Voltage dependence of Hodgkin-Huxley rate functions for a multi-stage K channel voltage sensor within a membrane

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Abstract

The activation of a K channel sensor in two sequential stages during a voltage clamp may be described as the translocation of a Brownian particle in an energy landscape with two large barriers between states. A solution of the Smoluchowski equation for a square-well approximation to the potential function of the S4 sensor satisfies a master equation, and has two frequencies that may be determined from the forward and backward rate functions. When the higher frequency terms have small amplitude, the solution reduces to the relaxation of a rate equation, where the derived two-state rate functions are dependent on the relative magnitude of the forward rates (α and γ) and the backward rates (β and δ) for each stage. In particular, the voltage dependence of the Hodgkin-Huxley rate functions for a K channel may be derived by assuming that the rate functions of the first stage are large relative to those of the second stage - α ≫ γ and δ ≪ β. For a Shaker IR K channel, the first forward and backward transitions are rate limiting (α ≪ γ and δ ≪ β), and for an activation process with either two or three stages the derived two-state rate functions also have a voltage dependence that is of a similar form to that determined for the squid axon. The potential variation generated by the interaction between a two-stage K channel and a noninactivating Na channel is determined by the master equation for K channel activation and the ionic current equation when the Na channel activation time is small, and if δ ≪ β, and either α ≪ γ or α ≫ γ, the action potential may be described by the membrane voltage V and the open probability n.
INTRODUCTION

Based on the measurement of increased Na and K conductance during a depolarizing voltage clamp of the squid axon membrane, Hodgkin and Huxley (HH) proposed a model of the action potential which accounted for the threshold potential, the refractory period and the speed of transmission of the impulse [1]. The Na and K ionic conductance was described by activation variables \(m\) and \(n\) and an inactivation variable \(h\), that each satisfy a first-order rate equation with empirical transition rate functions that depend on the potential difference \(V\) across the membrane [1]. It was assumed that the voltage sensitivity of \(m\), \(n\) and \(h\) is dependent on the transverse movement of charged gating particles when the electric field within the membrane is changed. The existence of a delay in the increase of the K and Na conductance, and the transient nature of the Na conductance increase were represented by \(g_K \propto n^4\) and \(g_{Na} \propto m^3 h\). The HH model has been applied to ion channels in cardiac Purkinje fibres [2], toad myelinated neuron [3], gastropod neuron (including an A-type inactivating K channel) [4], and a bursting pacemaker neuron in the mollusc Aplysia [5].

The activation variable for the squid axon K channel satisfies a rate equation

\[
\frac{dn}{dt} = \alpha_n - (\alpha_n + \beta_n)n, \tag{1}
\]

where the empirical forward and backward rate functions are of the form

\[
\alpha_n = \frac{C(V - A)}{1 - \exp[-B(V - A)]}, \tag{2}
\]

\[
\beta_n = E \exp[-DV], \tag{3}
\]

\(V\) is the membrane voltage, and A to E are constants. The HH rate functions \(\alpha_n\) and \(\beta_n\) have been successful in describing activation in a wide variety of ion channels because \(\alpha_n\) represents the exponential dependence on \(V\) for small depolarizations and the almost linear dependence on \(V\) for large clamp potentials, and \(\beta_n\) represents the exponential voltage dependence of the rate constant for a large hyperpolarization. The expression for \(\alpha_n\) in Eq. (2) may be obtained from a solution to the Smoluchowski equation for the probability density of states of the voltage sensor, when the potential function is linear in the transverse coordinate \(Z\) [6, 7], and a rate equation for activation may be derived if there is a large diffusion or potential barrier between closed and open states [8]. However, in view of the presence of negative residues on the S2 and S3 segments within the voltage sensing domain (VSD), as well as induced charge at the dielectric boundary of the membrane, the potential function for the S4 sensor is a nonlinear function of \(Z\) for each potential \(V\) [9, 10].

By expressing a cloned Shaker IR K channel in Xenopus oocytes, contamination with endogenous currents is minimized, and permits a more accurate determination of the ionic and gating currents across the membrane [11]. Assuming that channel opening occurs when the voltage sensor in each of the four subunits is activated through two transitions between three states, followed by a cooperative transition, the model accounts for steady state and kinetic activation and deactivation for both ionic and gating currents, the measurement of
gating charge of 13e per channel during activation, and the rising phase of the gating current observed during depolarization.

Each of the four subunits of a voltage-dependent K channel has a voltage-sensing domain with transmembrane segments S1 to S4, and segments S5 to S6 that form the ion-conducting pore domain. The S4 segment moves transversely through a gating pore in response to a change in the potential difference across the membrane, and is stabilized by the interaction between its positively charged residues R1 to R4 and negatively charged amino acids on S2 and S3 segments [12, 13]. In the activated state, lipid phosphate groups on the membrane surface [14, 15, 16, 17]. The attached residues of the S4 segment sequentially translocate across a focused membrane field where the hydrophobic residue $F_{290}$ on the S2 segment of the K channel contributes to the energy barrier for transitions [18]. By measuring the effect of mutants on the gating current of the K channel, it has been shown that $F_{290}$ controls the transfer of the arginine R4 across the membrane field during the final gating transition, and has a significant effect on deactivation kinetics but only a small effect on activation time [19]. When the hydrophobic residue I287 on the S2 segment or V363 adjacent to the residue R1 on the S4 segment are replaced by the hydrophilic amino acid Thr, the rate of activation of the K channel is increased by a factor of two, and comparable to the activation rate in a Na channel [20]. Therefore, the first forward transition during activation and the first backward transition during deactivation are rate-limiting, and dependent on the hydrophobicity of residues on adjacent segments in the VSD.

Assuming that the energy of the voltage sensor is dependent on the Coulomb force between positive S4 residues and negative residues on neighboring segments, the electric field within the membrane and the dielectric boundary force on charged residues, the energy of each potential well and, therefore, the equilibrium distribution of gating charge for each voltage may be calculated for both wild-type and mutant Shaker K channels, and good agreement with experimental data is obtained by an appropriate choice of the positions of the negative residues and of the other parameter values [9]. The time-dependence of the survival probabilities of the closed states of the voltage sensor during a voltage clamp may be calculated from a solution of Smoluchowski’s equation with a potential function for the S4 sensor that may be derived by application of Poisson’s equation to a dielectric slab between solvent regions, and is in accord with a three-stage empirical model of the ionic and gating currents for a K channel [21, 22]. In particular, the voltage dependence of the forward and backward rate functions for each stage may be derived from the asymmetry of the energy barriers determined by the interaction between S4 residues in close proximity to residues on S2 and S3 segments.

In this paper, assuming that the K channel conductance is modulated by a single S4 sensor which activates in two sequential stages, it is shown that if the rate functions satisfy $\delta \ll \beta$, and either $\alpha \ll \gamma$ or $\alpha \gg \gamma$, the master equation that describes the dynamics of a voltage clamp may be reduced to a rate equation with derived forward and backward rate functions that are approximated by the functions $\alpha_n$ and $\beta_n$ for a squid axon K channel [1]. A three-stage model
of activation of a K channel may be determined that has the same derived rate functions and survival probability $n(t)$ during a voltage clamp as a two-stage model. If $δ ≪ β$, and either $α ≪ γ$ or $α ≫ γ$, the interaction between Na and K channels during a repetitive action potential may be described by two variables $V$ and $n$.

**THE VOLTAGE CLAMP OF A MULTI-STAGE K CHANNEL SENSOR**

It is assumed that a K channel voltage sensor is a Brownian particle that translocates across electrostatic barriers between potential wells by absorbing energy from the thermal environment [9]. The solution of the Smoluchowski equation for a square-well approximation to the potential function of the S4 sensor satisfies a master equation [10], and if activation occurs in two stages between three states (see Fig. 1) [11], the occupation probabilities of the closed states $n_1$, $n_2$ and the open state $n$ are determined by

\[
\frac{dn_1(t)}{dt} = -αn_1(t) + βn_2(t),
\]

\[
\frac{dn_2(t)}{dt} = αn_1(t) + δn(t) - (β + γ)n_2(t),
\]

\[
\frac{dn(t)}{dt} = γn_2(t) - δn(t).
\]

where the rate functions

\[
α(V) = α_0 \exp[q_α(V - V_0)/kT], \quad β(V) = β_0 \exp[-q_β(V - V_0)/kT],
\]

\[
γ(V) = γ_0 \exp[q_γ(V - V_0)/kT], \quad δ(V) = δ_0 \exp[-q_δ(V - V_0)/kT],
\]

$α_0, β_0, γ_0, δ_0$ are constants (ms$^{-1}$), $q_i$ is the equivalent charge for each forward or backward transition, $k$ is Boltzmann’s constant, $kT/e ≈ 25$ mV, $e$ is the electronic charge, $V_0$ is a constant and $V$ is the membrane voltage (in mV).

For a large hyperpolarized holding potential, we may assume that $n_1(0) = 1$, and $n(0) = n_2(0) = 0$, and if the K voltage sensor is depolarized to a clamp potential $V$ (activation), the solution of Eqs. (4) to (6) for the open state is (see Appendix 1)

\[
n_A(t) = \frac{αγ}{ω_1ω_2} + \frac{αγ}{ω_1(ω_1 - ω_2)} \exp(-ω_1t) - \frac{αγ}{ω_2(ω_1 - ω_2)} \exp(-ω_2t),
\]

where $ω_1$ and $ω_2$ ($≫ ω_1$) are solutions of the characteristic equation

\[
ω^2 - ω(α + β + γ + δ) + αγ + δ(α + β) = 0.
\]

However, if the S4 sensor is initially in the open state ($n(0) = 1$, and $n_1(0) = n_2(0) = 0$), and if the K sensor is repolarized to a clamp potential $V$ (deactivation), the solution is (see Appendix 1)

\[
n_D(t) = \frac{αγ}{ω_1ω_2} + \frac{δ(ω_2 - γ - δ)}{ω_1(ω_2 - ω_1)} \exp(-ω_1t) + \frac{δ(ω_1 - γ - δ)}{ω_2(ω_1 - ω_2)} \exp(-ω_2t).
\]
Assuming that the rate functions of the first step are larger than the second step ($\beta \gg \delta$ and $\alpha \gg \gamma$), it may be shown from Eq. (10) that $\omega_1 \approx \gamma\alpha/\alpha + \beta + \delta$ and $\omega_2 \approx \alpha + \beta \gg \omega_1$, and Eqs. (9) and (11) become

$$n_A(t) \approx \frac{\alpha\gamma}{\alpha\gamma + \delta(\alpha + \beta)}[1 \exp(-\omega_1 t)], \quad (12)$$

$$n_D(t) \approx \frac{\alpha\gamma + \delta(\alpha + \beta)\exp(-\omega_1 t)}{\alpha\gamma + \delta(\alpha + \beta)}. \quad (13)$$

Eqs. (12) and (13) are solutions of the rate equation [1]:

$$\frac{dn}{dt} = \alpha_n, n + (\alpha_n, n + \beta_n, n)n, \quad (14)$$

where

$$\alpha_{n,2}(V) = \frac{\omega_1 \alpha\gamma}{\alpha\gamma + \delta(\alpha + \beta)}, \quad (15)$$

$$\beta_{n,2}(V) = \frac{\omega_1 \delta(\alpha + \beta)}{\alpha\gamma + \delta(\alpha + \beta)}, \quad (16)$$

and, therefore, $\omega_1 = \alpha_{n,2} + \beta_{n,2}$, and $\beta_{n,2}/\alpha_{n,2} = (1 + \beta/\alpha)\delta/\gamma$. Substituting $\omega_1 \approx \gamma\alpha/(\alpha + \beta) + \delta$, we may write

$$\alpha_{n,2}(V) \approx \frac{\gamma}{1 + \beta/\alpha}, \quad (17)$$

$$\beta_{n,2}(V) \approx \delta, \quad (18)$$

which provide a good fit to the empirical rate functions $\alpha_n$ and $\beta_n$ for the squid axon K channel [1] (see Fig. 2). The rate functions are chosen so that $\gamma \approx \alpha_n$ for large depolarized potentials, the ratio $\beta/\alpha \approx \gamma/\alpha_n - 1$, and $\delta = \beta_n$. There is also good agreement between the survival probability of the state $n(t)$ during a voltage clamp calculated from a rate equation with $\alpha_n$ and $\beta_n$, and from the master equation solutions Eqs. (9) and (11) (see Figs. 3(a) and 3(b)), and therefore, an activation process for the voltage sensor with at least two stages provides a physical basis for the mathematical form and asymmetry of $\alpha_n$ and $\beta_n$, without assuming a constant electric field within the membrane. The function $\alpha_{n,2}(V)$ has the almost linear variation of $\gamma(V)$ for large depolarizing clamp potentials, and has the exponential variation of $\beta(V)$, as well as $\alpha(V)$ and $\gamma(V)$, for depolarizations near the resting state, whereas $\beta_{n,2}(V)$ has the exponential voltage dependence of $\delta(V)$.

However, if we assume that $\beta \gg \delta$ and $\gamma \gg \alpha$, a limiting case of the description of ionic and gating currents within the Shaker K channel [11], it may be shown that $\omega_1 \approx \alpha\gamma/(\gamma + \beta + \delta(\alpha + \beta)/(\gamma + \beta))$ and $\omega_2 \approx \gamma + \beta \gg \omega_1$, and Eqs. (19) and (20) become

$$n_A(t) \approx \frac{\alpha\gamma}{\alpha\gamma + \delta(\alpha + \beta)}[1 - \exp(-\omega_1 t)], \quad (19)$$

$$n_D(t) \approx \frac{\alpha\gamma + \delta(\gamma - \alpha)\exp(-\omega_2 t)}{(\gamma + \beta)^2} + \frac{\delta(\beta - \delta)\exp(-\omega_1 t)}{(\gamma + \beta)(\alpha + \delta)}. \quad (20)$$
and for a sufficiently large hyperpolarization, $\beta - \delta > \gamma - \alpha$, and we may write

$$n_D(t) \approx \frac{\alpha \gamma + \delta(\alpha + \beta) \exp(-\omega_1 t)}{\alpha \gamma + \delta(\alpha + \beta)}, \quad (21)$$

Eqs. (19) and (21) are also solutions of Eq. (14), and substituting $\omega_1 = \alpha \gamma / (\gamma + \beta) + \delta(\alpha + \beta) / (\gamma + \beta)$ into Eqs. (15) and (16),

$$\alpha_{n,2}(V) \approx \frac{\alpha}{1 + \beta/\gamma} \quad (22)$$

$$= \frac{\alpha_0 \exp[q_\alpha(V - V_0)/kT]}{1 + (\beta_0/\gamma_0) \exp[-(q_\beta + q_\gamma)(V - V_0)/kT]},$$

$$\beta_{n,2}(V) \approx \frac{\delta(\alpha + \beta)}{(\gamma + \beta)} \quad (23)$$

$$= \frac{\delta_0 \exp[-q_\delta(V - V_0)/kT](1 + (\alpha_0/\beta_0) \exp[(q_\alpha + q_\beta)(V - V_0)/kT])}{1 + (\gamma_0/\beta_0) \exp[(q_\gamma + q_\beta)(V - V_0)/kT]}.\,$$

For a large depolarizing potential $V$, $\alpha_{n,2}(V) \approx \alpha$ and $\beta_{n,2}(V) \approx \delta \alpha/\gamma$, whereas for a hyperpolarizing potential, $\alpha_{n,2}(V) \approx \gamma \alpha/\beta$ and $\beta_{n,2}(V) \approx \delta$, and have a similar form to the empirical rate functions for a delayed rectifier $K$ channel in a cardiac Purkinje fiber [23], which may be expressed as

$$\alpha_x = \frac{D \exp[C(V - A)]}{1 + \exp[-B(V - A)]}, \quad (24)$$

$$\beta_x = \frac{H \exp[-G(V - E)]}{1 + \exp[F(V - E)]}, \quad (25)$$

where $A$ to $H$ are constants.

For a Shaker $K$ channel [11], the derived rate functions $\alpha_{n,2}$ and $\beta_{n,2}$ may be approximated by functions $\alpha_H$ and $\beta_H$ which have the same mathematical form as the rate functions $\alpha_n$ and $\beta_n$ (see Fig. 4). Although there is good agreement for small depolarizations between the survival probability of the state $n(t)$ during a voltage clamp calculated from a rate equation with $\alpha_{n,2}$ and $\beta_{n,2}$, and from Eqs. (9) and (11) (see Figs. 5(a) and 5(b)), for larger depolarizations the higher frequency component of the solution makes a contribution, and therefore deviates from the two-state model. If $\beta(V)$ and $\gamma(V)$ are increased by a factor of 3, the rate equation provides a better fit to two-stage activation for larger depolarizations (see Fig. 5(c)). However, the rate equation cannot account for the rising phase of the $K$ channel gating current that has been observed in both Shaker and squid axon $K$ channels [11], and requires an activation model of the ion channel for which $\gamma > \alpha$ (see Appendix 2).

If the rate functions satisfy $\beta \ll \delta$ and $\alpha \ll \gamma$, from Eqs. (9) and (10),

$$\omega_1 \approx \alpha + \beta \delta / (\gamma + \delta) \ll \omega_2 \approx \gamma + \delta,$$

and

$$n_A(t) \approx \frac{\alpha \gamma}{\omega_1 \omega_2} + \frac{\alpha \gamma}{\omega_2} \left( \frac{-\exp(-\omega_1 t)}{\omega_1} + \frac{\exp(-\omega_2 t)}{\omega_2} \right). \quad (26)$$
The high frequency term is small for all potentials, and hence
\[ n_A(t) \approx \frac{\alpha \gamma}{\alpha \gamma + \delta (\alpha + \beta)} [1 - \exp(-\omega_1 t)]. \] (27)

For deactivation, the low frequency term in Eq. (11) is small, and
\[ n_D(t) \approx \frac{\alpha \gamma + \delta (\alpha + \beta) \exp(-\omega_2 t)}{\alpha \gamma + \delta (\alpha + \beta)}. \] (28)

Therefore, the ionic currents during a voltage clamp cannot be described by a rate equation, and similarly, if \( \beta \ll \delta \) and \( \alpha \gg \gamma \), the ionic currents have contributions from both low and high frequency terms and therefore require a master equation description.

If the S4 voltage sensor is activated in three stages between four states (see Fig. 6), the solution of the Smoluchowski equation for the activation landscape may be expressed as a master equation
\[
\begin{align*}
\frac{dn_1(t)}{dt} &= -\alpha_1 n_1(t) + \beta_1 n_2(t), \\
\frac{dn_2(t)}{dt} &= \alpha_1 n_1(t) + \beta_2 n_3(t) - (\beta_1 + \alpha_2) n_2(t), \\
\frac{dn_3(t)}{dt} &= \alpha_2 n_2(t) + \beta_3 n(t) - (\beta_2 + \alpha_3) n_3(t), \\
\frac{dn(t)}{dt} &= \alpha_3 n_3(t) - \beta_3 n(t).
\end{align*}
\] (29-32)

where the rate functions
\[ \alpha_i(V) = \alpha_{i0} \exp[q_{\alpha_i} V/kT], \quad \beta_i(V) = \beta_{i0} \exp[-q_{\beta_i} V/kT], \] (33)
for \( i = 1 \) to 3, \( \alpha_{i0} \) and \( \beta_{i0} \) are constants (ms\(^{-1}\)), and \( q_{\alpha_i} \) and \( q_{\beta_i} \) are the equivalent charges for the \( i \)th forward or backward transition for each stage.

Assuming that the rate functions for the last step of activation of the S4 sensor across the membrane are an order of magnitude smaller than previous steps (\( \beta_1, \beta_2 \gg \beta_3 \) and \( \alpha_1, \alpha_2 \gg \alpha_3 \)), the master equation may be reduced to (see Appendix 1)
\[ \frac{dn}{dt} = \alpha_{n,3} - (\alpha_{n,3} + \beta_{n,3})n, \] (34)

where
\[
\begin{align*}
\alpha_{n,3}(V) &\approx \frac{\alpha_3}{1 + (\beta_2/\alpha_2)(1 + \beta_1/\alpha_1)}, \\
\beta_{n,3}(V) &\approx \beta_3,
\end{align*}
\] (35-36)

which also provide a good fit to the rate functions \( \alpha_n \) and \( \beta_n \) for the squid axon K channel, and reduce to the expressions in Eqs. (17) and (18) when
\[ \beta_1 = 0. \] However, if it is assumed that the rate functions satisfy \( \beta_3 \ll \beta_1, \beta_2 \) and \( \alpha_1 \ll \alpha_2, \alpha_3 \), the master equation may be approximated by Eq. (34) where

\[
\alpha_{n,3}(V) \approx \frac{\alpha_1 \alpha_3}{\alpha_3 + (\beta_2/\alpha_2)(\alpha_3 \beta_1/\beta_2 + \beta_1)},
\]

(37)

\[
\beta_{n,3}(V) \approx \frac{\beta_3 (\alpha_1 + \beta_2 (\alpha_1 + \beta_1)/\alpha_2)}{\alpha_3 + (\beta_2/\alpha_2)(\alpha_3 \beta_1/\beta_2 + \beta_1)}.
\]

(38)

For a large depolarizing potential \( V \), \( \alpha_{n,3}(V) \approx \alpha_1 \) and \( \beta_{n,3}(V) \approx \beta_3 \alpha_1/\alpha_3 \), whereas for a hyperpolarizing potential, \( \alpha_{n,3}(V) \approx \alpha_1(\alpha_2/\beta_1)(\alpha_3/\beta_2) \) and \( \beta_{n,3}(V) \approx \beta_3 \).

If a two and three stage model of activation of a voltage sensor each reduce to the same rate equation, \( \alpha_{n,3}(V) = \alpha_{n,2}(V) \) and \( \beta_{n,3}(V) = \beta_{n,2}(V) \), and assuming \( \alpha_1 = \alpha_3 \beta_1/\beta_2 \), the rate functions \( \alpha = \alpha_1 \), \( \delta = \beta_3 \), \( \gamma = \alpha_3 \), \( \beta = \beta_2(\alpha_1 + \beta_1)/\alpha_2 \approx \beta_2(\alpha_3 \beta_1/\beta_2 + \beta_1)/\alpha_2 \), and hence the effective charge \( q_3 = q_3 + q_2 - q_2 \) has contributions from several transitions. That is, a three-stage model of activation of a K channel may be determined that has the same derived rate functions and survival probability \( n(t) \) during a voltage clamp as a two-stage model (see Figs. 7 and 8). The three-stage rate functions have a similar voltage dependence to those obtained from an electrostatic model of S4 activation [21], where approximately 1e is transferred across the membrane for each of the three activation steps per subunit, and provides a good fit to an empirical three-stage model of K channel activation [22].

**TWO-STAGE K CHANNEL SENSOR ACTIVATION AND THE ACTION POTENTIAL**

In this section, we consider the effect of two-stage activation of a K channel on potential oscillations across a membrane. The Shaker K channel permits the conduction of ions when the activation of each of several voltage sensors through a sequence of closed states is followed by a cooperative transition to the open state [11]. However, in order to simplify the analysis, it is assumed that (1) the K conductance is modulated by a single S4 sensor that activates in two stages (2) each ion channel is persistent, that is, does not inactivate and (3) the Na channel sensor has a time constant of activation that is small by comparison with other transitions. A more general analysis may take account of the effect of relaxing each of these assumptions on the membrane potential and the bifurcations of the system of equations.

The potential response of a membrane with Na, K and leakage ion channels may be described by the current equation

\[
C \frac{dV}{dt} = I - \bar{g}_{Na} m_{\infty}(V)(V - V_{Na}) - \bar{g}_K n(V - V_K) - \bar{g}_L (V - V_L),
\]

(39)

and the master equation for two-stage activation of the K channel sensor

\[
\frac{dn}{dt} = \phi_n(V) \left( \frac{1 - n}{1 + \rho_n \exp[-(q_\gamma + q_\beta)(V - V_0)/kT]} - n \right),
\]

(40)

\[
\frac{dn_1}{dt} = \phi_1(V) \left( \frac{1 - n}{1 + \rho_1 \exp[q_\alpha + q_\beta](V - V_0)/kT]} - n_1 \right),
\]

(41)
where Eq. 5 has been eliminated by application of \( n_2 = 1 - n_1 - n \). The ion conductance and \( V_i \) is the equilibrium potential for each ion (Na, K and leakage), \( \rho_1 = \alpha_0/\beta_0, \rho_n = \delta_0/\gamma_0, \phi_1(V) = \alpha(V) + \beta(V), \phi_n(V) = \gamma(V) + \delta(V), C=1 \mu F/cm^2, \alpha_m = 0.1(V+V_m-25)/(1-exp[-0.1(V+V_m-25)]), \beta_m = 4 \exp[-(V+V_m)/18], \) and the steady state Na channel open probability is \( m_\infty(V) = \alpha_m/(\alpha_m + \beta_m) \), a good approximation to the activation variable \( m \) when the Na channel activation time is small. The functions \( \alpha_m \) and \( \beta_m \) have a similar mathematical form to \( \alpha_n \) and \( \beta_n \) and therefore we may assume that the Na channel voltage sensor also activates in two stages. We may define \( \alpha_{m,2} = 2.5 \exp[0.3(V+V_m-25)]/(1+\exp[-1.57(V+V_m-25)]) \) (see Eq. 17) such that \( \alpha_{m,2} \approx \alpha_m, \beta_{m,2} = \beta_m, \) and \( m_\infty(V) \approx \alpha_{m,2}/(\alpha_{m,2} + \beta_{m,2}) \).

The stationary points of the system of Eqs. (39) - (41) are given by the intersection of the \( V \), \( n \), and \( n_1 \) nullclines, and their stability may be determined from the characteristic equation (see Appendix 3). If the two-stage activation of the K channel satisfies \( \beta \gg \delta \) and either \( \alpha \gg \gamma \) or \( \gamma \gg \alpha \), the limit cycle solutions of Eqs. (11) and (39) provide a good fit to the solutions of Eqs. (49) - (41) - see Figs. 9 and 10 for the squid axon K channel, and Figs. 11 and 12 for the Shaker K channel, where the bifurcation analysis is generated by XPPAUT [24]. The range of currents for which there is a coexistence of a stable state and a stable limit cycle (bistability) is dependent on the parameters of the system, and in particular, \( q_0 \) and \( q_7 \), and the relative value of \( \alpha_0 \) and \( \gamma_0 \) (see Figs. 10 and 12).

**CONCLUSION**

A two-stage model of the activation of a voltage sensor in each of the four subunits of a Shaker K channel, followed by a cooperative transition, can describe the kinetic activation and deactivation of both ionic and gating currents [11], but does not account for the success of the two-state Hodgkin-Huxley model [11]. In this paper, we show that if the rate functions satisfy \( \delta \ll \beta \) and either \( \alpha \gg \gamma \) or \( \gamma \gg \alpha \), the solution of a two-stage model of the opening of an ion channel during a voltage clamp may be approximated by the solution \( n \) of a rate equation where the backward transition rate is an exponential function of \( V \), and the forward rate may be expressed as an exponential-quasilinear function of \( V \), and have a similar form to the empirical rate functions \( \alpha_n \) and \( \beta_n \) of the squid axon K channel, and the functions \( \alpha_x \) and \( \beta_x \) of a delayed rectifier K channel in a cardiac Purkinje fiber [11] [23].

The derived rate functions account for the asymmetry of \( \alpha_n \) and \( \beta_n \) in terms of the effective charge for the transitions of the activation process, which may be calculated from the voltage-dependence of the barrier heights for each stage of activation of the S4 sensor [21]. If the opening of a K channel occurs upon the activation of each of four independent two-stage voltage sensors, the K conductance may be expressed as \( g_k n^4 \), where \( n \) is the solution to the corresponding rate equation with derived rate functions \( \alpha_{n,2} \) and \( \beta_{n,2} \). Whereas the K channel ionic current is a function of the activation variable \( n \), during the voltage clamp of a two-stage opening of an ion channel, the gating current has a contribution from each transition and exhibits a rising phase for a large depolarization when \( \gamma > \alpha \), and therefore is in accord with experimental data from a Shaker K
channel [11]. The potential oscillation generated by the interaction between a two-stage K channel and a noninactivating fast Na channel is determined by the master equation for K channel activation and the ionic current equation, and if the K channel rate functions satisfy the above inequalities, the solution is determined by only two variables V and n.

If the S4 sensor is activated through three transitions where the first forward transition and the first backward transition (from the activated state) are rate limiting (α₁ ≪ α₂, α₃ and β₁ ≪ β₁, β₂), the master equation during a voltage clamp also reduces to a rate equation. A three-stage model may be determined that has the same derived rate functions and survival probability for the activated state as a two-stage model of voltage sensor activation. The rate function inequalities are supported by recent experiments that have shown that the activated state as a two-stage model of voltage sensor activation. The rate function time is reduced when the residue V363 adjacent to R1 on the S4 segment and the residue I287 on the S2 segment are replaced by the hydrophilic amino acid Thr [20], and that the deactivation time for the first backward transition is dependent on the hydrophobicity of the residue F²⁹⁰ on the S2 segment of a K channel subunit [18, 19].

APPENDIX 1

For a large hyperpolarized holding potential, we may assume that n₁(0) = 1, and n(0) = n₂(0) = 0, and if a K voltage sensor is depolarized to a clamp potential V (activation), by application of matrix methods and the initial condition, the solution of Eqs. [4] to [6] for two-stage activation is

\[ n_{1A}(t) = \frac{\beta_1}{\omega_1 \omega_2} - \frac{\alpha \gamma - \omega}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) + \frac{\alpha \gamma - \omega}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \]

\[ n_{2A}(t) = \frac{\alpha \gamma}{\omega_1 \omega_2} - \frac{\alpha \gamma}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) + \frac{\alpha \gamma}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \]

\[ n_{A}(t) = \frac{\alpha \gamma}{\omega_1 \omega_2} + \frac{\alpha \gamma}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) - \frac{\alpha \gamma}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \]

where \( \omega_1 \) and \( \omega_2 \) (\( \gg \omega_1 \)) are solutions of

\[ \omega^2 - \omega (\alpha + \beta + \gamma + \delta) + \alpha \gamma + \delta (\alpha + \beta) = 0. \]

However, if the S4 sensor is initially in the open state (\( n(0) = 1 \)), and \( n_{1}(0) = n_{2}(0) = 0 \), and if the K sensor is repolarized to a clamp potential V (deactivation), the solution is

\[ n_{1D}(t) = \frac{\beta_1}{\omega_1 \omega_2} - \frac{\beta_1}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) - \frac{\beta_1}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \]

\[ n_{2D}(t) = \frac{\beta_1}{\omega_1 \omega_2} + \frac{\beta_1}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) + \frac{\beta_1}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t), \]

\[ n_{D}(t) = \frac{\beta_1}{\omega_1 \omega_2} + \frac{\beta_1}{\omega_1 (\omega_1 - \omega_2)} \exp(-\omega_1 t) + \frac{\beta_1}{\omega_2 (\omega_1 - \omega_2)} \exp(-\omega_2 t). \]
If a three stage K voltage sensor is depolarized to a clamp potential \( V \) from a hyperpolarized potential \( (n_1(0) = 1, n_2(0) = n_3(0) = 0) \), the solution of Eqs. (29) - (32) for the activated state is

\[
n_A(t) = \frac{\alpha_1 \alpha_2 \alpha_3}{\omega_1 \omega_2 \omega_3} - \frac{\alpha_1 \alpha_2 \alpha_3 \Sigma_{i=1}^{3} a_i}{\omega_1 \omega_2 \omega_3} \exp(-\omega_i t)
\]

where \( a_i = 1/(\omega_i \Pi_{j \neq i}(\omega_j - \omega_i)) \), the frequencies \( \omega_1 < \omega_2 < \omega_3 \) are solutions of

\[
\omega^3 - e_1 \omega^2 + e_2 \omega - e_3 = 0,
\]

and

\[
e_1 = \Sigma_{i=1}^{3}(\alpha_i + \beta_i) = \Sigma_{i=1}^{3} \omega_i
\]

\[
e_2 = \alpha_1(\alpha_2 + \alpha_3 + \beta_2 + \beta_3) + \alpha_2(\alpha_3 + \beta_3) + \alpha_3(\beta_2 + \beta_3)
= \omega_1 \omega_2 + \omega_2 \omega_3 + \omega_1 \omega_3
\]

\[
e_3 = \alpha_1 \alpha_2 \alpha_3 + \beta_1(\alpha_1 \alpha_2 + \beta_2(\alpha_1 + \beta_1)) = \omega_1 \omega_2 \omega_3.
\]

The higher frequency terms have small amplitude and \( n_A(t) \) satisfies a rate equation with time constant \( \omega_1 = \alpha_{n,3}(V) + \beta_{n,3}(V) \) where

\[
\alpha_{n,3}(V) \approx \frac{\alpha_3}{1 + (\beta_2/\alpha_2)(1 + \beta_1/\alpha_1)}, \quad (42)
\]

\[
\beta_{n,3}(V) \approx \beta_3. \quad (43)
\]

However, if \( \alpha_1 \ll \alpha_2, \alpha_3 \) and \( \beta_3 \ll \beta_1, \beta_2, \)

\[
\omega_2 + \omega_3 \approx \Sigma_{i=1}^{2}(\alpha_i + \beta_i), \quad (44)
\]

\[
\omega_2 \omega_3 \approx \alpha_3 \alpha_2 + \alpha_3 \beta_1 + \beta_1 \beta_2, \quad (45)
\]

and the rate functions

\[
\alpha_{n,3}(V) \approx \frac{\alpha_1 \alpha_3}{\alpha_3 + (\beta_2/\alpha_2)(\alpha_2 \beta_2 + \beta_1)}, \quad (46)
\]

\[
\beta_{n,3}(V) \approx \frac{\beta_3(\alpha_1 + \beta_2(\alpha_1 + \beta_1)/\alpha_2)}{\alpha_3 + (\beta_2/\alpha_2)(\alpha_2 \beta_2 + \beta_1)}. \quad (47)
\]

**APPENDIX 2**

When a constant potential difference is applied to the membrane, the movement of gating charge within the channel is comprised of a fast component,
considered to be a redistribution of the charge of the S4 sensor within energy wells, and a slow component generated by transitions between stationary states. The gating charge $Q_i$ associated with the transition to the $i$-th state from the first state is the change in charge induced in the surrounding solvent \[25\], and may be expressed as $\sum_{j=2}^{\infty} Q_j$ where $Q_j$ is the gating charge for the transition from the $(j - 1)$th state to the $j$-th state. The observable gating charge $Q_g(t)$ for each subunit of the K channel is also dependent on the survival probability for each state, and if the voltage sensor activates in two steps, $Q_{g,2}(t) = Q_2 n_2(t) + (Q_2 + Q_3)n(t)$, and the gating current

$$I_{g,2}(t) = Q_2 \frac{dn_2}{dt} + (Q_2 + Q_3) \frac{dn}{dt}. \quad (48)$$

From the solution of Eqs. (4) to (6) in Appendix 1, we may write

$$\frac{dI_{g,2}}{dt}(t) = \frac{\alpha(\gamma Q_3 - (\alpha + \beta)Q_2)\omega_1}{\omega_1 - \omega_2} \left[ \omega_1 \exp(-\omega_1 t) - \omega_2 \exp(-\omega_2 t) \right] + \frac{\alpha Q_2 \omega_1 \omega_2}{\omega_1 - \omega_2} \left[ \exp(-\omega_1 t) - \exp(-\omega_2 t) \right]$$

and therefore

$$\frac{dI_{g,2}}{dt}(0) = \alpha(\gamma Q_3 - (\alpha + \beta)Q_2). \quad (51)$$

For hyperpolarized potentials, $\beta \gg \alpha, \gamma$ and the gating current decreases initially, whereas for depolarized potentials, $\beta$ is small and an initial rising phase in the relaxation of the gating current is dependent on the relative magnitude of the forward rate functions $\alpha$ and $\gamma$. For the two-stage activation model of the squid axon K channel, $\alpha \gg \gamma$, and hence $dI_{g,2}/dt(0) < 0$ and there is no rising phase of the gating current for each potential, whereas for the two-stage activation model of the Shaker K channel for which $\gamma > \alpha$, $dI_{g,2}/dt(0) > 0$ and the gating current has a rising phase for larger depolarizations.

If the voltage sensor activates in three steps, $Q_{g,3}(t) = Q_2 n_2(t) + (Q_2 + Q_3)n_3(t) + (Q_2 + Q_3 + Q_4)n(t)$, the gating current

$$I_{g,3}(t) = Q_2 \frac{dn_2}{dt} + (Q_2 + Q_3) \frac{dn_3}{dt} + (Q_2 + Q_3 + Q_4) \frac{dn}{dt}. \quad (52)$$

and it may be shown from Eqs. (29) - (40) and the initial conditions $n(0) = 1$, and $n_1(0) = n_2(0) = n_3(0) = 0$, that

$$\frac{dI_{g,3}}{dt}(0) = \alpha_1(\alpha_2 Q_3 - (\alpha_1 + \beta_1)Q_2), \quad (53)$$

and therefore, if the gating current has a rising phase for larger depolarizations, $\alpha_1 < \alpha_2$.

**APPENDIX 3**

Stationary points of the system of Eqs.(39) - (41) are given by the intersection of the $V$, $n$, and $n_1$ nullclines:

$$\frac{I - g_L(V - V_L) - g_{Na}m_{\infty}(V)(V - V_{Na})}{g_K(V - V_K)} = \frac{\alpha \gamma}{\alpha \gamma + \alpha \delta + \beta \delta}. \quad (54)$$
and it is assumed that the parameters are chosen so that there is only one solution. The stability of the stationary point may be determined by assuming that \( V = V_s + \tilde{V} \quad n = n_s + \tilde{n} \quad and \quad n_1 = n_{1s} + \tilde{n}_1 \), where \((V_s, n_s, n_{1s})\) is the stationary solution, and defining the matrix of coefficients \( M \) of the linearized equations in \((\tilde{V}, \tilde{n}, \tilde{n}_1)\),

\[
M = \begin{pmatrix}
R & -g_K(V_s - V_K)/C & 0 \\
\phi_{ns}n_{1\infty s}'(1 - n_s) & -\phi_{ns} - \phi_{n\infty s} & -\phi_{ns}n_{\infty s} \\
\phi_{1s}n_{1\infty s}'(1 - n_s) & -\phi_{1s}n_{1\infty s} & -\phi_{1s}
\end{pmatrix}
\]

where \( R = [-g_L - g_K n_s - g_{Na}m_{\infty s} - g_{Na}m_{\infty 1}(V_s - V_{Na})]/C \), \( n_{\infty}(V) = \gamma/(\gamma + \delta) \), \( n_{1\infty}(V) = \beta/(\alpha + \beta) \), \( \phi_{ns} = \phi_n(V_s) \), \( \phi_{1s} = \phi_1(V_s) \), \( n_{\infty} = n_{\infty}(V_s) \), \( n_{1\infty} = n_{1\infty}(V_s) \), \( n'_{\infty} = n'_\infty(V_s) \), \( n'_{1\infty} = n'_{1\infty}(V_s) \) and \( m_{\infty} = m_{\infty}(V_s) \). The eigenvalues may be determined from the characteristic equation

\[
det(M - I\omega) = \omega^3 + f_1\omega^2 + f_2\omega + f_3 = 0,
\]

where

\[
f_1 = \phi_{ns} + \phi_{1s} - R, \\
f_2 = -R(\phi_{ns} + \phi_{1s}) - \phi_{ns}\phi_{1s}(1 - n_{\infty s}n_{1\infty s}) + (g_K/C)(V_s - V_K)\phi_{ns}n_{\infty s}'(1 - n_{1s}), \\
f_3 = (g_K/C)(V_s - V_K)\phi_{ns}\phi_{1s}[n_{\infty s}'(1 - n_{1s}) - n_{\infty s}n_{1\infty s}'(1 - n_s)] + R\phi_{ns}\phi_{1s}(n_{\infty s}n_{1\infty s} - 1).
\]

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Figure 1: Two-stage activation model of a K channel voltage sensor, where $n_1$, $n_2$, and $n$ are state occupation probabilities, and $\alpha$, $\beta$, $\gamma$ and $\delta$ are voltage-dependent rate functions between states.

\[
\begin{array}{c}
\alpha & \gamma \\
\beta & \delta \\
n_1 = n_2 = n
\end{array}
\]

Figure 2: The derived rate functions $\alpha_{n,2}$ and $\beta_{n,2}$ (solid line) in Eqs. (17) and (18) provide a good approximation to the HH rate functions (ms$^{-1}$) $\alpha_n = 0.01(V + 50)/(1 - \exp(-0.1(V + 50)))$ and $\beta_n = 0.125 \exp(-(V + 60)/80)$ (dotted line) when the rate functions for two-stage activation (ms$^{-1}$) are $\alpha(V) = 3.2 \exp(0.3(V - V_0)/25)$, $\beta(V) = 8.8 \exp(-1.4(V - V_0)/25)$, $\gamma(V) = 0.233 \exp(0.25(V - V_0)/25)$, $\delta(V) = 0.125 \exp(-0.312(V - V_0)/25)$, $V_0 = -57.9$. 

\[15\]
Figure 3: Survival probability of the state $n(t)$ during a voltage clamp for a two-stage activation model of a K channel (solid line) and for the HH squid axon model (dotted line) during (a) activation and (b) deactivation (see Fig. 2).
Figure 4: The derived rate functions $\alpha_{n,2}$ and $\beta_{n,2}$ (solid line) in Eqs. (15) and (16) are calculated for the rate functions determined experimentally for the Shaker K channel [11]: $\alpha(V) = 1.12 \exp(0.25V/25)$, $\beta(V) = 0.373 \exp(-1.6V/25)$, $\gamma(V) = 2.8 \exp(0.32V/25)$, $\delta(V) = 0.021 \exp(-1.1V/25)$ (ms$^{-1}$), and may be approximated by the rate functions $\alpha_H = 0.0195(V + 45.8)/(1 - \exp(-0.15(V + 45.8)))$ and $\beta_H = 0.135 \exp(-0.052(V + 45.8))$ for single transition activation (dotted line).
Figure 5: Survival probability of the state $n(t)$ during a voltage clamp for a two-stage model of a K channel (solid line) and HH model (dotted line) during (a) activation and (b) deactivation, for the rate functions of Fig. 4, and (c) activation when $\beta(V)$ and $\gamma(V)$ are increased by a factor of 3, and the rate functions $\alpha_H = 0.02(V + 45.8)/(1 - \exp(-0.15(V + 45.8)))$ and $\beta_H = 0.12\exp(-0.057(V + 45.8))$. 
Figure 6: Three-stage activation model of a K channel voltage sensor, where $n_1$, $n_2$, $n_3$, and $n$ are state occupation probabilities, and $\alpha_i$, $\beta_i$ for $i = 1$ to 3 are voltage-dependent rate functions between states.

\[
\begin{align*}
\alpha_1 & \quad \alpha_2 & \quad \alpha_3 \\
& \quad n_1 = n_2 = n_3 = n \\
\beta_1 & \quad \beta_2 & \quad \beta_3
\end{align*}
\]

Figure 7: The derived rate functions $\alpha_{n,2}(V)$ and $\beta_{n,2}(V)$ are calculated for a two-stage K channel (solid line), where $\alpha(V) = 1.12 \exp(0.25V/25)$, $\beta(V) = 0.74 \exp(-1.6V/25)$, $\gamma(V) = 5.7 \exp(0.32V/25)$, $\delta(V) = 0.021 \exp(-1.1V/25)$ (ms$^{-1}$)), and $\alpha_{n,3}(V)$, $\beta_{n,3}(V)$ are calculated for a three-stage K channel (dotted line) where the rate functions $\alpha_1(V) = 1.15 \exp(0.25V/25)$, $\alpha_2(V) = 22.8 \exp(0.52V/25)$, $\alpha_3(V) = 5.7 \exp(0.32V/25)$, $\beta_1(V) = 1.42 \exp(-0.7V/25)$, $\beta_2(V) = 5.7 \exp(-0.7V/25)$, $\beta_3(V) = 0.022 \exp(-1.1V/25)$. 
Figure 8: Survival probability of the state $n(t)$ during a voltage clamp for a two-stage (solid line) and three-stage (dotted line) models of activation of a K channel voltage sensor during (a) activation and (b) deactivation, for the rate functions of Fig. 7.
Figure 9: The limit cycle solution of Eqs. (39) - (41) (solid line) provide a good fit to the solution of Eqs. (14), (17), (18) and (39) (dotted line) when $\beta \gg \delta$, and $\alpha \gg \gamma$. The rate functions $\alpha(V) = 6.4 \exp(0.3V/25)$, $\beta(V) = 17.6 \exp(-1.4V/25)$, $\gamma(V) = 0.233 \exp(0.25V/25)$, $\delta(V) = 0.125 \exp(-0.312V/25)$, $g_K = 34 \text{ mS/cm}^2$, $g_{Na} = 15 \text{ mS/cm}^2$, $g_L = 0.1 \text{ mS/cm}^2$, $V_K = -72 \text{ mV}$, $V_{Na} = 55 \text{ mV}$, $V_L = -49.4 \text{ mV}$, $I = 98 \mu A/\text{cm}^2$, $V_m = 58$. 
Figure 10: The bifurcation diagram for Eqs. (39) - (41) when $\beta \gg \delta$, and $\alpha \gg \gamma$ (see Fig. 9).
Figure 11: The limit cycle solution of Eqs. (39) - (41) (solid line) may be approximated by the solution of Eqs. (14), (22), (23) and (39) (dotted line) when $\beta \gg \delta$, and $\gamma \gg \alpha$. The rate functions $\alpha(V) = 1.1 \exp(0.25(V - V_0)/25)$, $\beta(V) = 0.74 \exp(-1.6(V - V_0)/25)$, $\gamma(V) = 5.6 \exp(0.32(V - V_0)/25)$, $\delta(V) = 0.021 \exp(-1.1(V - V_0)/25)$, $g_K = 17 \text{ mS/cm}^2$, $g_{Na} = 9 \text{ mS/cm}^2$, $g_L = 0.1 \text{ mS/cm}^2$, $V_K = -81 \text{ mV}$, $V_{Na} = 46 \text{ mV}$, $V_L = -58.4 \text{ mV}$, $I = 35 \mu\text{A/cm}^2$, $V_m = 52.5$. 
Figure 12: The bifurcation diagram for Eqs. (39)–(41) when $\beta \gg \delta$, and $\gamma \gg \alpha$ (see Fig. 11).