na^+ channel gating, it has been assumed that the DIII sensor is the slowest to deactivate, and therefore, the time course of recovery from inactivation is given by Eq. (67) (see Fig. 20). However, by comparison, for a 15-state master equation model of the Na^+ channel, the deactivation rates between inactivated states are equal and, thus, the first closed-state probability during a hyperpolarizing clamp potential has contributions from several exponential terms but may also be approximated by a biexponential function (see Fig. 21) [6].

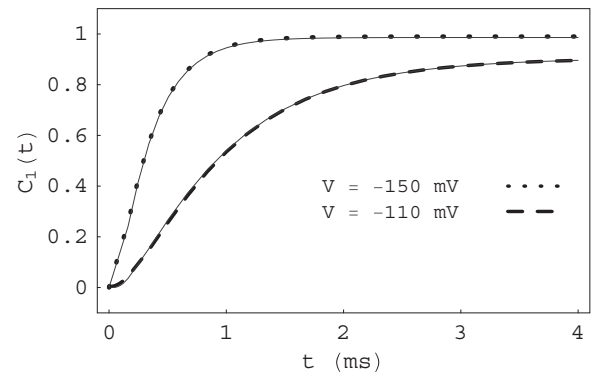


FIG. 21. During a hyperpolarizing clamp potential, the first closed-state probability $C_1(t)$ (solid line) determined from the numerical solution of a 15-state master equation for a Na^+ channel [6], may be expressed as a sum of exponential terms, but is approximated by the biexponential function $0.91 - 1.3 \exp(-1.24t) + 0.4 \exp(-4.2t)$ for $V = -110$ mV, and by $0.99 - 1.42 \exp(-3.55t) + 0.43 \exp(-11.7t)$ for $V = -150$ mV.

IV. CONCLUSION

Hodgkin and Huxley described the time-dependence of the Na^+ current in the squid giant axon membrane during a depolarizing clamp, in terms of the expression $m(t)^3h(t)$ where the activation variable $m(t)$ and inactivation variable $h(t)$ satisfy rate equations [1]. An alternative description of the Na^+ current in nerve and muscle membranes is provided by a master equation for coupled channel activation and inactivation processes where the backward inactivation rate to the open state is small, but the recovery rate to closed states increases as the activation sensors in the domains DI, DII, and especially DIII, deactivate. This model accounts for the small Na^+ current during repolarization of an inactivated channel, the saturation of the rate of recovery from inactivation for large hyperpolarized potentials, and the delay in the time-course of the recovery from inactivation [5]. If inactivation of the Na^+ channel is a two-stage process, the model can also account for the kinetics and voltage dependence of Na^+ inactivation for wild-type and mutant channels [6].

In this paper, we consider the coupling between two voltage sensors that regulate the Na^+ channel conductance and a two-stage inactivation process, where the first forward and backward inactivation transitions are rate-limiting, ensuring that the inactivation decay during a depolarizing voltage clamp is exponential. As the Na^+ current following inactivation is close to zero until the S4 sensors of the DIII, and either DI or DII domains deactivate, we have assumed that $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$. If σ_2 and σ_3 are small, the eigenvalues of the reduced six-state master equation that satisfies microscopic reversibility are approximated by the solutions of the characteristic equation for $\sigma_1 > \sigma_2, \sigma_3 = 0$. Therefore, the slowest relaxation ω_1 is determined by the inactivation rate ρ_1 when the inactivation rate is uniform between states, and by a linear combination of ρ_1 , ρ_2 , and ρ_3 , when the inactivation rate increases with S4 activation, where the exponential voltage dependence of ρ_k , for each k , saturates for a large depolarizing potential [1]. For a hyperpolarizing clamp of the Na^+ channel, the rate of recovery from inactivation is dependent on the backward inactivation rate σ_1 to the first closed state, as well as the rate functions of the DIII S4 sensor between inactivated states. The voltage dependence of the derived rate functions for inactivation and recovery from inactivation have a similar form to empirical expressions for Na^+ channels in the squid axon [1], hippocampal neurons [5], and Nav1.4 channels [6].

For a hyperpolarizing clamp potential, as the recovery rate $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$, it may be assumed that the deactivation rate functions between closed and open states are greater than those

between inactivated states ($\beta_O > \beta_{B2}, \beta_C > \beta_{B1}$), in order to satisfy microscopic reversibility. Therefore, the closed-state terms with eigenvalues of the characteristic equation that are determined by the roots of the polynomial $F(\omega)$ have an amplitude that are close to zero, and as the DIII S4 sensor is the slowest to deactivate ($\beta_{B1} \ll \beta_{B2}$) [10,11], the time-dependence of the recovery from inactivation is biexponential, and therefore, in agreement with the kinetic data from Nav1.4 channels [6].

For a depolarizing clamp of a Na^+ channel, assuming that $\beta_O > \beta_{B2}$ and $\beta_C > \beta_{B1}$, each term of the open-state probability with eigenvalue $\lambda = -\omega$, where ω approximates the roots ω_{2G} and ω_{3G} of the polynomial $G(\omega)$, also has an amplitude close to zero. A further simplification is possible when it is assumed that the activation sensors are independent ($\alpha_C = 2\alpha_O, \beta_O = 2\beta_C$) and it may be shown that the time-dependence of the open state $O(t) = m(t)^2h(t)$. When the rates δ_{i2} and δ_{i3} are small, the analytical solution provides a good approximation to the numerical solution of the corresponding master equation that satisfies microscopic reversibility, and the HH expression for the open-state probability is still valid for larger values of δ_{i2} and δ_{i3} , comparable to those determined for phenomenological master equation models of the Na^+ channel. Assuming that the inactivation rate from the open state is larger than the rate from closed states, the time-dependence of the probability for entry into the inactivated state has the largest contribution from the ω_1 and ω_2 terms and, therefore, may be approximated by a biexponential function, as described experimentally for Na^+ channels [6].

In most nerve membrane Na^+ channels, the activation of three voltage sensors regulate the Na^+ channel conductance, and by application of similar constraints on the activation and deactivation rate functions for inactivated and closed states, the time-dependence $m(t)^3h(t)$ of the Na^+ current may be derived from the solution to an eight state master equation for coupled activation and inactivation, and is also a good approximation when there is a separate opening step that follows the activation of cooperative sensors. For models of the Na^+ channel where the inactivation rate from the closed or open states increases as the S4 sensors activate, a more general form for the HH expression for the open state probability may be derived where $m(t)$ and $h(t)$ are dependent on both activation and inactivation processes.

APPENDIX

The Jacobian matrix of the six-state system of Eqs. (18) to (23) is

$$M = \begin{pmatrix} -(\alpha_C + \rho_1) & \beta_C & 0 & \sigma_1 & 0 & 0 \\ \alpha_C & -(\alpha_O + \beta_C + \rho_2) & \beta_O & 0 & \sigma_2 & 0 \\ 0 & \alpha_O & -(\beta_O + \rho_3) & 0 & 0 & \sigma_3 \\ \rho_1 & 0 & 0 & -(\alpha_{B1} + \sigma_1) & \beta_{B1} & 0 \\ 0 & \rho_2 & 0 & \alpha_{B1} & -(\alpha_{B2} + \beta_{B1} + \sigma_2) & \beta_{B2} \\ 0 & 0 & \rho_3 & 0 & \alpha_{B2} & -(\beta_{B2} + \sigma_3) \end{pmatrix}.$$

Assuming that the recovery rate $\sigma_2, \sigma_3 = 0$, and that the solution is a sum of terms of the form $\exp(\lambda t)$, where $\lambda = -\omega$ is an eigenvalue, the characteristic equation is

$$F(\omega)G(\omega) - \sigma_1[\rho_1 E(\omega)R(\omega) + \rho_2 \alpha_C \beta_{B1} S(\omega)] = 0, \quad (\text{A1})$$

where $F(\omega)$ and $G(\omega)$ are given by Eqs. (28) and (30), and

$$\begin{aligned} E(\omega) &= \omega^2 - (\alpha_O + \beta_O + \beta_C + \rho_2 + \rho_3)\omega \\ &\quad + (\beta_C + \rho_2)(\beta_O + \rho_3) + \rho_3 \alpha_O, \\ R(\omega) &= \omega^2 - (\beta_{B1} + \alpha_{B2} + \beta_{B2})\omega + \beta_{B1} \beta_{B2}, \\ S(\omega) &= \omega^2 - (\beta_O + \beta_{B2} + \rho_3)\omega + \beta_{B2}(\beta_O + \rho_3) \\ &\quad + \alpha_O \beta_{B2} \rho_3 / \rho_2. \end{aligned}$$

The constant term of Eq. (A1) is zero, and hence $\lambda = 0$ is an eigenvalue. The nonzero eigenvalues of the characteristic equation for Eqs. (18) to (23) are $\lambda_j = -\omega_j$ for $j = 1$ to 5, and assuming that the difference between the roots of $F(\omega)$ and $G(\omega)$ is sufficiently large, the magnitude of the σ_1 term in Eq. (A1) is small relative to either $F(\omega)$ or $G(\omega)$, and ω_j may be approximated by the roots of Eqs. (28) and (30), defined such that $\omega_{1F} \leq \omega_{2F} \leq \omega_{3F}$, and $\omega_{1G} \leq \omega_{2G} \leq \omega_{3G}$ (see Figs. 5 and 13). From Eq. (A1), as the coefficient of ω is approximately $f_3 g_2 + f_2 g_3$,

$$\omega_1 \omega_2 \omega_3 \omega_4 \omega_5 \approx f_3 g_2 + f_2 g_3, \quad (\text{A2})$$

and therefore,

$$\omega_1 \approx \frac{f_3}{\omega_{2F} \omega_{3F}} \frac{g_2}{\omega_{2G} \omega_{3G}} + \frac{f_2}{\omega_{2F} \omega_{3F}} \frac{g_3}{\omega_{2G} \omega_{3G}}, \quad (\text{A3})$$

where $g_2 \approx \omega_{2G} \omega_{3G}$ and $f_2 \approx \omega_{2F} \omega_{3F}$. If β_h is the rate of inactivation and α_h is the rate of recovery from inactivation, $\omega_1 = \alpha_h + \beta_h \approx \omega_{1G} + \omega_{1F}$. To ensure that the Na^+ current recovers from inactivation when the S4 sensors that regulate Na^+ conductance deactivate, it may be assumed that $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$ and therefore, to satisfy microscopic reversibility, from Eqs. (26) and (27), $\beta_{B1} \alpha_C < \beta_C \alpha_{B1}$ and $\beta_{B2} \alpha_O < \beta_O \alpha_{B2}$. If the Na^+ channel is depolarized to a clamp potential V from a large hyperpolarized holding potential [$C_1(0) = 1$ and $C_2(0) = O(0) = B_1(0) = B_2(0) = B_3(0) = 0$], the solution of Eqs. (18) to (23) for $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$, when σ_2, σ_3 are sufficiently small, may be approximated by the solution of the corresponding master equation for which $\sigma_1 > \sigma_2, \sigma_3 = 0$. We may define $\omega_k \approx \omega_{kF}, \omega_{k+2} \approx \omega_{kG}$, for $k = 2, 3$ and therefore, from Eqs. (28) and (82), $|F(\omega_2)|, |F(\omega_3)| \ll |F(\omega_4)|, |F(\omega_5)|$ and $|B_{k2}|, |B_{k3}| \ll |B_{k4}|, |B_{k5}|$ for each k . Therefore, to satisfy the initial conditions, $k_5, k_6 \approx 0$ and

$$k_2 + k_3 + k_4 = -k_1 \sigma_1 \beta_{B1} \beta_{B2},$$

$$k_2 \omega_1 + k_3 \omega_2 + k_4 \omega_3 = 0,$$

$$k_2 \omega_1^2 + k_3 \omega_2^2 + k_4 \omega_3^2 = 1,$$

with the solution

$$\begin{aligned} k_2 &= \frac{1 - k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_2 \omega_3}{(\omega_2 - \omega_1)(\omega_3 - \omega_1)}, \\ k_3 &= -\frac{1 - k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_1 \omega_3}{(\omega_2 - \omega_1)(\omega_3 - \omega_2)}, \\ k_4 &= \frac{1 - k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_1 \omega_2}{(\omega_3 - \omega_2)(\omega_3 - \omega_1)}. \end{aligned}$$

From Eq. (42),

$$k_1^{-1} = \sum_{j=1}^2 (C_{js} + B_{js}) + O_s + B_{3s},$$

and assuming that $\rho_k \sigma_1$ terms are small, for each k , from Eqs. (29), (31), and (78),

$$\begin{aligned} C_{1s} + C_{2s} + O_s &= \sigma_1 \beta_{B1} \beta_{B2} [E_0 + \alpha_C (\beta_O + \rho_3) + \alpha_C \alpha_O] \\ &\approx f_2 g_3, \\ \sum_{j=1}^3 B_{js} &\approx [(\alpha_{B1} + \sigma_1)(\alpha_{B2} + \beta_{B2}) + \beta_{B1} \beta_{B2}] f_3 \\ &\approx f_3 g_2, \end{aligned}$$

where $f_2 \approx \omega_{2F} \omega_{3F}$, $g_2 \approx \omega_{2G} \omega_{3G}$. Therefore,

$$\begin{aligned} k_1^{-1} &\approx \sigma_1 \beta_{B1} \beta_{B2} \omega_{2F} \omega_{3F} + f_3 \omega_{2G} \omega_{3G}, \\ k_1 O_s &\approx \frac{\alpha_C \alpha_O}{\omega_{2F} \omega_{3F}} \frac{\alpha_h}{\alpha_h + \beta_h}, \\ k_1 \sigma_1 \beta_{B1} \beta_{B2} \omega_{2F} \omega_{3F} &\approx \frac{\alpha_h}{\alpha_h + \beta_h}. \end{aligned}$$

From the open-state probability in Eq. (38),

$$\begin{aligned} O(t) &\approx \frac{\alpha_C \alpha_O}{\omega_2 \omega_3} \frac{\alpha_h}{\alpha_h + \beta_h} + \frac{\alpha_C \alpha_O}{(\omega_2 - \omega_1)(\omega_3 - \omega_1)} \frac{\beta_h \exp(-\omega_1 t)}{\alpha_h + \beta_h} \\ &\quad \times \left\{ 1 - \frac{\omega_3 - \omega_1}{\omega_3 - \omega_2} \exp[-(\omega_2 - \omega_1)t] \right. \\ &\quad \left. + \frac{\omega_2 - \omega_1}{\omega_3 - \omega_2} \exp[-(\omega_3 - \omega_1)t] \right\}. \quad (\text{A4}) \end{aligned}$$

If it is assumed that $\rho_1 = \rho_2 = \rho_3$, and the two activation sensors are independent ($\alpha_C = 2\alpha_m, \alpha_O = \alpha_m, \beta_C = \beta_m, \beta_O = 2\beta_m$), from Eq. (28) we may write $\omega_{1F} = \rho_1$, $\omega_{2F} = \alpha_m + \beta_m + \rho_1$, $\omega_{3F} = 2(\alpha_m + \beta_m) + \rho_1$, $\omega_{2F} - \omega_{1F} = \omega_{3F} - \omega_{2F} = \alpha_m + \beta_m$, $\omega_{3F} - \omega_{1F} = 2(\alpha_m + \beta_m)$, and

$$\begin{aligned} O(t) &\approx \left(\frac{\alpha_m}{\alpha_m + \beta_m} \right)^2 \frac{\alpha_h}{\alpha_h + \beta_h} + \frac{\beta_h \exp(-\omega_1 t)}{\alpha_h + \beta_h} \\ &\quad \times \left(\frac{\alpha_m}{\alpha_m + \beta_m} \right)^2 \{1 - \exp[-(\alpha_m + \beta_m)t]\}^2, \quad (\text{A5}) \end{aligned}$$

and hence

$$O(t) \approx m(t)^2 h(t), \quad (\text{A6})$$

$$m(t) = \frac{\alpha_m}{\alpha_m + \beta_m} \{1 - \exp[-(\alpha_m + \beta_m)t]\}, \quad (\text{A7})$$

$$h(t) = \frac{\alpha_h + \beta_h \exp[-(\alpha_h + \beta_h)t]}{\alpha_h + \beta_h}. \quad (\text{A8})$$

It may also be shown from the closed-state probabilities in Eqs. (36) and (37) that $C_1(t) = [1 - m(t)]^2 h(t)$, and $C_2(t) = 2m(t)[1 - m(t)]h(t)$. However, if $\rho_1 < \rho_2 < \rho_3$, assuming that $\alpha_C = 2\alpha_O$, $\beta_O = 2\beta_C$ and $\omega_{1F} \approx \Delta_{1F}$, $\omega_{2F} = \alpha_O + \beta_C + \Delta_{2F}$, $\omega_{3F} = 2(\alpha_O + \beta_C) + \Delta_{3F}$ are roots of the polynomial F in Eq. (28),

$$\Delta_{1F} \approx \frac{\alpha_O^2 \rho_3 + 2\alpha_O \beta_C \rho_2 + \beta_C^2 \rho_1}{(\alpha_O + \beta_C)^2}, \quad (\text{A9})$$

$$\Delta_{1F} + \Delta_{2F} + \Delta_{3F} \approx \rho_1 + \rho_2 + \rho_3, \quad (\text{A10})$$

$$\begin{aligned} 3\Delta_{1F} + 2\Delta_{2F} + \Delta_{3F} &\approx \frac{\alpha_O}{\alpha_O + \beta_C}(\rho_1 + 2\rho_2 + 3\rho_3) \\ &+ \frac{\beta_C}{\alpha_O + \beta_C}(3\rho_1 + 2\rho_2 + \rho_3), \end{aligned} \quad (\text{A11})$$

with the solution

$$\Delta_{2F} \approx \frac{\alpha_O(\rho_2 + 2\rho_3) + \beta_C(2\rho_1 + \rho_2)}{\alpha_O + \beta_C} - 2\Delta_{1F}, \quad (\text{A12})$$

$$\Delta_{3F} \approx \frac{(\rho_1 - \rho_3)(\alpha_O - \beta_C)}{(\alpha_O + \beta_C)} + \Delta_{1F}. \quad (\text{A13})$$

Defining $\Delta = \Delta_{2F} - \Delta_{1F}$, as $\omega_{3F} - \omega_{1F} \approx 2(\omega_{2F} - \omega_{1F})$, Eq. (A4) may be expressed as $O(t) \approx m(t)^2 h(t)$, where h is defined in Eq. (A8) and

$$m(t) = \frac{\alpha_O}{\alpha_O + \beta_C + \Delta} \{1 - \exp[-(\alpha_O + \beta_C + \Delta)t]\}, \quad (\text{A14})$$

a more general form of the HH expression for m in Eq. (A7). If the Na^+ channel is hyperpolarized to a clamp potential V from a large depolarized holding potential [$C_1(0) = C_2(0) = O(0) = B_1(0) = B_2(0) = 0$ and $B_3(0) = 1$], the solution of Eqs. (18) to (23) for $\sigma_1 > \sigma_2 > \sigma_3 \approx 0$ may be approximated by the solution of a master equation for which $\sigma_1 > \sigma_2, \sigma_3 = 0$, and the deactivation rate functions satisfy $\beta_O > \beta_{B2}, \beta_C > \beta_{B1}$, in order to satisfy microscopic reversibility. For a hyperpolarizing potential, we may define $\omega_k \approx \omega_{kG}$, and $\omega_{k+2} \approx \omega_{kF}$, for $k = 2, 3$, and as ω_{2F} and ω_{3F} are roots of $F(\omega)$, assuming that the difference between the roots of $F(\omega_j)$ and $G(\omega_j)$ is sufficiently large, from Eqs. (28) and (59), $|F(\omega_4)|, |F(\omega_5)| \ll |F(\omega_2)|, |F(\omega_3)|$, and hence $|C_{24}| \gg |C_{22}|, |C_{23}|, |C_{25}|$ and $|O_4|, |O_5| \gg |O_2|, |O_3|$. Therefore, to satisfy the initial conditions, $k_5, k_6 \approx 0$,

$$\begin{aligned} k_2 + k_3 + k_4 &= 0, \\ k_2\omega_1 + k_3\omega_2 + k_4\omega_3 &= 0, \\ k_2\omega_1^2 + k_3\omega_2^2 + k_4\omega_3^2 &= -1, \end{aligned}$$

with the solution

$$\begin{aligned} k_2 &= -\frac{1}{(\omega_2 - \omega_1)(\omega_3 - \omega_1)}, \\ k_3 &= \frac{1}{(\omega_2 - \omega_1)(\omega_3 - \omega_2)}, \\ k_4 &= -\frac{1}{(\omega_3 - \omega_2)(\omega_3 - \omega_1)}. \end{aligned}$$

From Eq. (36), as $\rho_k \approx 0$ for each k , the closed-state probability,

$$\begin{aligned} C_1(t) &\approx \frac{\beta_C \beta_O}{\beta_C \beta_O + \alpha_C \beta_O + \alpha_C \alpha_O} \\ &+ \sum_{j=1}^3 k_{j+1} \frac{\sigma_1 \beta_{B1} \beta_{B2} E(\omega_j)}{F(\omega_j)} \exp(-\omega_j t), \end{aligned} \quad (\text{A15})$$

and as $\beta_O \gg \beta_{B2}, \beta_C \gg \beta_{B1}$, we may write $E(\omega_j) \approx \beta_C \beta_O$, $F(\omega_j) \approx \omega_j(\beta_C \beta_O + \alpha_C \beta_O + \alpha_C \alpha_O)$, and Eq. (A15) becomes

$$\begin{aligned} C_1(t) &\approx \frac{\beta_C \beta_O}{\beta_C \beta_O + \alpha_C \beta_O + \alpha_C \alpha_O} \\ &\times \left[1 - \frac{\sigma_1 \beta_{B1} \beta_{B2}}{\omega_1(\omega_2 - \omega_1)(\omega_3 - \omega_1)} \exp(-\omega_1 t) \right. \\ &+ \frac{\sigma_1 \beta_{B1} \beta_{B2}}{\omega_2(\omega_2 - \omega_1)(\omega_3 - \omega_2)} \exp(-\omega_2 t) \\ &\left. - \frac{\sigma_1 \beta_{B1} \beta_{B2}}{\omega_3(\omega_3 - \omega_1)(\omega_3 - \omega_2)} \exp(-\omega_3 t) \right]. \end{aligned} \quad (\text{A16})$$

If it is assumed that $\alpha_{B2} + \beta_{B1} \gg \sigma_1$ and $\omega_{1G} = \Delta_{1G} < \omega_{2G} = \alpha_{B2} + \beta_{B1} + \Delta_{2G} \ll \omega_{3G} = \alpha_{B1} + \beta_{B2} + \Delta_{3G}$ are the roots of the polynomial $G(\omega)$ in Eq. (30), it may be shown that

$$\begin{aligned} \Delta_{1G} &\approx \frac{\sigma_1 \beta_{B1} \beta_{B2}}{(\alpha_{B2} + \beta_{B1} + \Delta_{2G})(\alpha_{B1} + \beta_{B2} - \Delta_{2G})}, \\ \Delta_{2G} &\approx \frac{D_1 - \sqrt{D_1^2 - 4D_2}}{2}, \\ \Delta_{3G} &\approx \sigma_1 - \Delta_{1G} - \Delta_{2G}, \\ \bar{\Delta}_{1G} &= \frac{\sigma_1 \beta_{B1} \beta_{B2}}{(\alpha_{B2} + \beta_{B1} + \Delta_G)(\alpha_{B1} + \beta_{B2} + \sigma_1 - \Delta_G)}, \end{aligned}$$

where $D_1 = \alpha_{B1} + \beta_{B2} - \alpha_{B2} - \beta_{B1} + \sigma_1$, $D_2 = \alpha_{B1} \beta_{B2} - \alpha_{B1} \beta_{B1} - \alpha_{B2} \beta_{B2} - \bar{\Delta}_{1G}(\alpha_{B1} + \beta_{B2}) + \sigma_1 \beta_{B2}$, the value of the parameter Δ_G is chosen so that $\bar{\Delta}_{1G} \approx \Delta_{1G}$, and if $\alpha_{B1}, \Delta_{2G} \ll \beta_{B2}$, we may write

$$\alpha_h \approx \Delta_{1G} \approx \frac{\sigma_1 \beta_{B1}}{\alpha_{B2} + \beta_{B1} + \Delta_{2G}}.$$

However, the HH rate function $\alpha_m \approx 0$ for a hyperpolarizing potential, and assuming that the DIII S4 sensor is the slowest to deactivate [10,11], $D_1 \approx \beta_{B2} \gg D_2 \approx (\alpha_{B1} - \alpha_{B2} + \sigma_1 - \bar{\Delta}_{1G})\beta_{B2}$ and therefore, $\Delta_{2G} \approx D_2/D_1 = \alpha_{B1} - \alpha_{B2} + \sigma_1 - \bar{\Delta}_{1G}$, $\Delta_{3G} = \alpha_{B2} - \alpha_{B1}$, $\omega_{3G} = \alpha_{B2} + \beta_{B2}$, $\omega_{1G}\omega_{2G} = \sigma_1 \beta_{B1} \beta_{B2}/\omega_3 \approx \sigma_1 \beta_{B1}$, and ω_{1G}, ω_{2G} are solutions of

$$\omega^2 - \omega(\alpha_{B1} + \beta_{B1} + \sigma_1) + \sigma_1 \beta_{B1} = 0.$$

From Eq. (A16), as $\omega_1, \omega_2 \ll \omega_3$, and $\omega_1 \omega_2 \omega_3 = \sigma_1 \beta_{B1} \beta_{B2}$,

$$\begin{aligned} C_1(t) &\approx \frac{\beta_C \beta_O}{\beta_C \beta_O + \alpha_C \beta_O + \alpha_C \alpha_O} \\ &\times \left[1 - \frac{\omega_2}{\omega_2 - \omega_1} \exp(-\omega_1 t) + \frac{\omega_1}{\omega_2 - \omega_1} \exp(-\omega_2 t) \right], \end{aligned}$$

and if the two activation sensors are independent ($\alpha_C = 2\alpha_m, \alpha_O = \alpha_m, \beta_C = \beta_m, \beta_O = 2\beta_m$), we may write

$$\begin{aligned} C_1(t) &\approx \left(\frac{\beta_m}{\alpha_m + \beta_m} \right)^2 \left[1 - \exp(-\omega_1 t) \right. \\ &\times \left. \left(1 + \frac{\omega_1 \{1 - \exp[-(\omega_2 - \omega_1)t]\}}{\omega_2 - \omega_1} \right) \right]. \end{aligned} \quad (\text{A17})$$

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