Theoretical & computational Neuroscience:

Programming the Brain

(BM 6140)

2-credit

Quantitative analysis of AP : Hodgkin-Huxley

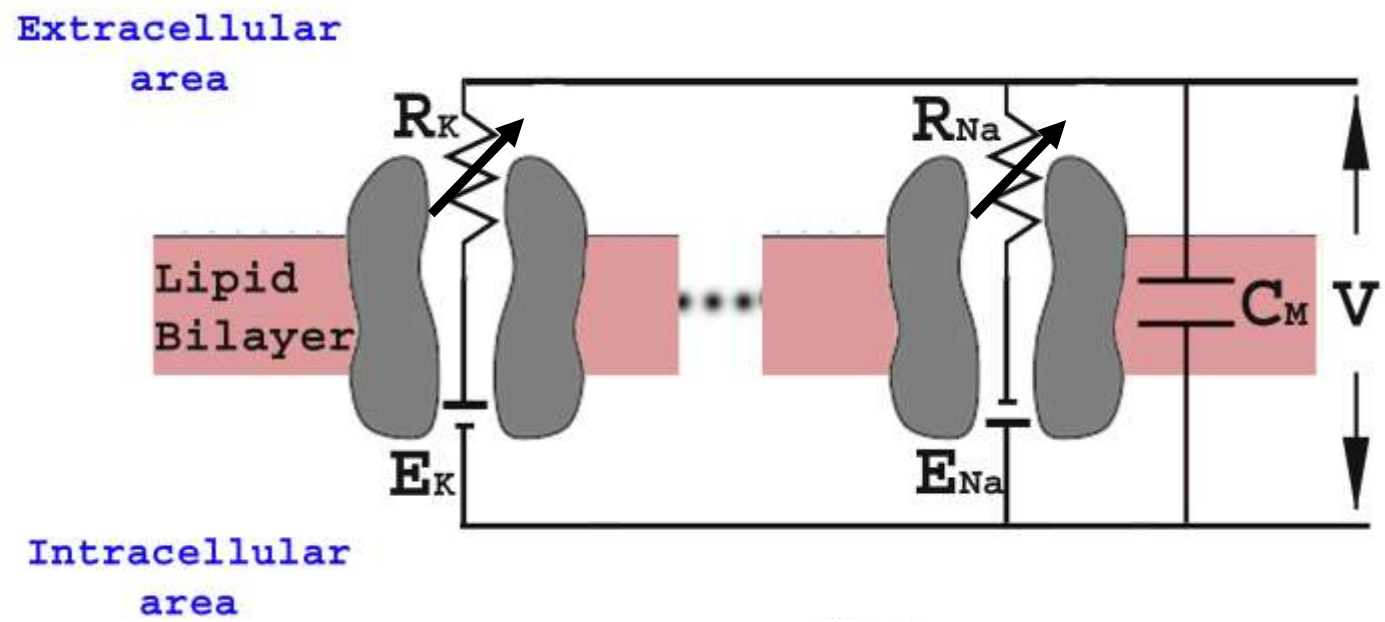
- Hodgin, Huxley 1952, series of papers
- Nobel prize (1963) in physiology or medicine

Take a step back in time !

You don’t know this is the model…. At most you have a hunch

You have to characterize the behaviour of cross membrane elements !!

So how will you go about the job ?



Observe I /p , o/p relationship we are engineers ☺

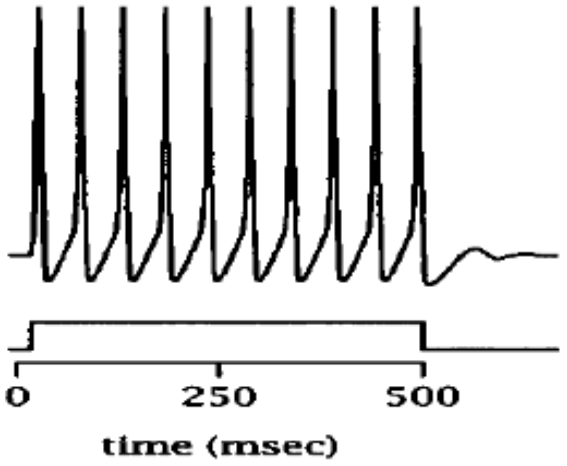
I/p current and measure voltage

Problem

i/p constant currents, but voltage does not stabilize to a value..

You get noisy fluctuations or oscillations (spikes)

So how do I get a I vs V relation ?



Try constant V and measure I ?

Can we keep V constant ?

Can you really keep V constant ?

Flow of current deposits charge inside the cell and modifies voltage..

What is the solution ?

Voltage clamp

- H&H knew that permeability changes and sodium current in particular was responsible for AP
- But the "explosive character" of action potentials made it very difficult to study I-V relationships : Hence the need to keep potential under control.

- Huxley, 2002, TINS, From overshoot to voltage clamp

- Kenneth Cole designed the first voltage clamp system
- Uses 2 electrodes, one to measure voltage and the other to inject sufficient current into cell to keep the voltage constant. The injected current serves as proxy for the actual cross membrane currents
- Additional benefits of the voltage clamp system
- *Space clamping the inside of the cell*
- *Capacitive currents are eliminated*

What do you notice ?

V / I
evolves
with time

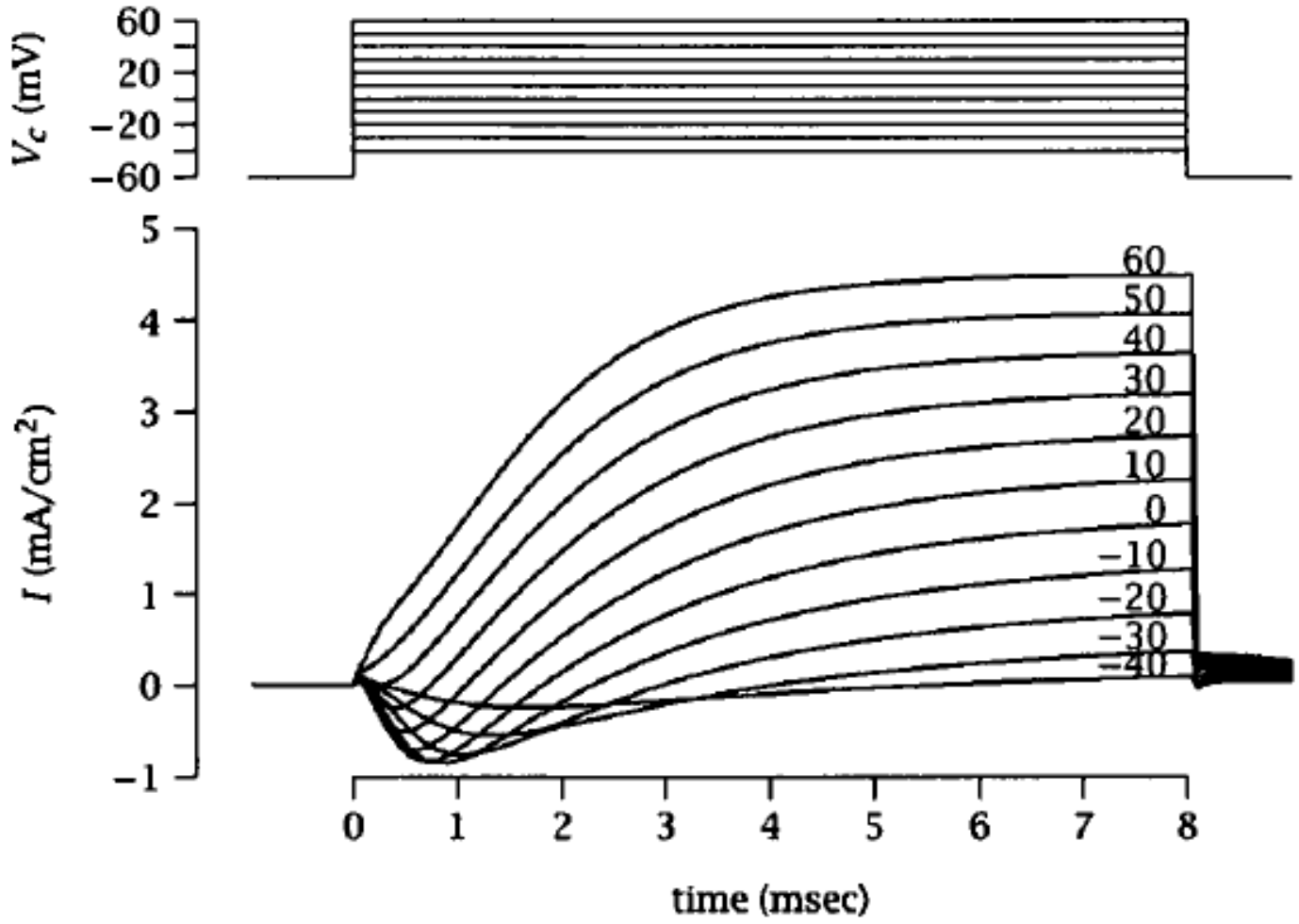
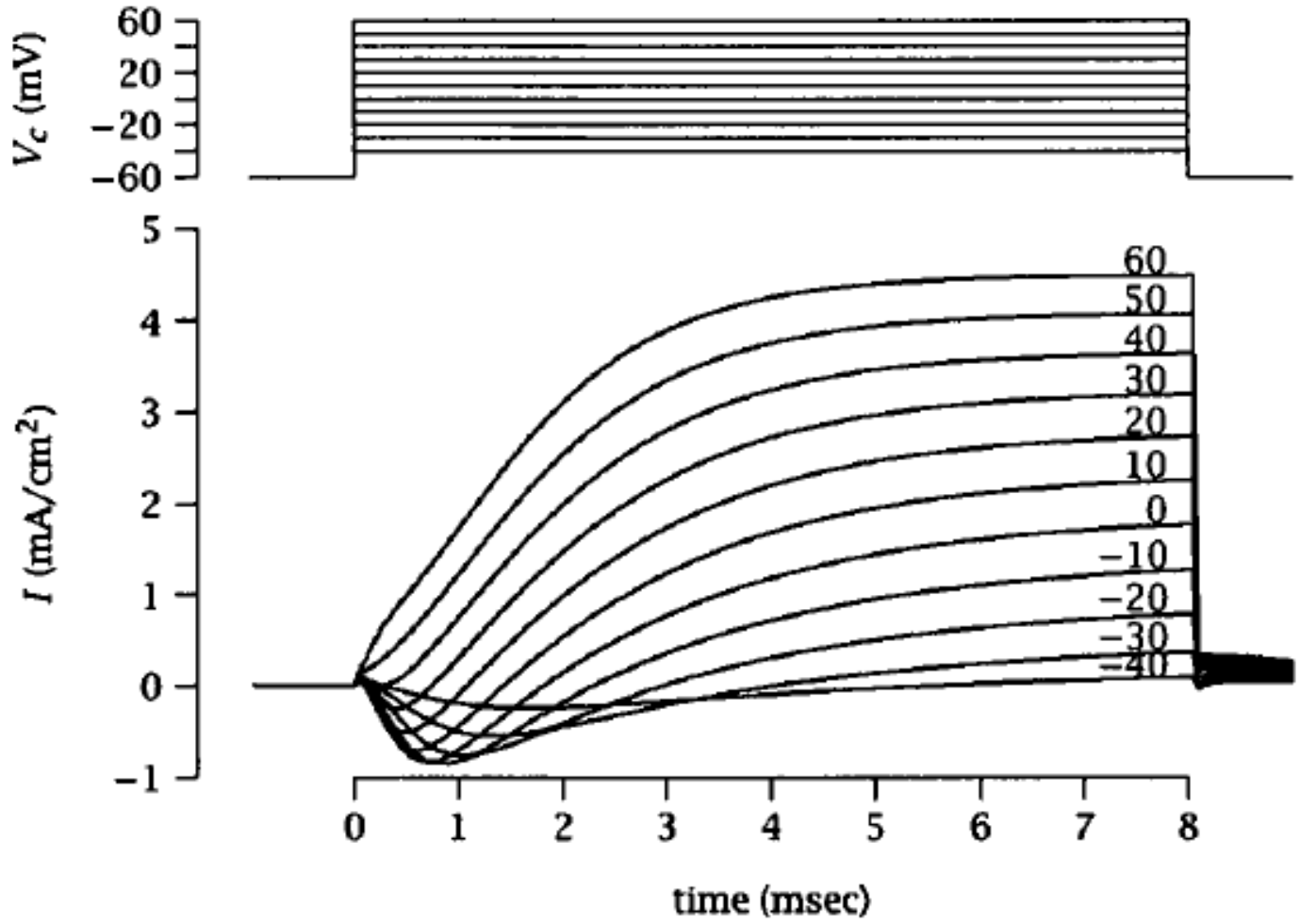


Figure 6.2 Currents measured with voltage clamp of squid axon. Membrane potential was held at −60 mV and then stepped (at 0 msec) to various potentials (shown at the right of each trace) for 8 msec before stepping back to −60 mV.

Observations



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Observations

- Early inward current
- Late outward current
- How do we know if they are carried by the same ionic species or different species ?

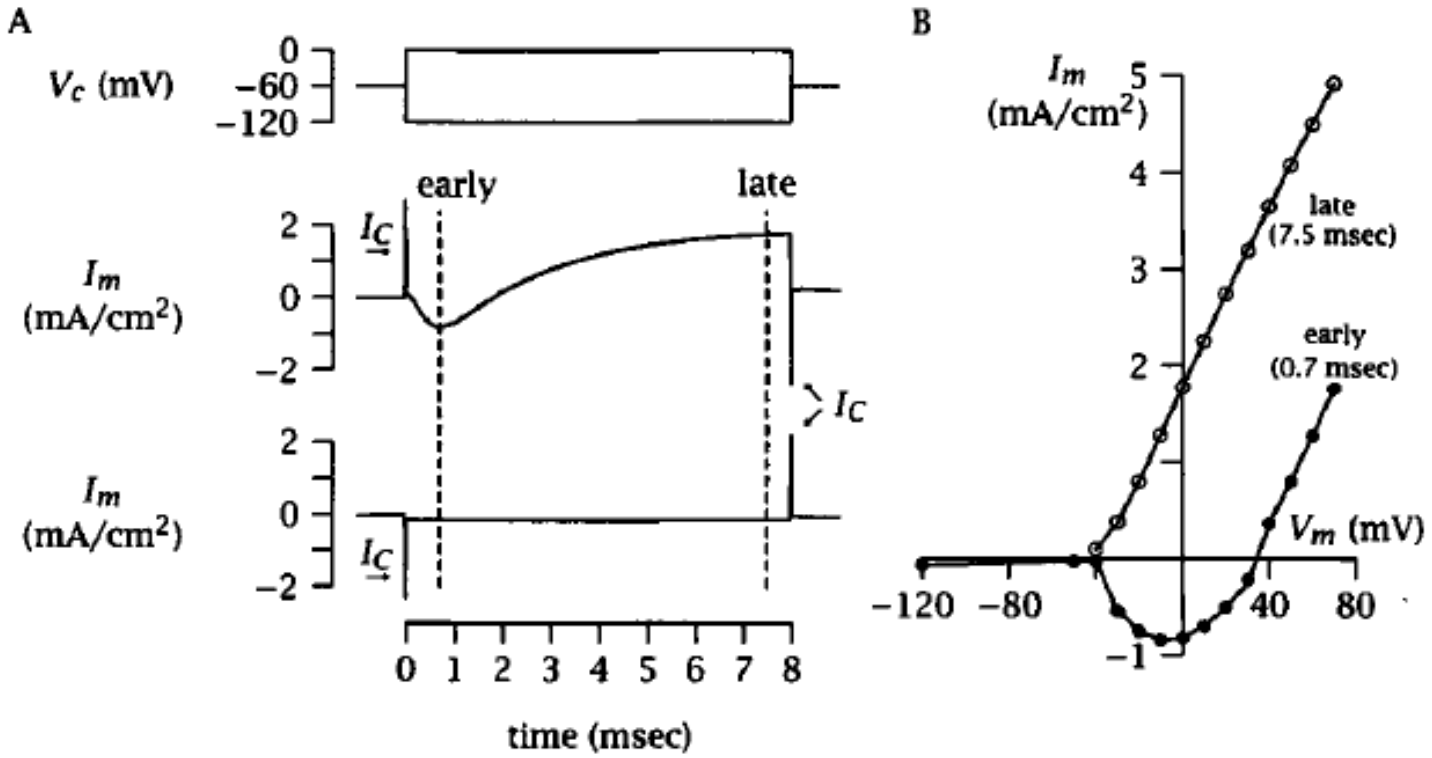


Figure 6.3 Early and late currents of a squid axon when the voltage is stepped from −60 mV to 0 mV or −120 mV (A); and the current-voltage relations of the early and late currents (B).

Tinker with ion concentrations

- Change Na^+ concentration or apply TTX(abolish Na^+ currents)
- Change K^+ concentrations or apply TEA(abolish K^+ currents)
- What would you see in each case ?

$$I = I_K + I_{Na}$$

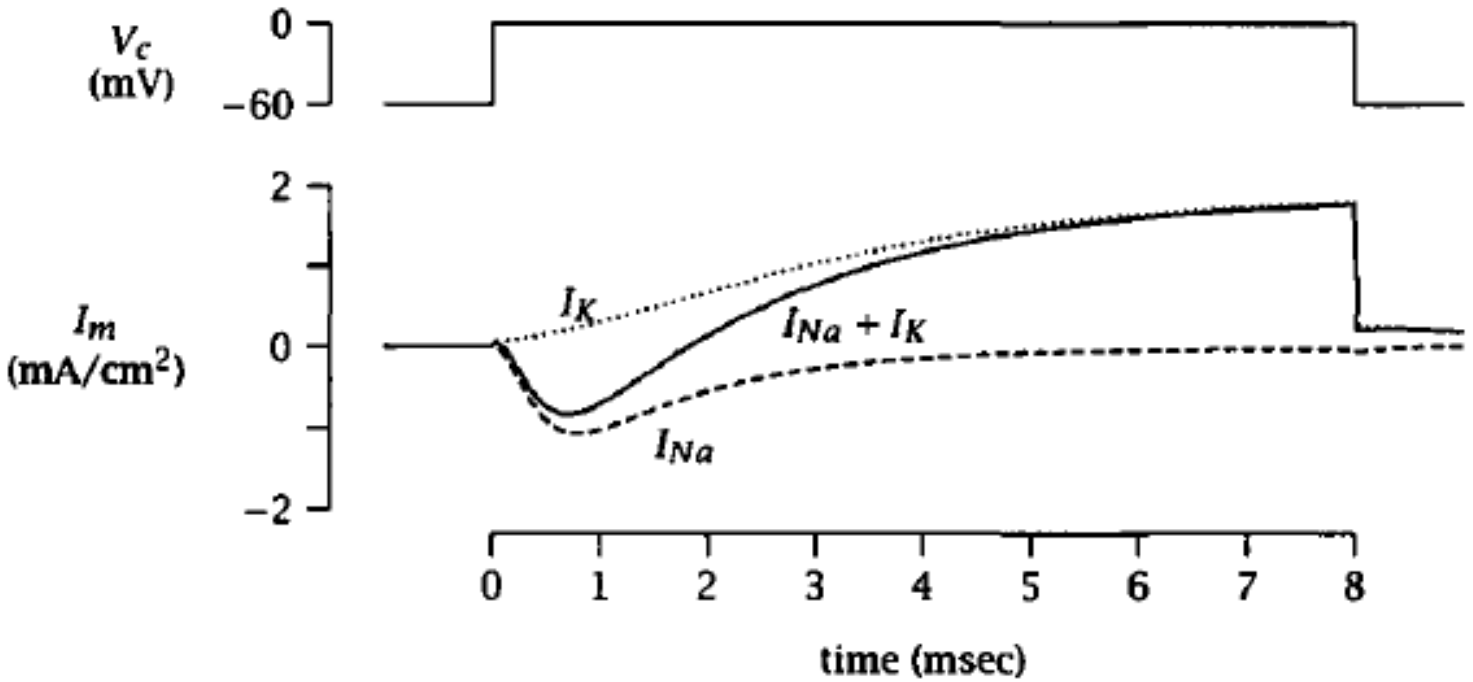


Figure 6.4 Separation of membrane current (solid trace) into Na^+ (dashed trace) and K^+ (dotted trace) currents. I_K is obtained in the presence of TTX or when $[Na^+]_{out} = 0$; I_{Na} is obtained in the presence of TEA. The voltage is stepped from −60 mV to 0 mV for 8 msec.

Plot of early and late currents (peak values) vs voltage

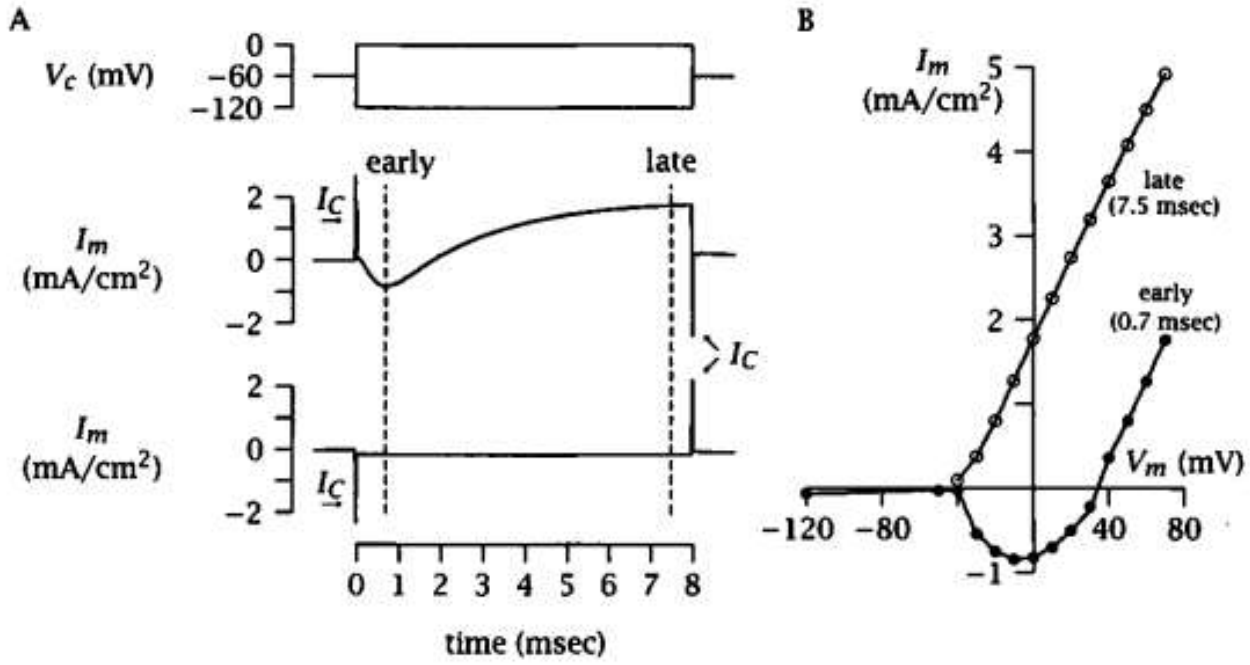


Figure 6.3 Early and late currents of a squid axon when the voltage is stepped from −60 mV to 0 mV or −120 mV (A); and the current-voltage relations of the early and late currents (B).

What can you say about g_{Na} and g_k ?

$g(t) = I(t) / (V - E)$?
Hence g(t) is of the same form as I(t) ?

- Think ! !
- How do we know that the channel behaves like a resistor ?? In other words, how do we know Ohm's law holds ? Can we test this ?

Get multiple (I,V) measurements keeping the conductance (g) constant !

If linear, say ! Ohm !

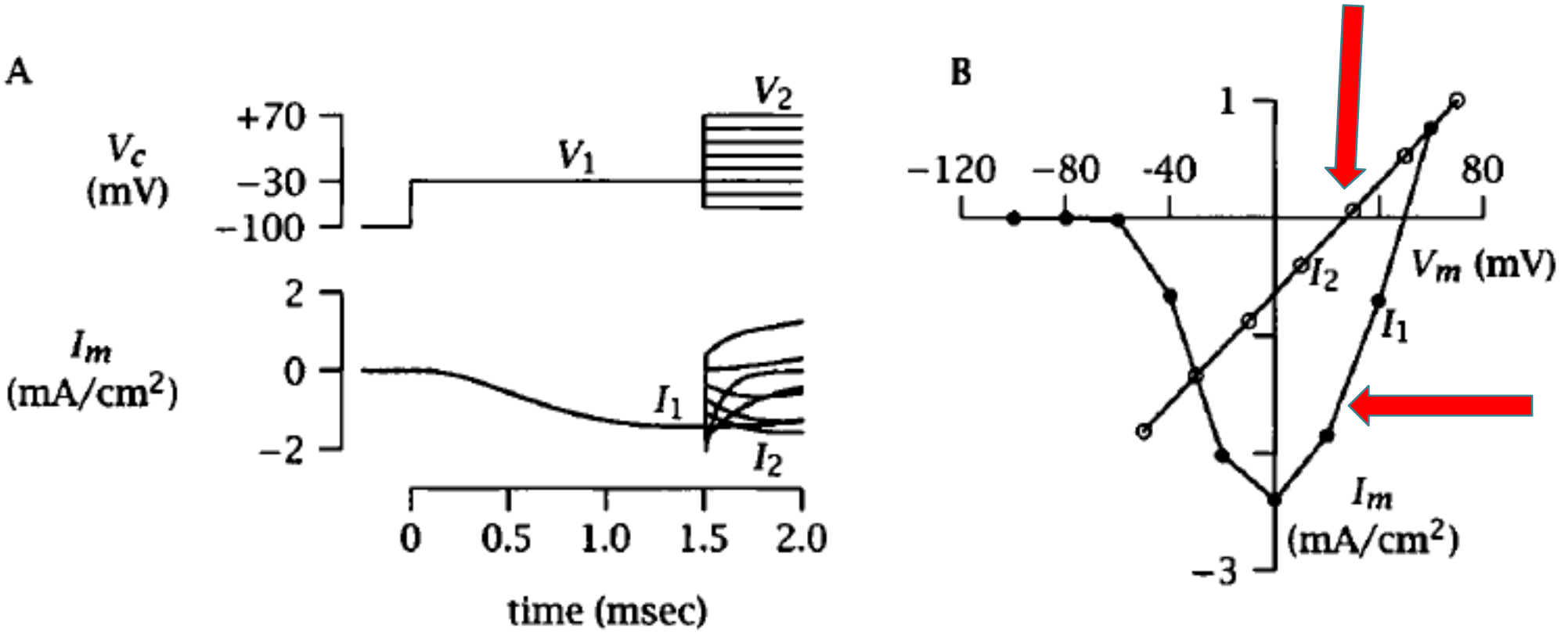
But how to keep g constant ?

Measure instantaneous change in current in response to change in clamp voltage

- Use 2 clamp voltages (see next page for waveforms)
- Of course, there is the assumption that ion channel conductance does not change instantaneously !

OMG! It's Ohm!

- I2,V2 pts on this curve were measured immediately after V2 is turned ON.
- So g does not have sufficient time to change.
- If Ohm, V2-Ena = g(I2) must be a straight line, which is what we see



I1,V1 pts on this curve were measured at different clamp voltages V1, hence g is not constant. So I1 vs V1 is non linear

Figure 6.5 Instantaneous current-voltage relation (B) obtained with voltage clamp for the early inward channel (A). Closed circles indicate normal peak inward current for various depolarizations. Open circles indicate variation of I_2 with V_2 as shown in the intersection on right. $I_2 - I_1$, instantaneous step of current produced by voltage step $V_1 - V_2$. Duration of first pulse = 1.5 msec. (After Hodgkin and Huxley 1952b.)

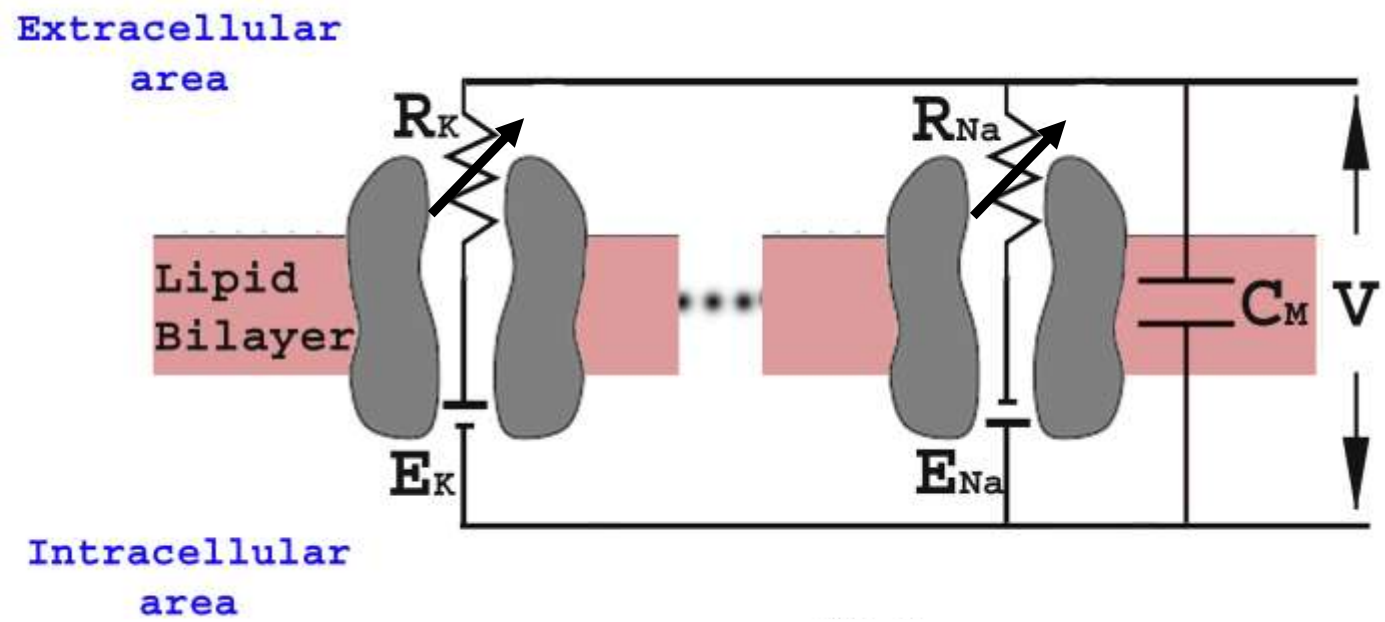
What do the points I2=I1 signify ?

V2 = V1 => No change in g , hence no change in I as well

V2 = Ena => although g changes, there is no driving force (V2-Ena = 0)

Implications

- Ion channel = Resistor { Na and K channels in Nodes of Ranvier are an exception }
- g(t) = I(t) / (V – E) {I is a proxy for conductance save for the scaling by V-E}



Time course of g and its peak values

g is obviously a function of V,t

Traces of g are similar for all Vs and scale as predicted by ohm's law. Hence g is function of V and not I

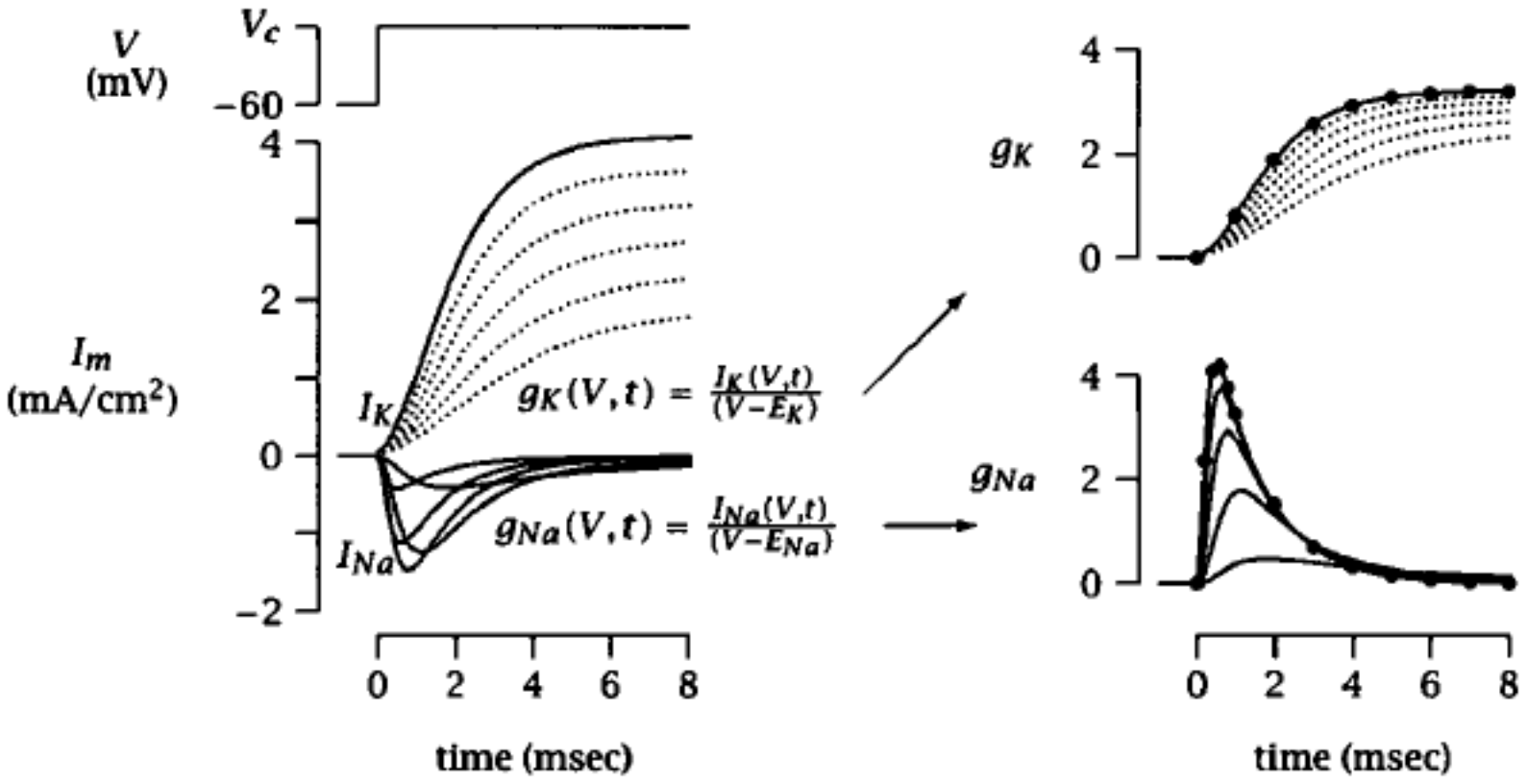


Figure 6.7 Time course of g_K (dashed traces) and g_{Na} (solid traces) at various voltages (V_c) obtained from I_K and I_{Na} traces, according to Ohm's law.

What should be the form of g(t) for K and Na ?

HH equations

$g_K \sim (1 - \exp(-t/\tau))$

g_{Na} ~ product of $1 - \exp(-t/\tau)$ and $\exp(-t/\tau)$

So what would be the form of $\frac{dg}{dx}$?

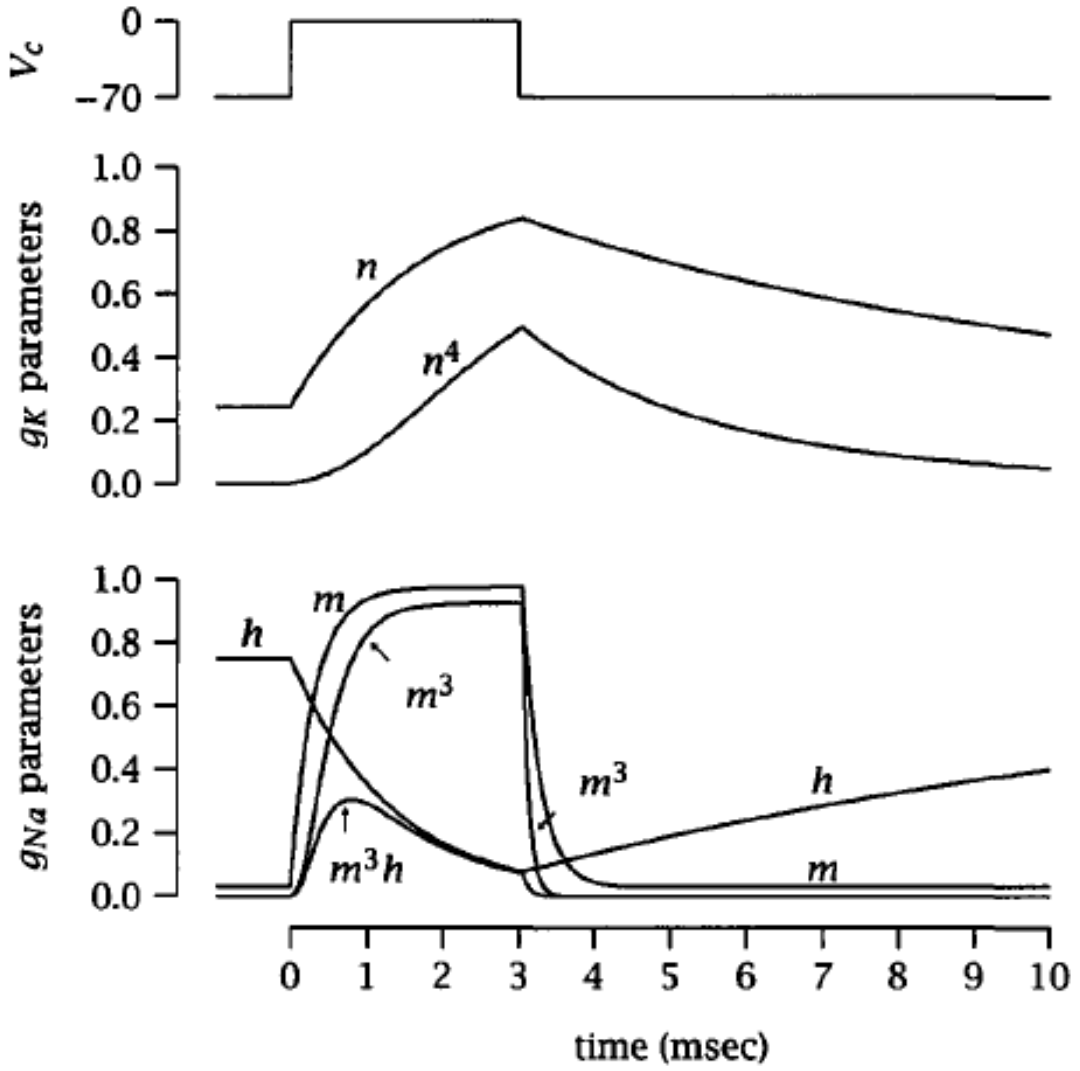


Figure 6.9 Time courses of n, n^4, m, m^3, h, and m^3h following a depolarizing voltage step (from −70 mV to 0 mV; duration of the step is 3 msec). n and m follow the $(1 - e^{-t/\tau})$ time course (activated by depolarization), whereas h follows the $e^{-t/\tau}$ time course (inactivated by depolarization).

HH equations

$g_k = \bar{g}_k n^4$

$g_{Na} = \bar{g}_{Na} m^3 h$ H—H realised that the curves were better represented by powers of exponentials rather than exponentials themselves

Where m,h,n can be described as variables following first order kinetics

$x \overset{\beta,\alpha}{\longleftrightarrow} 1 - x$

Hodgkin-Huxley equations

After fitting curves, HH obtained

$$I_{inj} = C_m.\frac{dV}{dt} + I_{ion}(V,t)$$

$$I_{ion}(V,t) = I_{Na}(V,t) + I_K(V,t) + g_L.(V - E_L)$$

$$I_{Na}(V,t) = m^3(V,t).h(V,t).\overline{g}_{Na}.(V - E_{Na})$$

$$I_K(V,t) = n^4(V,t).\overline{g}_K.(V - E_K)$$

$$\frac{dm}{dt} = \frac{m_\infty(V) - m}{\tau_m(V)}$$
$$\frac{dn}{dt} = \frac{n_\infty(V) - n}{\tau_n(V)}$$
$$\frac{dh}{dt} = \frac{h_\infty(V) - h}{\tau_h(V)}$$

$$where\ x_\infty = \frac{\alpha_x}{\alpha_x + \beta_x}\ and\ \tau_x = \frac{1}{\alpha_x + \beta_x}$$

Note that $h_\infty < h_0, n_\infty > n_0$ and $m > m_0$

Q

Which of these variables express dependence of g on

- Time
- Voltage

What does $\overline{g}_{Na}$ represent

Temporal evolution of g is described by properties of gating variables' like

- Forward , backward rates α, β
- OR
- Steady state (in)activation $x_\infty and\ rate\ \tau_x\ at\ which\ it\ aproaches\ steady\ state$

Voltage dependence of g

Characterized by describing voltage dependence of α, β or x_∞, τ_x

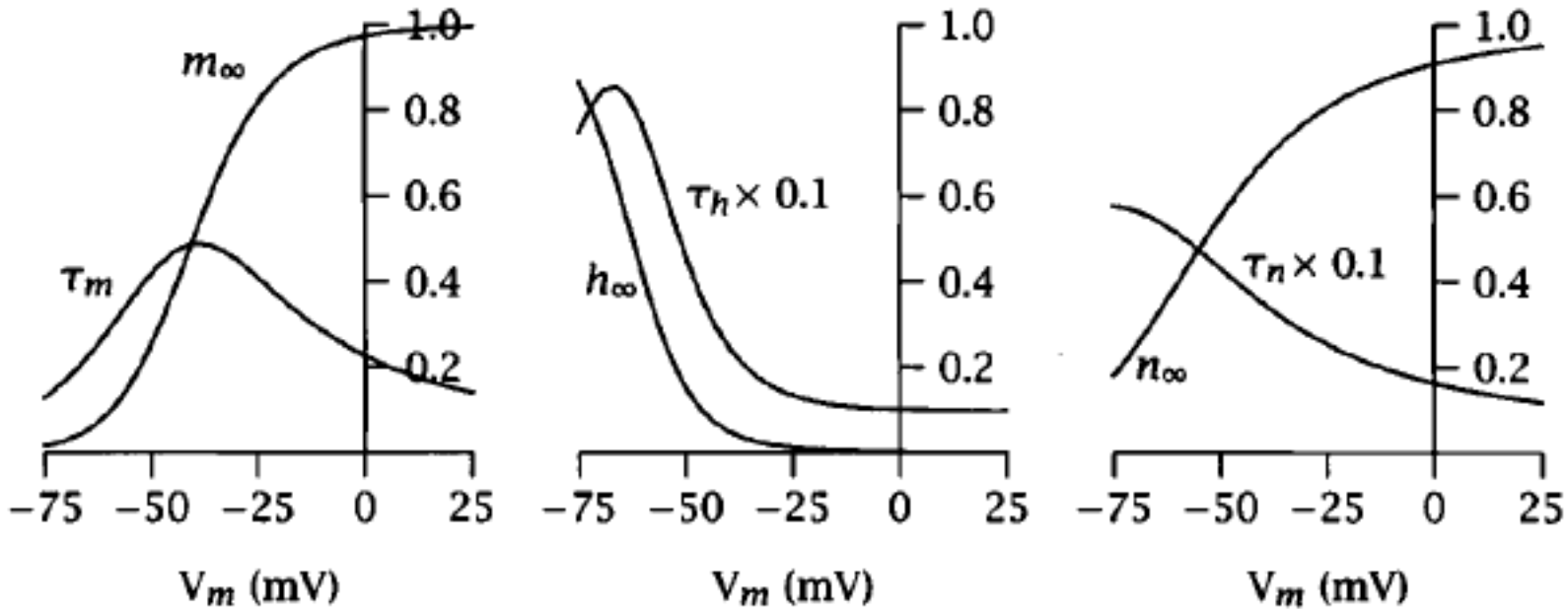


Figure 6.12 Steady-state activation curves (n_∞, m_∞, and h_∞) and the voltage dependence of the time constants of the Hodgkin and Huxley model.

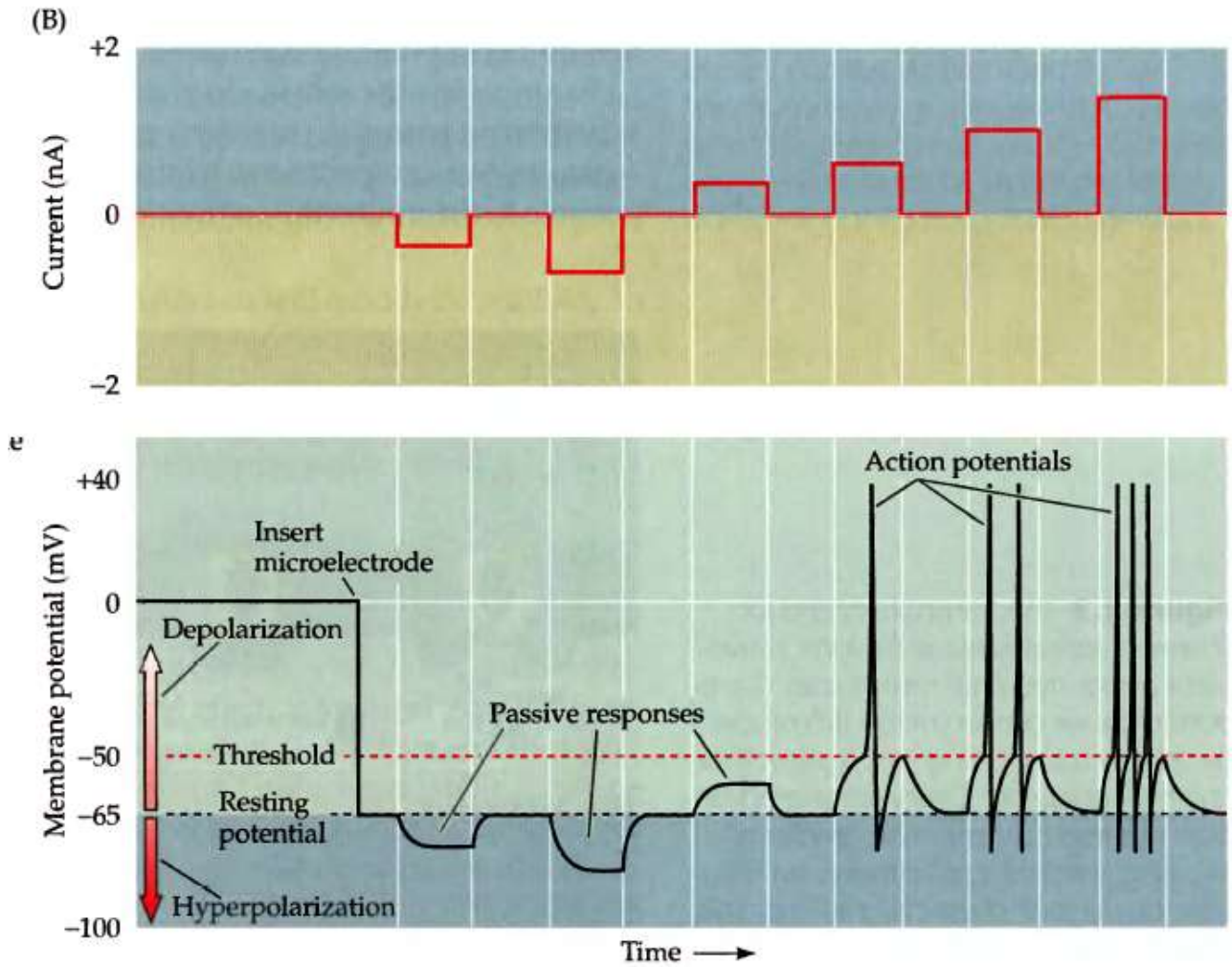
Physical significance

- So far, only curve fitting
- HH also speculated on physical significance too in their ‘gate model’.
- E.g. Opening of K channel requires 4 gating particles (hence its prob is n^4) and the availability of gating particles is given by the exponential form (derived from Boltzmann’s equations)
- Interested ? see the original HH papers, a series of 5 papers published in 1952

So where is the Action Potential ?

We have been working on voltage clamp all the while !!

- Numerical integration of HH equations to see the AP. HH show the same in their original papers
- HH also made simplifications and predicted the conduction velocity in a squid axon (and it matched well)


(B)
+2
Current (nA)
0
–2
e
+40
Membrane potential (mV)
0
–50
–65
–100
Insert microelectrode
Depolarization
Threshold
Resting potential
Hyperpolarization
Passive responses
Action potentials
Time