Voltage gating by molecular subunits of Na\(^+\) and K\(^+\) ion channels: higher-dimensional cubic kinetics, rate constants, and temperature

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Fohlmeister JF. Voltage gating by molecular subunits of Na\(^+\) and K\(^+\) ion channels: higher-dimensional cubic kinetics, rate constants, and temperature. J Neurophysiol 113: 3759–3777, 2015. First published April 1, 2015; doi:10.1152/jn.00551.2014.—The structural similarity between the primary molecules of voltage-gated Na and K channels (alpha subunits) and activation gating in the Hodgkin-Huxley model is brought into full agreement by increasing the model’s sodium kinetics to fourth order (m³ → m⁴). Both structures then virtually imply activation gating by four independent subprocesses acting in parallel. The kinetics coalesce in four-dimensional (4D) cubic diagrams (16 states, 32 reversible transitions) that show the structure to be highly failure resistant against significant partial loss of gating function. Rate constants, as fitted in phase plot data of retinal ganglion cell excitation, reflect the molecular nature of the gating transitions. Additional dimensions (6D cubic diagrams) accommodate kinetically coupled sodium inactivation and gating processes associated with beta subunits. The gating transitions of coupled sodium inactivation appear to be thermodynamically irreversible; response to dielectric surface charges (capacitive displacement) provides a potential energy source for those transitions and yields highly energy-efficient excitation. A comparison of temperature responses of the squid giant axon (apparently Arrhenius) and mammalian channel gating yields kinetic Q₁₀ = 2.2 for alpha unit gating, whose transitions are rate-limiting at mammalian temperatures; beta unit kinetic Q₁₀ = 14 reproduces the observed non-Arrhenius deviation of mammalian gating at low temperatures; the Q₁₀ of sodium inactivation gating matches the rate-limiting component of activation gating at all temperatures. The model kinetics reproduce the physiologically large frequency range for repetitive firing in ganglion cells and the physiologically observed strong temperature dependence of recovery from inactivation.

Voltage-gating sodium and potassium ion channels; molecular alpha and beta subunits; rate constants and kinetics; energy efficiency and temperature Q₁₀

On the molecular level, virtually all voltage-gated ion channels in nerve and muscle share a similarly structured alpha subunit (e.g., Hanlon and Wallace 2002; Jan and Jan 1997; MacKinnon 1991, 1995). This large protein molecule includes four repeated and nearly identical substructures that consist of parallel arrays of membrane-spanning alpha helices to form the channel wall and pore (for ion conduction) and four “voltage sensors” (for gating the channel). The similarities in the number of molecular substructures (4) and the number of components implied by the Hodgkin-Huxley equations (exponents 3 and 4), as well as the parallel natures of the molecular arrangement and of the model gating, suggest that the model kinetics may directly apply in molecular alpha unit gating; the small numerical discrepancy is easily disposed.

The kinetic equations of Hodgkin and Huxley, however, are in a highly condensed form, which masks analytically important details that are implied in the model interpretation. Among the hidden details are numerous nonconducting states of gating and the multiple kinetic paths to the conducting channel. With four parallel subprocesses inferred in alpha unit gating (also in n⁴), there are 16 possible combinations of the individual subprocess states. Each combination is a unique molecular conformation, and collectively they constitute the 16 states of activation gating of the molecule as a whole. These hidden states emerge in “cubic kinetic diagrams,” which are mathematically equivalent to parallel kinetics and in which each subprocess is assigned a new geometrical dimension (thus, 3D for m³; 4D for n⁴, as well as for alpha unit gating). The specific combinations of substates appear at the cube corners (i.e., the whole channel states of gating); the gating transitions occur on the cube edges. This explicit expression of every state and every transition renders the gating process fully transparent and can easily be extended to higher dimensions (here to 6D) to assess both the kinetic connectivity of sodium inactivation gating and temperature-response phenomena possibly due to beta subunit activity.

METHODS

Experimental Data and Computations

All analysis is based on experimental data of nerve impulse trains obtained from rat and cat retinal ganglion cells, throughout the temperature ranges of 7.0–37.1°C. Figure 1A gives two examples of impulse trains and action potentials obtained at 37 and 16.7°C. These and all animal data analyzed herein are from a previous study (Fohlmeister et al. 2010) that received ethical approval and gives the experimental methods.

Model results were obtained from a combination of single- and multicompartment computer simulations. The single-compartment runs employed a four-pole Runge-Kutta routine with variable integration step. The multicompartment simulations employed the computer
Fig. 1. Simulating experiment. A: experimental impulse trains and action potentials (AP), stimulated and recorded at the soma of a rat “type 1” (beta) retinal ganglion cell at 37 and 16.7°C; the injected currents were 240 and 80 pA (these were minimal currents to induce repetitive firing, in steps of 40 pA). B: temperature series of phase plots: 37 (boldface), 29.9, 23.3, 16.7 (boldface), 13.9, 9.8, and 7.5°C, in descending order of ordinate excursion. The plots are of impulse trains generated by the compartmentalized cell in the right-hand panel of the figure, with nonuniform channel-density distribution and stimulated and recorded at the soma. The rate constants for 37°C, and for kinetics m4, n4, and independent Na-inactivation h, are:

\[
\begin{align*}
\alpha_m(V) &= 87.88\left[\frac{1 + \exp\left[-\left(V + 51.5\right)/5\right]}{1 + \exp\left[-\left(V + 10\right)/2\right]}\right] + 3.534 \cdot \left(1 - \exp\left[-\left(V + 10\right)/2\right]\right) \\
\beta_m(V) &= 258.5\left[1 + \exp\left[0.055 \cdot \left(V + 70\right)\right]\right] \\
\alpha_n(V) &= 1.891 \cdot \exp\left[-\left(V + 52\right)/20\right] \\
\beta_n(V) &= 28.365\left[1 + \exp\left[-\left(V + 22\right)/10\right]\right] \\
\alpha_h(V) &= 2.8\left[1 + \exp\left[-0.4 \cdot \left(V - 10\right)\right]\right] + 0.04 \cdot \left(V + 62\right)\left[1 - \exp\left[-\left(V + 62\right)/2\right]\right] \\
\beta_h(V) &= 3.24\left[1 + \exp\left(V + 40\right)/100\right]
\end{align*}
\]

The “IS-SD break” is a local passive response to the electrotonic (axial) current, arising from an AP on the trigger segment that is about to invade the soma; the Gm, ratio of trigger seg.-to-soma = 9. Note the abrupt onset of nonzero dV/dt at 37°C, despite gating kinetics that are at basis Hodgkin-Huxley (McCormick et al. 2007); thus the phenomenon does not require new mechanisms (cf Naundorf et al. 2006). Gm at the soma = 41 mS/cm² (37°C; see RESULTS for kinetic and conductance Q10 s and further details). Right: an example of a traced retinal ganglion beta cell from the cat used in the multicompartment model simulations. An AP, having just departed the impulse trigger zone, is simultaneously propagating upward on the axon and beginning to invade the soma-dendritic region (gray scale in mV; 37°C; the arrows indicate the AP peaks); this time-point lies on the “shoulder” of the IS-SD break in the phase plot (in B), which is just before the onset of regenerative soma excitation. Surface areas are: soma = 1,379 μm²; total dendritic = 4,963 μm²; 105 dendritic processes; 762 dendritic compartments; range of dendritic diameters is 0.19–2.23 μm. The axon (76 total compartments) consists of the proximal initial segment (l = 45 μm; d = 1.2 μm), followed by the thinner “trigger zone” (90 μm; 0.7 μm), and the axon proper (1 mm; 1 μm). Channel densities and further details are given in Fohlmeister et al. (2010).

program NEURON (Carnevale and Hines 2006) with traced retinal ganglion cells (e.g., Fig. 1, right); the fixed integration step was adjusted for permissible maximum error of 0.01%, resulting in steps of 0.0001 ms at 37°C. Channel densities were distributed differentially among the major neural subdivisions of dendrites, soma, initial segment, trigger segment, and axon proper (Fig. 1 legend and Fohlmeister et al. 2010). The cytoplasmic resistivity was temperature-adjusted (Q10 = 0.8); widely spaced examples are: R = 136.6 Ω·cm [37.1°C], and 234.9 Ω·cm [13.9°C] (Robinson and Stokes 1954; Trevelyan and Jack 2002).

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The kinetic order of activation gating becomes a significant variable when the kinetic order is changed (see Table 1). The base model’s kinetic structure, which is based on congruence between the rate constants and the parameters for the Hodgkin-Huxley model in 4th order kinetics (HH m4, n4). The rate constants in 4th order kinetics for Na-activation ( parameters), and K currents, for kinetic orders 1 through 6, with the order-specific coefficients, Co, in Table 1; the analytic functions of voltage depend critically on the specific kinetic order. Figure 2, A and B, shows rate constants for kinetic orders 1 through 6 that are equivalent to those of the base model; Table 1 gives the analytic functions and lists the parameters.

This “equivalence” of the rate constants is based on congruence of their phase plots. Figure 2C shows 12 superposed phase plots of steady-state segments of model impulse trains. The plots consist of the Na-channel series, m3, n3 [x = 1, 2, ..., 6; each with n3 kinetics for the K-channel gating, which includes the base model (m4, n4)], plus the K-channel series, n4 (in this case, with the corresponding m4). Although the phase plots cannot in principle be absolutely identical, it is perhaps remarkable that virtual phase plot congruence is achieved even with first-order kinetics for Na-activation gating. For K-channel gating, in contrast, a first-order virtual fit was unattainable and is approximate for second- and third-order kinetics.

The rate constants in Fig. 2 are for 37°C. Changing temperature affects only the overall magnitudes of the rate constants (the coefficients, Co, in Table 1); the analytic functions of voltage dependence and the other numerical parameters are temperature independent; this pattern was found to hold throughout (cf. Fohlmeister et al. 2010).

Voltage Clamp, Energy Efficiency

Figure 3, A and B, shows simulated voltage clamp records of Na and K currents, for kinetic orders 1 through 6, with the order-specific rate constants in Table 1 and Fig. 2. All records were generated with the single voltage step command: $-68 \, \text{mV} \rightarrow 0 \, \text{mV}$ (tail currents at $-68 \, \text{mV}$). The well-known increase in the delay to rapid current rise seen with increasing kinetic order (the “sigmoidality”) dates to Hodgkin and Huxley’s (1952) choices of m3 and n4. Note, however, that the rate of change in sigmoidality slows considerably for kinetic orders

Table 1. Rate constants as functions of kinetic order (37°C)

<table>
<thead>
<tr>
<th>Kinetics</th>
<th>Na Activation</th>
<th>K Channel</th>
<th>GNa</th>
<th>Gk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha_{m,\text{act}}$ (A)</td>
<td>$\beta_{m,\text{act}}$ (B)</td>
<td>$\alpha_n$</td>
<td>$\beta_n$</td>
</tr>
<tr>
<td>Base (m3, n4)</td>
<td>3.152</td>
<td>52.0</td>
<td>0.15</td>
<td>60.0</td>
</tr>
<tr>
<td>m4, n4</td>
<td>35.0</td>
<td>0.1</td>
<td>60.0</td>
<td>20.0</td>
</tr>
<tr>
<td>m4, n6</td>
<td>41.2</td>
<td>0.0655</td>
<td>60.0</td>
<td>20.0</td>
</tr>
<tr>
<td>m4, n6</td>
<td>41.2</td>
<td>0.053</td>
<td>60.0</td>
<td>20.0</td>
</tr>
<tr>
<td>m4, n6</td>
<td>52.0</td>
<td>20.0</td>
<td>22.0</td>
<td>—</td>
</tr>
<tr>
<td>HH (m4, n4)</td>
<td>38.6</td>
<td>0.08</td>
<td>60.0</td>
<td>20.0</td>
</tr>
<tr>
<td>HH (h)</td>
<td>60.0</td>
<td>18.0</td>
<td>30.0</td>
<td>—</td>
</tr>
</tbody>
</table>

Parameters for equivalent rate constants for sodium and potassium channel gating when the kinetic order is changed in a “Base” model (Fohlmeister 2009); the analytic equations of the rate constants are:

$$
\alpha_{m,n}(V) = \frac{C_o \cdot (V + A)}{1 - \exp(-B \cdot (V + A))}
$$

$$
\beta_{m,n}(V) = C_o \cdot \exp(-V + C)\cdot D
$$

$$
\alpha_n(V) = 2.101 \cdot \exp(-V + A)/B
$$

$$
\beta_n(V) = 31.52/(1 + \exp[-(V + C)/10])
$$

These equations are for 37°C. Changing temperature affects only the overall magnitudes of the rate constants (the coefficients, Co, in Table 1); the analytic functions of voltage dependence are unchanged, whereas the numerical parameters of those functions depend critically on the (specified) kinetic order. Figure 2, A and B, shows rate constants for kinetic orders 1 through 6 that are equivalent to those of the base model; Table 1 gives the analytic functions and lists the parameters.

This “equivalence” of the rate constants is based on congruence of their phase plots. Figure 2C shows 12 superposed phase plots of steady-state segments of model impulse trains. The plots consist of the Na-channel series, m3, n3 [x = 1, 2, ..., 6; each with n3 kinetics for the K-channel gating, which includes the base model (m4, n4)], plus the K-channel series, n4 (in this case, with the corresponding m4). Although the phase plots cannot in principle be absolutely identical, it is perhaps remarkable that virtual phase plot congruence is achieved even with first-order kinetics for Na-activation gating. For K-channel gating, in contrast, a first-order virtual fit was unattainable and is approximate for second- and third-order kinetics.

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The phase plot series in Fig. 1B was obtained from steady-state segments of tonic nerve impulse trains, generated in multicompart ment computer simulations, using the traced ganglion cell in Fig. 1, right. The gating rate constants are given in the legend of Fig. 1 and differ from those in Table 1 and Fig. 2 (see RESULTS). The “IS-SD (initial segment/soma-dendritic) break,” also called “initial segment/dendritosomatic inflection,” is an initial deviation from the balance of $I_{Na}$ and $I_{cap}$ at the soma, during which $I_{cap}$ responds instead to the local electrotonic current that arises from the initiation of the action potential on the neighboring trigger segment; it is expressed in a more abrupt upswing in $dV_m/dt$. The prominence of the IS-SD break, as well as neural morphology, varies substantially among retinal ganglion cells, as does the distribution of ion channels to a lesser extent. Wider ranges of morphology parameters and channel densities are given in Fohlmeister et al. (2010, Table 1).

The method of fitting phase plot simulations to phase plots derived from experimental impulse trains is given in the subsection: “Phase plots from digitized data” in Fohlmeister et al. (2010, p. 1360). The fitting was carried out by adjusting individual model parameters that are sensitive to specific curve segments of the phase plots. Although the eye was an important judge of the quality of fit, quantitative accuracy and uniqueness of the fits were determined by a generalization of the Nelder-Mead simplex method, which is “well suited for optimizing objective functions that are noisy or discontinuous at the solution”; see the subsection: “Minimizing the residual error: uniqueness of the solutions” (ibid., pp. 1361-62). This property was particularly useful in fitting experimental data that appear to contain an abrupt onset of nonzero $dV_m/dt$ in the action potential or its phase plot (cf McCormick et al. 2007; Naundorf et al. 2006), which the present modeling suggests is an effect of the IS-SD break (e.g., Fig. 1B, for 37°C). The superposed phase plots in Fig. 3C also show that the curve of that onset is independent of the kinetic order.

**Equivalent Diagrams of the Hodgkin-Huxley Kinetic Construct**

The Hodgkin-Huxley kinetic structure for activation gating can be cast in at least three types of kinetic diagram, that are mathematically equivalent in form but cast in at least three types of kinetic diagram, that are mathematically
and this occurs in “cubic kinetic diagrams”: therefore, those combinations must each be expressed separately and explicitly, formations and that every mathematical combination of the channel’s sub-processes, in parallel (Fig. 4, “3D” box; or for 6 subprocesses, Fig. 4, “3D” box, bottom). Each subprocess is associated with a dimension of the cube, one to one. Because each subprocess switches between two states (nonconducting and potentially conducting), there are 23 (= 8) distinct triplet combinations of subprocess states, and this full complement of the whole channel’s states of gating is uniquely distributed among the eight corners of the cube. Gating transitions occur, reversibly, on all 12 cube edges (i.e., rate constants $\alpha_m$ and $\beta_m$ occur pairwise on every edge). Each subprocess (primary dimensional direction of the cube) is associated with four such transition pairs, one pair in conjunction with each of the four possible combinations of states of the two remaining subprocesses.

Cubic kinetics (as also Hodgkin-Huxley’s parallel construct and, more importantly, the molecular structure of the channel alpha subunits) all virtually imply a parallel mechanism of gating, with the caveat that an ion-conducting pore is formed when, and only when, all subprocesses (of the channel) have each individually switched to their potentially conducting state. Thus, almost all combinations of the individual subprocess states (cube corners) represent a nonconducting channel, except for the single conducting combination (e.g., state G7* in Fig. 4, “3D” box). Note that both parallel and cubic kinetic diagrams are amenable to nonuniform subprocesses (i.e., subprocesses with differing rate constants $\alpha_m$ and $\beta_m$). This nonuniformity, however, excludes the sequential diagram: the key that permits an equivalent sequential diagram remains a mathematically condensed one (albeit in alternative form) and that a fully expanded expression of the model gating must be still more highly detailed. Consider, therefore, that physically, the channel’s states of gating are molecular conformations and that every mathematical combination of the channel’s subprocess states represents a unique conformation. To be fully detailed, therefore, those combinations must each be expressed separately and explicitly, and this occurs in “cubic kinetic diagrams”:

Remaining (for the moment) with the example of $m^3$, that gating system unfolds into an ordinary three-dimensional kinetic cube (Fig. 4.

$\frac{dI}{dt} = I_{m} + I_{K} + I_{Na} + I_{Ca}$

Fig. 3. Voltage clamp records at 37°C. A and B: simulated Na and K currents under voltage clamp for kinetic orders 1–6. The single clamp pulse is: $-68 \text{ mV} \rightarrow 0 \text{ mV} \rightarrow -68 \text{ mV}$, and rate constants of Table 1 and Fig. 2 (boldfaced curves are for $m^3$, $n^3$; see text for sigmoidality). Inset in A: sodium tail currents from steady state $0 \text{ mV} \rightarrow -68 \text{ mV}$ (expanded ordinate, contracted abscissa). Membrane currents associated with action potentials in multicompartment simulations of the mammalian (C) and Hodgkin-Huxley models (D), at 6.3°C and 4th-order kinetics ($m^4$, $n^4$) throughout. Rate constants are given in Table 1: cell morphology and channel distribution of Fig. 1. A high degree of temporal overlap of $I_{m}$ and $I_{K}$ (D) indicates low energy efficiency in excitation (see RESULTS and DISCUSSION).
tools in visualization and computation, provided the uniformity condition is satisfied.

Cubic Kinetic Diagrams in Higher Dimensions

The four repeat components of the channel-molecular alpha subunit, like the n<sub>4</sub> kinetics of K-channel gating in the Hodgkin-Huxley model, suggest a four-dimensional cube ("4D" in Fig. 4, below left). Additional gating phenomena attributed to beta subunits, as well as kinetically coupled Na-inactivation gating, further increase the cubic dimensionality to at least six (6D). There is no a priori restriction on the number of dimensions, which equals the number of first-order kinetic processes under consideration.

A cube of arbitrary dimension D is constructed by "dragging" a copy of a "(D - 1) cube" into a new direction (a new dimension) while retaining the original (D - 1) cube; the process of dragging creates a new set of edges, while the number of cube corners is doubled (see Fig. 4). The process begins with a first-order kinetic diagram (a "1D cube," or line) dragged at right angles into a second dimension to create a square ("2D cube"); the 3D cube results by dragging the "2D cube" into the third dimension, etc. (the dimensions are taken to be mutually orthogonal in a D-dimensional Euclidean space). In general, the number of cube corners is 6<sub>D</sub>, there are D edges entering each corner; the number of cube edges is 12·2<sub>(D-1)</sub>.

Figure 4 gives several forms of kinetic diagram for six parallel processes of activation gating, including the 6D cube. Gating transitions are implied pairwise (i.e., reversibly) on all cube edges (a later exception will be the transitions of kinetically coupled Na-inactivation gating). In all cubic projections (herein), the rate constants α<sub>m,n</sub> are implied to point in the upward direction (rightward on the horizontal edges), and β<sub>m,n</sub> downward (leftward when horizontal); differential line thickness serves ease of visualization only. The 6D cubic diagram has 6<sub>4</sub> = 64 corners (states of gating), each corner formed by 6 edges; there are 6·2<sub>(6-1)</sub> = 192 cube edges (paired kinetic transitions). G* is the conducting state in each diagram; all remaining corners are nonconducting states. Selected corners are marked with base-10 integers ("0" through "63 - 1"); the circled state ("47"), whose binary equivalents give the specific states-of-gating for each dimension [see "Numerical note (binary numbers)" in METHODS and Fig. 7B]. Kinetic cubes can be combined in "parallel," e.g., 4D + 2D (below top left). The conducting state of all parallel diagrams is given by the product of their individual "end states," thus: G* = G<sub>15</sub>·g<sub>3</sub> (cubes 4D + 2D), G* = m<sup>3</sup> ("3D" box), and G* = Π<sub>i=1</sub> m<sub>i</sub> (top right box; G* = m<sup>6</sup> for uniform rate constants).

Fig. 4. Mathematically equivalent kinetic diagrams of the Hodgkin-Huxley model’s kinetic structure. The “3D” box (bottom left) shows the equivalent “parallel,” “sequential,” and “cubic” diagrams for the 3rd-order HH sodium-activation kinetics (m<sup>3</sup>). All others are generalized to 6th-order kinetics (6D). All edges of cubic diagrams represent paired rate constants (as drawn in the 2D and 3D cubes); the α<sub>m,n</sub> are upwardly directed (rightward on the horizontal edges), and β<sub>m,n</sub> downward (leftward when horizontal); differential line thickness serves ease of visualization only. The 6D cubic diagram has 2<sup>6</sup> = 64 corners (states of gating), each corner formed by 6 edges; there are 6·2<sub>(6-1)</sub> = 192 cube edges (paired kinetic transitions). G* is the conducting state in each diagram; all remaining corners are nonconducting states. Selected corners are marked with base-10 integers ("0" through "63 - 1"); the circled state ("47"), whose binary equivalents give the specific states-of-gating for each dimension [see "Numerical note (binary numbers)" in METHODS and Fig. 7B]. Kinetic cubes can be combined in “parallel,” e.g., 4D + 2D (below top left). The conducting state of all parallel diagrams is given by the product of their individual "end states," thus: G* = G<sub>15</sub>·g<sub>3</sub> (cubes 4D + 2D), G* = m<sup>3</sup> ("3D" box), and G* = Π<sub>i=1</sub> m<sub>i</sub> (top right box; G* = m<sup>6</sup> for uniform rate constants).
flow of increasing state population is from lower left to upper right, and the general flow during repolarization is in the reverse direction (upper right to lower left). When the combined gating by alpha subunit plus (two) beta subunits is considered, their inherently unequal rate constants are distributed among the dimensions in the 6D cubic diagram, with dimensions 1–4 reserved (arbitrarily) for alpha unit gating throughout.

Numerical note (binary numbers): The cube corners can be numbered, so that the binary equivalents yield an informative characteristic of the digit within the binary (increasing right to left); the numbered dimensions are identified at the lower right.

Characteristic Temperature Response: Alpha and Beta Unit Gating

When the temperature is reduced from 37°C, the response of mammalian channel gating (rate constants) is “Arrhenius” initially, down to ~23.3°C [i.e., uniformly logarithmic as a function of inverse absolute temperature, (°K)⁻¹, with constant Q₁₀ ≡ 2]. The temporal duration (width) of the action potentials increases from ~0.2 ms (37°C) to ~0.7 ms (23.3°C). However, when the temperature is further reduced, below 23.3°C, the action potentials widen more rapidly and reach ~6 ms duration at 10°C (Fohlmeister et al. 2010, Fig. 1).

In striking contrast, the channel gating in the squid giant axon generates action potentials of width ~2.5 ms at 6.3°C (experimentally, and the Hodgkin-Huxley model). This relatively short duration falls on the extrapolation of the initially Arrhenius mammalian response (Fohlmeister 2009, Fig. 4). It thus appears that the squid axon may respond with constant Q₁₀ throughout, whereas mammalian gating shows an upturn in its kinetic Q₁₀ from a constant value for T > 23.3°C, to sharply increasing Q₁₀(T) for T < 23.3°C (Fohlmeister et al. 2010) with no salient effects, which depend only on the kinetic Q₁₀. 
limiting in that upper temperature range. Activation gating in the squid axon may be limited to the alpha subunit (suggested by its Arrhenius continuation at low temperatures), whereas an additional process of high $Q_{10}$ in mammalian gating (rate-limiting for $T < 23.3^\circ$C) is provisionally associated with the channels' polypeptide beta subunits. Beta subunits are associated with a variety of gating effects (Catterall 1995; Hanlon and Wallace 2002; see DISCUSSION) and, as additional dimensions in the gating kinetics, are highly flexible in regard to voltage dependence (see RESULTS). They are the only channel entities that are molecularly distinct from the alpha subunits, which make them plausible candidates for independent temperature response.

Thus, the following are among the questions addressed in RESULTS: 1) How does a weakly (or non)voltage-sensing beta unit process, acting in parallel, effectively slow the much stronger, primary voltage-sensing mechanism of the alpha unit gating? 2) What are the attendant consequences for inactivation gating in the sodium channel? Finally, 3) how does beta unit activity manifest itself for $T > 23.3^\circ$C, where its fast rates are masked by the slower alpha unit gating?

RESULTS

Kinetic $Q_{10}$ for Alpha and Beta Unit Gating

Activation gating of both Na and K channels was treated as by the alpha subunit alone and with one (or two) beta subunit processes added in parallel. The voltage dependence of the rate constants was determined from phase plot fitting (see below); the coefficients of the rate constants were tested for their ability to satisfy the combined following three temperature-response constraints (see METHODS):

1) The alpha and the beta subunits are assumed to respond independently to temperature change, each with a constant kinetic $Q_{10}$ throughout the range of 8–37°C.

2) The width (temporal duration) of the action potentials generated by the alpha subunit alone at 6.3°C (Hodgkin-Huxley’s experimental temperature) should match the width of the action potential of the Hodgkin-Huxley model (~2.5 ms, near its base).

3) The combined gating by alpha and beta subunits should yield action potentials of widths that match those of experimental mammalian action potentials at all temperatures.

Combined, these constraints are satisfied by the unique solution of kinetic $Q_{10} = 2.2$ for alpha unit gating and kinetic $Q_{10} = 14$ for the beta unit processes in all models.

Constraint 3 alone is also satisfied when the temperature-dependent $Q_{10}(T)$ in Fig. 5A (filled circles) is uniformly applied to all active gating processes (thus to both alpha and beta subunits, or to alpha unit gating alone when beta units are missing). This variable $Q_{10}(T)$ reflects the non-Arrhenius nature of the temperature response in mammalian gating.

Rate Constants for Alpha Unit Activation Gating

In highly energy-efficient excitation (which includes all present models at mammalian temperatures), the curve segment of $dV/dt > 0$ in phase plots (i.e., the upper half-curve) is almost exclusively determined by the Na current of the action potential’s regenerative phase (Fig. 6C). Curve fitting that segment of experimentally determined phase plots yields rate constants for alpha unit gating as shown in Fig. 6A (continuous curves):

The voltage dependence of the rate constants, specifically in the range of negative $V$, suggests that the gating transitions occur by crossing a molecular energy ridge, with the membrane electric field controlling the ridge height to be overcome (Fig. 6D). The rate constant $\alpha_{\text{m}}(V)$ is curve-fit (in this voltage range) by a steeply increasing “ridge function,” which approximates an exponential increase with declining ridge height. The ridge function is amended with an asymptotically linear function that arises in a voltage range (positive $V$) in which the ridge effect is no longer observed (Fig. 6D). This asymptotic component is in the form of Hodgkin-Huxley’s $\alpha_{\text{m}}(V)$ (Fig. 6A, dashed curve). The combined functions closely approximate the behavior of an electric field-dependent Boltzmann factor of a two-state system separated by an energy barrier; the rate constants yield steady state, $m_{\infty}(V)$, and time constant, $\tau_{\text{m}}(V)$, given in Fig. 6B.

The K-channel rate constants (not plotted), which are derived from the phase plot segment of $dV/dt < 0$, are of similar functional constructs. The full expressions of the rate constants are given in Table 2 (also Fig. 1 legend for another form of Na-inactivation gating, see below).

Beta Unit Gating: Rate Constants and Rate-limiting Effects

Unlike the (necessarily) strong constraints on alpha unit gating, the voltage dependence of parallel added beta unit processes was found to be largely unconstrained. This flexibility allows beta unit processes to serve multiple functions, without their potentially divergent voltage dependences degrading the system’s ability to generate action potentials (see DISCUSSION). Restricted here to temperature effects, possible voltage dependences considered are “flat” rate constants (no voltage dependence) and rate constants responsive to dielectric surface charge (Table 2; see below).

Figure 5B shows phase plots at four temperatures. The plots include excitation by three mammalian models: “4D” (alpha unit alone), “5D” and “6D” (alpha, plus one and two beta units), and by the Hodgkin-Huxley model. The widths of the action potentials are (about) inversely proportional to the peak ordinate excursions (min-to-peak $dV/dt$) and are ~0.2 ms (37°C) and ~0.5 ms (25.7°C) for all models. Thus, the presence or absence of beta units has no effect on impulse width above room temperature (specifically $T > 23.3^\circ$C). This implies that the rate-limiting step of the gating lies with the alpha subunit under normal operating conditions (e.g., Bähring and Covarrubias 2011; Irvine et al. 1999; Wang et al. 2005).

For $T < 23.3^\circ$C, in contrast, Fig. 5C shows equal widths of ~2.5 ms at two significantly different temperatures, namely 6.3°C for the mammalian “4D” and Hodgkin-Huxley models (no beta units) and 13.9°C for the “5D” and “6D” models [which include beta unit(s)]. This difference reflects the rate-limiting effect of the beta units at low temperatures.

Beyond impulse width, the shapes of the phase plots (thus of the action potentials) distinctly differ between Hodgkin-Huxley and mammalian models (including the “4D” version, whose kinetic structure and order are identical to HH). This suggests preparation-specific differences in the rate constants of alpha unit gating, which may be another effect of the beta subunits (possibly including also the exposing of the above energy ridge, see DISCUSSION).
Sodium-inactivation Gating (General Results)

Sodium-inactivation gating was treated both as an independent first-order kinetic process (i.e., with the “h”-variable; Figs. 1B and 5, B and C) and as functionally coupled to sodium-activation gating (without an h-variable; Table 2; e.g., Armstrong and Bezanilla 1977). General restrictions arise from their distinctive kinetic topologies and, independently, from their phase plot analyses. Two fundamental differences were found, although the response to temperature change is common:

Equilibrium. Independent (h-variable) inactivation is an equilibrium process with locally balanced transitions [rate constants $\alpha_h(V)$ and $\beta_h(V)$]. In contrast, the kinetic topology of the coupled cubic (or equivalent sequential) diagrams admits both balanced (equilibrium) and locally unbalanced inactivation transitions. Phase plot exploration of these topological possibilities shows the several transitions of inactivation gating to be virtually unidirectional (e.g., the continuous vertical arrows in Fig. 7A), with possibly a small degree of equilibrating return transitions (dashed vertical arrows, see below).

Rate constants. The voltage dependences of the independent h-variable rate constants, $\alpha_h(V)$ and $\beta_h(V)$, are necessarily both strong and tightly constrained (Fig. 1 legend). In contrast, effective inactivation gating occurs for a wide range of voltage dependences in the coupled system, including voltage-independent (flat) inactivation rate constants (e.g., Fig. 4 in Zagotta and Aldrich 1990). Phase plot analysis narrows this range and shows a preferred voltage dependence that suggests response to dielectric surface charges (capacitive displacement and gating currents; see below; cf Sheets and Hanck 1995). In all cases, the inactivation rate constants of the coupled system are denoted $\alpha_i$ and $\beta_i$.

Temperature response. The kinetic $Q_{10}$ of Na-inactivation gating follows the $Q_{10}$ of the rate-limiting component of activation gating at all temperatures and for all models: Thus, when activation gating is by the alpha unit alone, the inactivation $Q_{10} = 2.2$ throughout. When beta unit(s) are present, the inactivation kinetic $Q_{10} = 2.2$ for $T > 23.3^\circ$C (alpha unit is rate-limiting), and $Q_{10} = 14$ for $T < 23.3^\circ$C (beta units are rate-limiting). Only the coefficients of the rate constants
VOLTAGE GATING BY CHANNEL MOLECULAR SUBUNITS

Table 2. Rate constants for 6D channel gating with kinetically coupled Na inactivation (37°C)

<table>
<thead>
<tr>
<th>Subunit</th>
<th>α_{m,n}</th>
<th>β_{m,n}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–4 (alpha)</td>
<td>44.178</td>
<td>0.2</td>
</tr>
<tr>
<td>5 (beta, surface charge)</td>
<td>8.0288</td>
<td></td>
</tr>
<tr>
<td>6 (α_5, β_5, surface charge)</td>
<td>0.66907</td>
<td></td>
</tr>
<tr>
<td>1–4 (alpha)</td>
<td>4.1233</td>
<td>0.2</td>
</tr>
<tr>
<td>5, 6 (beta, surface charge)</td>
<td>0.00589</td>
<td></td>
</tr>
<tr>
<td>5, 6 (beta, flat)</td>
<td>11.894</td>
<td></td>
</tr>
</tbody>
</table>

Parameters of the gating rate constants, α_{m,n} and β_{m,n,1}, associated with kinetically coupled Na inactivation (37°C); the analytic functions of the rate constants are:

\[
\alpha_{m,n} = A/\{1 + \exp[-B \cdot (V_m + C)] + D \cdot (V_m + E)/1 - \exp[-F \cdot (V_m + E)]\}
\]

\[
\beta_{m,n} = G \cdot (V_m + H)/1 - \exp[J \cdot (V_m + H)]
\]

The α_{m,n} (V) [alpha unit] consist of 2 component functions: an initial rise function (parameters A, B, C) and an asymptotically linear increasing function (parameters D, E, F; see text, and Fig. 6A). Two sets of rate constants are listed for both beta unit and for inactivation gating (α_5, β_5); these are dielectric surface charge-responsive functions and "flat" numbers with no voltage dependence. Typical conductances at 37°C, used with these rate constants are [e.g., Fig. 8, (mS/cm^2): G_Na = 58.2, G_K = 64.1, G_Ca = 0.72, G_K,Ca = 0.25, G_L = 0.51; V_L = −63.3 mV. conductance Q_{10} = 1.97 (T > 23°C; increasing for T < 23°C).

Sodium Inactivation Coupled to Alpha Unit Activation Gating

Molecular inactivation gating almost certainly occurs on the cytoplasmic channel surface, where it is without direct access to the (intra)membrane electric field; it is therefore likely that its voltage dependence is relatively weak (Armstrong et al. 1973). Although simultaneous voltage insensitivity and kinetic independence are possible for beta unit processes (see above), that combination cannot occur for sodium-inactivation gating (see DISCUSSION). Inactivation is therefore likely to be coupled to the voltage-sensitive alpha subunit (e.g., the kinetics of Fig. 7, A and B). Coupling replaces the need for direct voltage sensing, as the transient voltage clamp Na currents show in Fig. 7C, which employed flat (voltage-insensitive) rate constants α_1 and β_1 (Table 2).

Figure 7A shows the activation gating by the alpha subunit, twice represented in sequential diagrammatic form (see METHODS). The upper sequential bank represents the inactivated channel; all states of the lower sequential bank are noninactivated. Unidirectional transitions at all locations of inactivation gating yield the best fit to the phase plots of experimental impulse trains [rates α_1 for G5^+ → I(5), and β_1 for I(j) → C(j), j = 1, 2, 3, 4; continuous vertical arrows in Fig. 7A]; these also yields the lowest channel-density requirement (see below). Locally equilibrating return transitions can also be fit to a maximum of −0.1β_1 and −0.1α_1, respectively (dotted arrows in Fig. 7A; cf Bähring and Covarrubias 2011; Clancy and Rudy 2002; Wang et al. 2005).

The “5D cubic” diagram (Fig. 7B) yields equivalent results to the “double sequential” of Fig. 7A but is more generally applicable; if the rate constants of alpha unit gating are non-uniform, the 5D cubic diagram must replace the double sequential. Two 4D “subcubes” replace the two sequential banks: States 16 through 31 collectively represent the inactivated channel (these define one of the 4D subcubes). Dimension 5 (which connects the two subcubes) serves the transitions of Na-inactivation gating. As in Fig. 7A, there is only one transition to the inactivated state, either exclusive or dominant, namely G*(15) → 31 (which is α_1). The remaining transitions in dimension 5 (those originating in states 16–30 and terminating on states 0–14) are return transitions from the inactivated states (β_1 exclusive or dominant; there are 15 such transitions). As in Fig. 7A, equilibrating return transitions of < 0.1β_1 and < 0.1α_1 may apply.

Figure 7, C and D, gives, respectively, the Na current under voltage clamp and the corresponding time course of inactivation; both simulations employ flat (voltage independent) inactivation rate constants (Table 2). The curves in Fig. 7D are plots of the changing fraction of Na channels that are inactivated [= Σ(normalized populations of states I-1 through I-5) in A, Σ(states 16 through 31) in B]; these sums are conceptually equivalent to “1−h” in kinetically independent h-variable inactivation gating. Note the induced effective voltage dependence (in Fig. 7D), due to the coupling, despite the flat rate constants.

Qualitatively similar records are generated also with inactivation rate constants of a wide range of voltage dependences. This flexibility, found here under voltage clamp, parallels that found also in the phase plot analysis (above). It implies that heterogeneous molecular processes are possible in inactivation gating (as also in beta unit activity) without system failure and without substantially affecting the shape of the action potentials.

The lowest channel density requirement for excitation occurs with exclusively unidirectional inactivation transitions (Fig. 7, A and B, continuous arrows only) and with the
dielectric charge-sensitive inactivation rate constants; the open 
channel Na conductance in single compartment simulations 
was $G_{Na} = 23 \text{ mS/cm}^2$ at 23.3°C ($G_{Na} = 57.4 \text{ mS/cm}^2$ at 37°C; conductance $Q_{10} = 1.95$). To maintain the same amplitude in 
the action potentials, $G_{Na}$ must be doubled with the flat rate 
constants in use. The $G_{Na}$ requirement also increases with 
equilibrating return transitions; for fractional magnitudes of 
0.08 (as above), the required $G_{Na}$ increases by factors of 1.67 
(for the dielectric charge sensitive) and 1.97 (flat rate con-
stants), relative to their respective nonequilibrated Na-channel 
conductances.

**Coupled Na Inactivation with Beta Unit Gating: 6D Cubic 
Kinetics**

The inclusion of beta unit gating involves a further increase 
in cubic dimensionality (to at least 6D; cf Fig. 4). Although 
arbitrary, dimensions 1–4 will be reserved for alpha unit 
gating, dimension 5 serves a beta unit, and in the sodium 
channel dimension 6 serves coupled inactivation gating. Each 
dimension is associated with a specific pair of rate constants 
($\alpha_{m,n}, \beta_{m,n}$) and a specific kinetic $Q_{10}$. The difference in $Q_{10}$ 
alone, between alpha and beta unit gating, is sufficient to 
disallow the type of double-sequential diagram of Fig. 7A (see 
**DISCUSSION**).

The conversion of the 6D cubic diagram (in Fig. 4), from 
one that serves six first-order processes of activation gating to 
one that encompasses coupled Na-inactivation gating (in di-
menion 6), is accomplished by the following two changes:

1) The explicit conducting state of the Na channel, $G^*$, 
changes from 63 to 31, and all left-end states in dimension 6 
represent the inactivated channel (including state 63). These 
states encompass a 5D subcube (the one composed of all thin 
edges on the left in Fig. 4).

2) The presence of beta unit gating creates two possibilities 
for the transition to the inactivated channel:

- $G^*(31) \rightarrow 63$ only.
- $G^*(31) \rightarrow 63$ and $G_{Alpha}(15) \rightarrow 47$. 

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**Fig. 7. Alpha unit gating with kinetically coupled Na inactivation.** A: (double-)sequential kinetic diagram for alpha unit activation gating; the top bank contains 
the inactivated states; the bottom bank is the noninactivated channel. Locally balancing inactivation transitions (dotted arrows) are possible at low rates and with 
increased $G_{Na}$ (see text). B: 5D cubic kinetic diagram (required for nonuniform alpha unit rate constants; otherwise equivalent to A, see text). The numbered 
dimensions (bottom right) are associated with molecular subunits (schematic section at top right). The left 4D subcube (states 16–31) is the inactivated channel. 
The transition to inactivation is $G^*(15) \rightarrow 31$, which is equivalent to $G_{5}^* \rightarrow I-5$ in $A$ ($\alpha_{I}$ dominant). All other transitions in dimension 5 are return transitions 
from an inactivated state (15 transitions, $\beta_{I}$ dominant; only one is shown). As in $A$, the dominant unidirectional transitions ($\alpha_{II}$ or $\beta_{I}$) may be locally balanced 
at low rates, which requires increased $G_{Na}$. Cube corners are numbered, whose binary equivalents code for the state-of-gating, e.g., $G^*(15)$ = “01111”: the alpha 
unit is conducting (“11111”); the left-most “0” (dimension 5) implies not inactivated [see “Numerical note (binary numbers)” in METHODS]. C: simulated voltage 
clamp records of Na current, generated in 20 mV increments from a holding potential of $-55 \text{ mV}$, with coupled inactivation gating by “flat” rate constants (Table 
2); the highest test potential is $+75 \text{ mV}$. $D$: voltage dependence of coupled inactivation gating with flat rate constants (Table 2). Continuous curves give the 
changing fraction of Na channels that are inactivated [$= \Sigma(n)$ (all inactivated state populations)] under the voltage clamp protocol in $C$; this fraction is conceptually 
equivalent to “1 – h” for kinetically independent inactivation gating. Dashed curves show the fraction of inactivation associated with closed states in the 
avtivation gating, at 3 voltages [i.e., $\Sigma$ (states I-1 through I-4) in $A$, or $\Sigma$(16 through 30) in $B$].
These transitions are dominantly one way (unpaired $\alpha_i$ only). State 47 is circled in Fig. 4.

The two transitions in $b$ apply if inactivation gating occurs with indifference to the state of the beta subunit (i.e., is coupled strictly to the alpha subunit). Each of the four numbered states (15, 31, 47, 63) represent all (four) alpha unit processes in their potentially conducting conformation; states GAlpha(15) and 47 with nonconducting beta unit; states G*31 and 63 with the beta unit also potentially conducting (47 and 63 are inactivated channel states; G*31 the ion-conducting channel).

Alternatively, $a$ applies if the transition to the inactivated state requires that all subunits of activation gating, both alpha and beta units, are in their potentially conducting states; this is a direct extension of the pattern in Fig. 7, $A$ and $B$. The difference in the phase plots generated by $a$ and $b$ is insufficient to decide between the two (see DISCUSSION). In either case, all remaining transitions in dimension 6 are unpaired return transitions from the inactivated channel ($\beta_i$ only); there are 31 such transitions for $a$ and 30 for $b$.

**Rate Constants of Coupled Na Inactivation: Dielectric Surface Charge**

The channel-surface location of Na inactivation (also of the beta subunits) expose them to dielectric charges associated with the capacitive displacement current (also gating currents). The displacement-induced surface charge varies directly (for constant membrane capacitance) with the membrane potential, $V$. For an electrically charged gating particle, rate constants that are otherwise flat are amended with a linear voltage dependence (see Table 2). These linear rate constants increase the transition rates, both to inactivation when depolarized and from the inactivated state upon repolarization (see DISCUSSION). Relative to the flat, these rate increases offer significant reductions in the minimally necessary channel densities for excitation: $G_{Na}$ is reduced to $\sim 50\%$, $G_K$ to 67%, with negligible effect on phase plots.

Similar changes to the rate constants of beta unit gating offer no advantage in $G_{Na}$ or $G_K$ and also have negligible effects in phase plots. Dielectric charge-sensitive rate constants were nevertheless employed, in both Na inactivation and beta unit gating, to generate the action potentials and phase plots in Fig. 8 (continuous curves). Computer programs to generate these are given as supplemental data.1

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1 The online version of this article contains supplemental material.
**Sleepy Sodium Channels (7–8°C)**

The sodium channels of mammalian retinal ganglion cells become sleepy in the low temperature range of 7–8°C (Fohlmeister et al. 2010; cf Matteson and Armstrong 1982), and the cells cease to generate action potentials below 7°C. The present analysis suggests a smaller effect also in K-channel gating. One characteristic of “sleepiness” lies in the repetitive firing response, which changes from the regular, tonic adaptation (seen at 9.8°C in Fig. 9B) to an inherent tendency to subthreshold oscillation with intermittent phase-locked spiking, as seen in the records of Fig. 9A, at 7.2 and 7.8°C (cf Fohlmeister 2009).

Sleepiness manifests itself in a rapid broadening of the action potentials; their duration increases from 5 ms (9.8°C) to 12 ms (7.2°C; Fig. 9E). The rapid increase in width is reflected in the ordinate scales of the corresponding phase plots (Fig. 9, C and D). The superposed model data (heavy curves) were generated in 6D cubic kinetics, with alpha unit plus one beta unit and with dielectric charge-responsive coupled Na inactivation.

The widening of the action potentials is associated with a steep (non-Arrhenius) temperature response in gating (Fig. 9F). Sleepiness strongly affects alpha unit gating, whose kinetic Q10 becomes undefined for T < 8°C (cf Fohlmeister et al. 2010). After the usual Q10 was applied, the alpha unit rate constants were further reduced by factors of 14.6 (Na channel) and 6.2 (K channel) for the model records of Fig. 9, C and E (7.2°C). Na inactivation and beta unit gating, on the other hand, appear to continue unchanged with kinetic Q10 = 14.

**DISCUSSION**

The enduring, 60 yr old Hodgkin-Huxley model appears remarkably prescient today, in light of the more recently gained molecular understanding of voltage-gated ion channels. The model’s mathematically condensed forms of “m” (Na activation) and “n” (K-channel gating), intentionally or unintentionally, carry the implication of several (3 and 4) identical gating processes occurring in parallel, i.e., independently and concurrently.

The same implication is conveyed by the four (4) repeat segments of the channel-molecular alpha subunits, which form parallel structural arrays composed of trans-membrane alpha helices. That parallel structure strongly suggests that the four
voltage-sensing alpha helices contained in those arrays (segments “S4”, see below) respond simultaneously to the membrane potential and do so independently. This striking similarity between the Hodgkin-Huxley structure and the alpha subunits was explored by unfolding the model’s condensed mathematics and applying them to the molecule.

Fully unfolded, the Hodgkin-Huxley equations are represented in cubic kinetic diagrams; these diagrams make every state (or molecular conformation), and every transition of gating, explicit. The condensed form m³ expands into an ordinary 3D cube (Fig. 4, lower left box). The molecular alpha subunit, however, implies four processes (a 4D cube), as does the n⁴ of Hodgkin-Huxley’s K-channel gating. Sodium-activation gating was therefore also recast (from m³) to fourth-order kinetics, m⁴.

The cubic dimensionality was further increased to incorporate gating effects due to beta subunits, which are ubiquitous among mammalian voltage-gated Na and K channels (Catterall 1995; Hanlon and Wallace 2002). Although the beta subunits likely affect alpha unit gating directly (among other functions, see below), they are tested here independently as the possible source of the non-Arrhenius temperature response of mammalian excitation, which is contrary to the apparently Arrhenius source of the non-Arrhenius temperature response of mammalian excitation, which is contrary to the apparently Arrhenius response of the squid giant axon without beta subunits.

More fundamentally, cubic kinetics are readily generalized to incorporate the coupling of Na inactivation, directly to its activation gating. Kinetic coupling, however, lies beyond equivalence with Hodgkin-Huxley and leads to insights into the robustness, rate constants, and energy efficiency of the gating mechanism, as well as into refractoriness.

**Alpha Subunit Gating and Rate Constants**

The four repeat segments (substructures) of the alpha subunit, each typically consisting of six alpha helices, segments S1–S6 (originally sequenced by Noda et al. 1984), appear to be the only components of the channel molecule that span the membrane and, therefore, the only channel components directly exposed to the strong intramembrane electric field (scale of 10⁵ volts/cm). The alpha subunit alone, then, is likely responsible for all direct voltage sensing in the gating of the ion-conducting channel pore.

Gating currents and mutational analyses point to segment S4 (which contains the positively charged residues lysine or arginine at every third location) as the primary voltage sensor for channel gating (Papazian et al. 1991; Perozo et al. 1994; Shieh et al. 2000). The base sequence of segment S4 appears also to be conserved across the full spectrum of voltage-gated Na and K channels, and the segment’s positional rearrangement, in response to depolarization, has been confirmed by fluorescence techniques (Cha and Bezanilla 1997; Mannuzzu et al. 1996). Negative charges on segments S2 and S3 contribute to the gating mechanism by electrostatic interaction with S4 (Papazian et al. 1995; Seoh et al. 1996). Residues of S5 and S6 may contribute to ion selectivity (Heginbotham et al. 1994), with other elements of the same segments possibly acting as the activation “gate” that regulates ion access to the channel pore (Kanevsky and Aldrich 1999; Liu et al. 1997; Shieh et al. 1997).

As with Hodgkin-Huxley, the present analysis posits that each of the four repeat components of the alpha subunit switches between two structural states (nonconducting and potentially conducting). The channel conducts ions when all four components of the alpha subunit are in their potentially conducting state; all other combinations of states of the four components render the channel closed (nonconducting). The directness and simplicity of this mechanism are consistent with the sigmoidality under voltage clamp and with the all-or-none conductances seen in most single channel records (e.g., Sigworth and Neher 1980).

Although the (parallel) kinetics are shared by the Hodgkin-Huxley model and the alpha subunits, phase plot analysis of mammalian action potentials shows their rate constants differ in voltage dependence (e.g., “HH” and “4D,” in Fig. 5, B and C). Figure 6A shows αₘ(V) with initially delayed onset, followed by a steep increase within a relatively narrow range of V (including the threshold region), and ultimately increasing linearly for large depolarizations. A linearly increasing rate suggests gating that responds to voltage directly (the membrane electric field), which is also the form (asymptotically) of Hodgkin-Huxley (dashed curve in Fig. 6A).

The specific nonlinear behavior for negative V (the primary gating range), however, suggests that the gating transitions cross a molecular energy ridge that intervenes between the two energy minima of the nonconducting and potentially conducting conformations. The energy minima are likely engendered to a large extent by internal electrostatic interactions between the positive lysines and arginines of segment S4 and the negative charges on S2 and S3. Those interactions will be strongly affected by the membrane electric field, which drives the opposite charges in opposite directions. Indeed, it appears that one of the two molecular energy depressions, the nonconducting state, exists definitively only in the presence of strongly polarizing electric fields. The field thus also establishes the intervening energy ridge and indirectly controls the gating rate by changing the energy difference between the initial state (of the transition) and the ridge crest (Fig. 6D, multiple panels). Rates across simple ridge crests (“activated complexes”) increase exponentially with declining energy difference; here they are curve-fit by a steeply rising segment of the rate constants. The native channel, in the absence of any field, occurs in a single conformation only, that of all four molecular alpha unit components potentially conducting, with no secondary energy minima.

As the membrane is increasingly depolarized, the rate constant αₘ(V) plateaus briefly and then continues to increase linearly (Fig. 6A). These two features shape the phase plots from peak rate-of-rise, through the peak of the action potential. The definitive linear increase seems to arise with electric field reversal (i.e., for positive V, with a possible zero-point offset due to fixed membrane surface charges, e.g., Gilbert and Ehrenstein 1969). This appears also to be the only voltage range in which the gating (rate) responds to voltage directly. Figure 8, B and C (dashed curves), shows the phase plot distortion when this directly voltage-driven form of the rate constants is applied throughout.

**Robustness of Alpha Unit Gating**

When the alpha unit gating is unfolded in cubic kinetics, it becomes possible to assess its resistance against failure on the level of individual states of gating. In general, it was found that
fully one-quarter of the inactivation-coupled sodium system, eight states in Fig. 7B, can be nonfunctional (i.e., removed) without loss of normal excitation, including low frequency repetitive firing (shown in Fig. 10C). The sizes of the action potentials (and marginally their shapes) do vary somewhat, and this depends on the kinetic distances between the nonfunctional states and the conducting state (i.e., the number of kinetic steps):

Several of these phase plots are compared in Fig. 10A; these include the fully functioning alpha unit (heavy curve) and two plots with eight nonfunctional Na-channel states (intermediate line thicknesses); the small declines in size of the action potentials are readily reversed by modest increases in the channel density, G_Na. (The nonfunctional states occur pairwise in the diagram, noninactivated and inactivated, and were pairwise removed. Pair members are associated across dimension 5 in Fig. 7B and represent the same state of the alpha unit.)

The fourth phase plot in Fig. 10A (thinnest curve) was generated with 10 nonfunctional states (again pairwise removed). This creates a bifurcation (two stable singular points: at rest and suprathreshold) that is not correctable by increasing G_Na. The spiraling phase plot remains suprathreshold; its decaying oscillatory membrane potential is shown in Fig. 10B, right); hyperpolarizing current is required to recover the resting state.

Another type of potential malfunction might be a “reluctant component” in the alpha unit. Component 4 (for example) may transition to potentially conducting only after components 1, 2, and 3 have done so. For coupled Na inactivation, this was modeled by amending the rate constants of component 4: \( \alpha_{in}(V) \rightarrow [(7) + (15) + (23) + (31)] \cdot \alpha_{in}(V) \) and \( \beta_{in}(V) \rightarrow [(7) + (15) + (23) + (31)] \cdot \beta_{in}(V) \); the numbers in parentheses refer to normalized state populations in Fig. 7B. This malfunction consistently led to high-frequency repetitive firing (i.e., loss of low-frequency repetitive firing under current clamp). The corresponding changes for h-variable inactivation are: \( \alpha_{in}(V) \rightarrow m_1 \cdot m_2 \cdot m_3 \cdot \alpha_{in}(V) \) and \( \beta_{in}(V) \rightarrow m_1 \cdot m_2 \cdot m_3 \cdot \beta_{in}(V) \), which led to “impulse bursting” (Fig. 10D).

Thus, the reluctant model appears generally to affect repetitive firing, although it retains the ability to generate action potentials, with monostable resting state, and no change in required stimulus currents.

**Fig. 10. Robustness and energy efficiency, refractoriness, and recovery of excitation in the coupled inactivation model.**

A: phase plots (37°C) generated by the 5D cubic kinetic model of Fig. 7B (rate constants in Table 2) and the same model with 2 sets of 8 missing states in Na-channel gating (states 1, 3, 6, 14, 17, 19, 22, 30 and 7, 10, 11, 12, 23, 26, 27, 28 by decreasing line thickness). The 4th plot (thinnest line) of 10 missing states (5, 6, 7, 10, 12, 21, 22, 23, 26, 28) expresses the suprathreshold behavior of a bistable system (spiral to a stable state at \(-39.965 \text{ mV}\)); stable rest is \(-63.46 \text{ mV}\) (see text). B: APs of the 2 thinnest line phase plots, including the spiral. C and D: 2 forms of repetitive firing response (regular and bursting) generated with a “reluctant alpha unit component” in Na-channel gating (see text). E: highly energy efficient membrane currents during an AP, generated in 6D cubic kinetics with dielectric charge-responsive, coupled Na inactivation. F and G (top records): high frequency repetitive firing at 37°C (\(-770 \text{ imp/s, } I_{stim} = 22 \mu\text{A/cm}^2\)) in F and its failure at low temperatures in G (9.8°C, 1 \( \mu\text{A/cm}^2\)); note the time bases. Bottom records give the sodium inactivation (\\(I\\) sum over normalized populations of all inactivated states, 31–63 in Fig. 4), which must decline to the range of 0.45–0.5 to enable a subsequent AP. At 37°C, this level is reached in \(-1 \text{ ms}\); it requires \(-100 \text{ ms}\) at 9.8°C, as may be inferred by extrapolating the inactivation curve segment: 12–26 ms in G. Note also that inactivation is virtually complete at the peaks of the APs for 37°C, whereas at 9.8°C the fractional inactivation peaks at 0.798; it ultimately reaches 0.961 in steady state; incomplete inactivation has implications for energy efficiency in excitation (see text). H: repetitive pulse stimulation at 9.8°C (5 \( \mu\text{A/cm}^2\) pulses of 1 ms duration) at intervals of 60 (light curves) and 100 ms (heavy curves). Note that the highest attainable impulse frequency at 9.8°C is \(-10 \text{ imp/s, under both current clamp and repetitive pulse stimulation. The simulations employed coupled inactivation and beta units and surface charge-sensitive rate constants.}
Gating by the alpha subunit of K channels is even more robust (not plotted): The system remains monostable and retains low-frequency repetitive firing, with up to seven nonfunctional states (nearly half of the total of 16 states of gating), as well as with a reluctant component. The K channel’s “reluctant model” does, however, significantly reduce the depth of after-hyperpolarization; restoration of the original depth requires a near doubling of $G_K$ (factor of $\sim 1.9$ was found in RESULTS).

**Coupling of Na Inactivation to Alpha Unit Activation Gating**

Inactivation gating is commonly depicted as a flexibly tethered ball (“gating particle”) near the cytoplasmic pore opening of the channel; the gating particle occludes the pore to inactivate the channel (originally Fig. 12 in Armstrong and Bezanilla 1977). This design is based on the finding that the components of sodium inactivation are readily and selectively cleaved by cytoplasmic protease, without compromising the activation gating or the ability of the channel to conduct ions (Armstrong et al. 1973). The surface location places the gating particle outside the region of the strong intramembrane electric field, suggesting that inactivation gating may occur with little (or no) independent voltage sensing.

The transitions of inactivation gating cannot, however, be both voltage insensitive and kinetically independent. That combination is tantamount to no inactivation gating; it yields a bistable system, which, once depolarized, will not recover the resting state. Sodium inactivation therefore almost certainly needs to be coupled to a process that is voltage sensitive, most likely to the activation gating of the sodium channel’s alpha subunit (Groome and Winston 2013). The present modeling finds, as a striking consequence of the coupling requirement, that all transitions of inactivation gating are highly dominant in one direction (ideally unidirectional, e.g., Fig. 7, A and B) and that the direction reverses when the state of activation gating switches between the open pore and any closed-pore conformation of the alpha subunit.

This kinetically cyclic transition pattern, which replaces the need for direct voltage sensing, suggests that a binding site (for the gating particle) is created by the conducting pore configuration of the alpha subunit (cf Hoshi et al. 1990; Iasoff et al. 1991) and that the binding site collapses for all other alpha unit conformations. Note that the alpha subunit’s “conducting pore configuration” is represented by two states in Fig. 7B, namely $G^*(15)$ and $31$ [G$^*$ and I(5) in Fig. 7A], although 31 and I(5) are nonconducting (i.e., inactivated states). Nonrelease of the bound gating particle [in states 31, or I(5)] is the proximal cause for the (dominantly) unidirectional nature of the transitions to inactivation, $G^* \rightarrow 31$, or G$^*$ → I(5). The gating particle is ejected with the collapse of the binding site, and this is also inherently unidirectional. The transition rate for the removal of inactivation is then equivalent to the rate of ejection.

**Inactivation Gating: Refractoriness and Recovery of Excitability**

As experimental observations, healthy mammalian retinal ganglion cells have the capacity, at $37^\circ$C, to generate impulses at very high frequencies (>1,000 impulses/s) but are incapable of correspondingly (temperature-adjusted) high impulse rates at low temperatures, either in current clamp or by repetitive pulse stimulation. These response characteristics are inherent also in the kinetically coupled model with beta units. In the model, the primary cause of the low temperature failure is exceptionally slow recovery from Na inactivation, which prevents an immediate subsequent spike. This slow recovery has virtually no effect on the shape (or width) of the action potential that caused the inactivation.

Figure 10F shows simulated repetitive firing by the coupled model at $37^\circ$C, in response to a step to 22 $\mu$A/cm$^2$ of continuous stimulus current; the firing rate is $\sim 750$ impulses/s. The time course of the fraction of Na channels that are inactivated is shown below the voltage record. Figure 10G gives corresponding records for $9.8^\circ$C (stimulus step to 1 $\mu$A/cm$^2$), and here the repetitive firing fails. Note the slow decline of inactivation after its rapid rise due to an initial action potential; a second (aborted) action potential returns the slowly declining inactivation to a high level, from which it does not recover (for the duration of the stimulus).

If the second, aborted action potential is delayed (by applying a smaller stimulus step), the inactivation curve will continue to decline at its former slow rate. In general, it was found that the inactivation level must decline to $\sim 0.45$–0.5 (fraction of Na channels) so as to enable a subsequent definitive action potential and thus to sustain repetitive firing. To reach this level at $9.8^\circ$C requires a minimum interspike interval of $\sim 100$ ms; thus the highest frequency of repetitive firing, at that low temperature, is $\sim 10$ impulses/s.

At $37^\circ$C, the inactivation level repeatedly descends to $\sim 0.38$ during the high frequency firing in Fig. 10F; that level declines to virtually zero during low frequency firing at that temperature: Thus, for the example of an interspike intervals of 140 ms ($\sim 7$ impulses/s, $I_{stim} = 50$ nA/cm$^2$, $37^\circ$C), it decreases from a peak of $> 0.999$ during the action potentials, to $< 0.0011$ at its minimum. Both the model and retinal ganglion cells are capable of low frequency repetitive firing at all temperatures, including $9.8^\circ$C, which occurs in response to low constant stimulus currents.

Similar temperature patterns of inactivation decline occur also with repetitive pulse stimulation: Fig. 10H shows the model responses, at 9.8°C, to stimulus pulses spaced by 60 ms (thin curves) and by 100 ms (heavier curves). Note that the 60 ms spacing is inadequate to maintain repetitive firing.

The data presented in Fig. 10, F–H, were computed using the 6D cubic kinetic diagram (in Fig. 4) and under the condition of only one transition to inactivation, namely, “G*31” $\rightarrow$ “63” (rate constant $\alpha_1$; this is condition 2a in RESULTS: “Coupled Na inactivation with beta unit gating; 6D cubic kinetics”). This transition occurs when all components of activation gating, both alpha and beta units, are in (have reached) their potentially conducting states.

Virtually the same results occur also under condition 2b, with two transitions to inactivation, namely “GAlpha(15)” $\rightarrow$ “47,” in addition to “G*(31)” $\rightarrow$ “63.” This condition implies that the transition to inactivation requires only the alpha unit components to be potentially conducting (i.e., is independent to the state of beta unit gating).

The same results continue to occur when the 6D cubic diagram is replaced by a fifth-order diagram, either Fig. 7, A or B, and beta unit gating is added as a parallel kinetic process. The channel conductance is then given by the product $G^* = m_{Beta}G5$ (Fig. 7A), or $G^* = m_{Beta}[15]$ (Fig. 7B), where $m_{Beta}$ is the state variable of the beta unit gating (see Fig. 4 legend). Here
The non-Arrhenius mammalian response is then modeled by the Arrhenius response (where there is no evidence for beta units), with a lower Q_{10} for alpha and beta unit gating; slowing the alpha units, kinetic Q_{10} to augment the primary alpha unit activation gating. The above) is the need for a parallel secondary process of high temperature phenomena is presumptive, because the model of high kinetic Q_{10}; the instantaneous state of the beta units is irrelevant to this phenomenon. Invoking beta units as the definitive cause of the mammalian low temperature phenomena is presumptive, because the modeling of a phenomenon cannot alone determine, or confirm, the physical basis of the phenomenon. What seems to be certain (from the above) is the need for a parallel secondary process of high kinetic Q_{10} to augment the primary alpha unit activation gating. Beta units have been implicated in modifying the channel gating (see below); their impact is therefore profound and, as the only other channel components, are thus reasonable default candidates to provide the required secondary gating processes.

**Beta Subunits: Gating Functions**

Beta subunits have been associated with modified excitation as expressed in channel subtypes (Farmer et al. 2012; Gosselin-Badaroudine et al. 2012; Kisselbach et al. 2012; Watanabe et al. 2014). Studies using antibodies, or in situ hybridization, have identified numerous subtypes of mammalian channels, among them Na channels Nav1.1–1.6 (Boiko et al. 2001; Caldwell et al. 2000; Cramer et al. 2003; Khaliq et al. 2003), and K channels Kv1.1–1.6 (Henne et al. 2000; Höltje et al. 2007; Pollock et al. 2002). Although these and other subtypes yield a variety of spiking responses, the primary mechanism for generating the action potential almost certainly remains with the alpha subunit. Comparing the plots of 4D, 5D, and 6D in Fig. 5B shows the negligible effect of the beta units in this primary function.

Since alpha unit gating is readily modified by beta units, their presence, with independent kinetic Q_{10}, is likely also the cause of the non-Arrhenius deviation in mammalian gating at low temperatures (cf Beam and Donaldson 1983; Fohlmeister et al. 2010). The effect is virtually indifferent to the number of additional processes; a single beta unit, with kinetic Q_{10} = 14, yields the necessary rate-reducing effect for T < 23.3°C (Fig. 5A, filled circles). The comparison of the squid axon’s temperature response (where there is no evidence for beta units), with that of mammalian neurons, is consistent with the Arrhenius alpha unit gating of kinetic Q_{10} = 2.2 (Fig. 5A, dashed line). The kinetic slowing induced by beta units may be nothing more than an unavoidable side-effect; the effect is benign because it falls well outside the operating temperature range of mammals.

It may be, however, that an important evolutionary function for beta subunits is to increase energy efficiency in excitation; note that the Hodgkin-Huxley model is very poor in energy efficiency (late Na current in Fig. 3D; cf Fohlmeister 2009; Sengupta et al. 2010). To improve this, the beta subunits need to directly alter the rate constants of alpha unit gating. Note that the mammalian rate constants result in almost optimized energy efficiency; Fig. 3C shows virtually no Na current after the action potentials’ rising phase.

It may further be that squid axon gating must forgo the benefit of energy efficiency because of the axon’s need for fast excitation, which would be significantly slowed by beta unit processes in the squid’s low temperature environment. The relatively fast response of the unfettered alpha unit gating, at low temperatures, is seen in the data of the squid giant axon at 6.3°C (the experimental temperature of Hodgkin-Huxley, leftmost diamond symbol in Fig. 5A). This falls on the Arrhenius continuation (dashed line) of the mammalian gating response at higher temperatures. One may also speculate that Beta units foster sleepiness in excitation at very low temperatures. Thus, virtually all Na channels in retinal ganglion cells fall sleepy below 8°C, whereas only a small subpopulation of those of the squid axon do so below 6.3°C (Matsen and Armstrong 1982).

Another distinctive observational division between the ranges above and below room temperature lies in the repetitive firing by retinal ganglion cells, which tends, stochastically, to be increasingly irregular with increasing T > 23.3°C (Fig. 1A), whereas for T < 23.3°C it is more typically regular and is often slowly adapting (Fig. 9B; the full range of temperatures is given in Fohlmeister et al. 2010, Fig. 1). The irregular firing at the normal (i.e., high) operating temperatures may be purposeful; it can maintain a high level of signal strength in a nervous system that typically tends to adapt rapidly to an unvarying signal (e.g., Clay et al. 2012). It is thus possible that the fast beta unit background activity at the higher temperatures introduces beneficial noise to the rate-limiting alpha unit gating.

**Thermodynamic Considerations: Dielectric Surface Charge**

The pattern of unidirectional (i.e., unpaired) kinetic transitions for coupled sodium inactivation gating is a pure modeling result: It was virtually impossible to find rate constants for locally paired transitions, even though that inherently offers a larger number of adjustable parameters. On the other hand, rate constants were readily found for the one-way transitions, as shown by their flexible voltage dependence. The inactivation kinetics appear, therefore, to be thermodynamically (virtually) irreversible.

The second law of thermodynamics thus requires an external energy source to drive each traversal of the gating cycle. During an action potential, or under voltage clamp, that energy source is the changing electric field within the membrane. In the single spontaneously stable steady state, namely the resting neuron, all transitions of inactivation gating arise from gating states that are virtually depopulated. Those “states of origin” consist of all inactivated states (except one), plus the conducting...
state. All of these states have fractional populations in the range $10^{-5}$–$10^{-2}$, which virtually eliminates spontaneous cycling.

Beyond this fundamental consideration, the electrostatic forces of induced dielectric charges are likely involved in driving the gating particle of inactivation. Dielectric charges include the shifting charged molecular residues that generate gating current (Armstrong and Bezanilla 1977, Fig. 12), as well as the surface charge associated with capacitive current, which is proportional to the membrane potential. Thus, the flat rate constants for coupled inactivation gating were amended with an asymptotically linear voltage dependence (Table 2), in effect an electrostatic attraction of the gating particle to the surface (its binding site) when depolarized, and a slowly increasing electrostatic repulsion from the surface with increasing membrane polarization or hyperpolarization. These modifications increase the already high energy efficiency of gating (see below) and may also contribute to satisfy the second law.

Energy Efficiency of Kinetically Coupled Na Inactivation Gating

The primary function of the sodium current, in action potentials, is to generate their rising phase; Na current that flows after the peak of the action potential is a functionally needless (energy-inefficient) source of increased ion pumping. The near absence of this late Na current is a key feature of all present (mammalian) models, at least for $T > 23.3^\circ C$ (e.g., Figs. 3C, 10E).

Kinetically independent (h-variable) Na inactivation generally resulted in lower energy efficiency than coupled Na inactivation. Late Na current is particularly pronounced in the Hodgkin-Huxley model (Fig. 3D) but can also be substantial in the mammalian h-variable model with beta unit gating, at the lower temperatures (e.g., the Na current at 13.9$^\circ C$ is similar to that in Fig. 9E). This late Na current is due to more-or-less incomplete inactivation of the Na channel, which occurs because the state variable, h(t), does not respond directly to the state of activation gating and responds relatively slowly to changes in the membrane potential, V.

In contrast, the direct coupling of inactivation to specific states of activation gating causes almost all Na channels to inactivate in the course of the action potential for $T > 23.3^\circ C$ (see results). This (almost) shuts down the Na current during the falling phase. Energy efficiency declines also for the coupled model at lower temperatures, and this is again directly related to incomplete inactivation during the action potential (see legend for Fig. 10, F and G).

Although this declining property occurs both with dielectric charge-responsive, or flat inactivation rate constants, the dielectric charge-responsive rate constants show a large gain over flat rate constants, in reducing the necessary channel densities for excitation: $G_{Na}$ is about halved, and $G_{K}$ is reduced to 67% (see results and Table 2). Thus, the same direction-specific driving energy that may help to mitigate concerns of “perpetual motion” in the kinetics also yields the most energy-efficient gating overall.

Summary

1) Complementary strengths of the Hodgkin-Huxley mathematics, and of the channel-molecular alpha subunits, are mutually exchanged on the basis of their identical respective structures. The activation gating, cast as four kinetic processes in parallel, is fully unfolded in 4D cubic kinetic diagrams, which make explicit all states and transitions of gating.

2) The gating function of the alpha subunit is highly failure resistant against partial loss of its mechanism. Alpha subunit gating is rate-limiting for $T > 23.3^\circ C$.

3) The rate constants of mammalian alpha unit gating reveal a molecular energy ridge between two conformational states; the closed-state conformation becomes unstable for depolarizations $> - 20$ mV.

4) Higher dimensional cubic diagrams (6D) encompass, further, coupled sodium inactivation to activation gating and general gating activity by beta subunits. The gating of coupled inactivation is found to be a (near) nonequilibrium process.

5) Strongly temperature-dependent beta unit activity (kinetic $Q_{10} = 14$) consistently induces the observed non-Arrhenius temperature response of mammalian gating and becomes rate-limiting for $T < 23.3^\circ C$. Sodium inactivation gating follows the kinetic $Q_{10}$ of the rate-limiting component of activation gating.

6) The combination of coupled Na inactivation and beta subunits uniquely yields exceptionally slow recovery from inactivation at low temperatures, as observed experimentally; this restricts repetitive firing to low impulse frequencies. At high (mammalian) temperatures the model yields fast recovery from inactivation and very high frequency repetitive firing for large stimuli (>1,000 impulses/s).

7) A specific voltage dependence for coupled Na inactivation and beta subunits uniquely yields exceptionally slow recovery from inactivation-induced dielectric charges, maximizes the already high energy efficiency of excitation and minimizes (needed) channel densities; it also provides an energy source for the nonequilibrium inactivation kinetics. Both mechanisms are otherwise flexible as to constraints on (or need for) independent voltage sensing.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

J.F.F. conception and design of research; J.F.F. analyzed data; J.F.F. interpreted results of experiments; J.F.F. prepared figures; J.F.F. drafted manuscript; J.F.F. edited and revised manuscript; J.F.F. approved final version of manuscript.

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