

Hodgkin-Huxley equations.

8-1. Today I'll give a historical perspective of the equations Hodgkin and Huxley developed to explain the ionic basis of the action potential. Experiments had suggested that the electrical behavior of the membrane could be represented by the parallel conductance model that we discussed previously. Hodgkin and Huxley turned out to be very lucky. The squid axon is permeable only to Na, K and Cl and [Cl] follows V_m passively, so the only ionic conductances involved were Na and K.

One observation H-H made was that changes in permeability depend on membrane potential and not on membrane current. This was a new idea for the time. For a fixed depolarization, Na current follows a time course whose form is independent of the current through the membrane. The current may reverse direction, but for a given voltage it follows the same time course. If voltage is restored, then the conductance returns.

H-H sought to explain the voltage dependent changes in membrane permeability from first principles. They proposed that permeability changes arise from the effect of the electric field on the orientation of molecules in the membrane having a charge or dipole. These molecules could be those making up the ion channel.

The electric field must do work to move charges. With depolarization, the dipole flips or charges move and this changes the configuration of the molecule. When all charged particles move to the "open state" the channel opens. This movement of charges in the membrane should cause a detectable current or "gating current" and such a gating current was eventually detected 20 years after the HH experiments.

8-2. How might we describe this quantitatively?

Suppose a gating particle can be in two states—open or closed. Suppose the transition from closed to open involves a conformational change that moves a charge of valence z from the inner membrane surface to the outer surface across a voltage drop E .

Then the ratio of the number of particles in the two states is given by the Boltzmann's equation which says that the probability of a particle being in a particular state depends on the energy difference between the two states. The energy difference is $w - zeE$ between open and closed states. We express the proportion outside to inside by $\frac{O}{C} = \exp\left(-\frac{w - zeE}{kT}\right)$ where

w =work required to move the charge when $E=0$ (non-electrical work) Conformational energy change (increase) that occurs without a change in voltage

zeE =electrical work or electrical energy increase when there is a potential difference. The movement of the gating charge $-zeE$ (move z inward, E is negative)

total energy change is $w - zeE$

z =valence, e =elementary charge, k -Boltzmann's constant and T =temperature Kelvin.

8.3 Given $\frac{O}{C} = \exp\left(-\frac{w - zeE}{kT}\right)$ we get $C = O \exp\left(\frac{w - zeE}{kT}\right)$.

Then the fraction in the O state is $O/(O+C)$ or

$$\frac{O}{O+C} = \frac{O}{O + O \exp\left(\frac{w - zeE}{kT}\right)} = \frac{1}{1 + \exp\left(\frac{w - zeE}{kT}\right)} \approx \text{Const} \exp\left(\frac{zeE}{kT}\right).$$

when E is large and negative so E dominates the conformational energy change w.

Now HH used voltage clamp to calculate the steady-state Na and K conductances at different potentials. They found that near rest,

for Na, there was an e-fold change in conductance for a 4 mV change in potential

for K, there was an e-fold change in conductance for a 5-6 mV change in potential.

Now $\frac{O}{O+C} \approx \text{Const} \exp\left(\frac{zeE}{kT}\right) = \text{Const} \exp\left(\frac{zEF}{RT}\right) \approx \text{Const} \exp\left(\frac{zE}{25}\right)$ because $e/k = F/R$ and

$F/RT \sim 25$ at room temperature ($e=1.6e-19$ coul/molecule, $k=1.38e-23$ Joule/molecule-°K, $F=9.65e4$ coul/mole, $R=8.31$ Joule/ mole-°K).

So for $\exp(zE/25)$ an e-fold change occurs when $zE=25$.

For Na, this means that $z \sim 6$ and for K $z \sim 4.5$

Therefore the molecule that moves for the Na channel has 6 charges or 3 dipoles, and for K 4.5 charges or 2.25 dipoles.

8-4. But there are problems with this analysis. What are they?

The analysis assumed that charges moves from one side of the membrane to the other. However charges may lie part way through the membrane and so won't move the full distance from one side to the other. There may be movements in charge part way across the membrane

Consequently there is little hope to get the time course of g_{Na} and g_K from first principles. Therefore HH sought to derive empirical relationships.

One difficulty with getting empirical relationships was that both g_{Na} and g_K rise with a delay when a depolarizing pulse is applied, but there is no delay when the membrane repolarizes. This means that first order equations won't work. It is not that simple. So how can this behavior be described empirically?

Well, suppose that Na and K pass through channels with "gates"

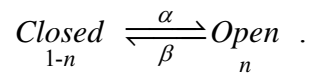
Suppose these gates are either open (permissive) or closed (non-permissive).

For a channel to be open, all gates must be open. If there are n gates with probability p of being open, then the probability of a channel being open is p^n .

Then the conductance = max conductance(when all are open) * p^n , or $g = \bar{g}p^n$.

8-5. How do we express this quantitatively?

Assume there is movement of a charged particle or gate between “closed” and “open” states



If the probability of being in the open state is n then this becomes $(1-n) \xrightleftharpoons[\beta]{\alpha} n$ where α, β are functions of voltage

We can form a differential equation from this $dn/dt = \alpha(1-n) - \beta n$.
In the steady-state $n = \alpha/(\alpha+\beta)$ which we define as $n_\infty \equiv \alpha/(\alpha+\beta)$.

The differential equation can be solved analytically. We re-write it as $dn/dt + (\alpha+\beta)n = \alpha$ and solve using $\exp((\alpha+\beta)t)$ as an integrating factor. We define $(\alpha+\beta)$ as $1/\tau$.

The solution is $n = n_\infty - (n_\infty - n_0) \exp(-t/\tau)$ where $n_\infty = \alpha/(\alpha+\beta)$ and $\tau_n = 1/(\alpha+\beta)$ and $n_0 =$ initial value of $n = \alpha_0/(\alpha_0+\beta_0)$. For Na and K, $n_0 \approx 0$.

8-6. Let's look at the solution $n = n_\infty - (n_\infty - n_0) \exp(-t/\tau)$

When activated from rest $n_0 \approx 0$ so when you depolarize $n \approx n_\infty (1 - \exp(-t/\tau))$

When you return to rest, $n_\infty \approx 0$ (final state has small n) so when you repolarize $n \approx n_0 \exp(-t/\tau)$

How do you get a delay with activation, but not with repolarization? Use the 4th power!
 $(1 - \exp(-t/\tau))^4$ has an inflection point that gives the delay on the rise. Conversely,
 $(\exp(-t/\tau))^4 = \exp(-4t/\tau)$ will just decay faster with no delay.

The 4th power implies that more than 1 charge or particle is moving. Why do we use the 4th power for potassium? Is this because the previous analysis said there was the movement of 4.5 particles? Or was the 5th power just too much computation for HH to do (no computers in 1952)? On the other hand Cole and Moore say we could use the 25th power. But basically we use the 4th power because it works!

8-7. Here are some plots that show the delays that the equations predict using various powers. The circles are experimental data for potassium and we see that the 4th power provides the best fit.

8-8. Here are some plots of n and n^4 for depolarizations and repolarizations. You can see the delay with the 4th power for the depolarization but no delay for the repolarization.

In the lower graphs we see the corresponding curves for Na where the activation variable is m and m is raised to the 3rd power. The inactivation variable is not raised to a power. We will discuss Na later.

8-9. So how do we get expressions for the voltage dependent rate parameters α and β ?

HH used the voltage clamp technique to do this.

1. Voltage clamp to large depolarizing potentials and measure the current. Rearrange the equation for current, $I_k = g_K(V - V_K)$, in terms of conductance, $g_K = I_k/(V - V_K)$ and solve for g_K from the current, the clamped voltage and the K reversal potential. (Note V is the clamped voltage level). This is illustrated in the next overhead **VC-4** from last quarter's class.

For large voltage steps, the conductance will reach a maximum level, corresponding to having every K channel open. We call this maximum conductance \bar{g} . Then the actual g_k during a voltage clamp step equals $\bar{g}n^4$ or $n = (g_K/\bar{g}_K)^{1/4}$ where n^4 represents the fraction of channels in the open state.

2. Once we have \bar{g}_K and have expressed n in terms of g_K and \bar{g}_K , we can substitute the above expression into the equation for n . Recall the equation for n derived earlier

$$n = n_\infty - (n_\infty - n_0) \exp(-t/\tau_n) \text{ to get } g_K = \left\{ (g_{K\infty})^{1/4} - \left[(g_{K\infty})^{1/4} - (g_{K0})^{1/4} \right] \exp(-t/\tau_n) \right\}^4 \text{ where}$$

$g_{K\infty}$ is the steady-state value of g_K for a given clamp potential (see next 2 overheads) and g_{K0} is the initial g_K value at rest (which typically is 0). (Note: the right hand side of the above equation is raised to the 4th power to get rid of the 1/4 power on the left side. Also the \bar{g}_K terms cancel from both sides of the equation).

3. Given the computed conductance time course and the calculated values of $g_{K\infty}$ for each voltage clamp step, we do curve fitting of the above equation for g_K to estimate τ_n . (See overhead 8-10).

4. Calculate n_∞ from $g_{K\infty} = \bar{g}_K n_\infty^4$ for each voltage clamp step V. (In the next overhead VC-4 from last quarter, we calculated the probability of a channel being open in this way, but I may not have alluded to the fact that this curve was n_∞^4 .) Anyhow this gives us n_∞ .

5. Given that $\tau_n = 1/(\alpha_n + \beta_n)$ and $n_\infty = \alpha_n/(\alpha_n + \beta_n)$ and τ_n and n_∞ are known, we can solve for α_n and β_n at each voltage clamp voltage V. ($\alpha_n = n_\infty/\tau_n$ and $\beta_n = (1 - n_\infty)/\tau_n$).

6. Finally, we fit a function to the α and β data points.

VC-4. (Overhead mentioned above. Illustrates the process of going from the current obtained with voltage clamp steps to the conductance, estimating the maximum conductance and the steady-state conductances for each voltage clamp step.

8-10. This figure is from HH. It shows the time course of the conductance for various voltage clamp steps as noted on the right of each curve. The voltages here are relative to 0 being rest. The steady-state conductance of the largest voltage clamp step is the \bar{g}_K . The other steady state voltages are the $g_{K\infty}$. τ_n is fit to each curve.

8-11. Plotted here are n_∞ , τ_n , α_n and β_n . Recall that $\tau=1/(\alpha+\beta)$, $n_\infty=\alpha/(\alpha+\beta)$ and so $\alpha= n_\infty/\tau$ and $\beta=(1 - n_\infty)/\tau$.

8-12. What functional form should be used to fit α and β ?

Occam's razor would say the simplest function that does the job!

One candidate might be one that resembles the constant field equation derived by Goldman for movements of a charged particle in a constant field as noted by HH. In this equation

$$I_K = \frac{zP_K EF^2}{RT} \frac{[K]_i - [K]_o \exp\left(-\frac{zFE}{RT}\right)}{1 - \exp\left(-\frac{zFE}{RT}\right)} \text{ or if we replace } [K]_i \text{ by } [K]_o \exp\left(-\frac{zE_K F}{RT}\right)$$

$$I_K = \frac{zP_K EF^2}{RT} [K]_o \exp\left(-\frac{zE_K F}{RT}\right) \left\{ \frac{1 - \exp\left(-\frac{zF(E - E_K)}{RT}\right)}{1 - \exp\left(-\frac{zFE}{RT}\right)} \right\}$$

(The substitution is just the Nernst equation).

This breaks down as noted by HH, so don't put too much stock in it. Nevertheless, HH used the resemblance to develop the following general functional form: $\frac{A(B + V)}{C + \exp((B + V)/D)}$ where C is often ± 1 . Because β is often $\ll \alpha$, HH used simpler expressions for β .

8-13. How about sodium? The analysis here is similar to that for K although inactivation is an additional complication.

We assume that $g_{Na} = \overline{g_{Na}} m^3 h$ where m is the activation variable and h is inactivation. One way to think of this is that there are 3 activation particles that have to move to the open state and 1 inactivation that must be in the open state for the channel to be open. Activation and inactivation particles thus have "open" and "closed" states or "permissive" and "non-permissive" states, just as for n with potassium. m opens with depolarization and h closes with depolarization.

We have similar equations for m and h

$$dm/dt = \alpha_m(1 - m) - \beta_m m \quad \text{and} \quad dh/dt = \alpha_h(1 - h) - \beta_h h$$

with similar solutions

$$m = m_\infty - (m_\infty - m_0) \exp(-t/\tau_m) \quad \text{and} \quad h = h_\infty - (h_\infty - h_0) \exp(-t/\tau_h)$$

and similar definitions

$$m_\infty = \alpha_m/(\alpha_m + \beta_m), \tau_m = 1/(\alpha_m + \beta_m) \quad \text{and} \quad h_\infty = \alpha_h/(\alpha_h + \beta_h), \tau_h = 1/(\alpha_h + \beta_h)$$

Then we do the voltage clamp to -100 mV as shown on the next overhead **VC-5** from last quarter. Clamping to -100 mV removes any inactivation. Then for a short period after the voltage jump, $h=1$ and the response is not affected by inactivation.

Current responses are shown in the middle of **VC-5**. Given the current we calculate the conductance by $g_{Na} = I_{Na}/(V - V_{Na})$ and this is shown on the bottom of **VC-5**. Note that the peak conductance reaches an asymptotic value for large depolarizing steps. We let g_{Na-bar} equal the peak conductance with the largest depolarizing step, and similar to K we have $g_{Na} = g_{Na-bar} m^3$ or $m = (g_{Na}/g_{Na-bar})^{1/3}$. In particular $m_{\infty} = (g_{Na\infty} / g_{Na-bar})^{1/3}$.

We study h by voltage clamping to various levels to get steady-state inactivation and then step to +100 mV to get full activation as shown on overhead **VC-6** from last quarter. The inactivation gate is assumed to be fully open at -100 mV and only partially open at other voltages. We measure the currents and compute g_{Na} as before. The peak g_{Na} going from -100 to +100 is full activation with no inactivation. The peak g_{Na} from -60 to +100 is full activation but with partial inactivation. We divide the peak g_{Na} at each initial clamp voltage by the peak g_{Na} when the initial clamp voltage is -100 mV. This gives us the probability that the inactivation gate is open and this is our h_{∞} .

Back to **8-13**. Now we assume that there is little activation and little inactivation at the start. This means that m_0 is approximately 0 and h_0 is approximately 1. Furthermore a (large) depolarizing step will give full inactivation, so h_{∞} is approximately 0. This simplifies our solutions given above for m and h to $m = m_{\infty} (1 - \exp(-t/\tau_m))$ and $h = \exp(-t/\tau_h)$, so an approximate expression for g_{Na} is

$$g_{Na} = g_{Na-bar} m_{\infty}^3 h_0 [1 - \exp(-t/\tau_m)]^3 \exp(-t/\tau_h).$$

The data are fit to this equation with values for τ_m and τ_h estimated from the data. (HH used double log paper! and compared results with known solutions.)

Then given m_{∞} and τ_m we can calculate α_m and β_m and similarly we calculate α_h and β_h . As with potassium the α and β are fit to the functional form given earlier.

8-14. So the voltage-dependent currents in the squid axon are represented as

$$I = g_{Na-bar} m^3 h (V - V_{Na}) + g_{K-bar} n^4 (V - V_K) + g_L (V - V_L)$$

where this last term is a leak current (or fudge factor) to make rest stable

m, n, h are calculated as above from the $\alpha, \beta,$ and τ s.

Note, the conductances g -bar are maximum conductances given as conductance densities in units of S/cm².

Other conductances in other preparations have been studied in a manner similar to that used by HH. Typically the activation variables (i.e., m or n) or inactivation variable (i.e., h) is described

in terms of a Boltzmann-like function or $1 / \left[1 + \exp\left(\frac{V - V_{1/2}}{k}\right) \right]$ where $V_{1/2}$ is the voltage where

the activation or inactivation variable = 0.5, and k determines the slope. This is just another sigmoid function. One hopes that experimental data for the time constants as a function of voltage are also available, along with the power (exponent) to be used for the state variables.