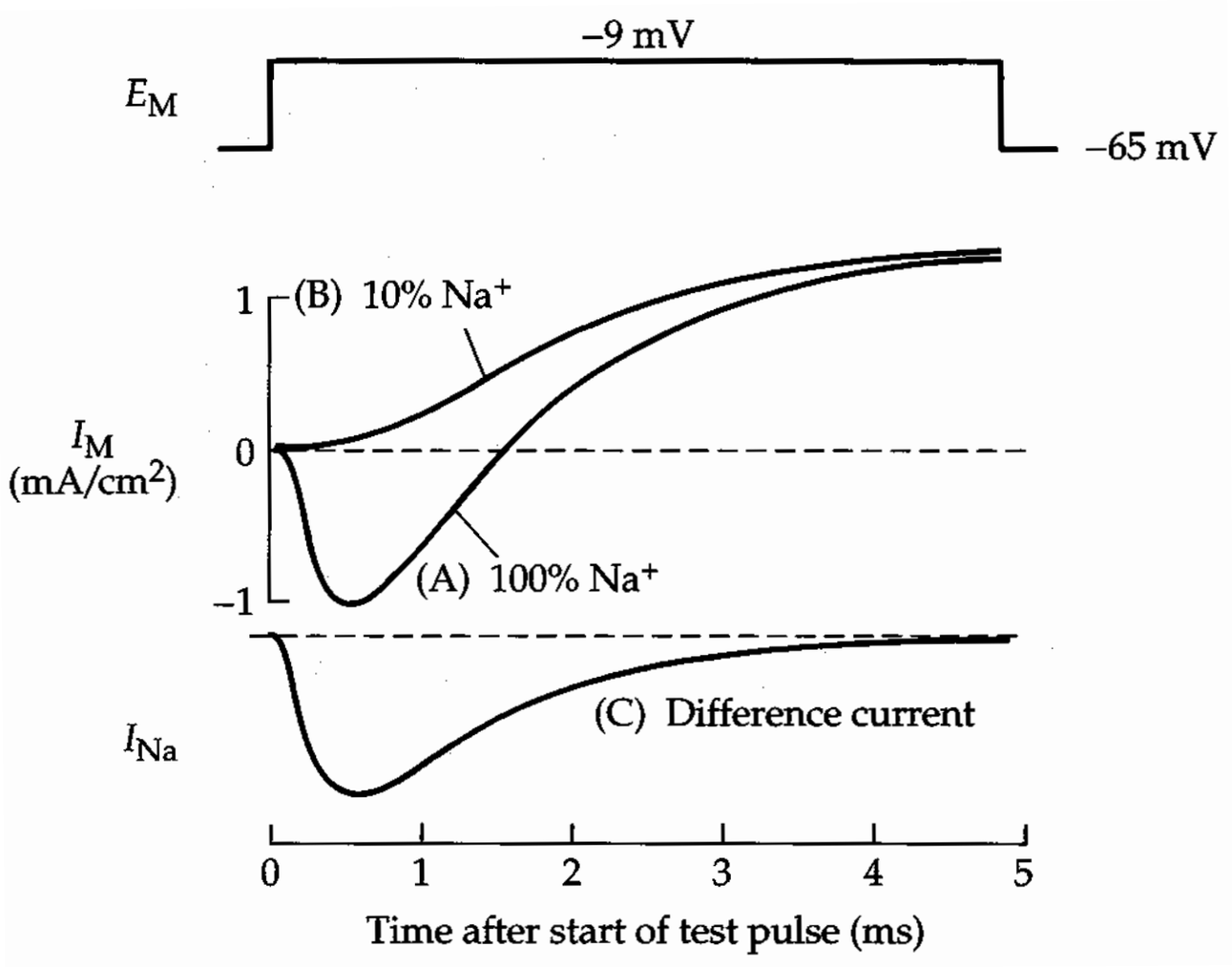
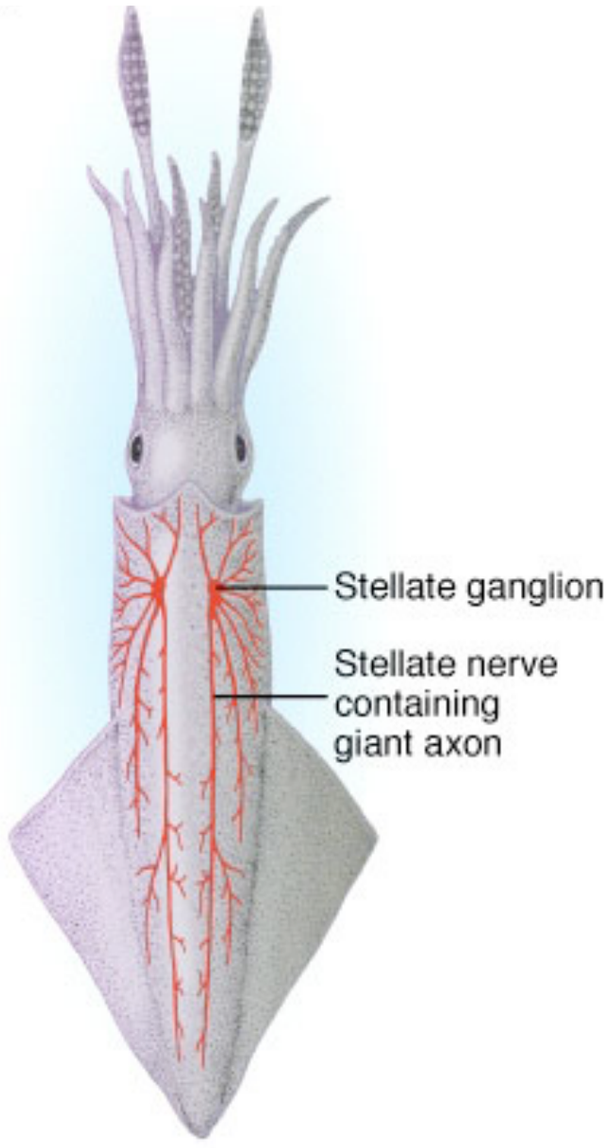
Cellular Biophysics & Modeling

Lecture 9

voltage-clamp recording and the
classical biophysics of the squid giant axon





dynamics of neuronal membrane potential

- current balance equation
- exponential relaxation
- current-voltage relations of ionic currents
- membrane bistability
- action potentials (excitability)
- repetitive spiking (oscillations)

measurement of ionic membrane currents

- current-clamp recording
- voltage-clamp recording

modeling with ODEs

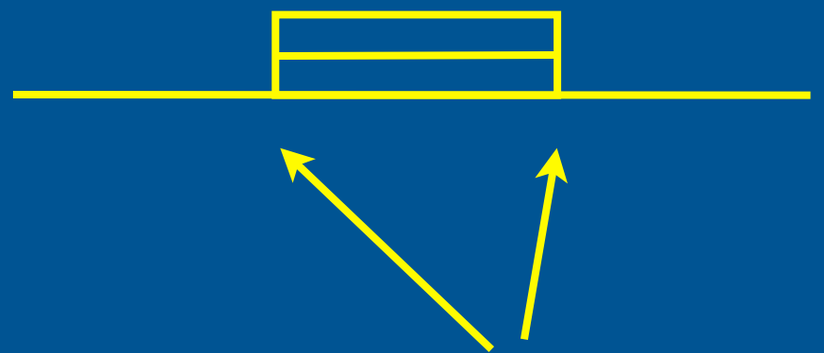
- scalar ODEs
- phase diagrams
- systems of ODEs
- phase plane analysis

membrane currents are measured
using the "voltage-clamp" technique

$$C\frac{dV}{dt} = I_{app} - I_{mem}$$

I_{app} such that $V = V_{com}$ $\rightarrow$

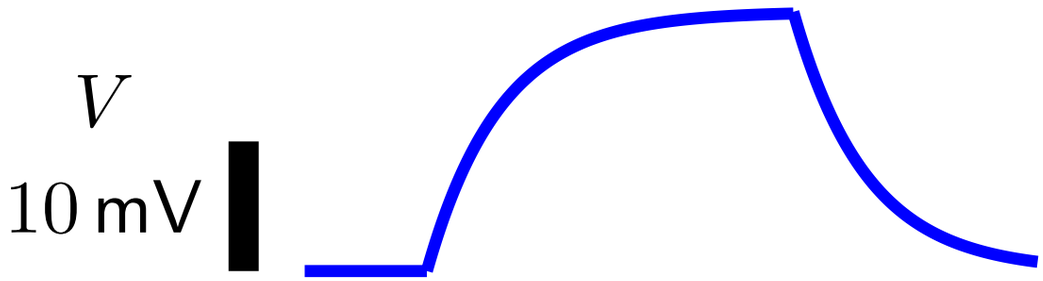
depolarizing
voltage pulses

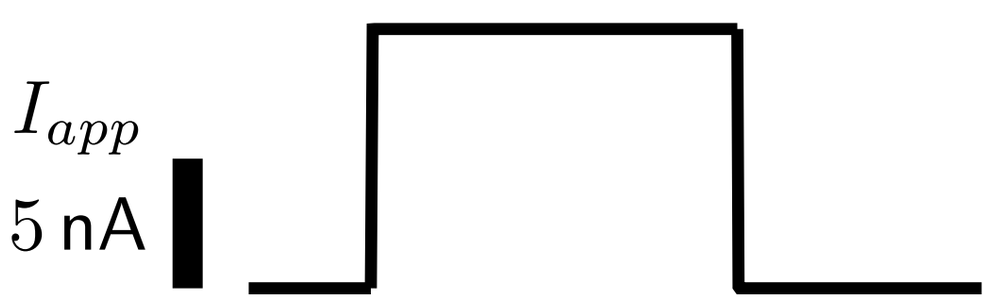


$\frac{dV}{dt} \approx 0$ and $I_{app} \approx I_{mem}$ except near jumps

The applied current required to maintain the command voltage "measures" the changing membrane currents evoked by the command voltage

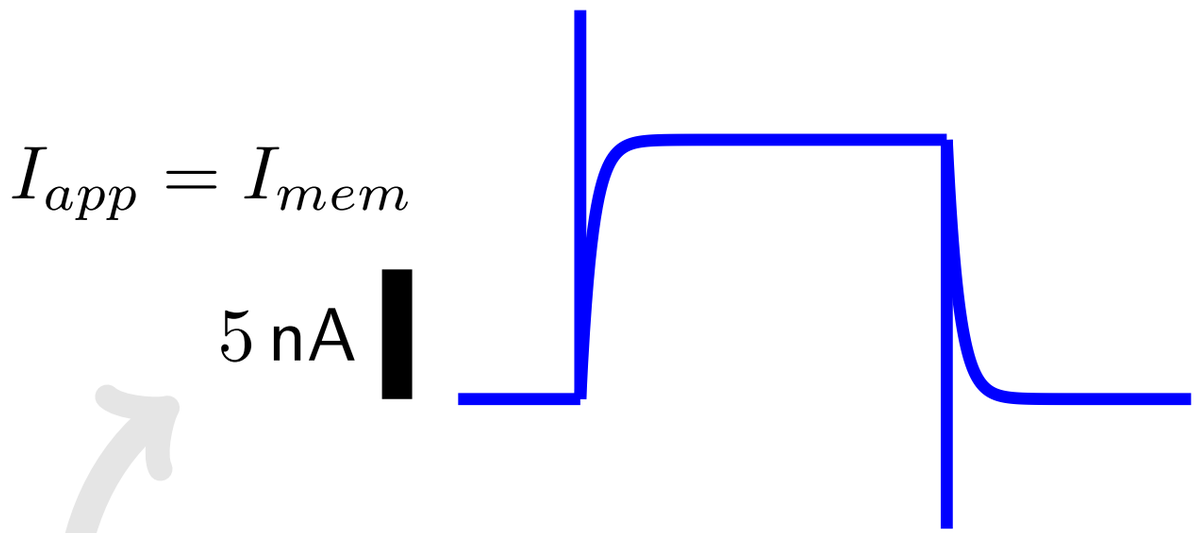
current clamp

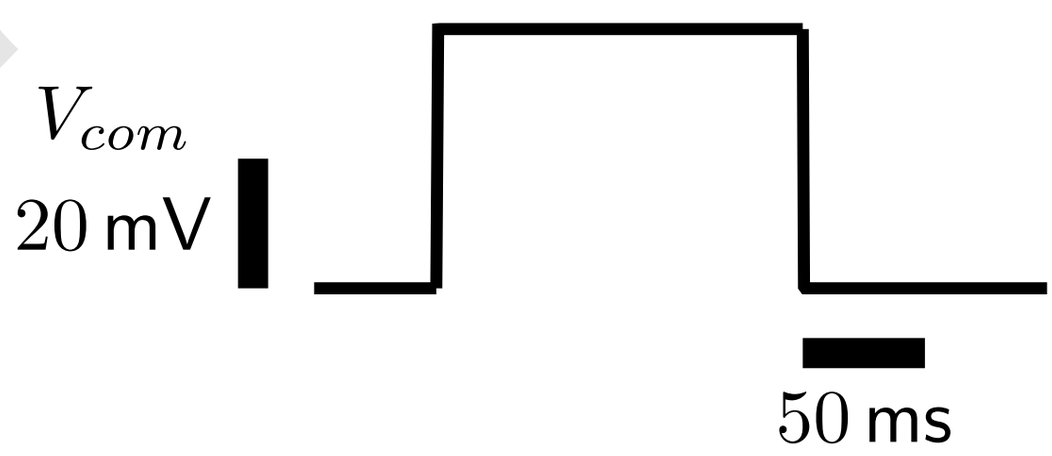




$$\frac{dV}{dt} = \frac{1}{C}\left[I_{app} - \underbrace{g\ (V - E)}_{I_{ion}}\right]$$

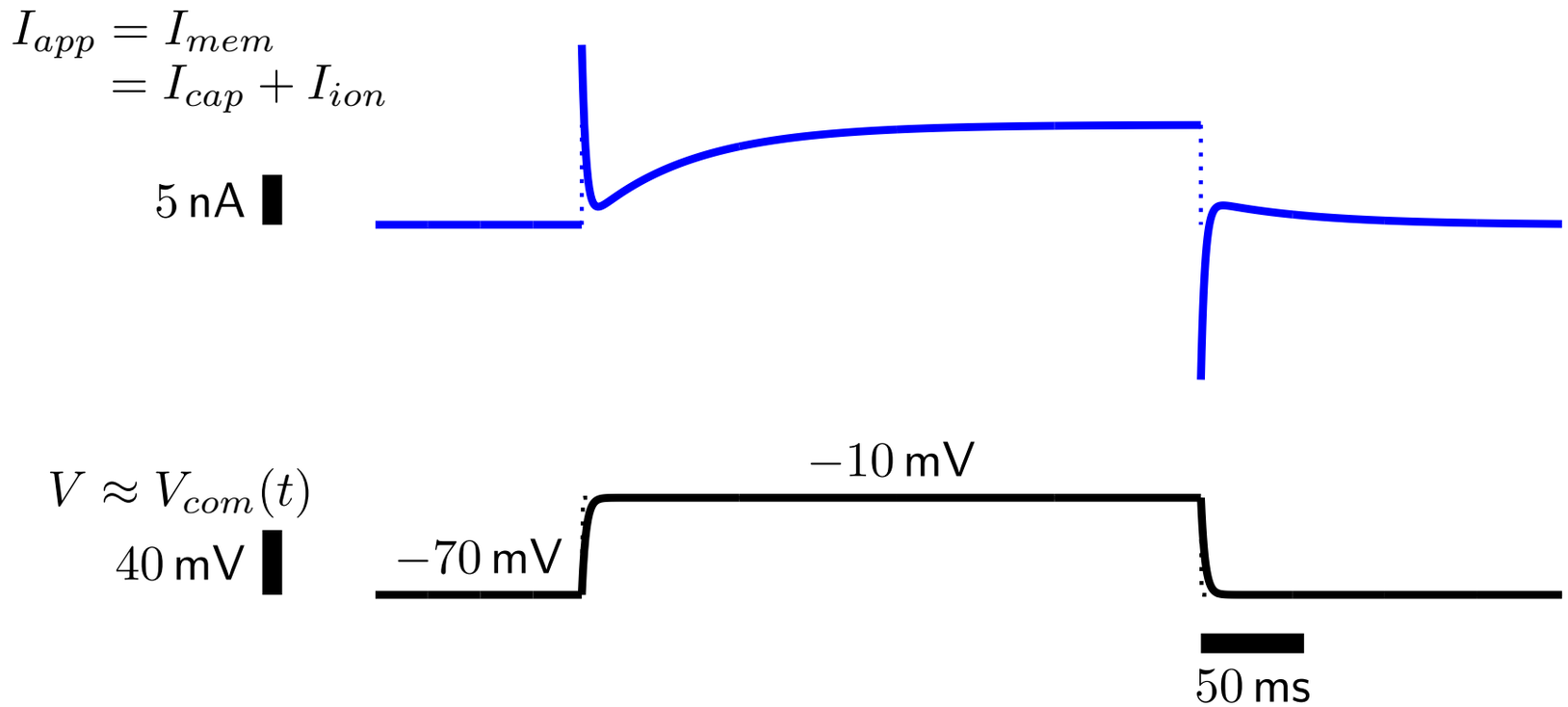
voltage clamp

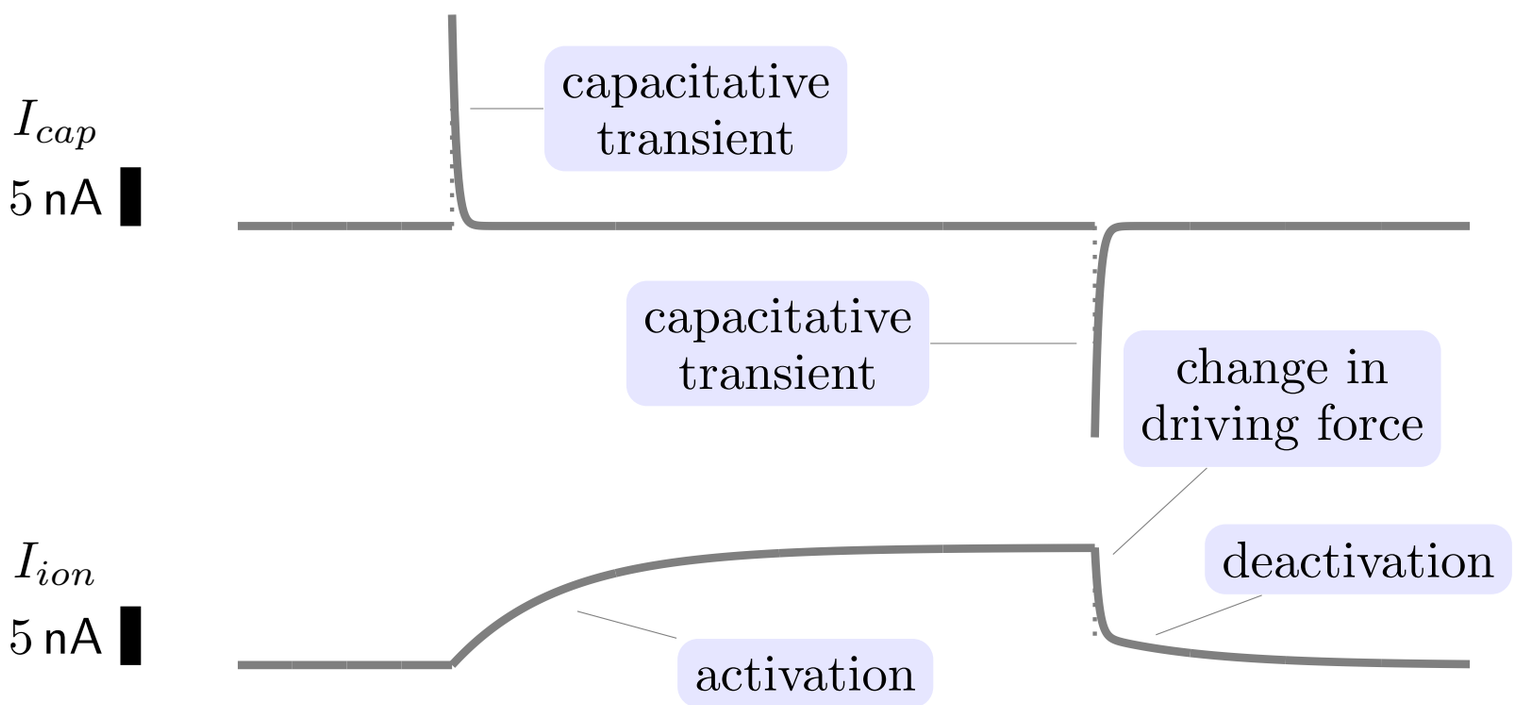




$$I_{app}(t) = \underbrace{C\frac{dV}{dt} + I_{ion}(V)}_{I_{mem}}$$

voltage-clamp recordings show ionic currents activate over time

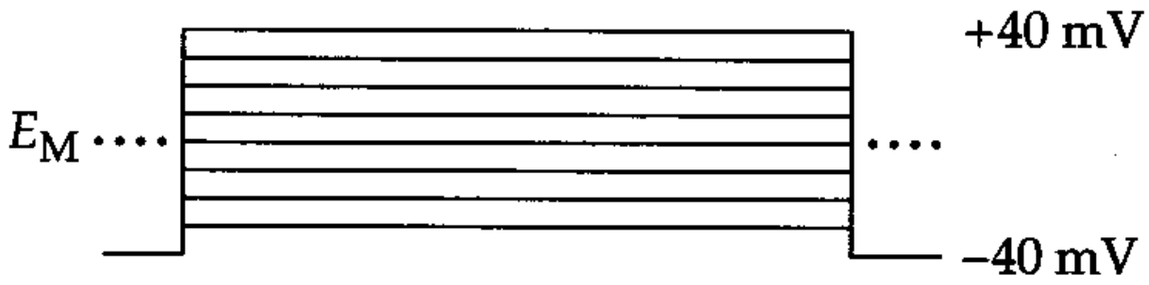




The “delayed-rectifier” potassium current

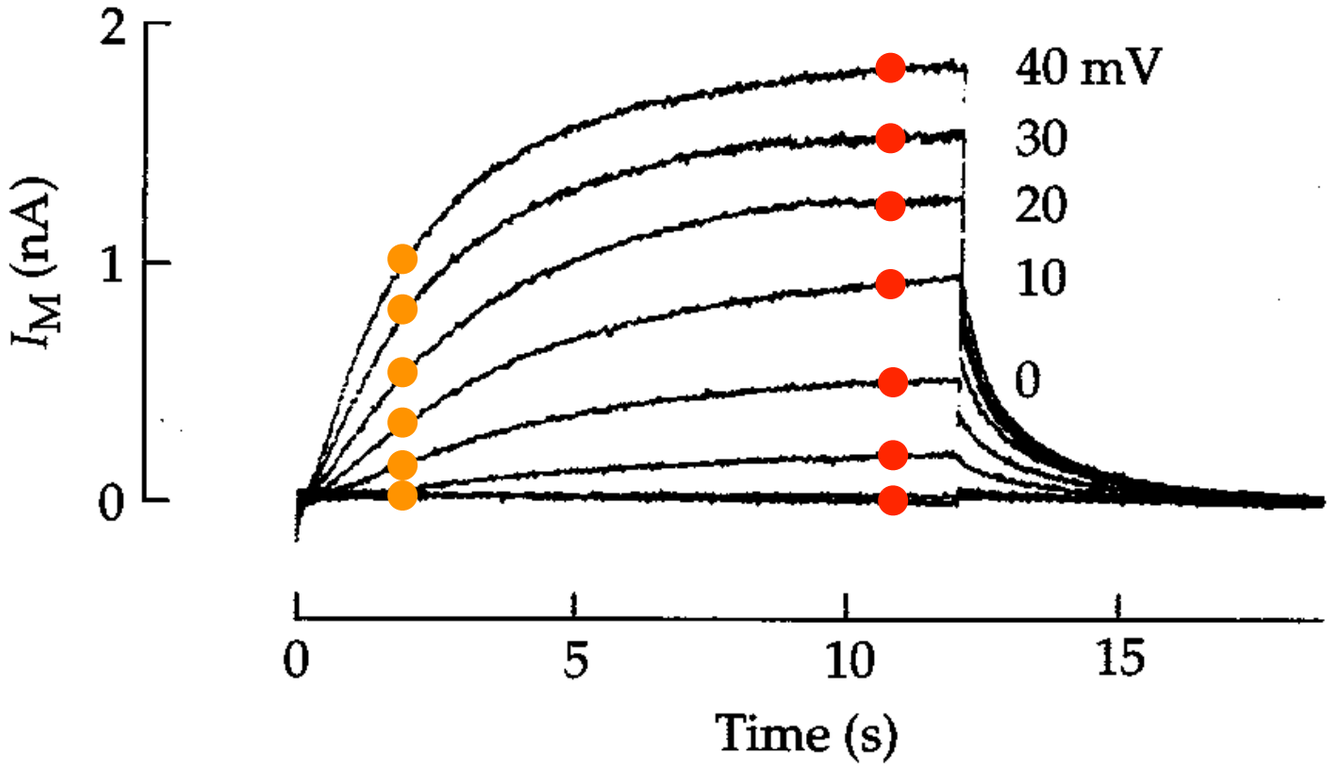
I_{Kv}

I_{Kdr}



initial currents

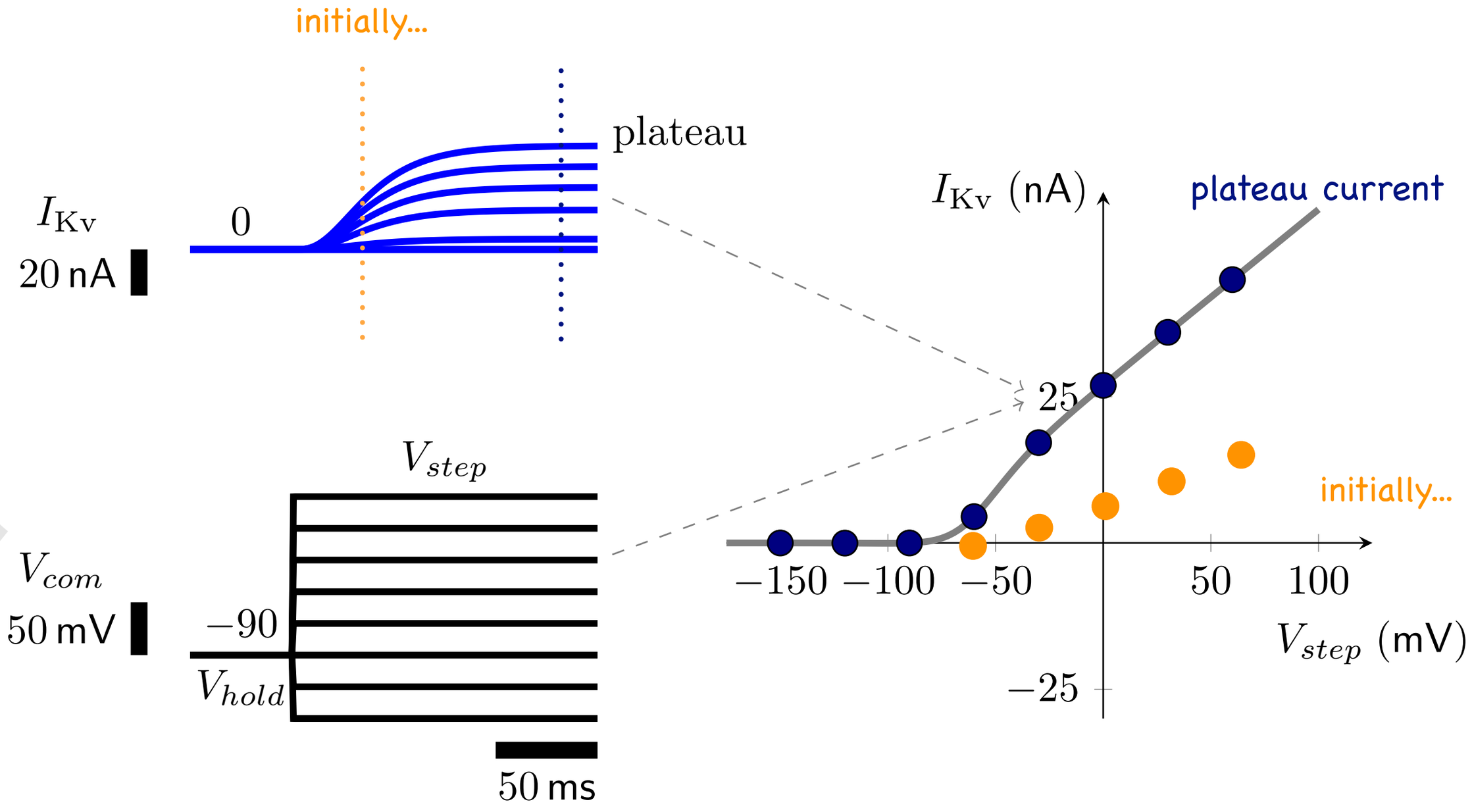
plateau currents



Slow Activation of I_K in Frog Heart

Ionic currents evoked by depolarizing voltage steps under voltage-clamp conditions. The outward currents are primarily I_{Ks} in slowly gated delayed-rectifier K channels. Note that the time scale is in seconds and compare with Figure 3.2B. T = 23°C. [From Giles et al. 1989.]

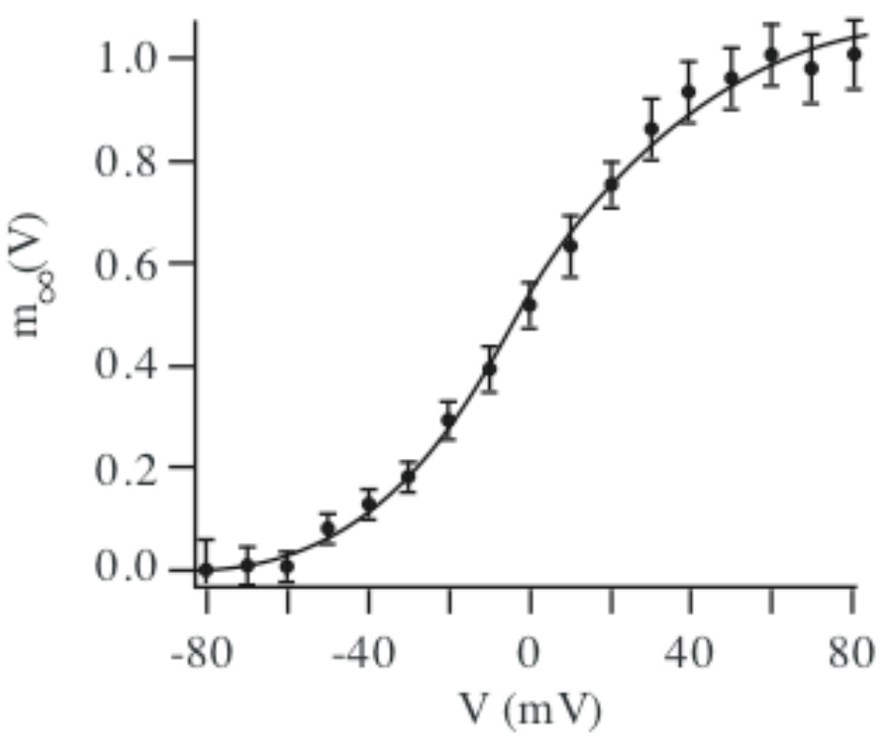
current-voltage relations usually show <u>maximum</u> current

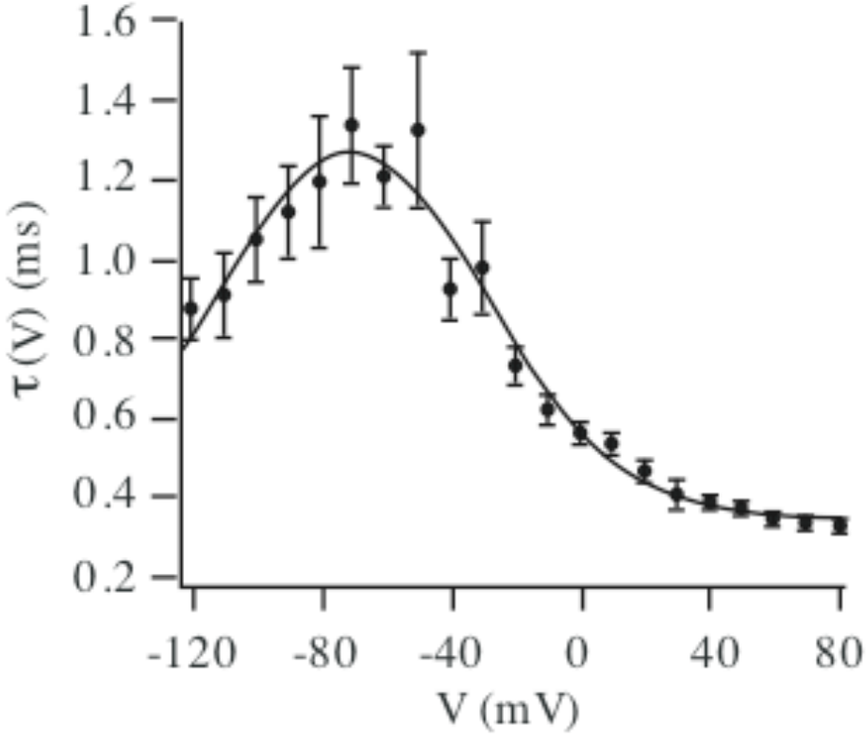


Ionic current activation is modeled using an ODE for gating variable dynamics

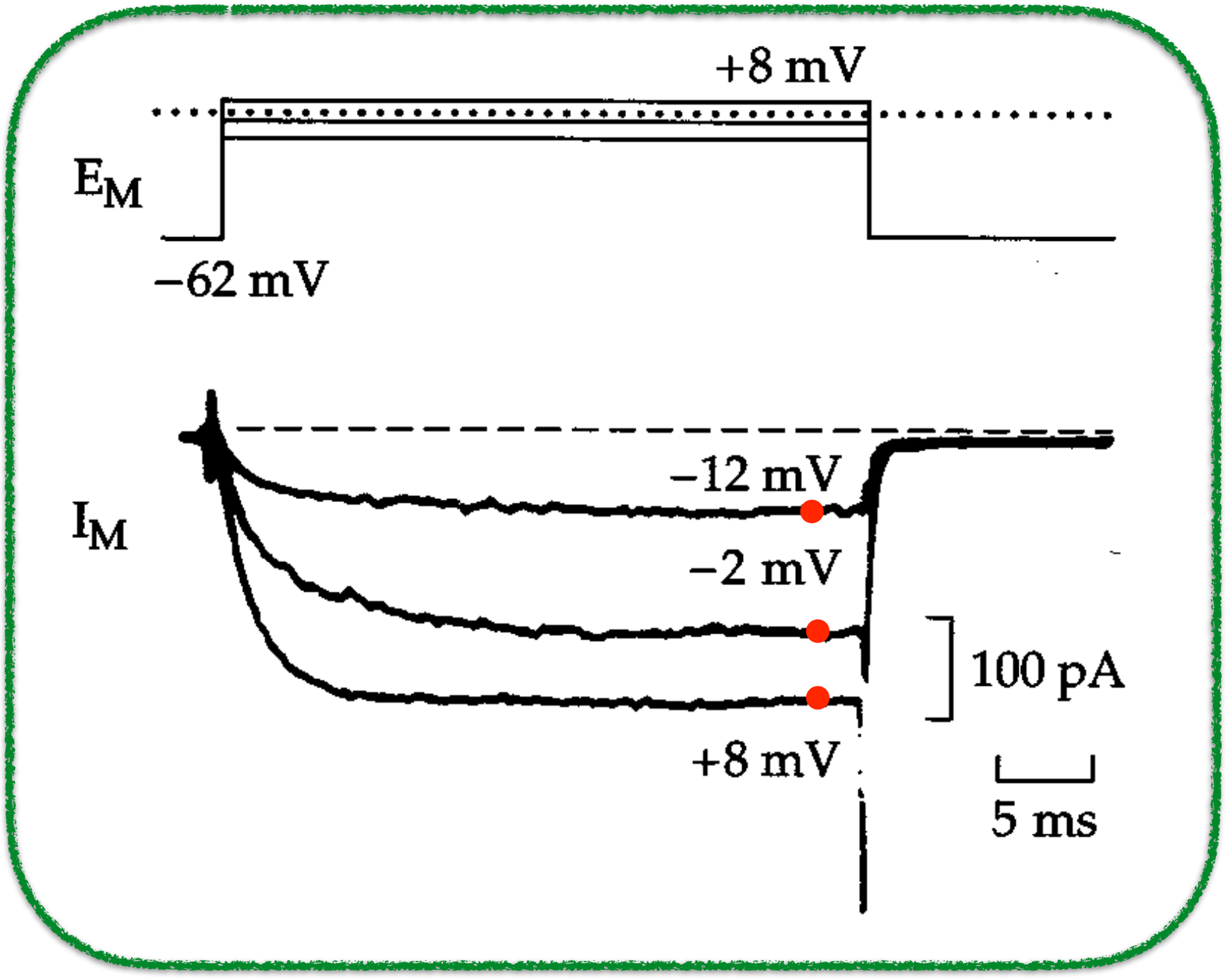
$$I_{K-DR} = g_K m^\eta \left(V - E_K\right)$$

$$\frac{dm}{dt} = -\frac{m - m_\infty(V)}{\tau_m(V)}$$



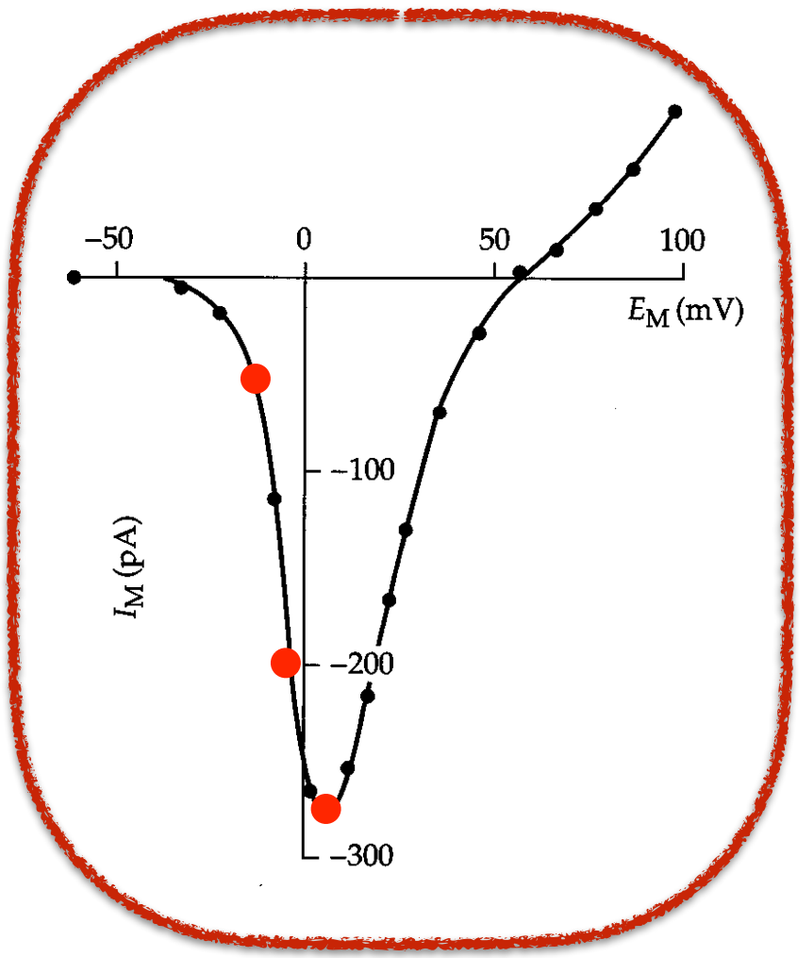


Ca^{2+} currents may also activate gradually



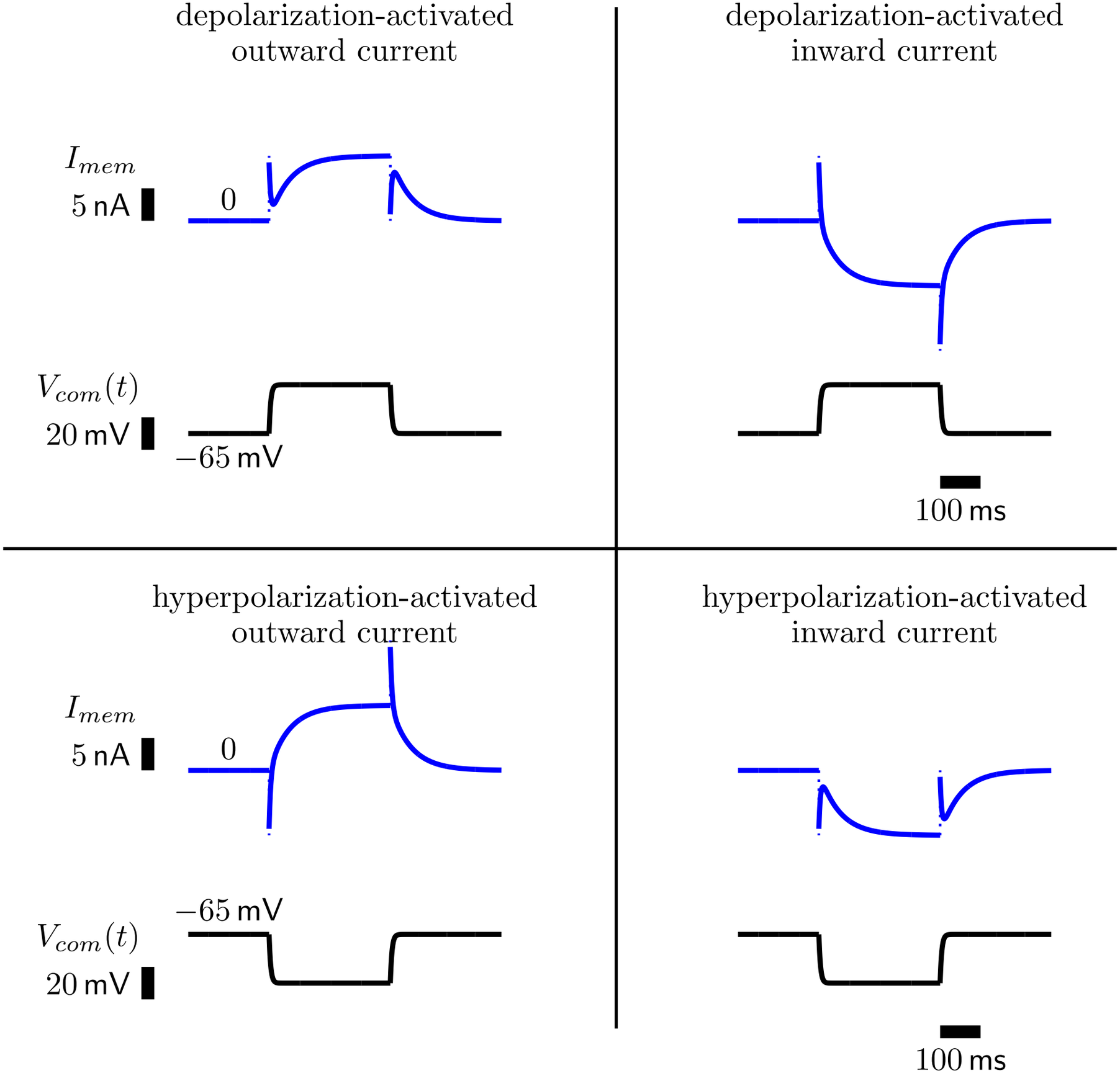
inward current
that activates with
depolarization

I-V relation plots the
"plateau" Ca^{2+} current

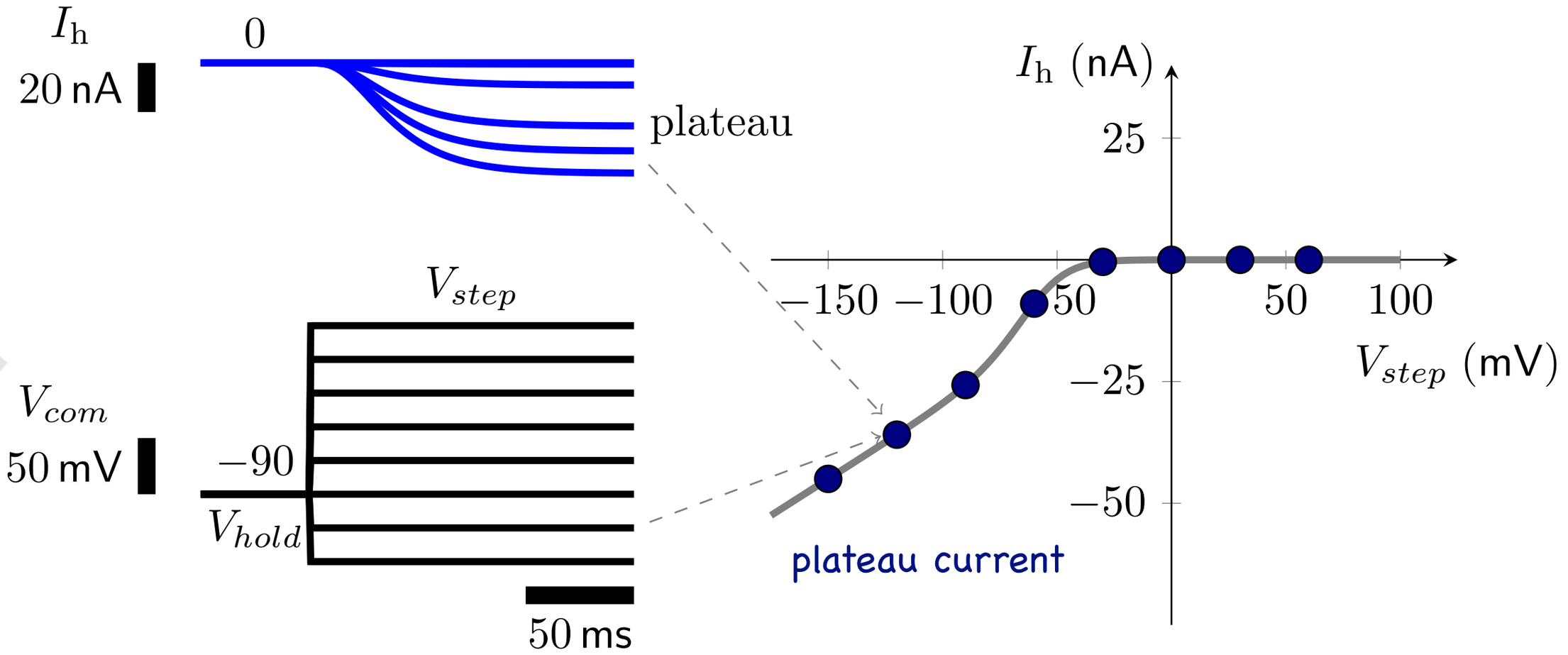


(B) Voltage-dependent activation of I_{Ca} in an isolated bovine chromaffin cell filled with CsCl, TEA, and EGTA and bathed in a solution containing TTX and 5 mM Ca. [From Fenwick et al. 1982b.] (C) Current-voltage relations for plateau current amplitudes measured in the chromaffin cell of part B. [From Fenwick et al. 1982b.]

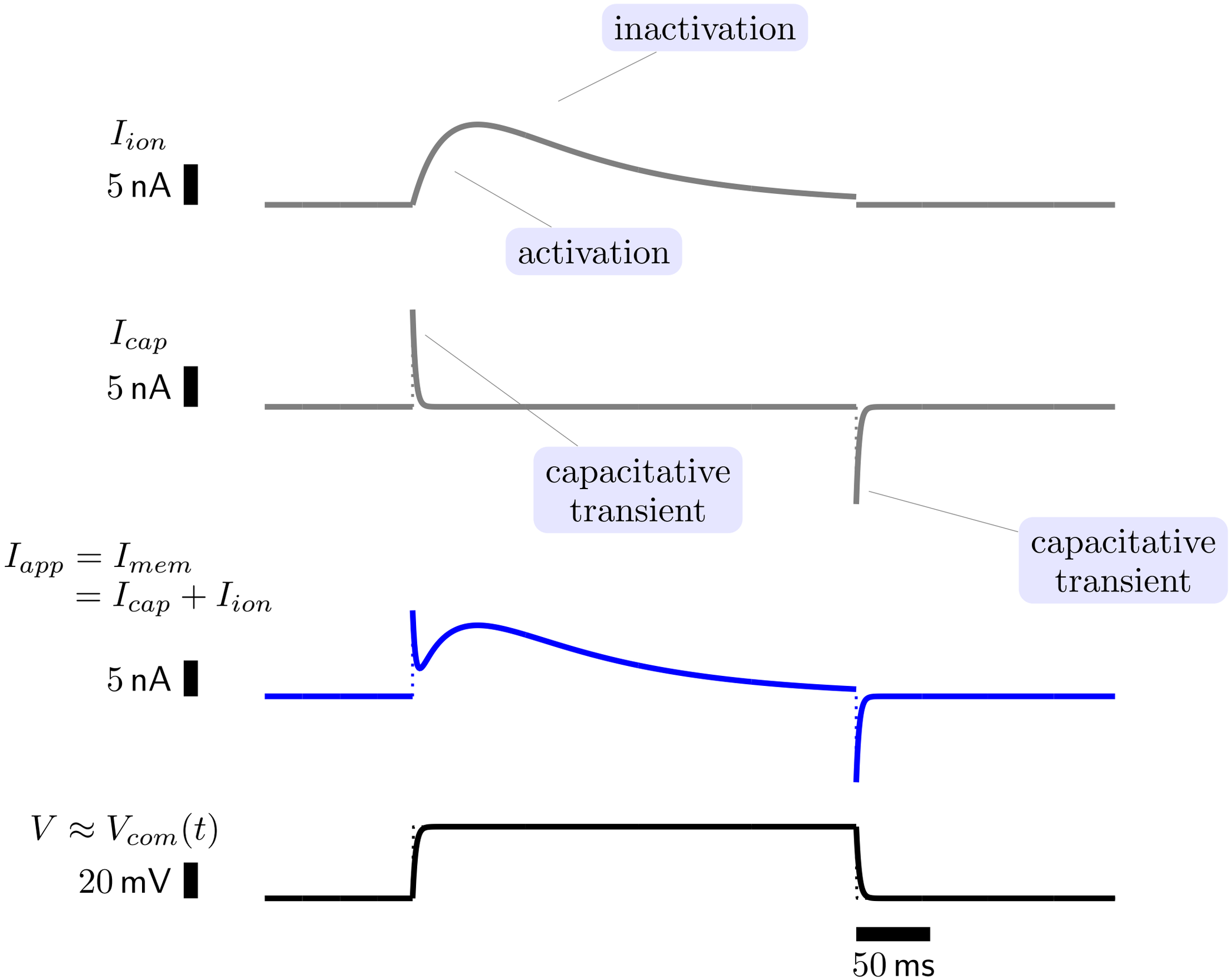
so far we have discussed <u>persistent</u> currents



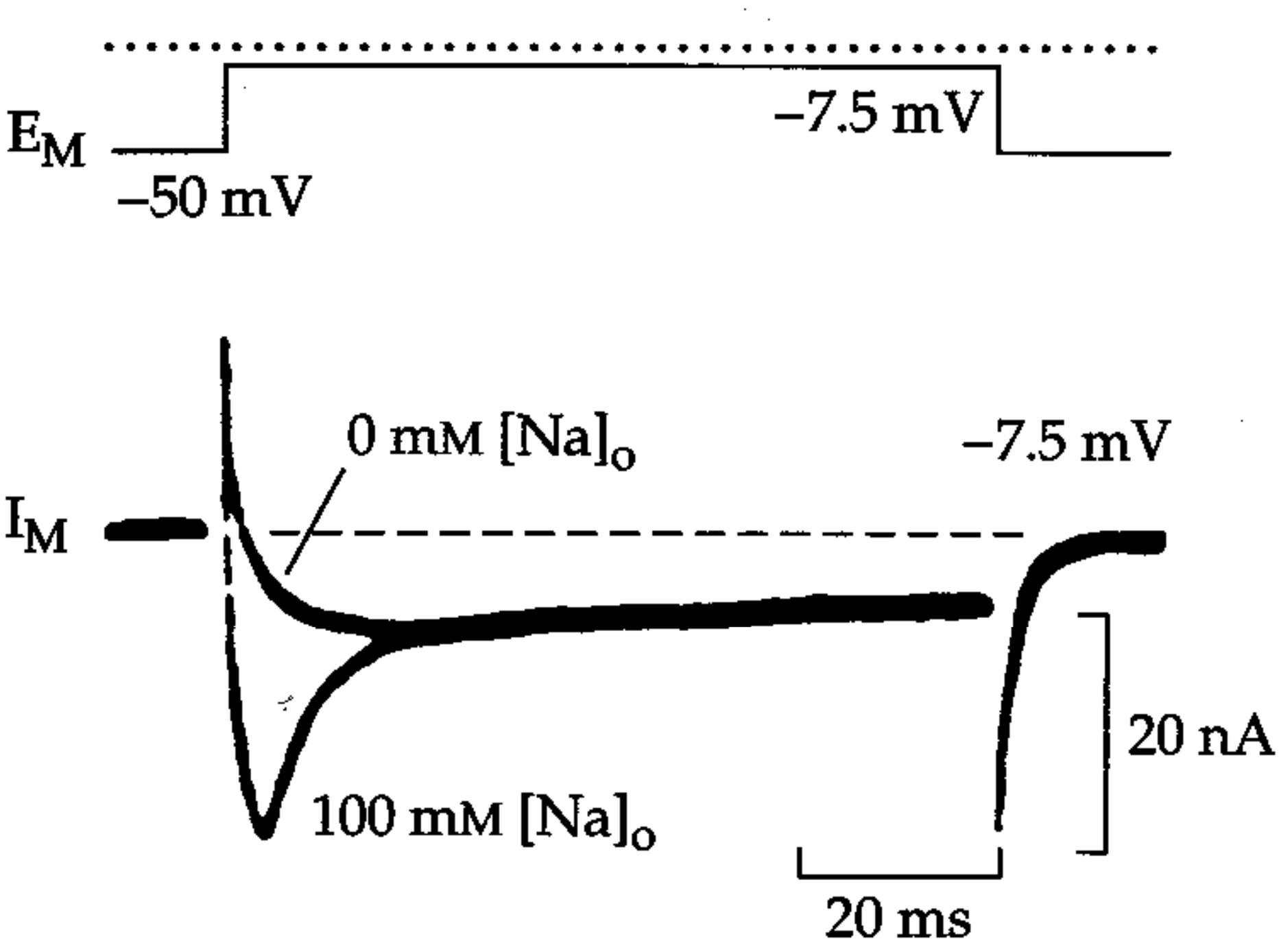
Is this current **activated by depolarization** or **hyperpolarization**?
Is this current **inward** or **outward**?



ionic currents are often <u>transient</u> (not persistent)



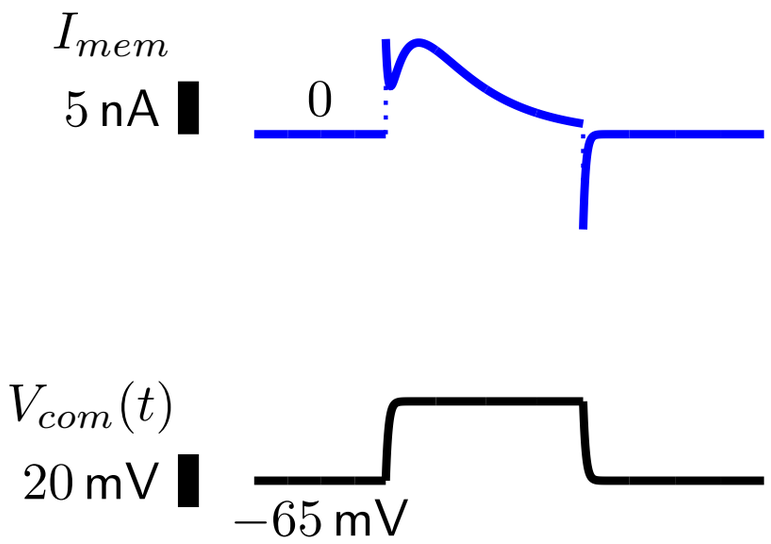
separation of Ca^{2+} and Na^{+} currents



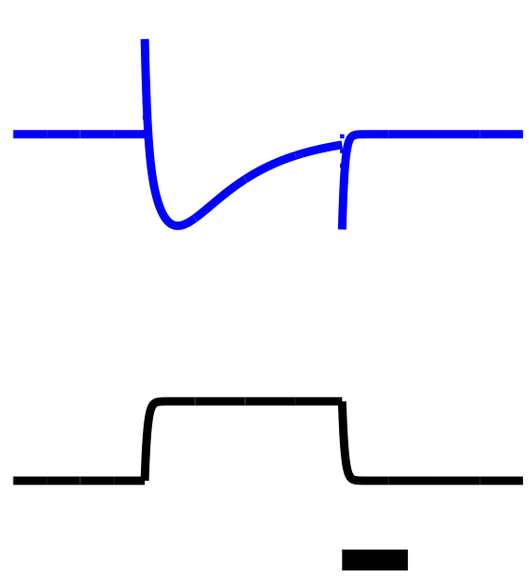
(A) Separation of I_{Na} and I_{Ca} in a snail neuron bathed in a medium with and without Na^+ ions. I_{Ca} is seen by itself in the Na^+-free medium containing 10 mM Ca. It has slower activation and inactivation kinetics than I_{Na}.

transient currents

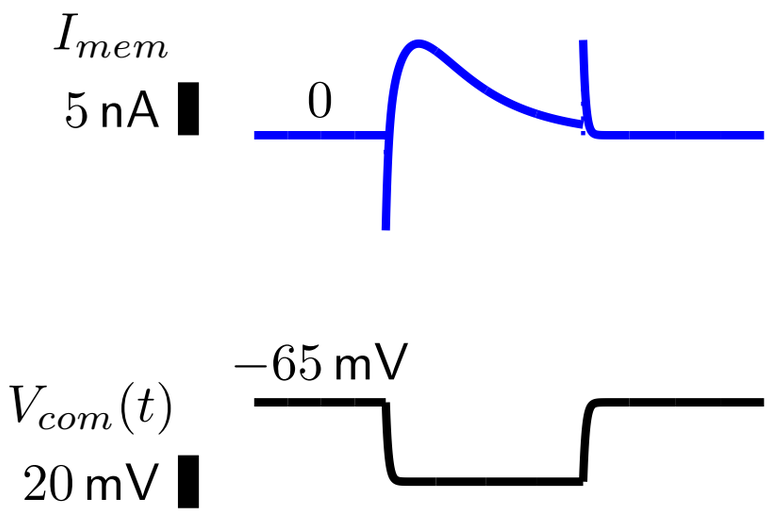
depolarization-activated
outward current



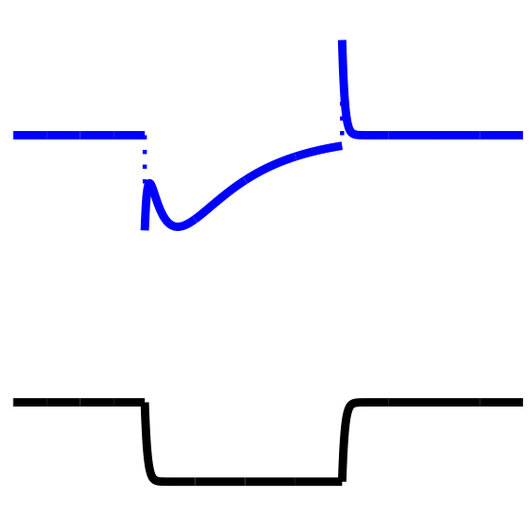
depolarization-activated
inward current



hyperpolarization-activated
outward current



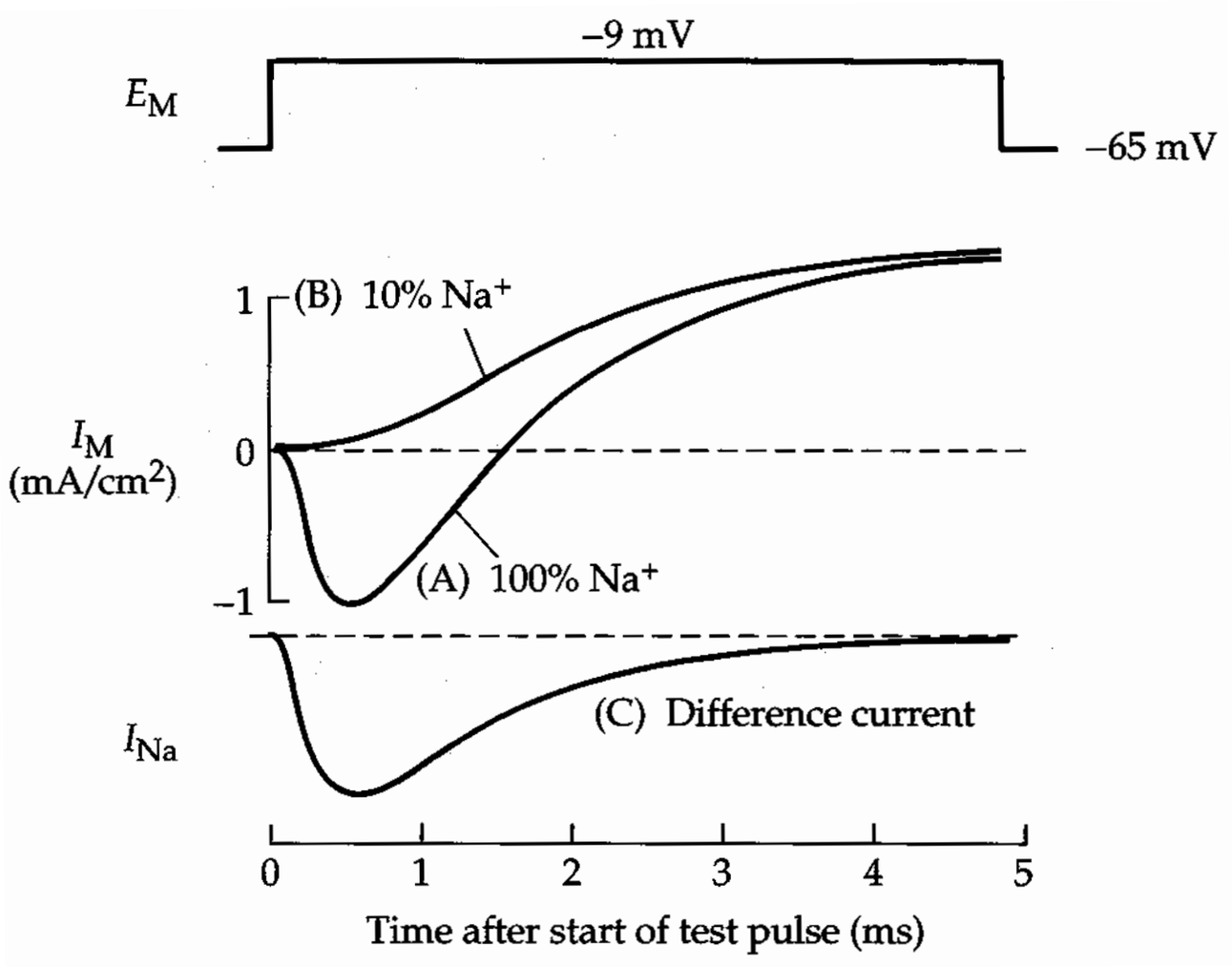
hyperpolarization-activated
inward current

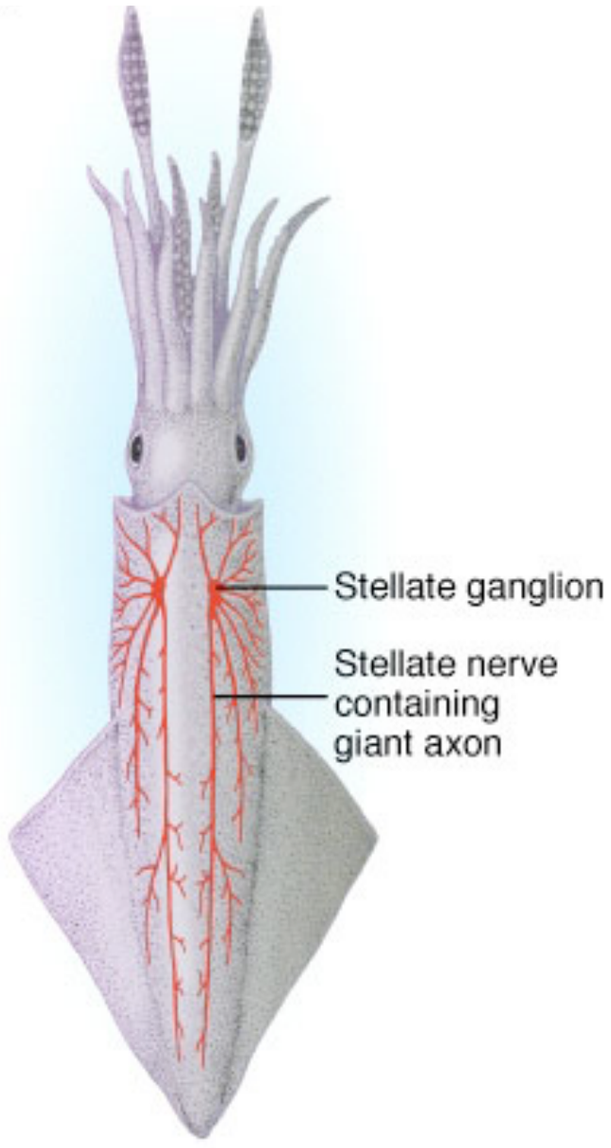


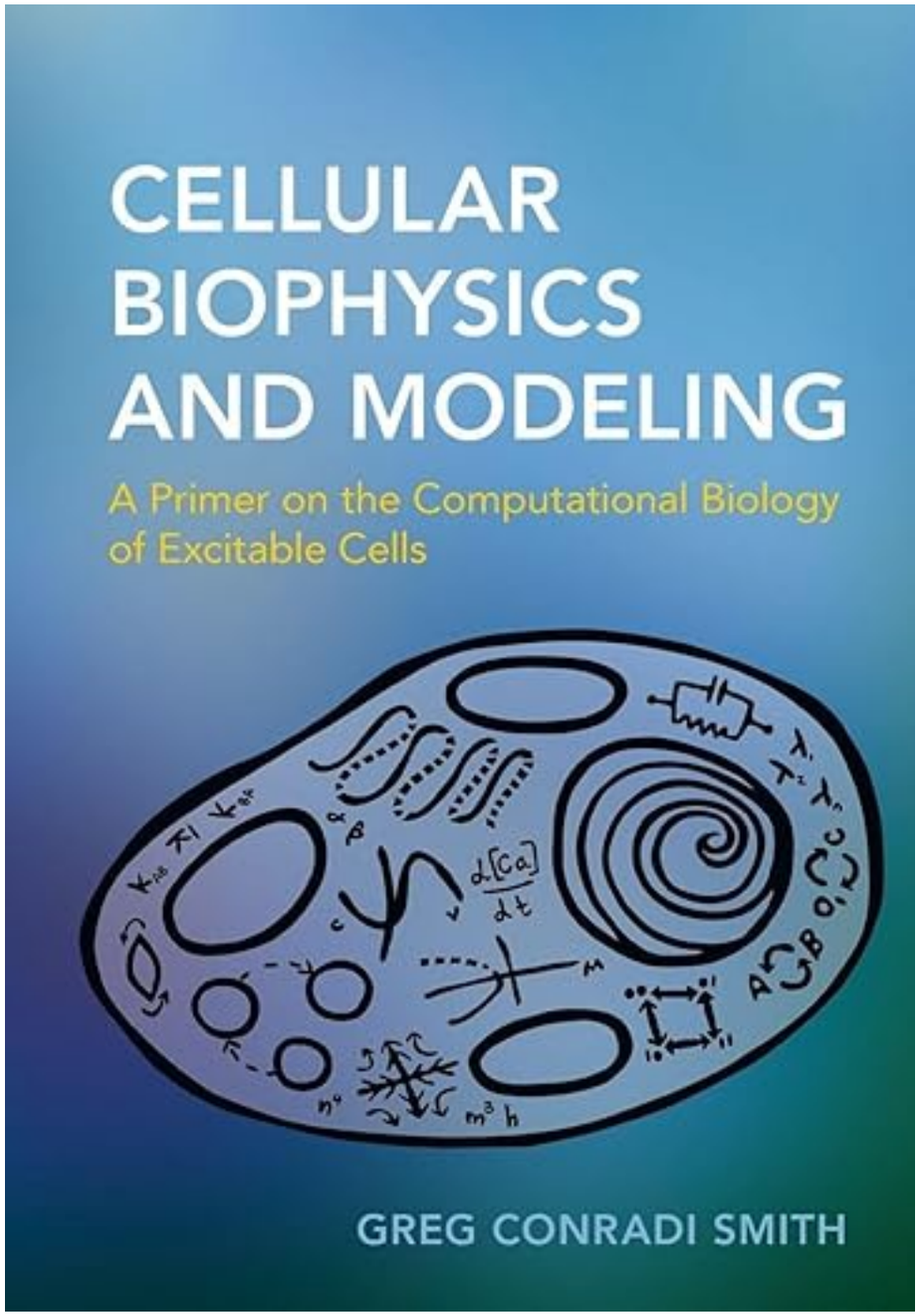
Cellular Biophysics & Modeling

Lecture 9

voltage-clamp recording and the
classical biophysics of the squid giant axon






CELLULAR
BIOPHYSICS
AND MODELING
A Primer on the Computational Biology
of Excitable Cells
GREG CONRADI SMITH

12 Voltage-Clamp Recording

Current-voltage relations are obtained using the electrophysiological technique of *voltage-clamp recording*. Voltage-clamp recording reveals that ionic currents are not always instantaneous functions of membrane voltage (as assumed in previous chapters). Rather, ionic currents activate (channels open) or deactivate (channels close) on a time scale of 5–50 ms. Voltage-clamp experiments also reveal slower processes of channel closure and recovery (inactivation and de-inactivation).

12.1 Current-Clamp and Voltage-Clamp Recording

The current balance equation for a neural membrane takes the form,

$$C\frac{dV}{dt} = I_{app} - I_{ion}(V)\,, \tag{12.1}$$

where $I_{ion}(V)$ is the total ionic membrane current. For a passive Ohmic current such as $I_{ion}(V) = I_L(V) = g_L(V - E_L)$, we are able to analytically calculate the membrane voltage $V(t)$ as a function of time (an exponential relaxation, recall Eq. 8.11). When $I_{ion}(V)$ is nonlinear, as was the case for the bistable neural membranes of Chapter 11, membrane voltage $V(t)$ may be simulated via numerical integration of the current balance equation (as in Fig. 11.4).

For both linear and nonlinear membranes, we have (up until now) thought of applied current $I_{app}(t)$ as a *stimulus*, and the resulting changes in neural membrane voltage $V(t)$ as the *response*. In Chapter 8 we learned that a positive applied current pulse will depolarize a passive membrane (Fig. 12.1, left). When the time course of applied current is chosen in advance (i.e., *clamped*), the result is a *current-clamp* recording of membrane voltage.

In **voltage-clamp recording**, the roles of applied current and membrane voltage are reversed (Fig. 12.1, right). Electronic instrumentation rapidly and precisely controls the applied current $I_{app}(t)$ so that membrane voltage $V(t)$ follows a **command voltage**, $V_{com}(t)$, that is chosen in advance to reveal the dynamics of membrane current.

13 Hodgkin-Huxley Model of the Action Potential

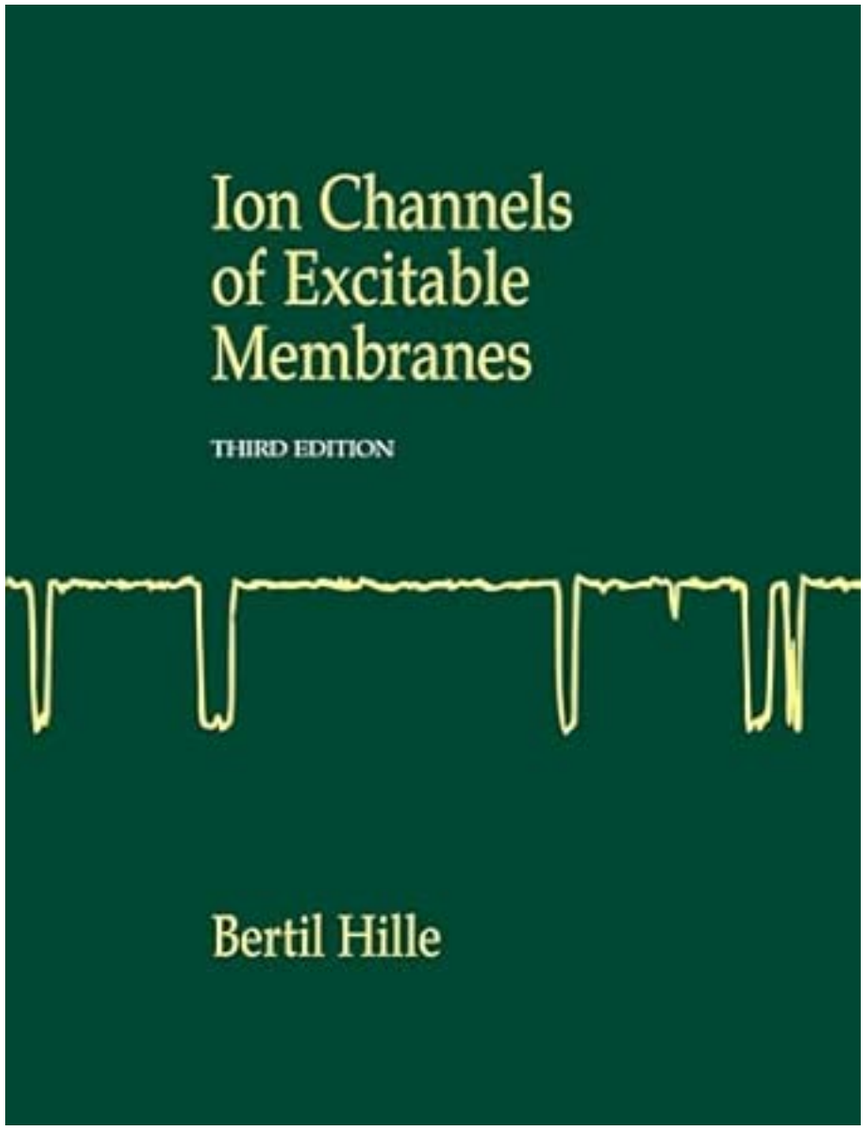
Alan Hodgkin and Andrew Huxley's Nobel Prize winning studies included seminal experimental observations of the action potential in the squid giant axon. Hodgkin and Huxley introduced the mathematical framework for modeling ionic currents of excitable membranes that has become standard.

13.1 The Squid Giant Axon

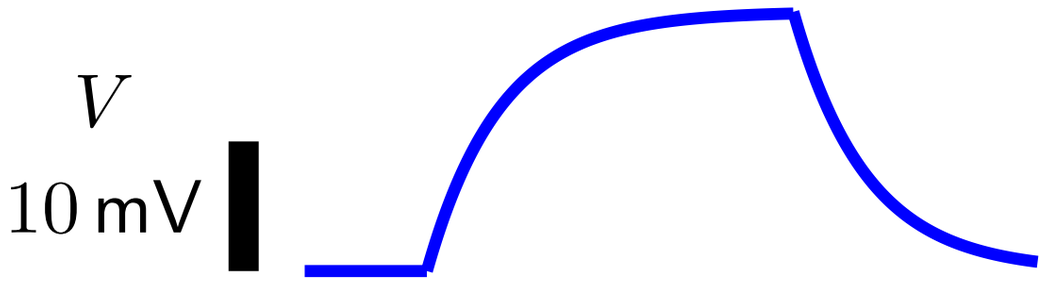
Alan Hodgkin and Andrew Huxley's studies of the dynamics of the action potential in neural membranes included the refinement of an experimental preparation: the squid giant axon.[1] The giant axon of a (normal sized) squid is a motoneuron that participates in escape reflex circuitry. The axon is many centimeters long (about half the squid's length) – originating in the stellate ganglion and innervating muscle cells in the mantle – and nearly a millimeter in diameter. The squid giant axon's diameter is a specialization that allows rapid propagation of action potentials.[2] The giant axon enabled experimental manipulation of intracellular and extracellular solutions and control of ionic concentrations on both sides of the nerve membrane. A wire could be threaded down the length of the large diameter axon leading to a *spatially clamped* electrical recording mode in which the giant axon was essentially uni-potential. This meant that wave phenomena related to the propagation of action potentials down the length of the axon would not confound electrical recordings.

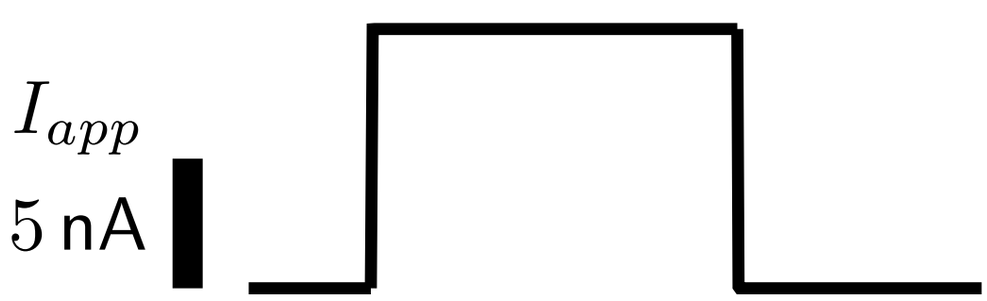
Current-Clamp Recording

Hodgkin and Huxley used both current-clamp and voltage-clamp recording techniques to study the action potential of the squid giant axon. To illustrate, Fig. 13.1 shows how the excitability of the squid giant axon may be triggered by brief pulses of depolarizing applied current ($I_{pulse} > 0$). For a **subthreshold** current pulse, the membrane responds passively (increasing while $I_{pulse} > 0$ and decreasing when I_{pulse} returns to zero). On the other hand, when **superthreshold** current is applied, an


Ion Channels
of Excitable
Membranes
THIRD EDITION
Bertil Hille

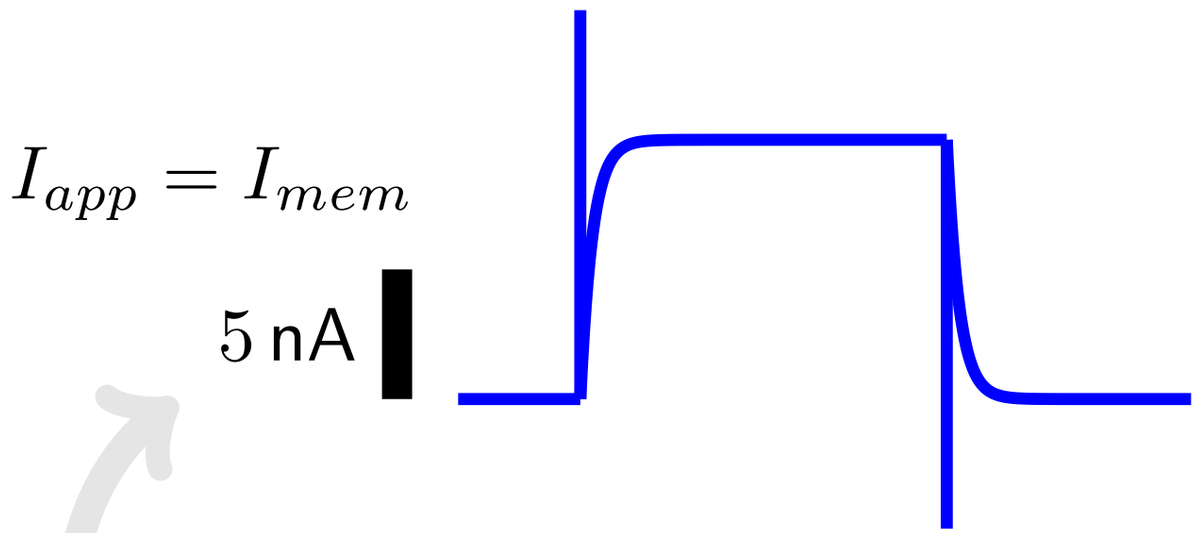
current clamp

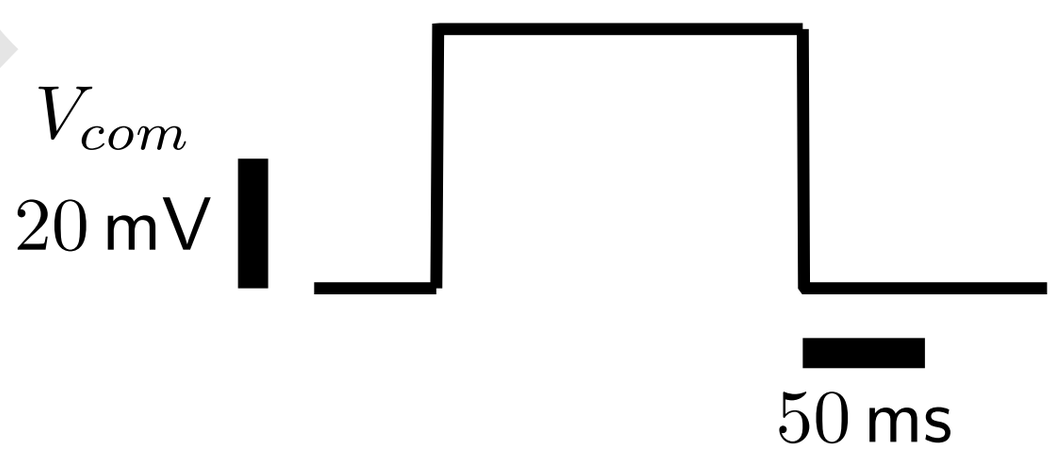




$$\frac{dV}{dt} = \frac{1}{C}\left[I_{app} - \underbrace{g\ (V - E)}_{I_{ion}}\right]$$

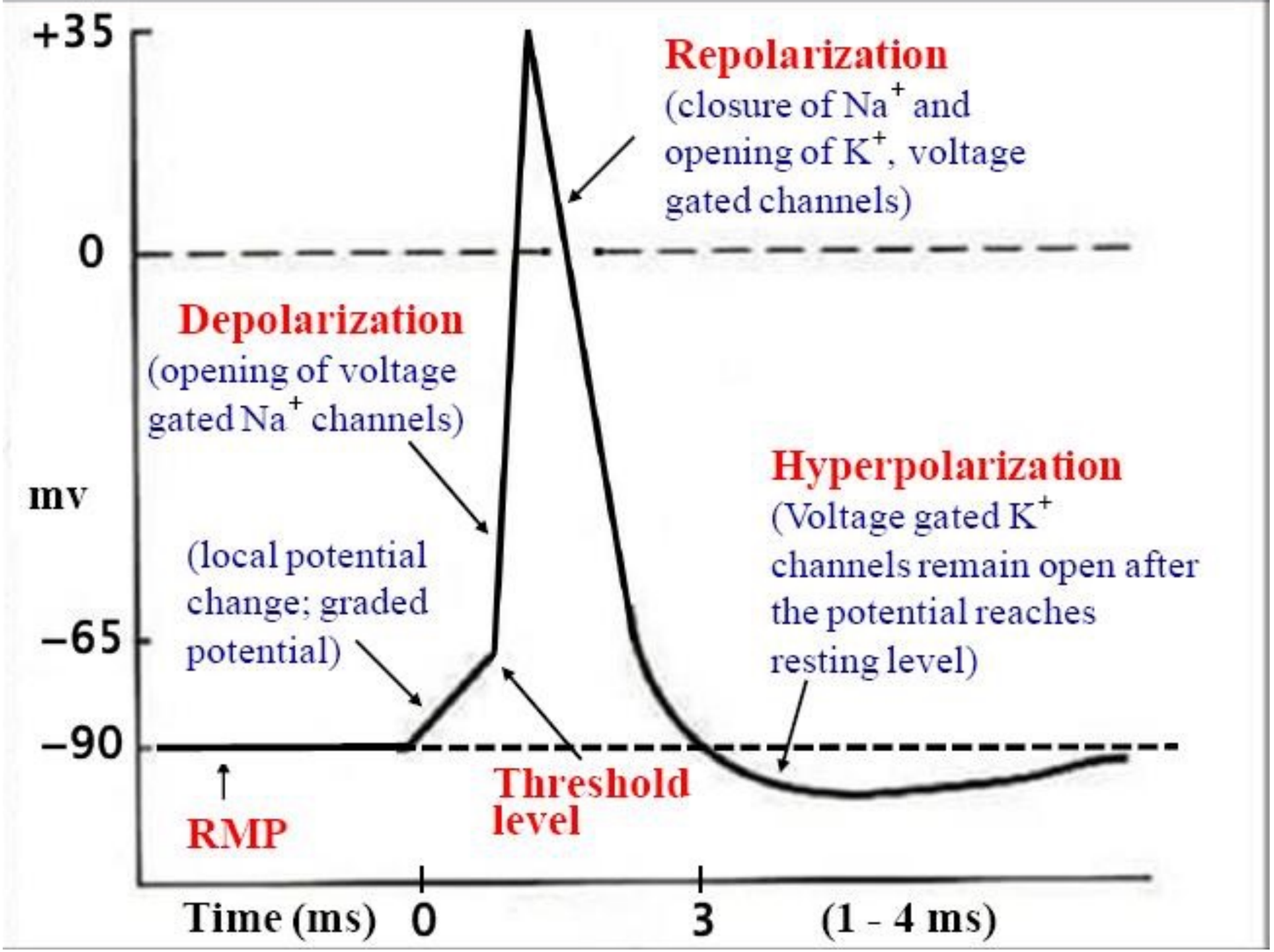
voltage clamp



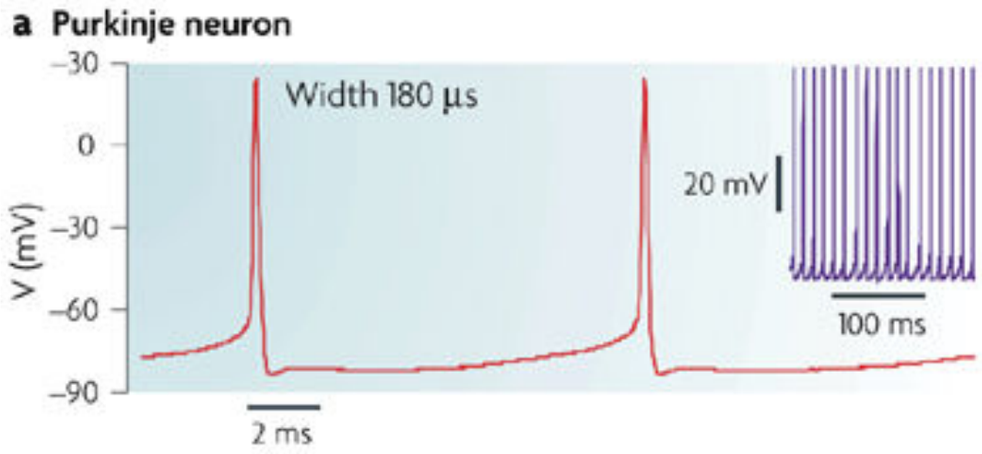


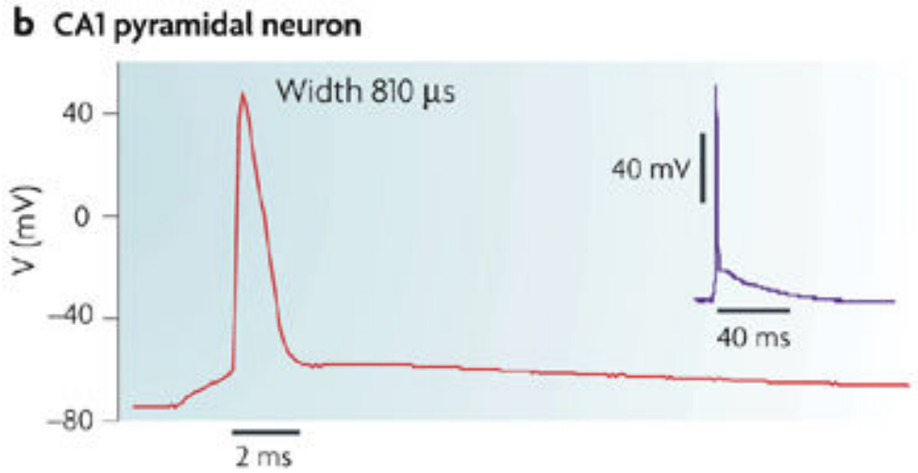
$$I_{app}(t) = \underbrace{C\frac{dV}{dt} + I_{ion}(V)}_{I_{mem}}$$

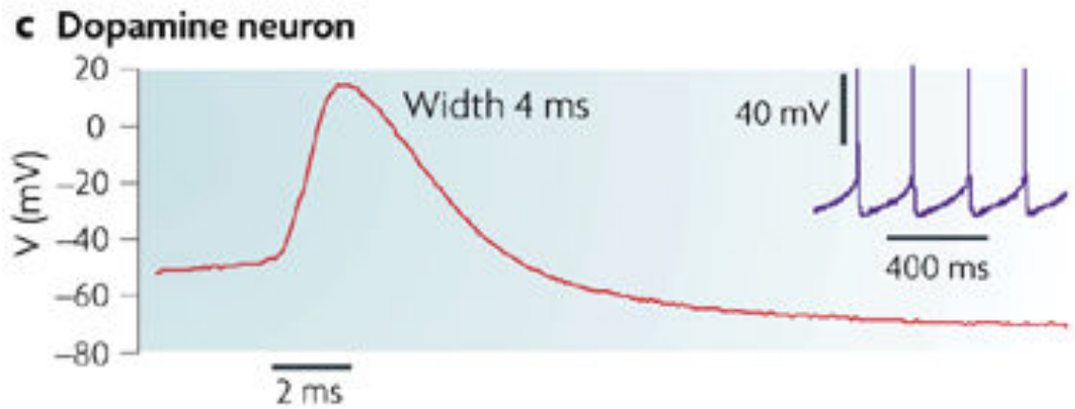
Action potential



action potentials (excitability)

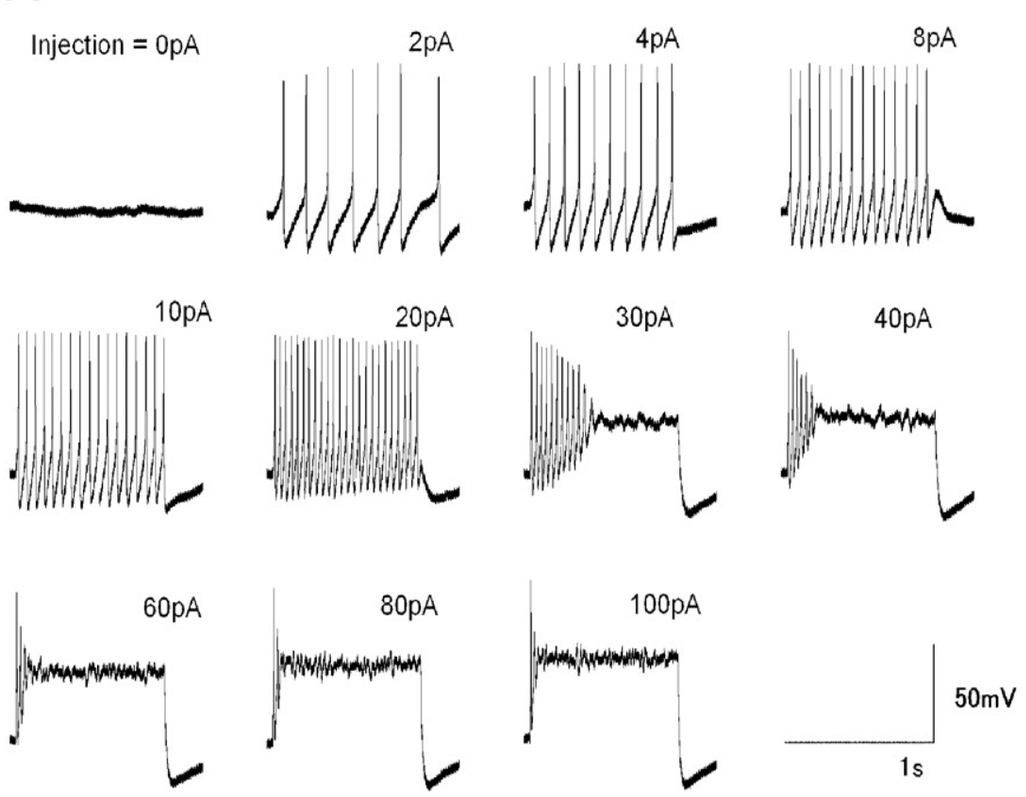




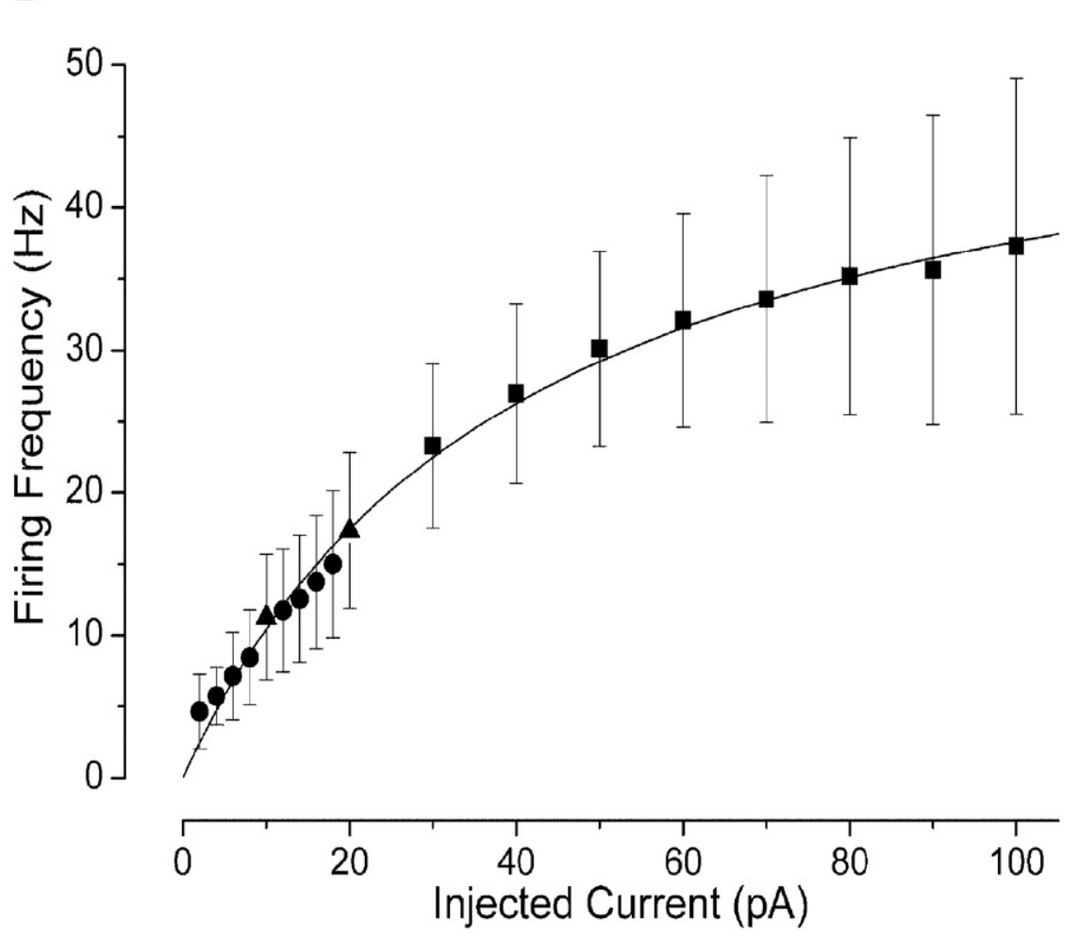


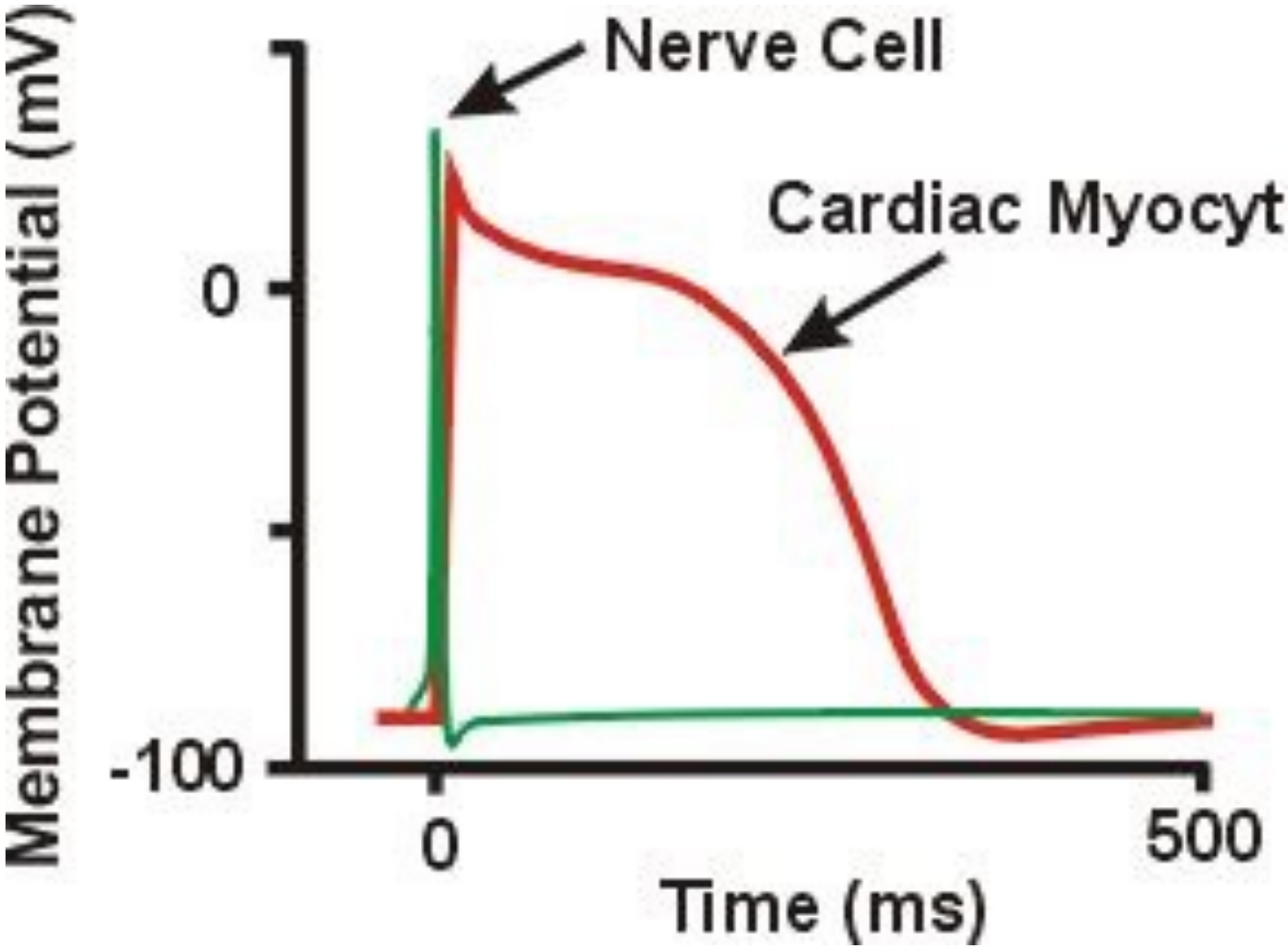
repetitive spiking (oscillations)

A



B




Nerve Cell
Cardiac Myocyte
Membrane Potential (mV)
0
-100
0
500
Time (ms)

We will study action potentials (excitability) and repetitive spiking (oscillations) exhibited by two classical experimental preparations and the associated membrane models

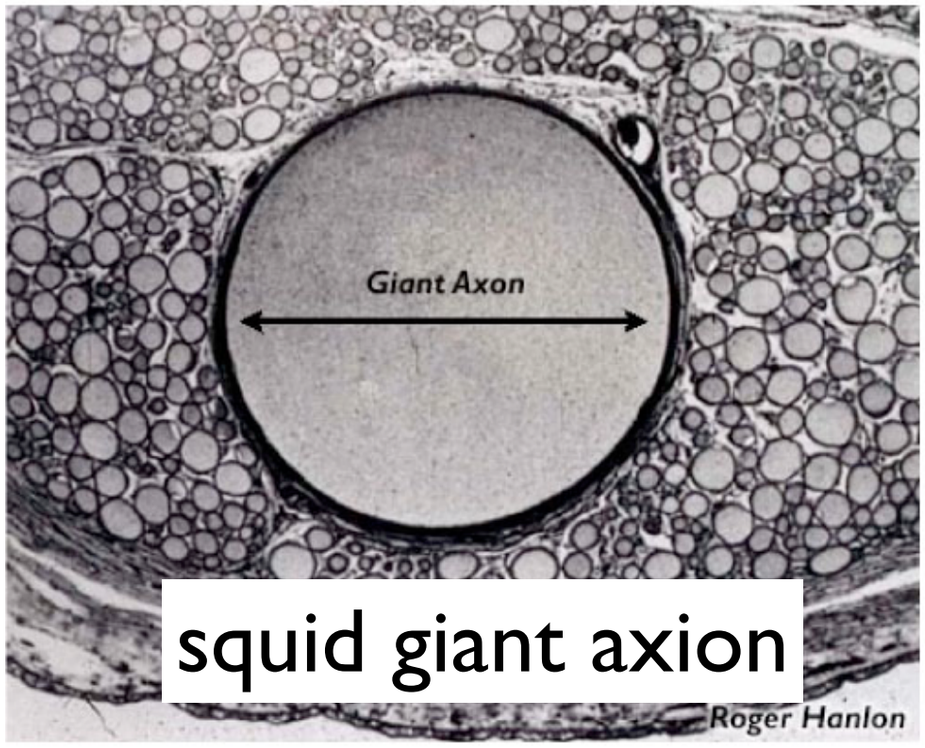
Hodgkin and Huxley's classical experiments with squid giant axon
(I_{leak}, $I_{Na\text{-}V}$, $I_{K\text{-}DR}$)

Morris-Lecar model of barnacle muscle fiber
(I_{leak}, $I_{Ca\text{-}V}$, $I_{K\text{-}DR}$)

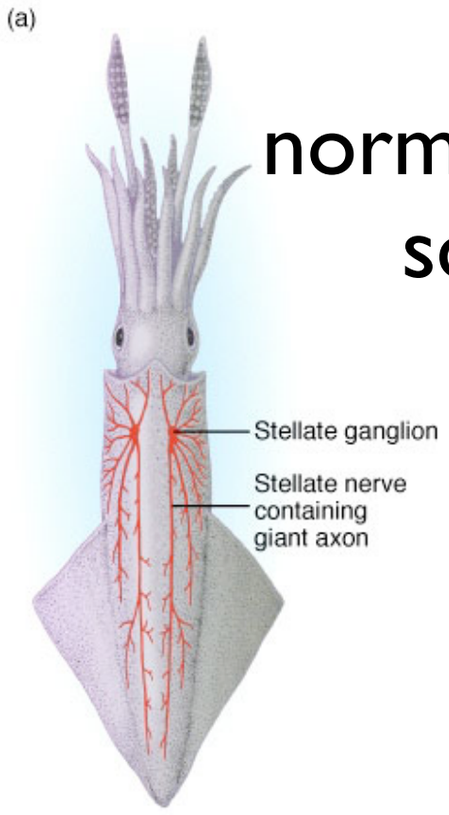
Hodgkin & Huxley's contributions

- Experimental observation of action potential in squid giant axon

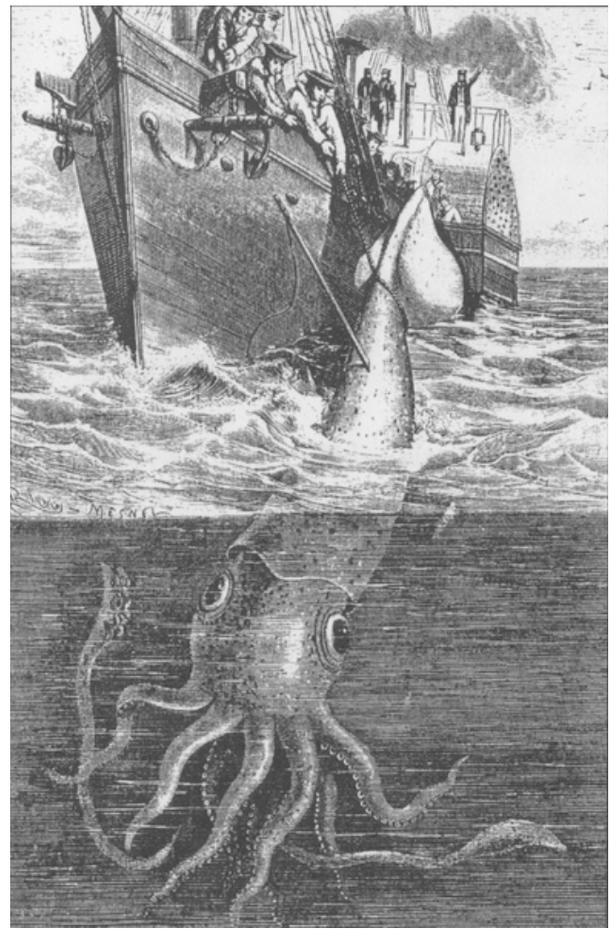
- Mathematical modeling that included current balance equation, voltage-gated ionic currents, and gating variables



squid giant axion



normal-sized squid

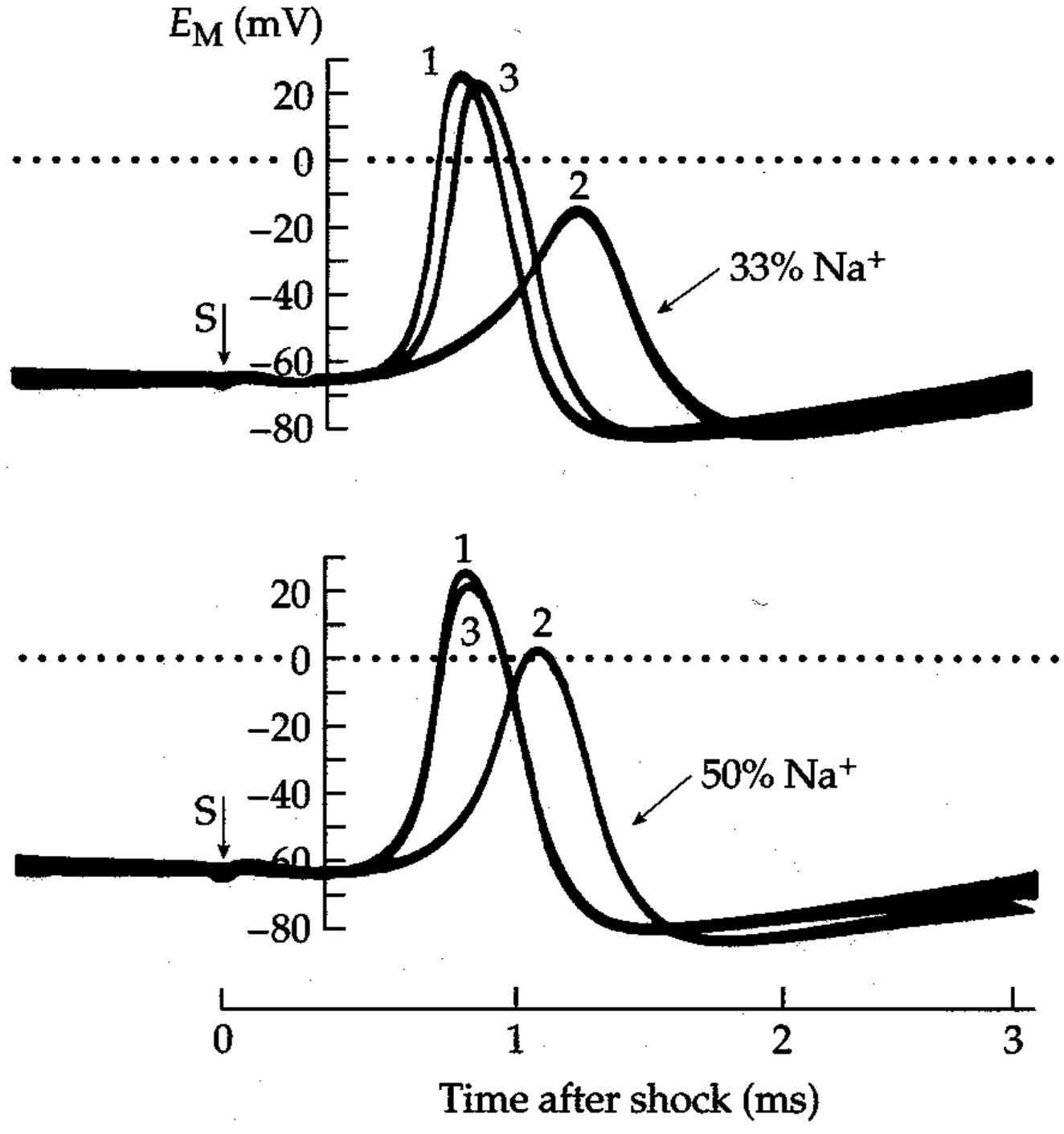


giant squid

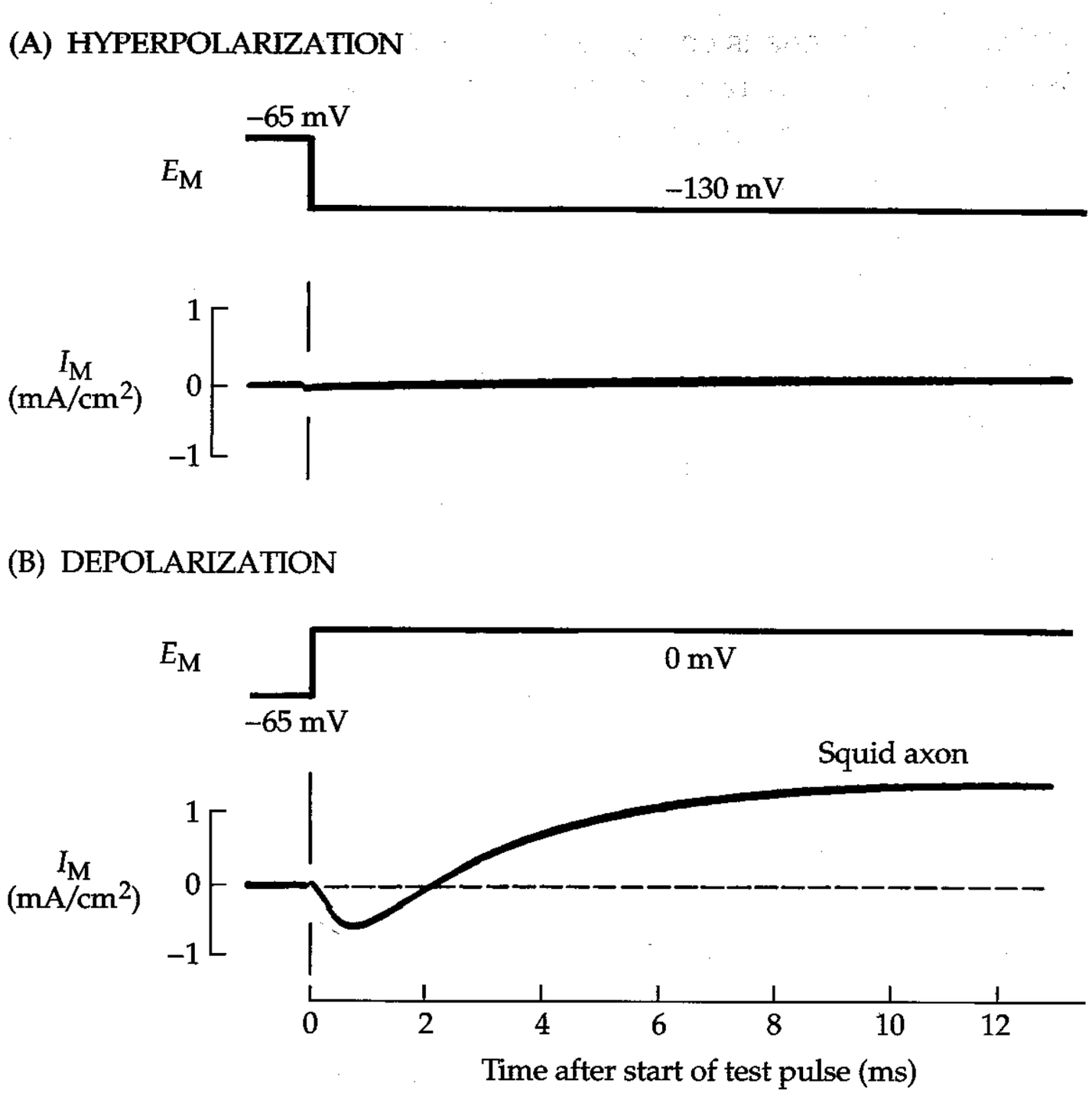
Cross section of stellate nerve showing differential axon diameters. The giant axon sends impulses to mantle muscles far from the stellate ganglion.

action potential suppressed when $[Na^+]_o$ is decreased

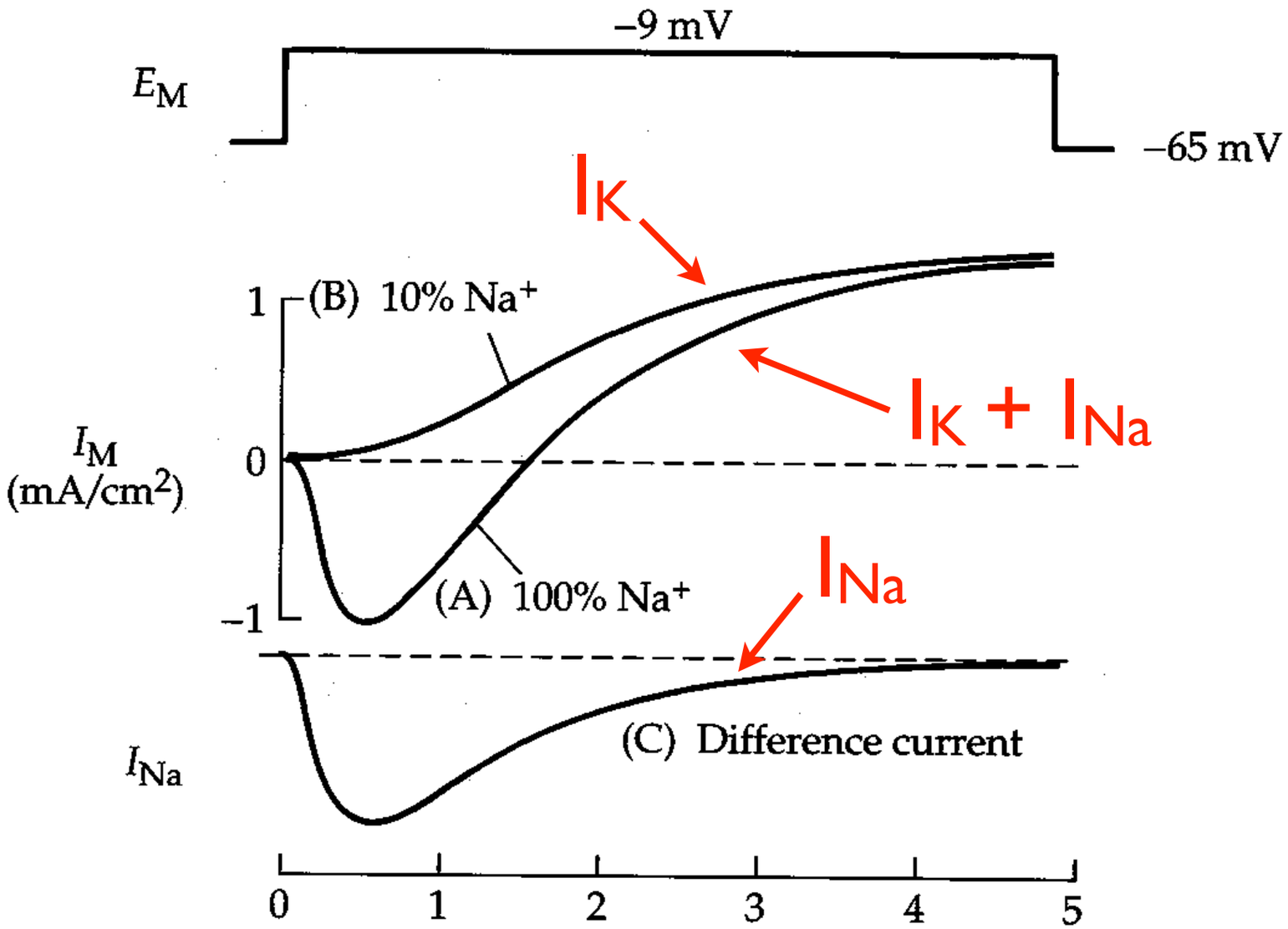
2.4 Na^+-Dependence of the Action Potential This is the first experiment to demonstrate that external Na^+ ions are needed for propagated action potentials. Intracellular potential is recorded with an axial microelectrode inside a squid giant axon. The action potential is smaller and rises more slowly in solutions containing less than the normal amount of Na^+. External bathing solutions: Records 1 and 3 in normal seawater; record 2 in low-sodium solution containing 1:2 or 1:1 mixtures of seawater with isotonic glucose. An assumed 15-mV junction potential has been subtracted from the voltage scale. [From Hodgkin and Katz 1949.]



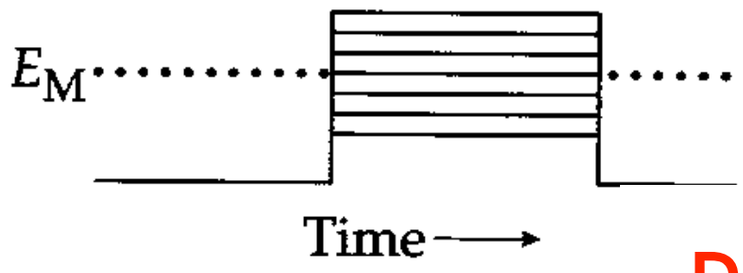
When I show you a figure, ask yourself:
Is this a "current clamp" or "voltage clamp" recording?

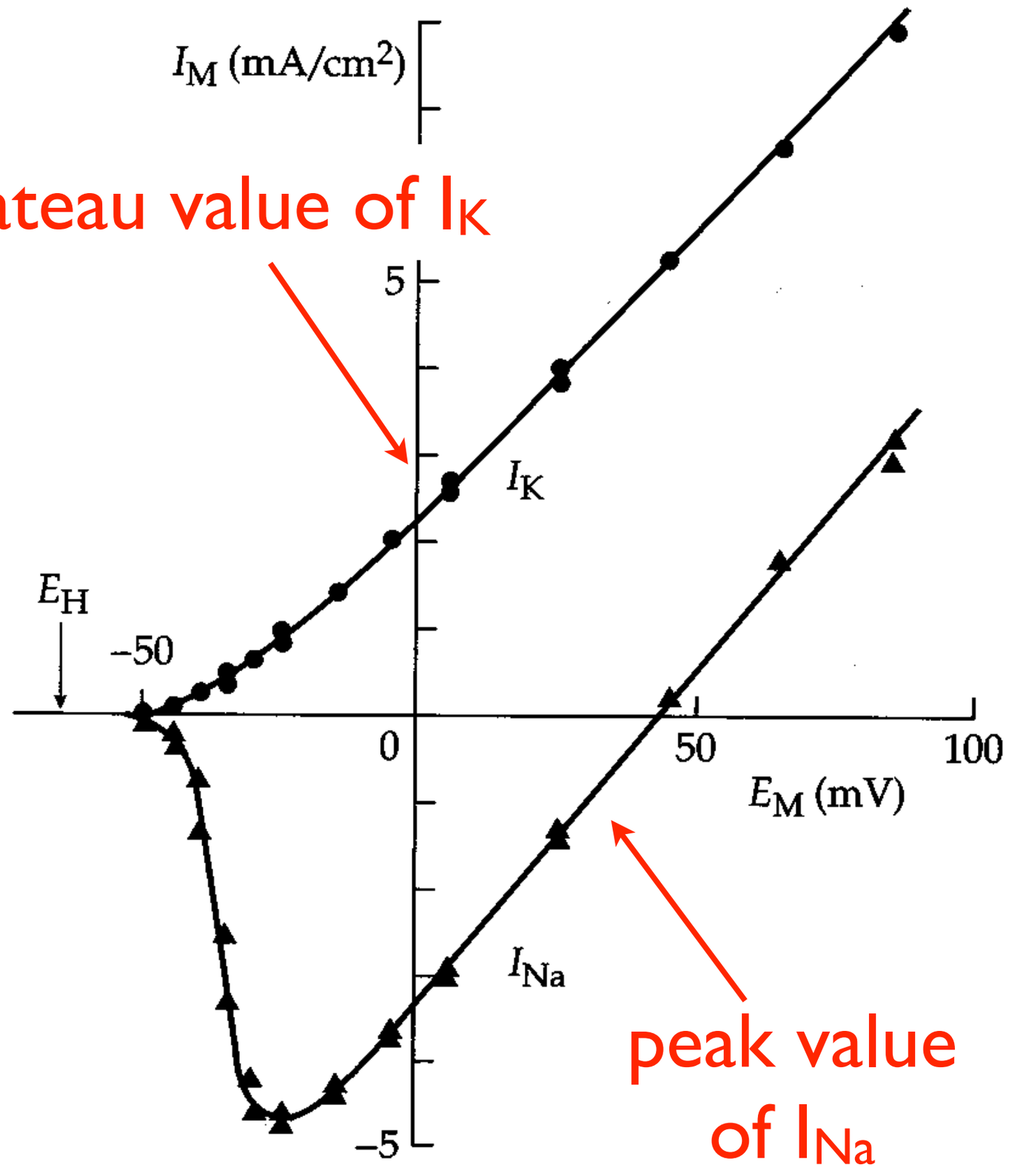


2.6 Voltage-Clamp Currents in a Squid Axon An axon is bathed in seawater and voltage clamped by the axial-wire method (see Figure 2.5). The membrane potential is held at –65 mV and then hyperpolarized in a step to –130 mV or depolarized in a step to 0 mV. Outward ionic current is shown as an upward deflection. The membrane permeability mechanisms are clearly asymmetrical. Hyperpolarization produces only a small inward current, whereas depolarization elicits a larger and biphasic current. $T = 3.8°C$ [Adapted from Hodgkin et al. 1952.]

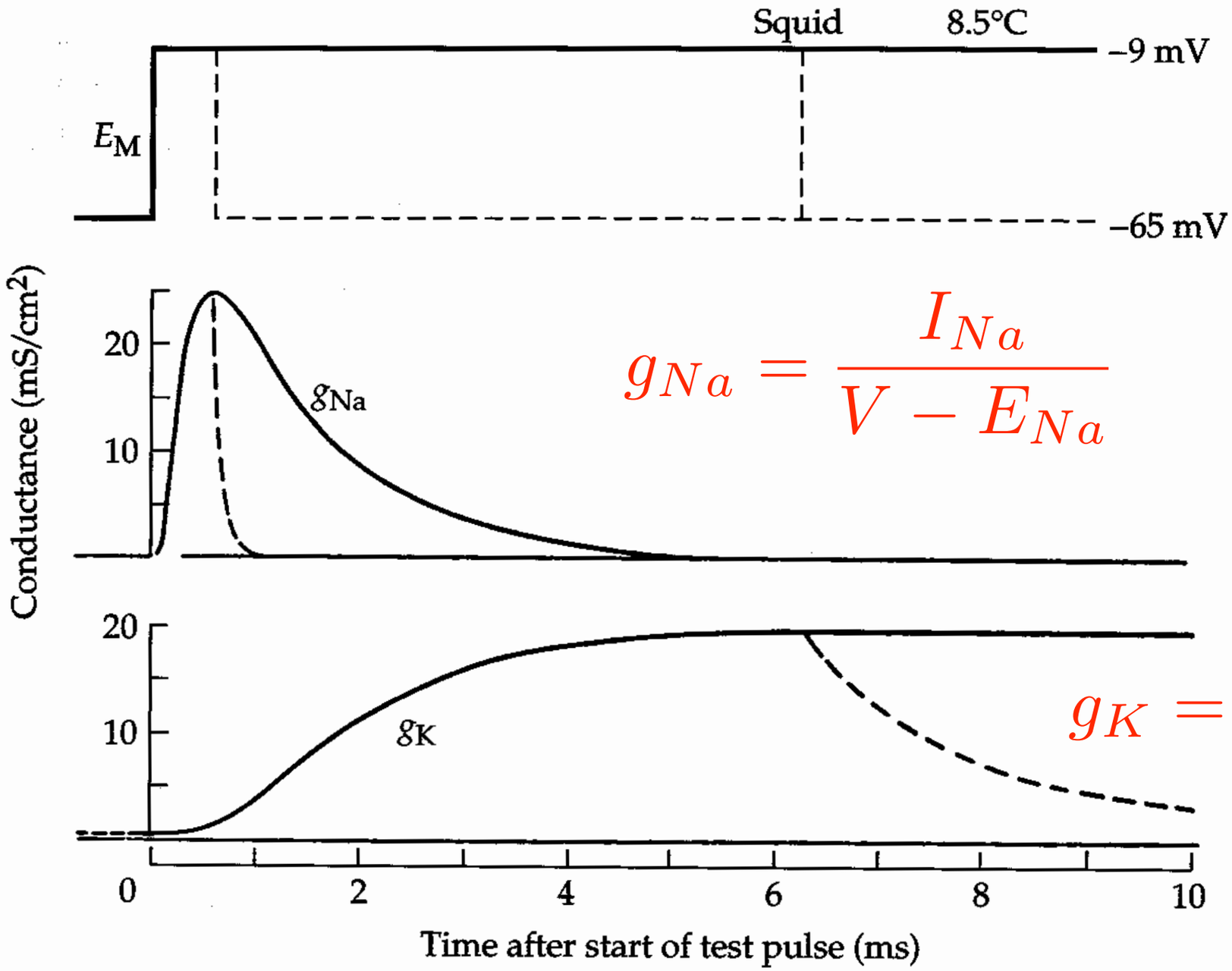


2.8 Separation of Na^+ and K^+ Currents An illustration of the classical ion substitution method for analyzing the ionic basis of voltage-clamp currents. Ionic currents are measured in a squid axon membrane stepped from a holding potential of –65 mV to –9 mV. The component carried by Na^+ ions is dissected out by substituting impermeant choline ions for most of the external sodium. (A) Axon in seawater, showing inward and outward ionic currents. (B) Axon in low-sodium solution with 90% of the NaCl substituted by choline chloride, showing only outward ionic current. (C) Algebraic difference between experimental records (A) and (B), showing the transient inward component of current due to the inward movement of external Na^+ ions. T = 8.5°C. [From Hodgkin 1958; adapted from Hodgkin and Huxley 1952a.]





2.9 Current-Voltage Relations of a Squid Axon The axon membrane potential is stepped under voltage clamp from the negative holding potential (E_H) to various test potentials, as in Figure 2.7. Peak transient Na^+ current (triangles) and steady-state K^+ current (circles) from each trace are plotted against the test potential. The nonlinearity of the two *I–E* relations between –50 to –20 mV reflects the voltage-dependent opening of Na and K channels by depolarizations, as explained in Figure 1.6. [From Cole and Moore 1960.]



$$g_{Na} = \frac{I_{Na}}{V - E_{Na}}$$

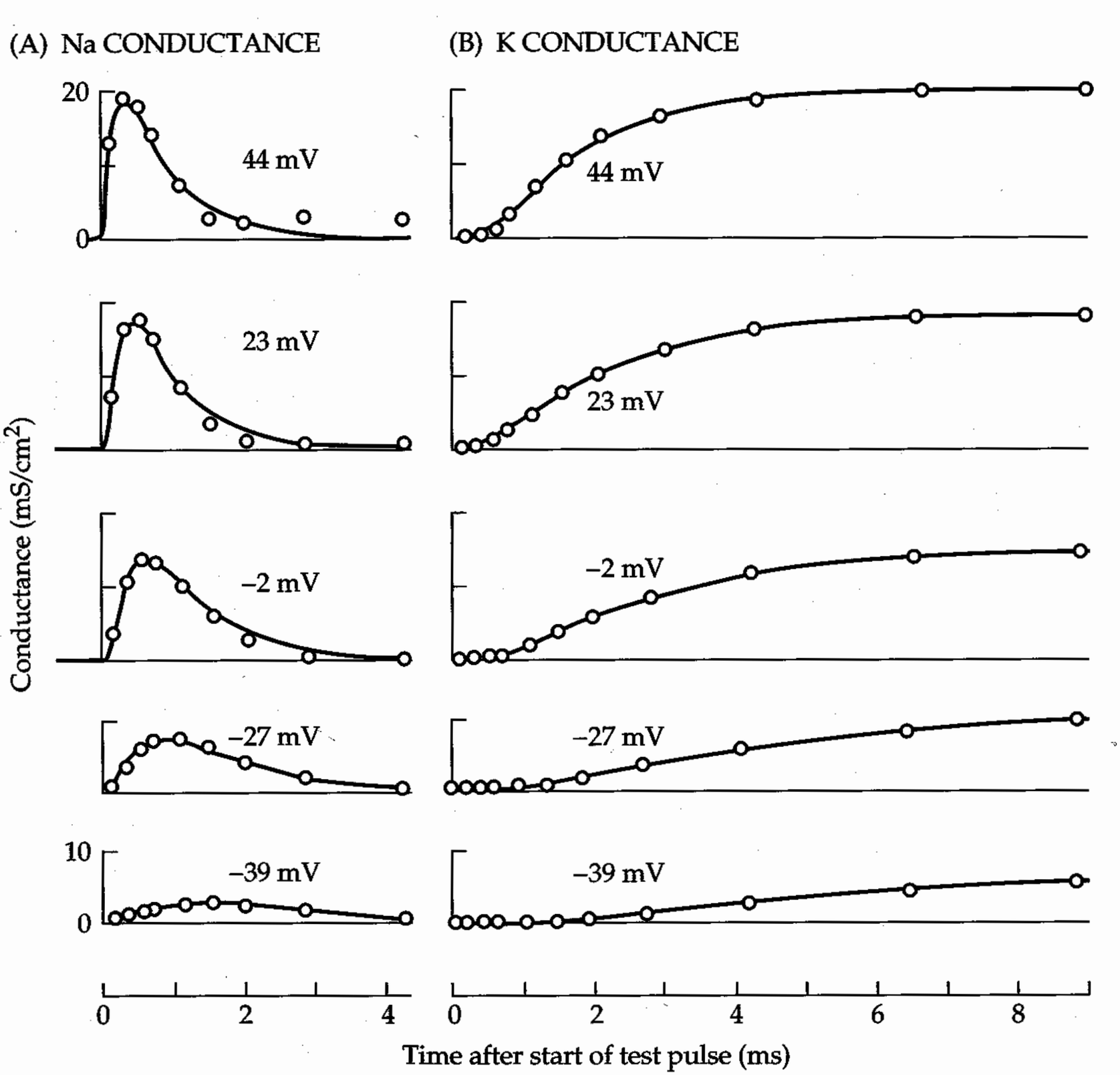
$$g_K = \frac{I_K}{V - E_K}$$

conductances calculated consistent with measured membrane currents assuming Ohmic form

2.11 Ionic Conductance Changes in a Squid Axon Time courses of sodium and potassium conductance changes during a depolarizing voltage step to –9 mV. Conductances calculated by Equations 2.2 and 2.3 from the separated current traces in Figure 2.8. Dashed lines show how g_{Na} decreases rapidly to resting levels if the membrane is repolarized to –65 mV at 0.63 ms when g_{Na} is high, and how g_K decreases more slowly if the membrane is repolarized at 6.3 ms when g_K is high. T = 8.5°C. [From Hodgkin 1958; adapted from Hodgkin and Huxley 1952a,b,d.]

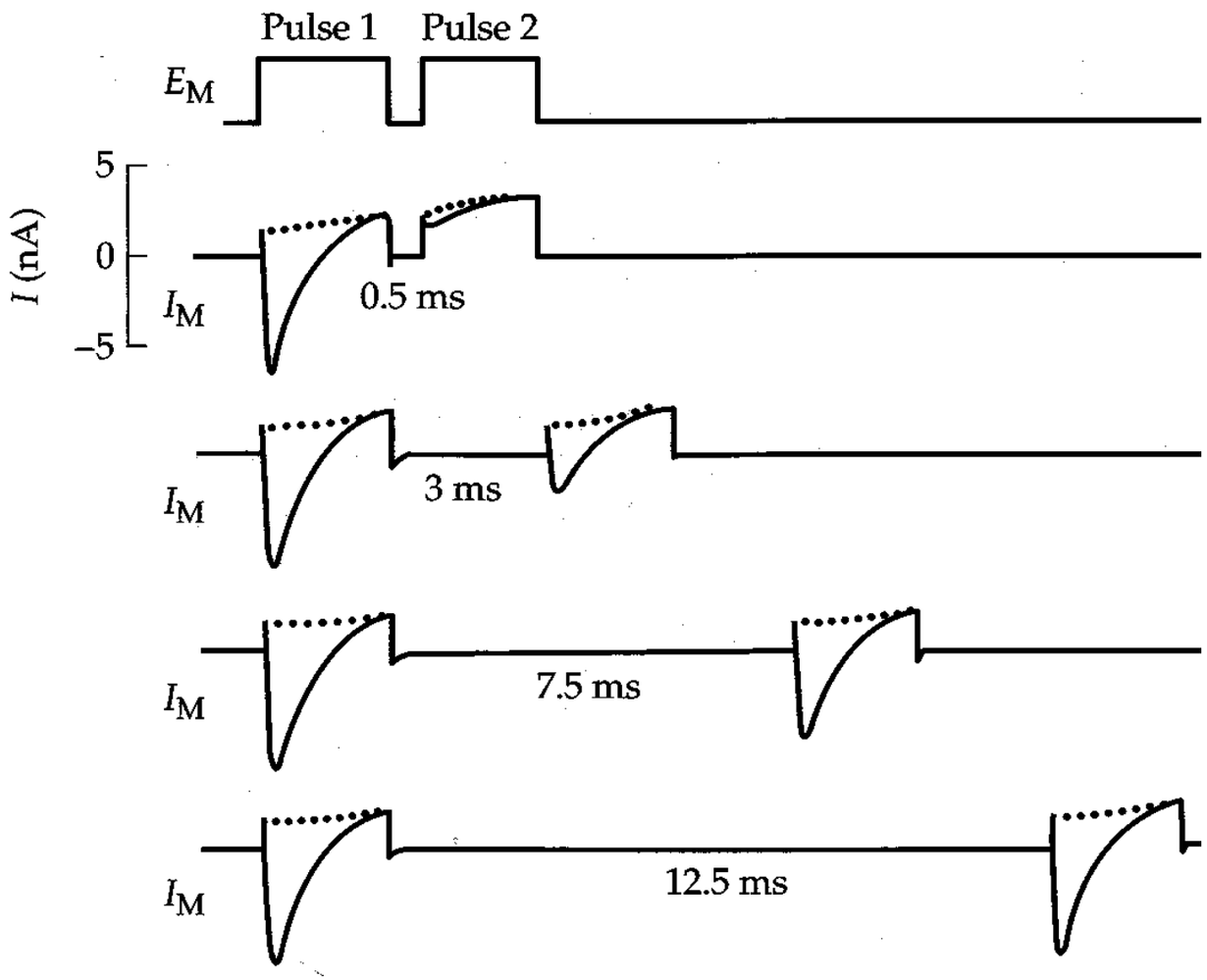
Aren't the Na plots upside down?

No, we are plotting <u>conductance</u> which is non-negative

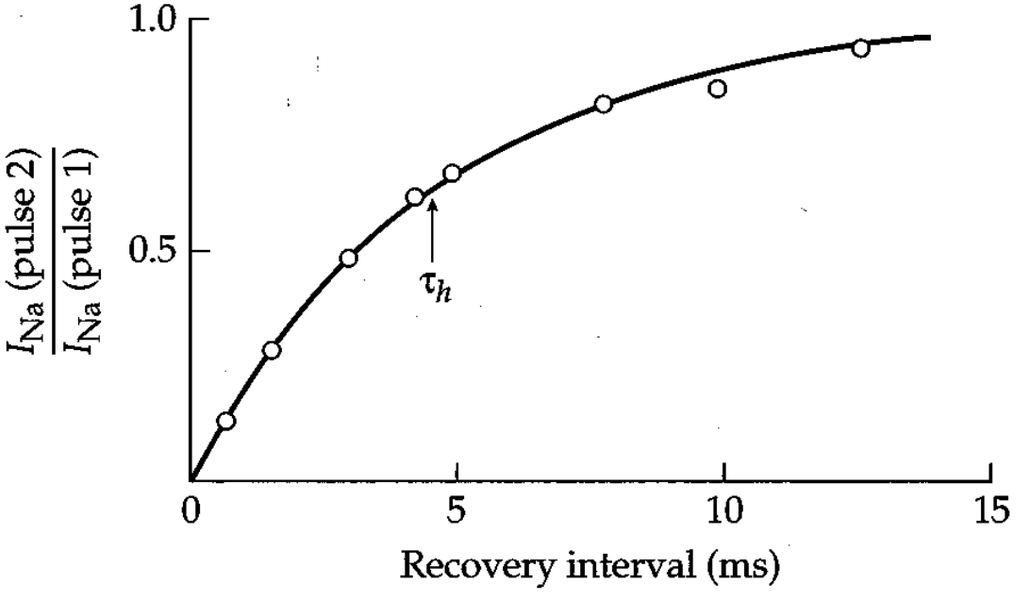


2.12 Conductance Changes at Many Voltages Time courses of g_{Na} (A) and g_K (B) during depolarizing steps to the indicated voltages. Circles are the ionic conductances measured in a squid giant axon at 6.3°C. Smooth curves are the conductance changes calculated from the Hodgkin-Huxley model. [From Hodgkin 1958; adapted from Hodgkin and Huxley 1952d.]

(A) TWO-PULSE EXPERIMENT



(B) RECOVERY CURVE



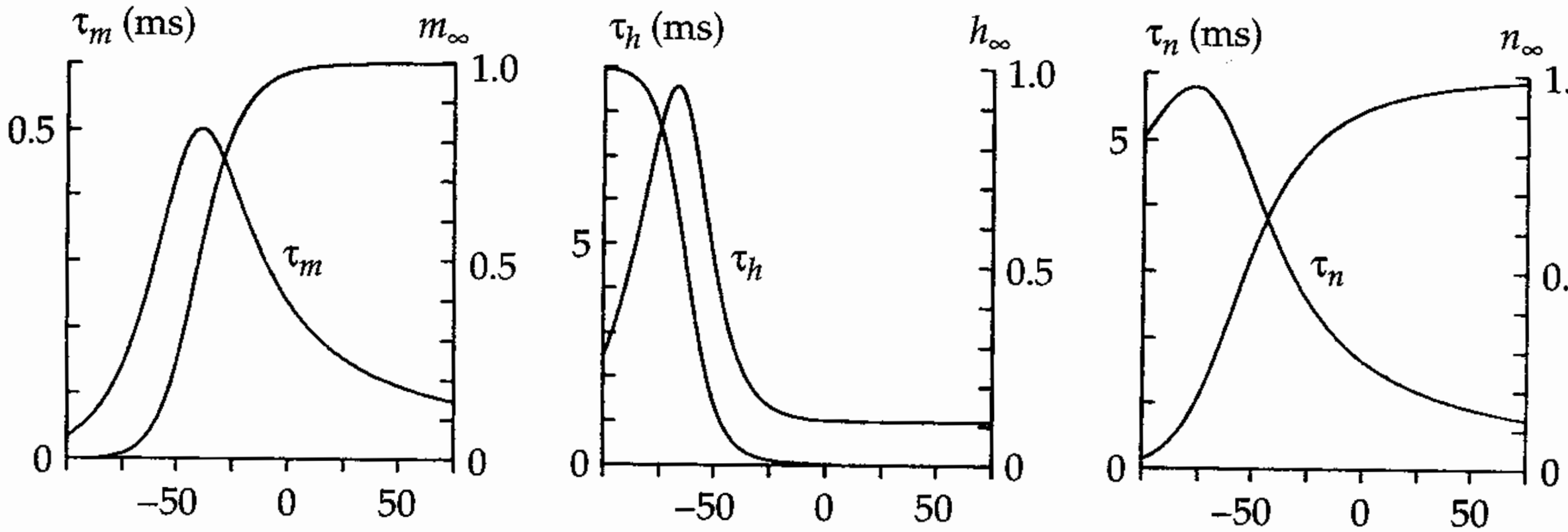
2.15 Recovery from Sodium Inactivation A two-pulse experiment measuring the time course of recovery from sodium inactivation in a frog node of Ranvier. (A) The first pulse to –15 mV activates and inactivates Na channels. During the interpulse interval, some channels recover from inactivation. The second pulse determines what fraction have recovered in that time. Dotted lines show the estimated contribution of potassium and leak currents to the total current. (B) Relative peak I_{Na} recovers with an approximately exponential time course (τ_h = 4.6 ms) during the interpulse interval at –75 mV. T = 19°C. [From Dodge 1963.]

Experimental measurements of the time constant for recovery of sodium current

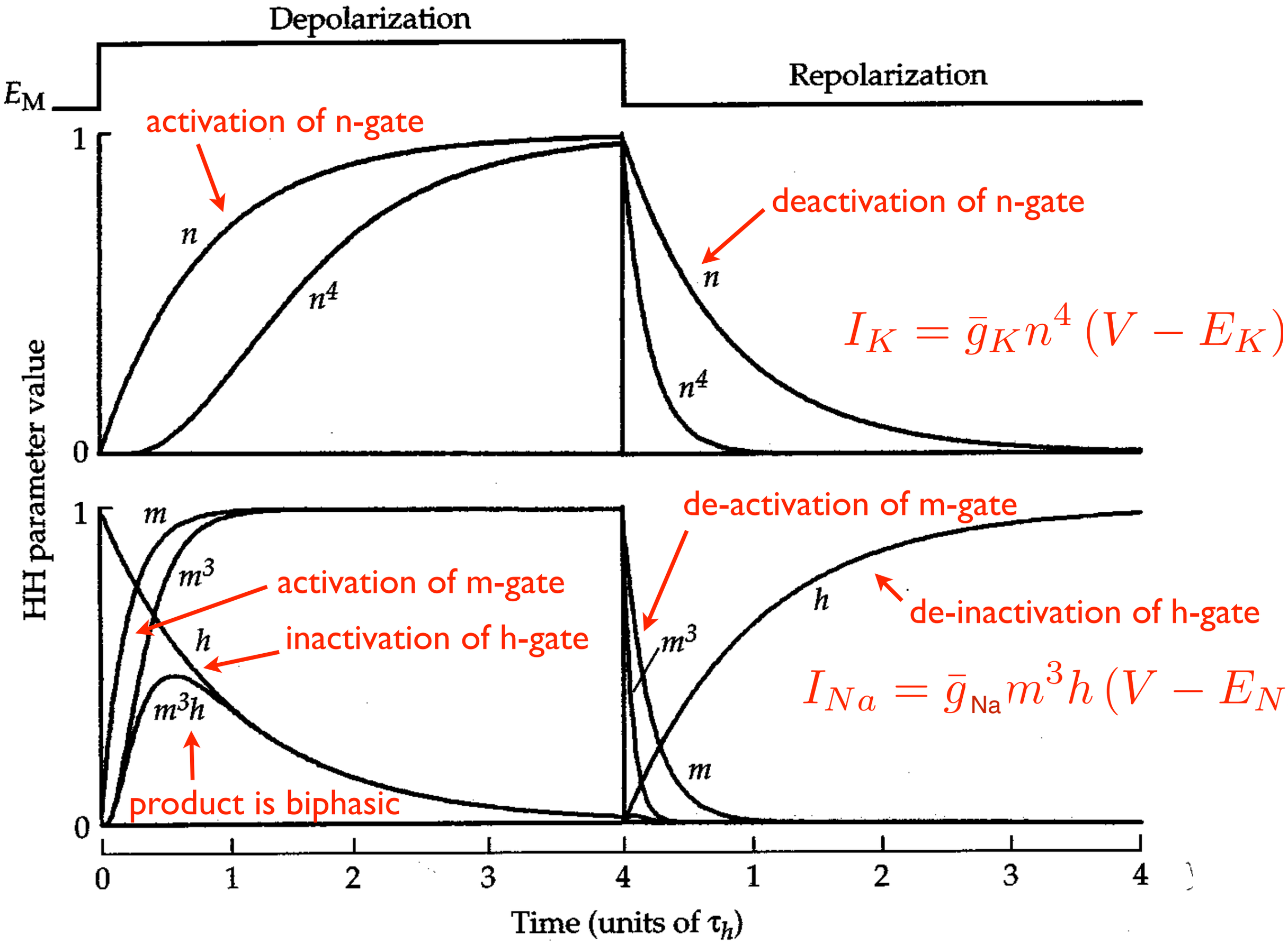
m
Na act

h
Na inact

n
K act



2.17 Voltage-Dependent Parameters of the HH Model Time constants τ_m, τ_h, and τ_n and steady-state values m_∞, h_∞, and n_∞ calculated from the empirical equations of the Hodgkin-Huxley model for squid giant axon membrane at 6.3°C. Depolarizations increase m_∞ and n_∞ and decrease h_∞. The time constants of relaxation are maximal near the resting potential and become shorter on either side. [From Hille 1970.]


Depolarization
Repolarization
E_M
activation of n-gate
deactivation of n-gate
1
0
n
n^4
$I_K = \bar{g}_K n^4 (V - E_K)$
HH parameter value
m
m^3
h
m^3h
activation of m-gate
inactivation of h-gate
de-activation of m-gate
de-inactivation of h-gate
product is biphasic
$I_{Na} = \bar{g}_{\mathrm{Na}} m^3 h (V - E_{Na})$
0 1 2 3 4 1 2 3 4
Time (units of τ_h)

$$I_{Na} = \bar{g}_{Na} m^3 h (V - E_{Na})$$

fast slow

$$I_K = \bar{g}_K n^4 (V - E_K)$$

fast

activation gate (m & n) fast

1

0

activated

activation

de-activation

de-activated

inactivation gate (h) slow

1

0

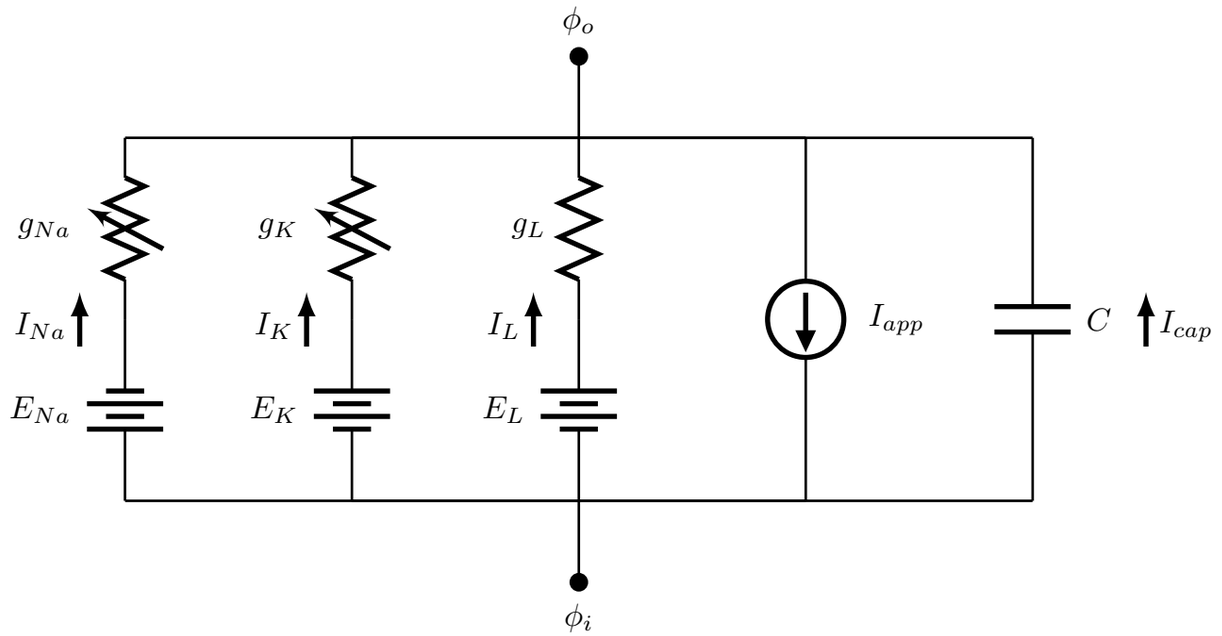
de-inactivated

inactivation

de-inactivation

inactivated

Hodgkin-Huxley model of action potential in squid giant axon

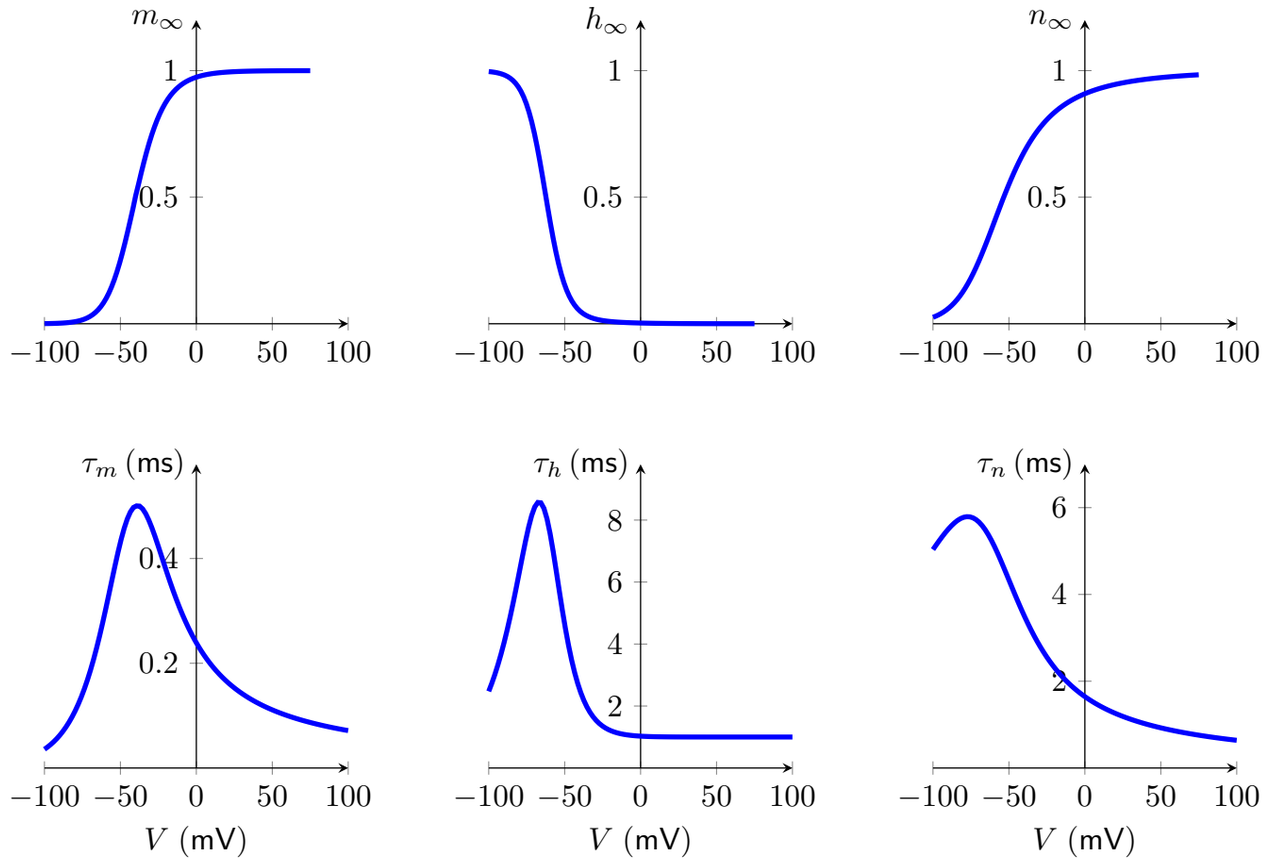


$$C\frac{dV}{dt} = I_{app} - g_{\mathrm{Na}} m^3 h \left(V - E_{\mathrm{Na}}\right) - g_{\mathrm{K}} n \left(V - E_{\mathrm{K}}\right) - g_{\mathrm{L}} \left(V - E_{\mathrm{L}}\right)$$

$$\frac{dm}{dt} = -\frac{m - m_\infty(V)}{\tau_m(V)}$$

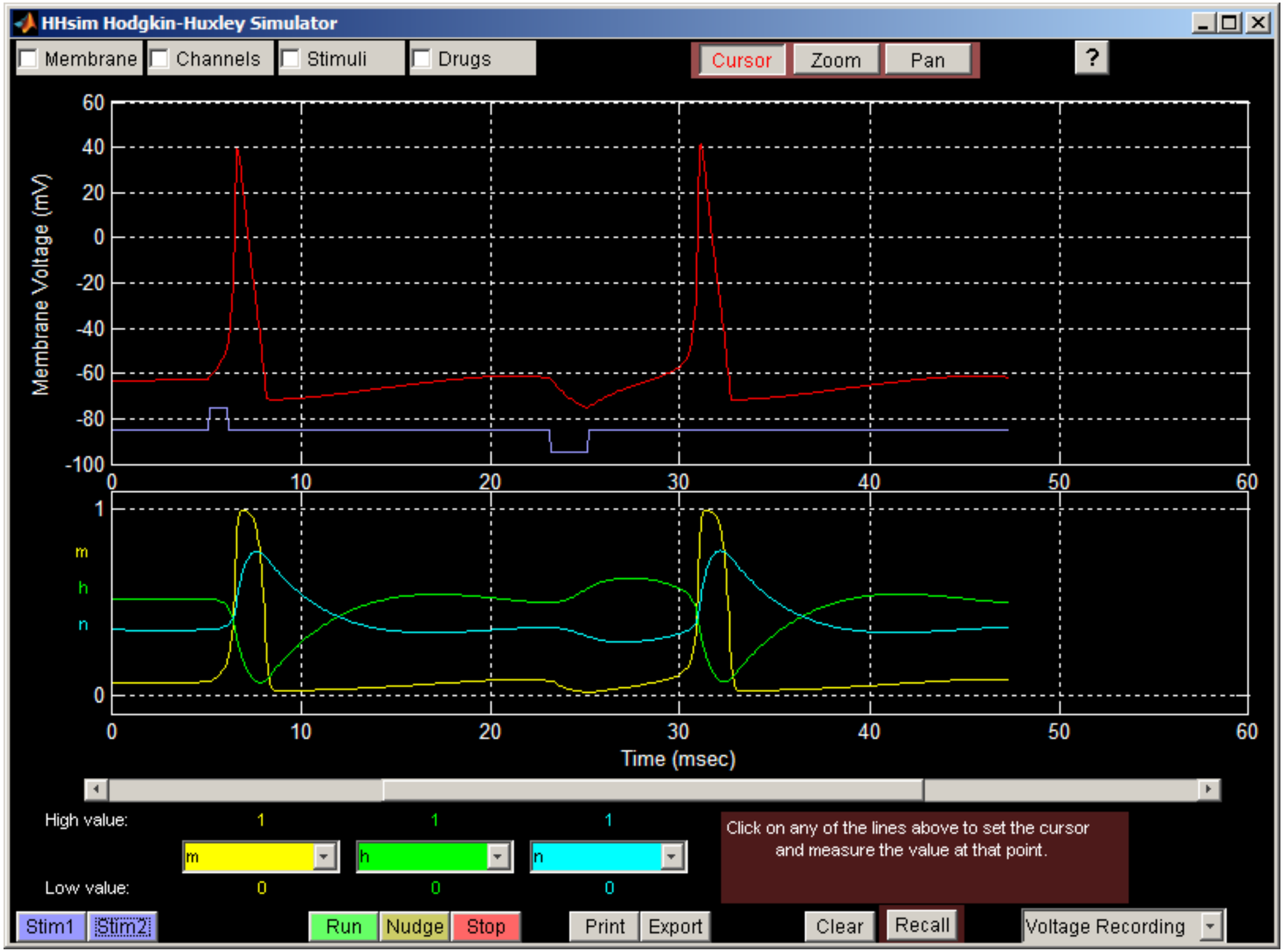
$$\frac{dh}{dt} = -\frac{h - h_\infty(V)}{\tau_h(V)}$$

$$\frac{dn}{dt} = -\frac{n - n_\infty(V)}{\tau_n(V)}.$$

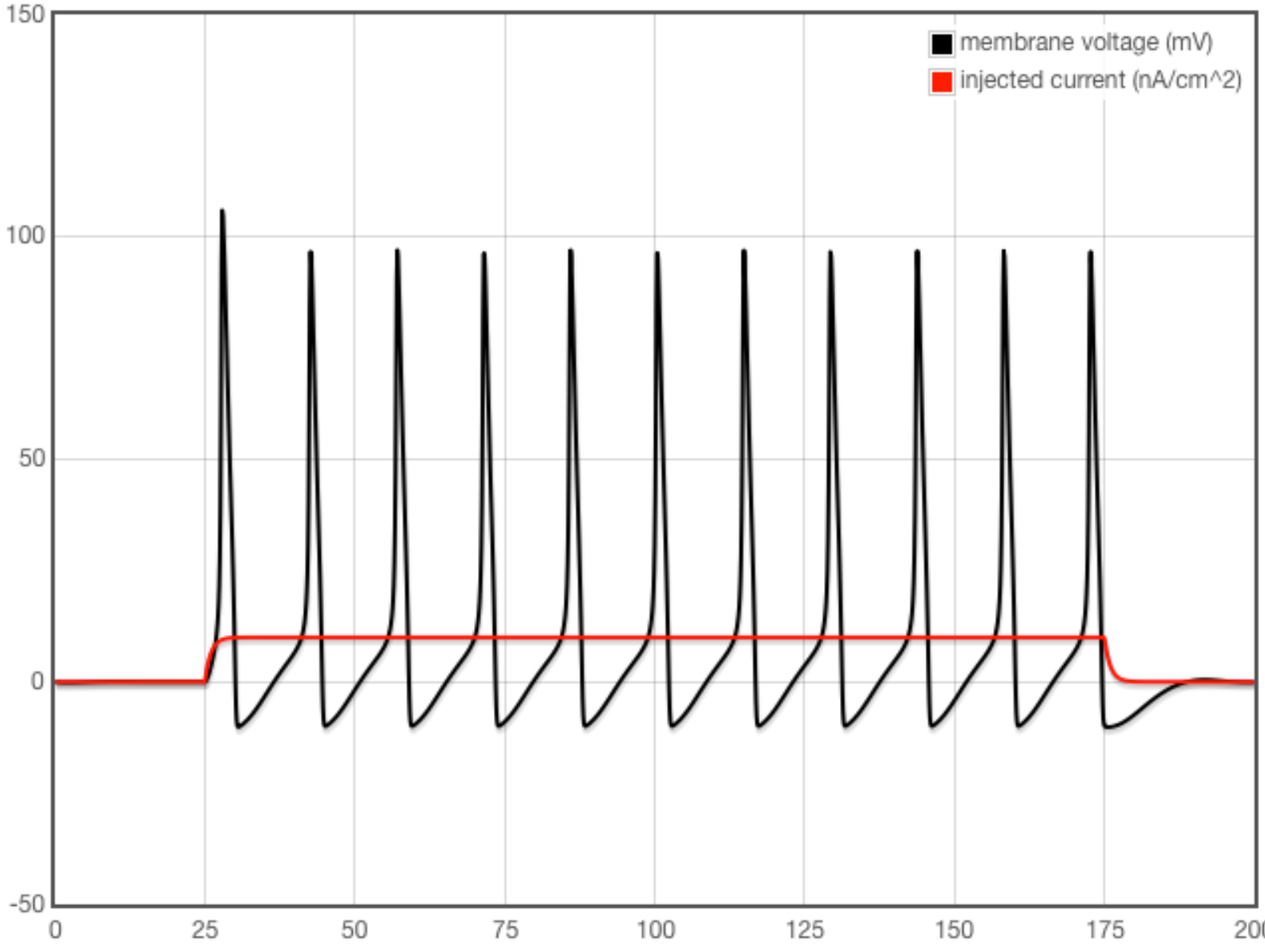


HHsim: Graphical Hodgkin-Huxley Simulator

http://www.cs.cmu.edu/~dst/HHsim/



Hodgkin-Huxley Simulation with Javascript



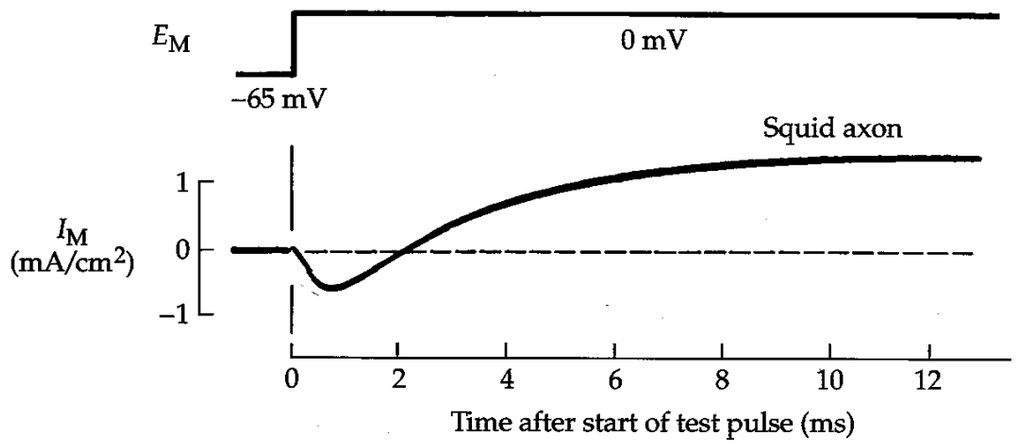
C (muF/cm^2):	1
GKMax (mS/cm^2):	36
GNaMax (mS/cm^2):	120
Gm (mS/cm^2):	0.3
EK (mV):	-12
ENa (mV):	115
VRest (mV):	10.613
V(0) (mV):	0
tStop (ms):	200
tInjStart (ms):	25
tInjStop (ms):	175
IDC (nA/cm^2):	10
IRand (nA/cm^2):	0
Itau (ms):	1

Start

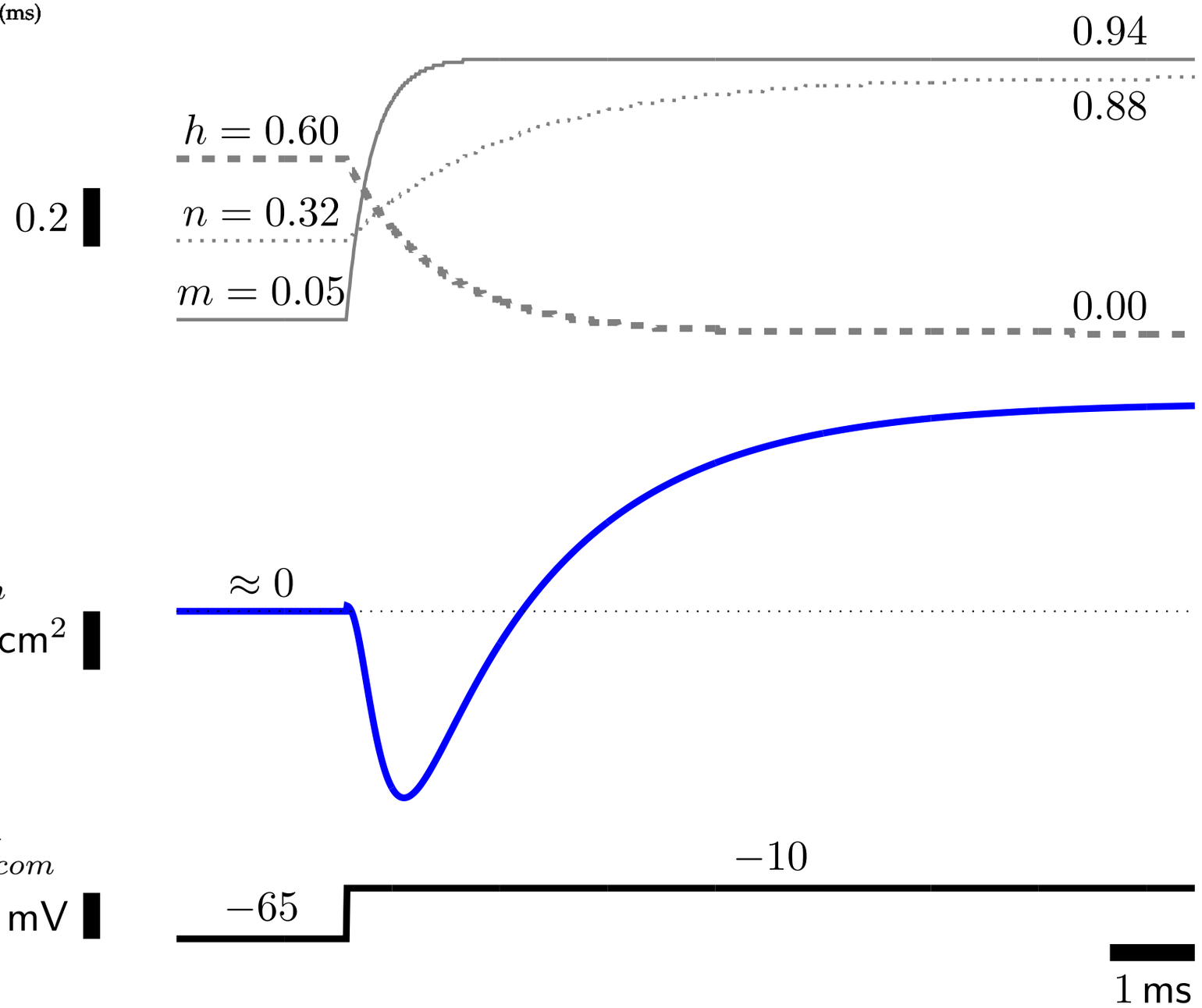
t=0s

A simple browser/Javascript based simulation of Hodgkin's & Huxley's formulas for action potential generation in the squid giant axon. I suppose the source code is mostly self-explaining. I use the parameters from Christof Koch's book Biophysics of Computation, chapter 6, the formulas for forward and backward rates, and the differential equations for the three gating variables. For both these equations and the transmembrane current equation, I use explicit (forward) Euler for discretization and a time step of 0.025ms. The current consists of a DC (IDC) and a random component (uniform distribution (-1; 1), scaled by IRand), lowpass-filtered (1st order RC) with Itau.
Please drop me an email if there's anything wrong or unclear.

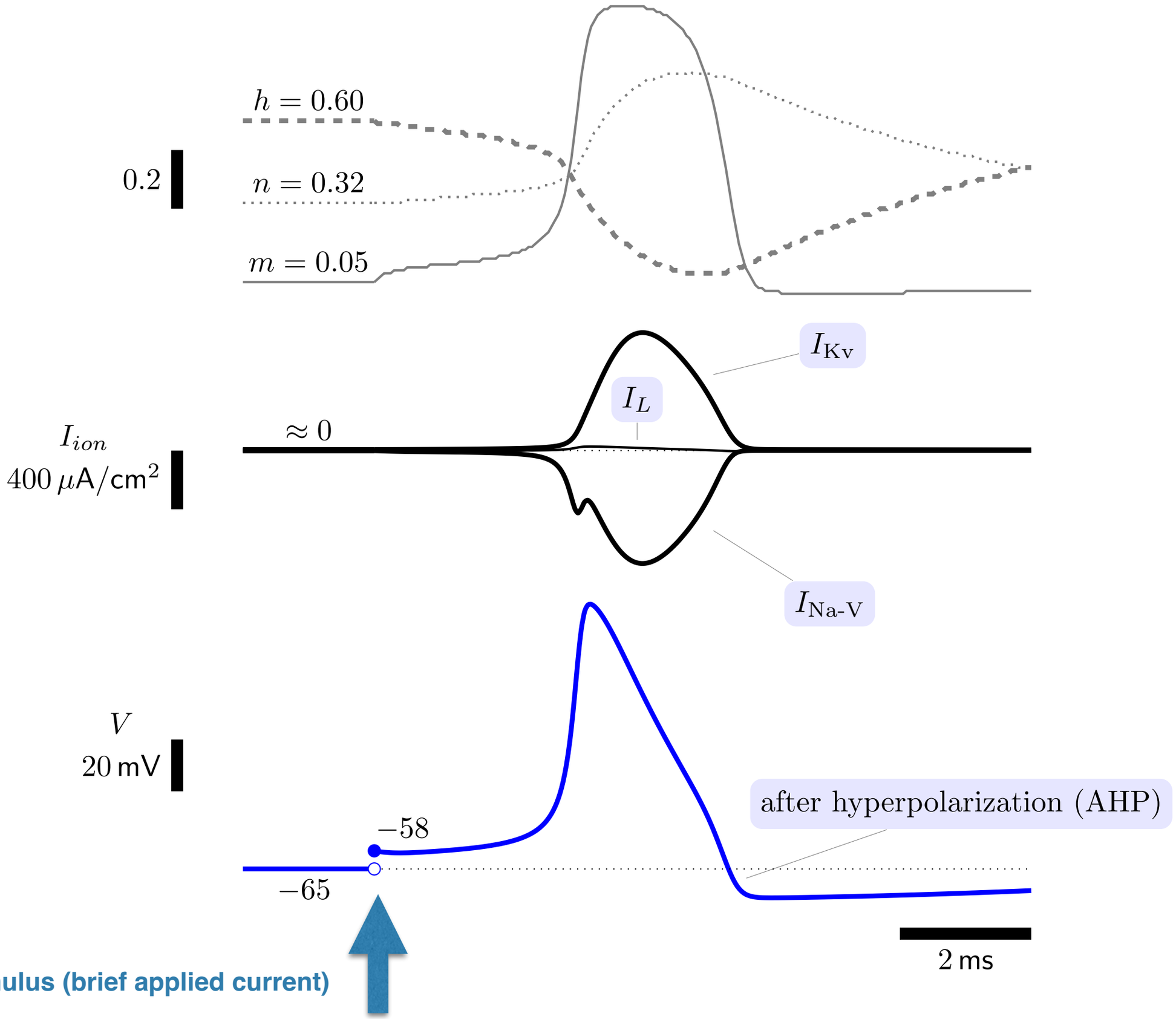
http://myselph.de/hodgkinHuxley.html



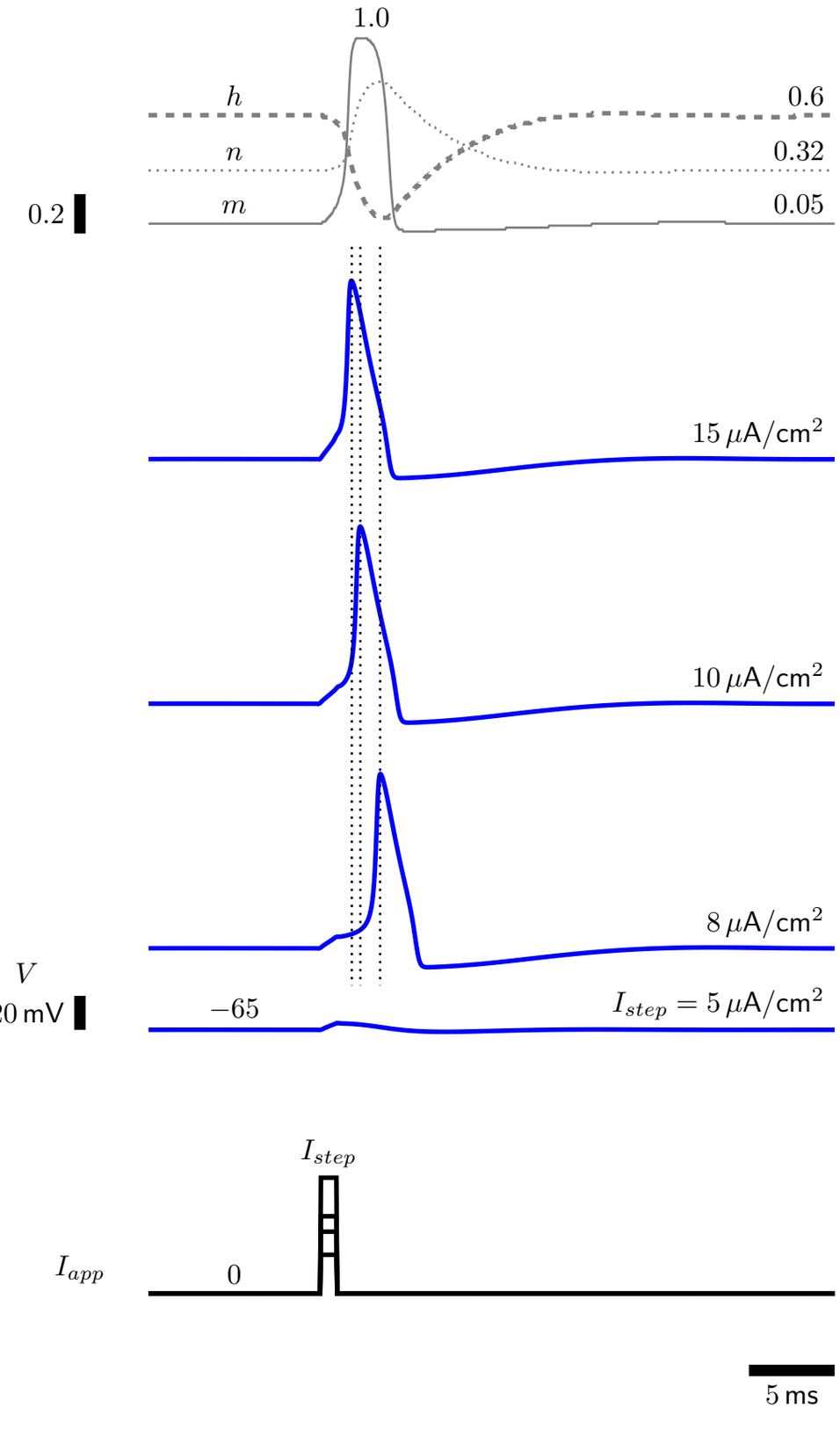
gating variable dynamics reproduce voltage-clamp recording (as expected)



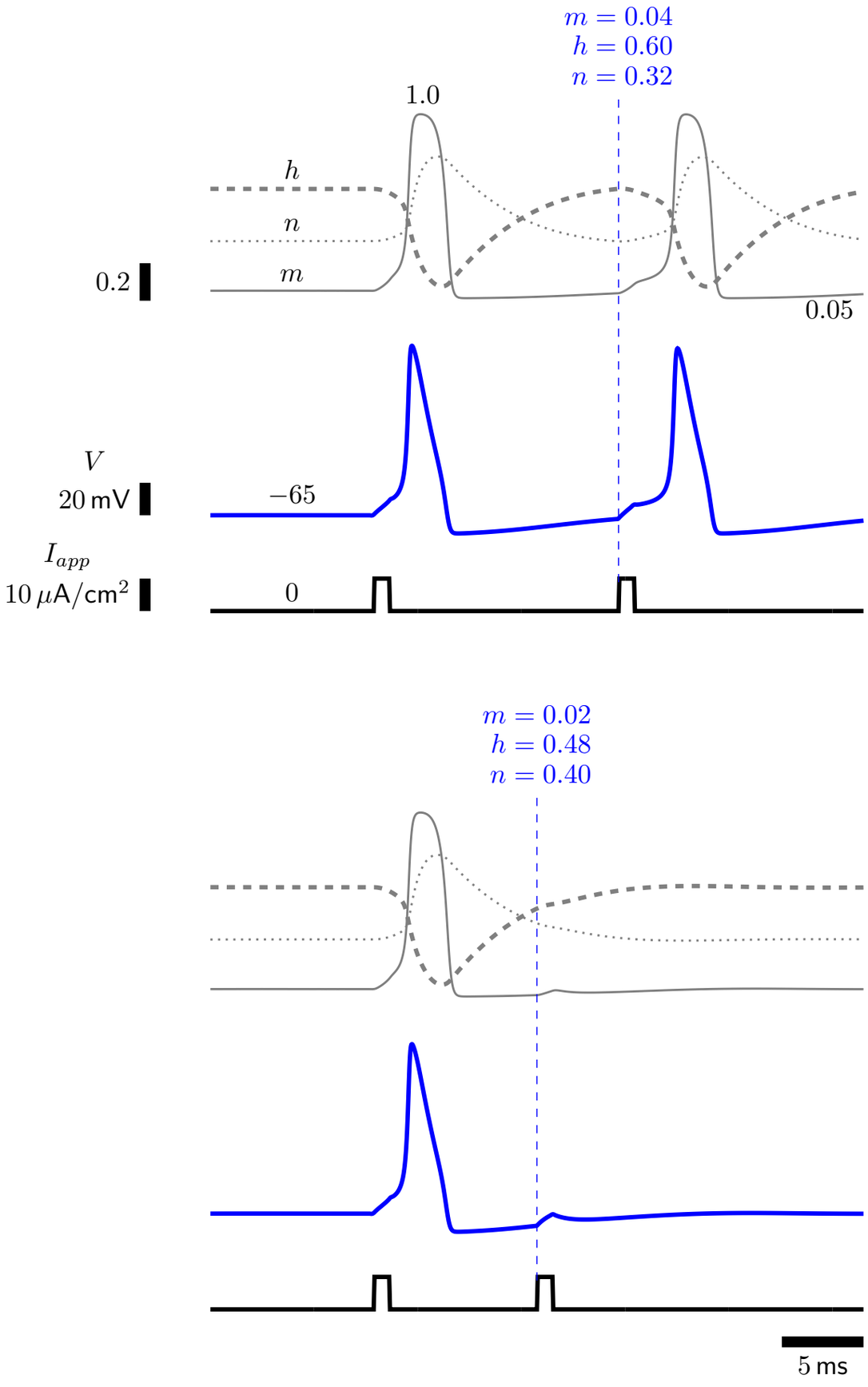
gating and currents predicted during action potential



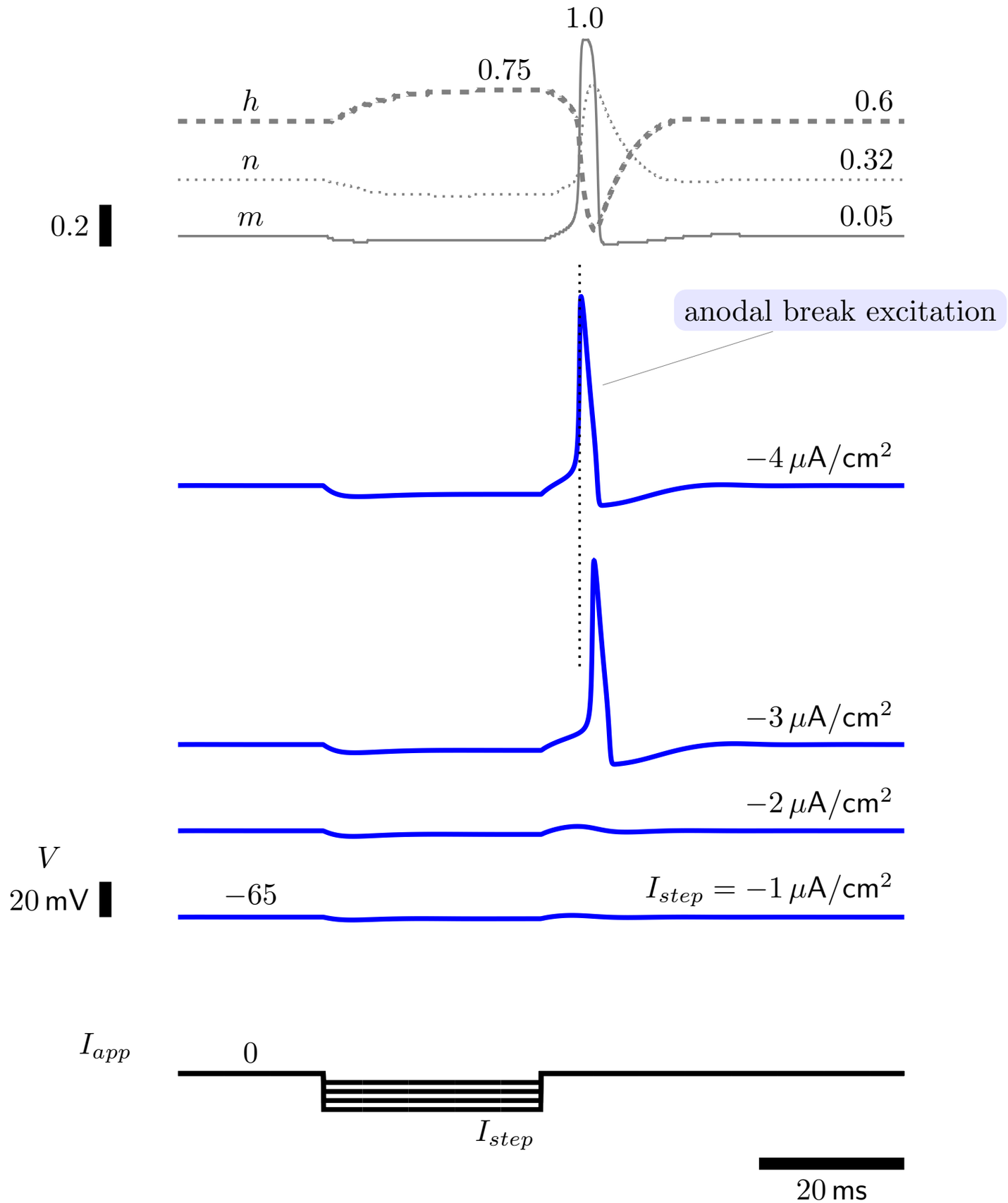
latency depends on stimulus



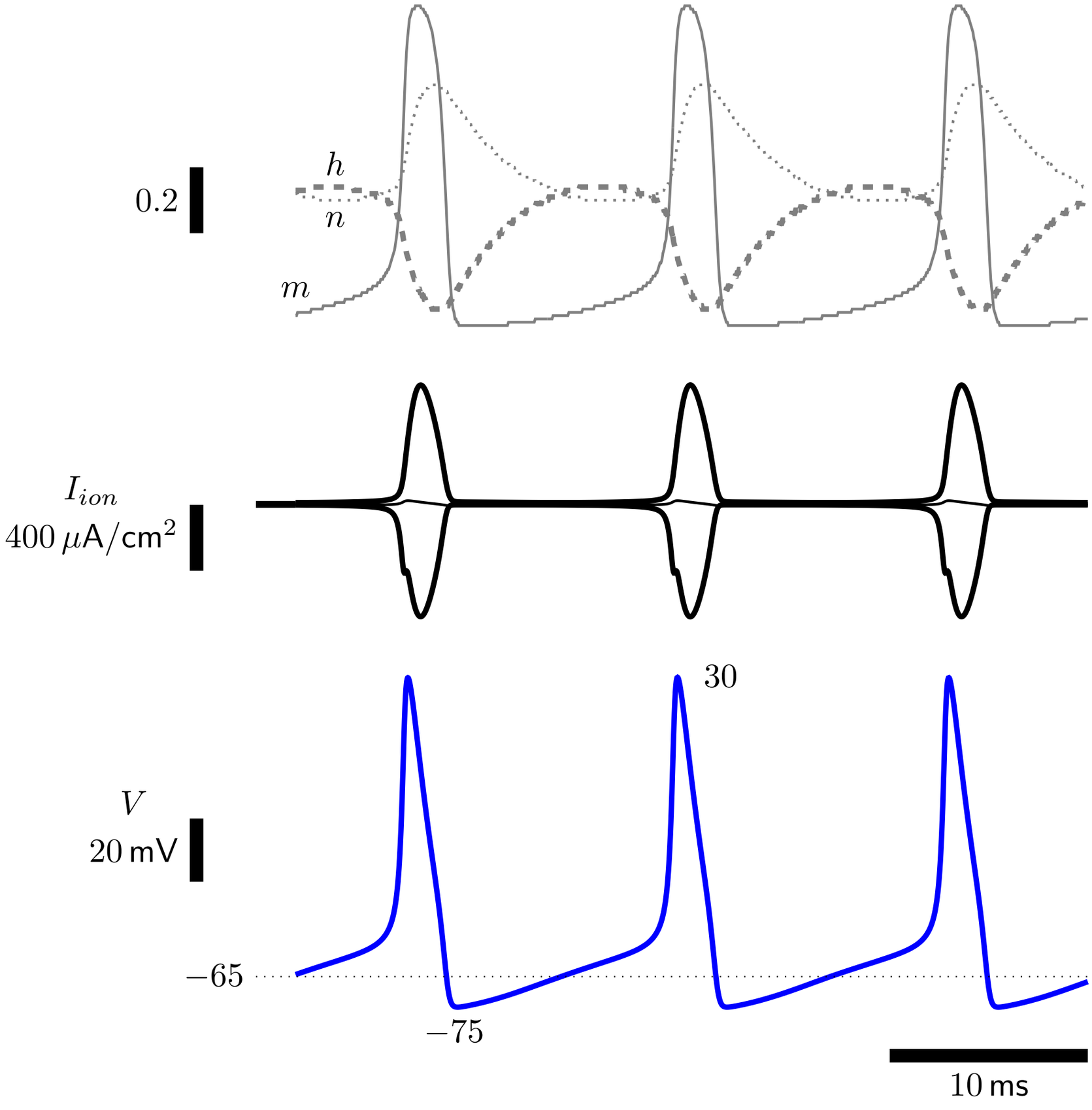
absolute refractory period



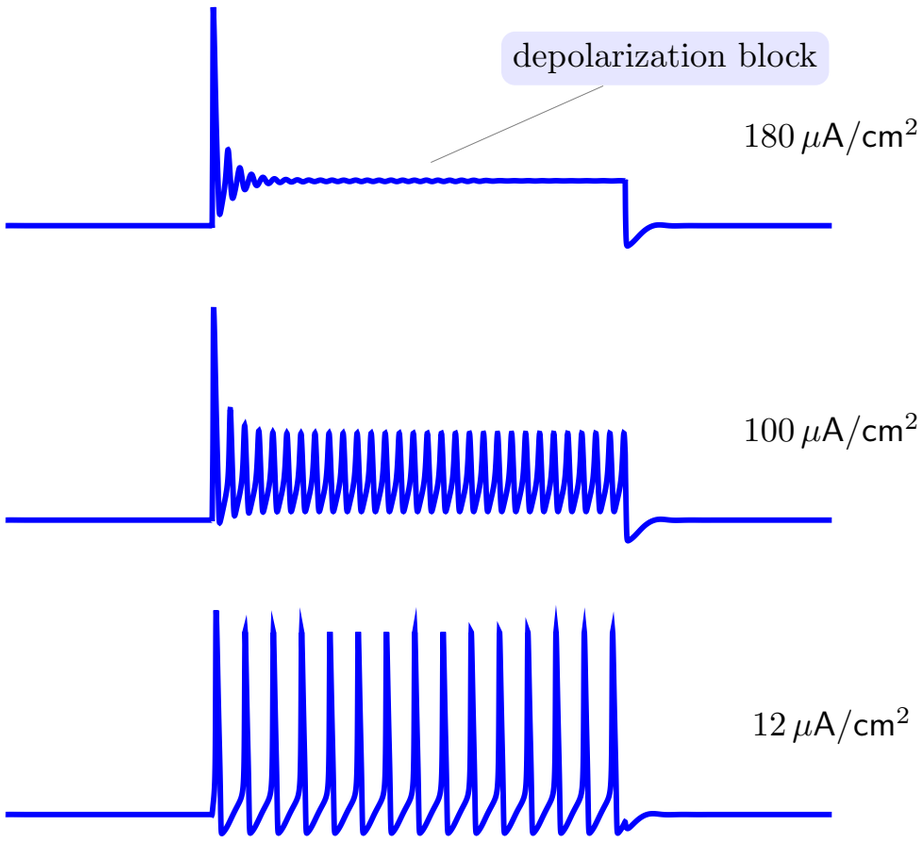
model suggests reason for post-anodal break excitation

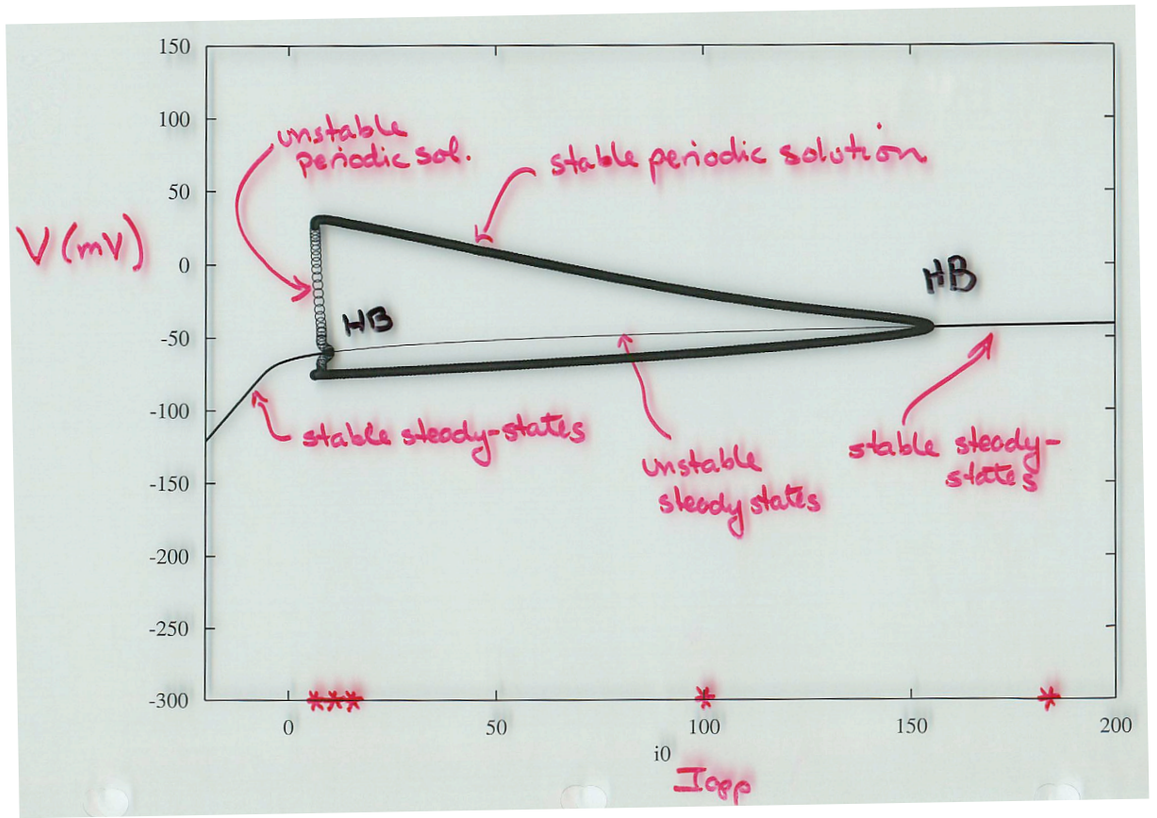


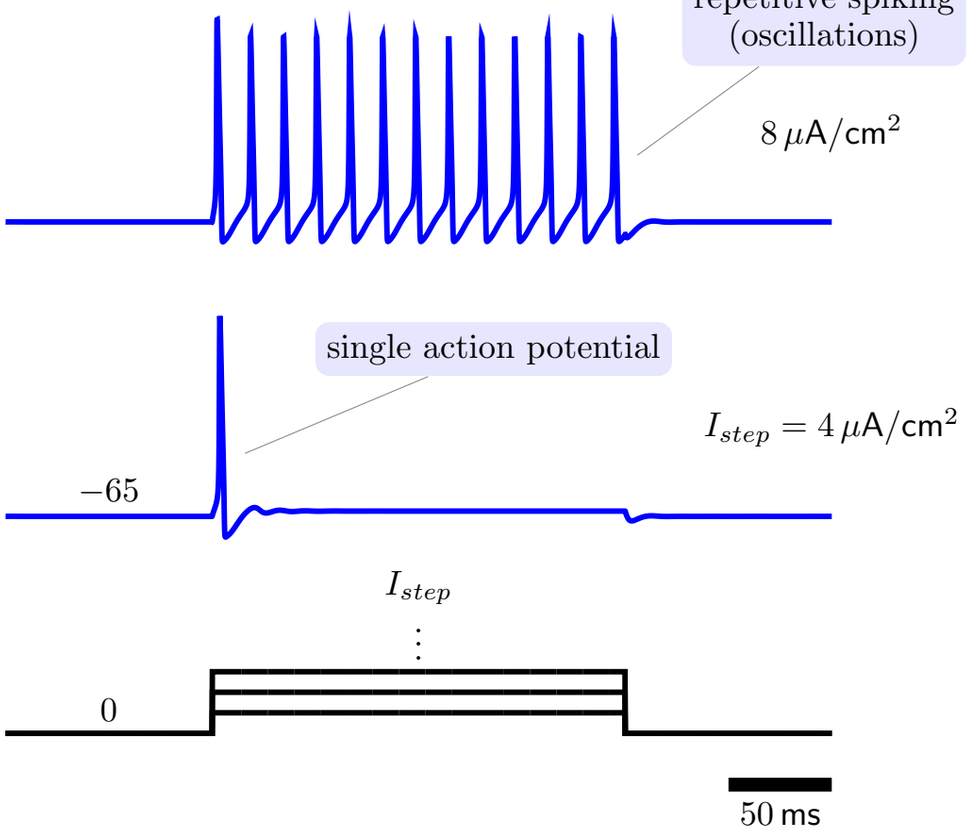
super-threshold applied current leads to repetitive spiking

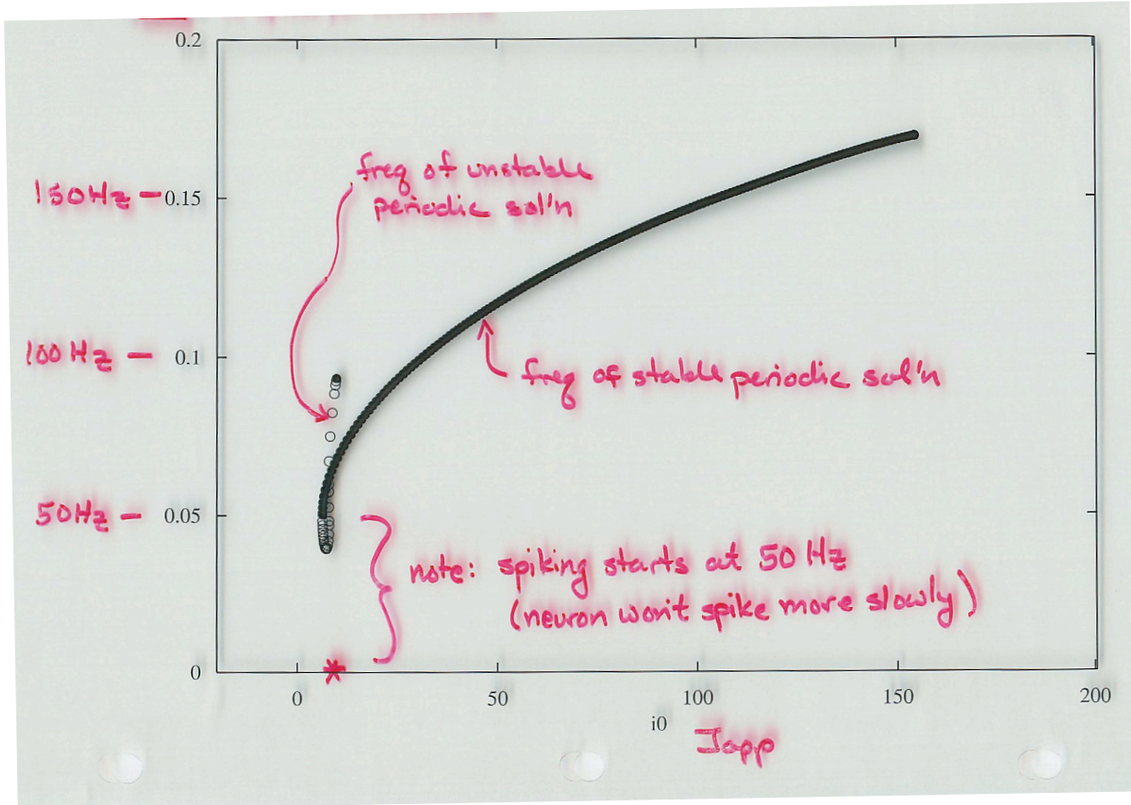


numerical bifurcation analysis of repetitive spiking









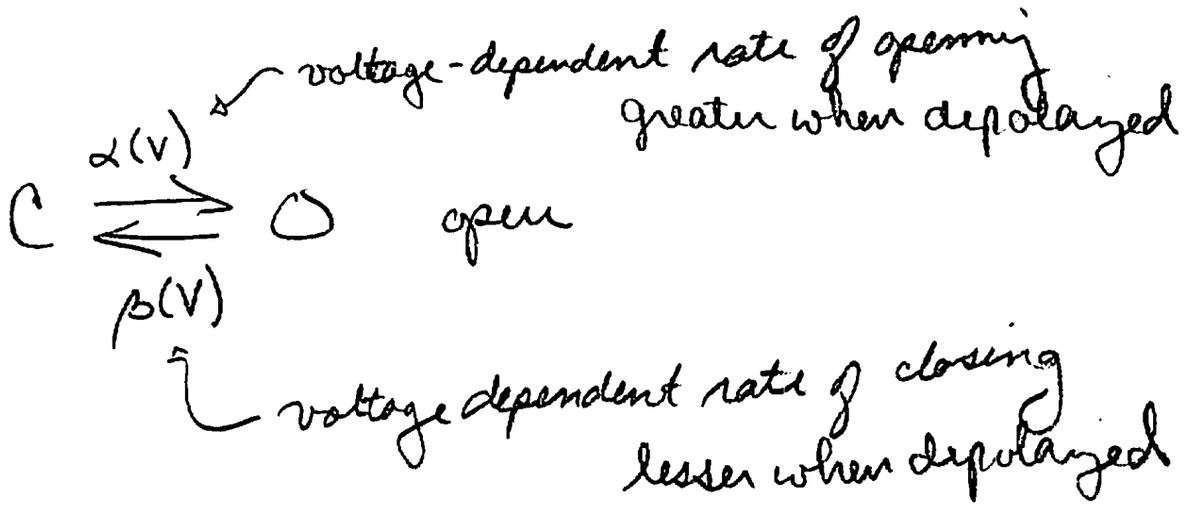
We can **numerically integrate** the HH model, that is, perform **simulations** and compare/contrast theory with experiment.

But how are we going to **analyze** and truly **understand** this model composed of **four** ODEs?

We will study the **Morris-Lecar model** of excitable barnacle muscle fibers in detail. It's a 3 variable model that we can reduce to 2 variables and study in the **phase plane**.

We will study membrane excitability and oscillations using **phase planes**, **linear stability analysis**, **bifurcation theory** for 2D systems, and the **Fitzhugh-Nagumo model**.

Justification for HH-style gating variable equations



$$\frac{dP_o}{dt} = \alpha(V)\,P_c - \beta(V)\,P_o$$

$$\frac{dP_c}{dt} = -\alpha(V)\,P_c + \beta(V)\,P_o$$

$$\Rightarrow \frac{dP_o}{dt} + \frac{dP_c}{dt} = 0$$

because $P_o + P_c = 1$

$$\frac{dP_o}{dt} = \alpha(V)\,(1-P_o) - \beta(V)\,P_o$$

(has to be open or closed)

$$P_o^{eq}: \quad 0 = \alpha(V)\,(1-P_o^{eq}) - \beta(V)\,P_o^{eq}$$

$$\alpha(V)\,P_o^{eq} + \beta(V)\,P_o^{eq} = \alpha(V)$$

$$P_o^{eq} = \frac{\alpha(V)}{\alpha(V)+\beta(V)}$$

$$\frac{dP_o}{dt} = -\frac{P_o - P_o^{eq}(V)}{\tau(V)} \quad \text{where } \tau(V) = \frac{1}{\alpha(V)+\beta(V)}$$