Action potentials and electrical excitability

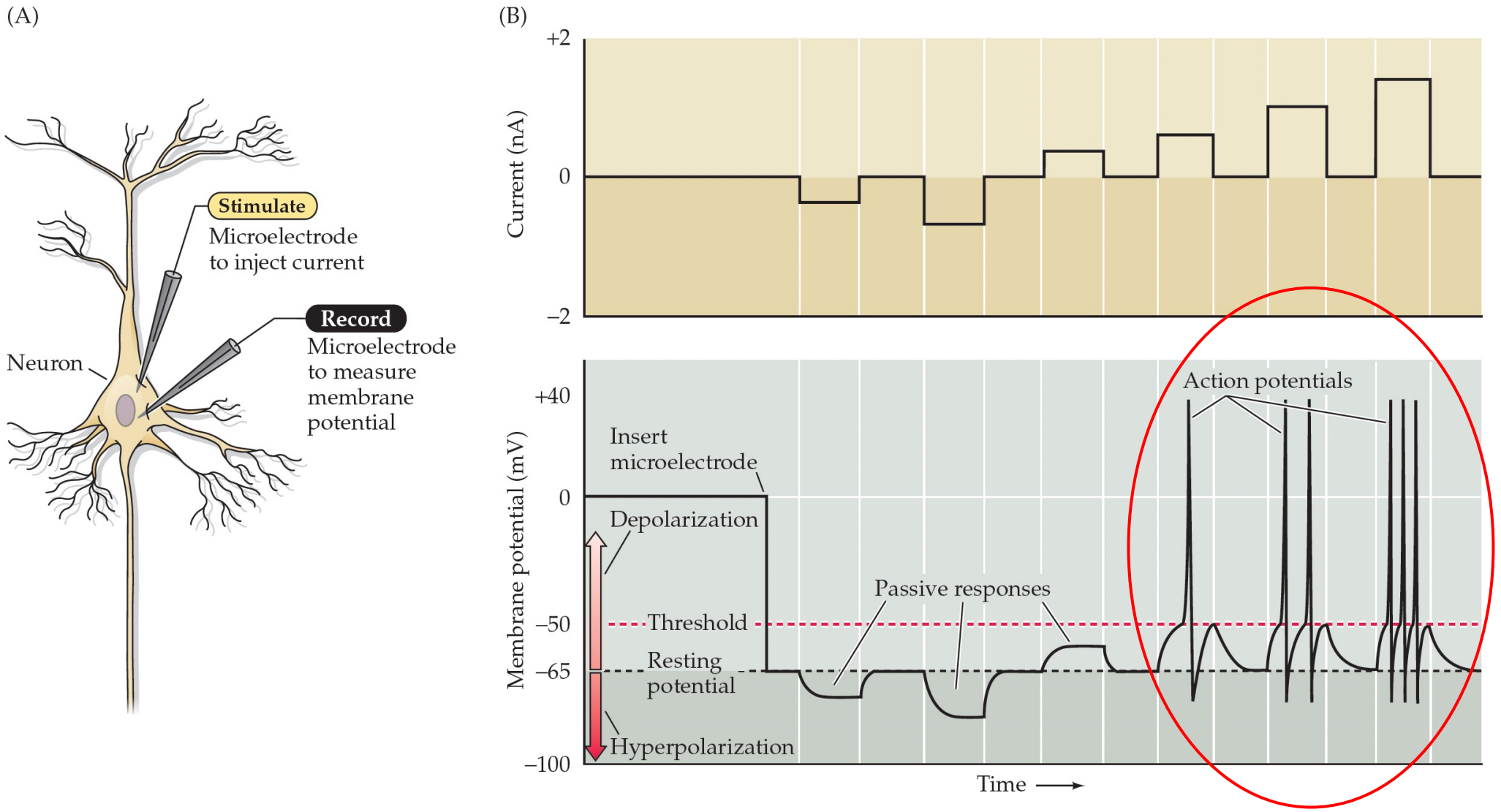
Prof. Pavan Ramdya

Reading: ***Purves* Chapter 3** all pp.; **Chapter 4** pp. 65-72

BIO-311

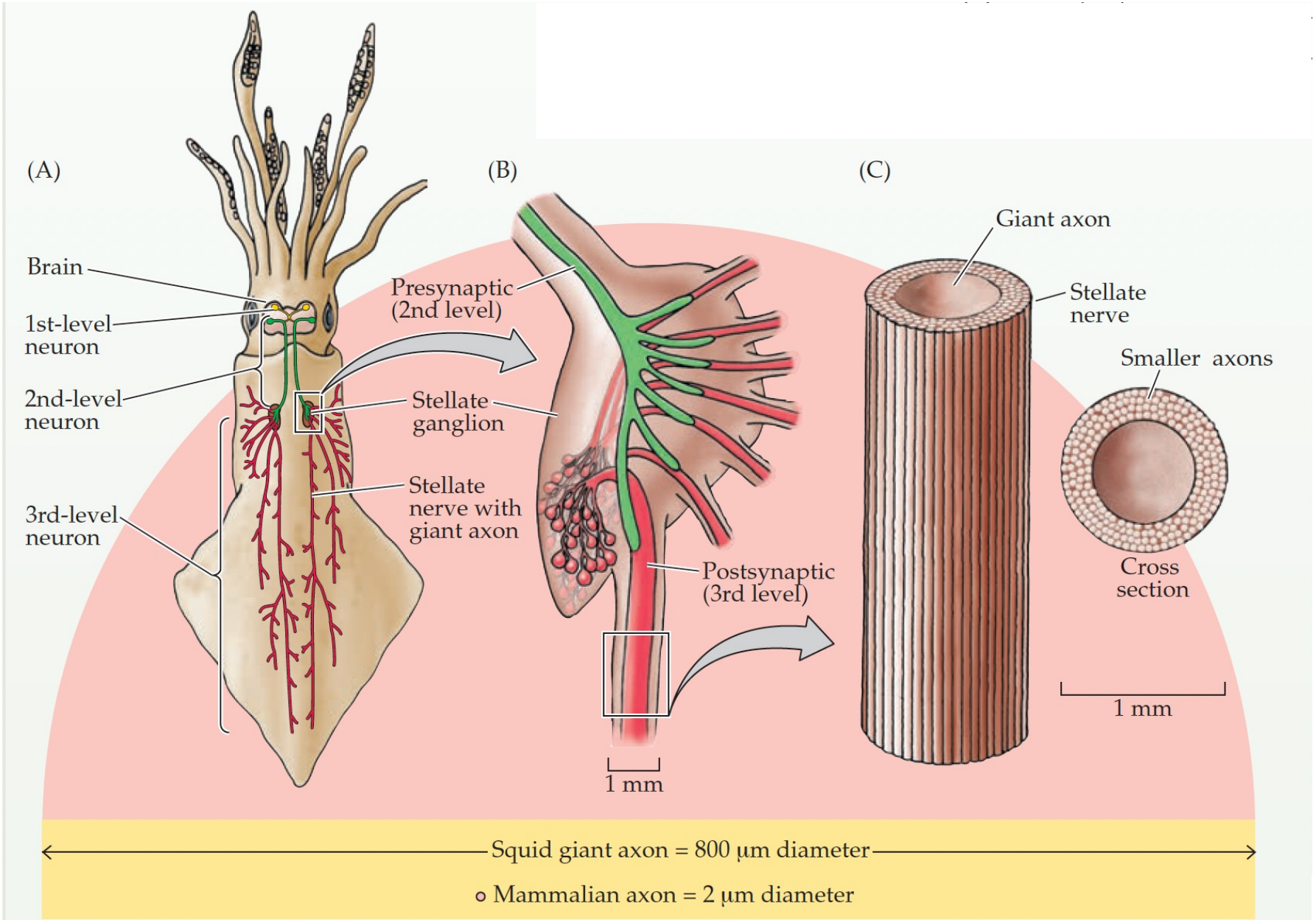
Image credit:
https://www.britannica.com/story/can-lightning-strike-the-same-place-twice

The action potential, an active membrane potential response



Purves, Figure 2.2

Insights drawn from the squid's 'giant axon'



Purves, Box 2A

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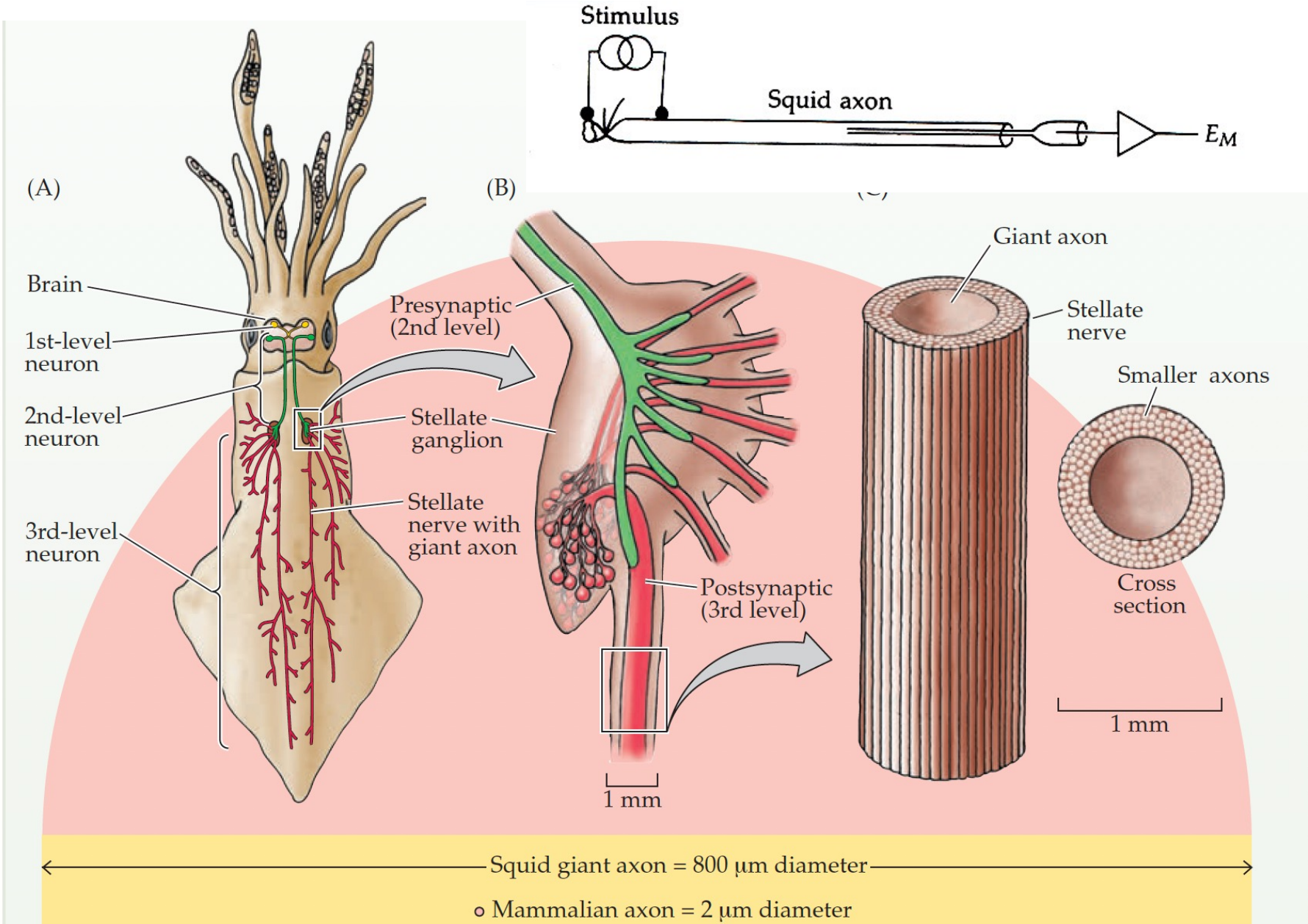
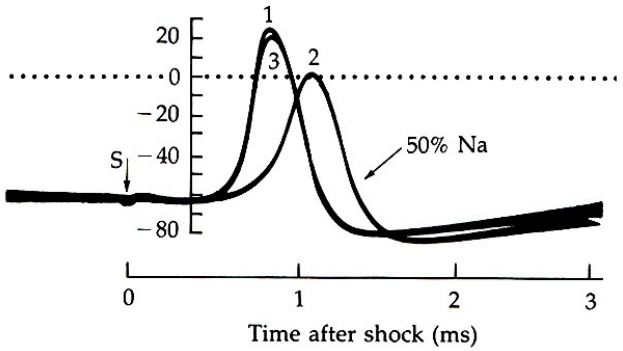


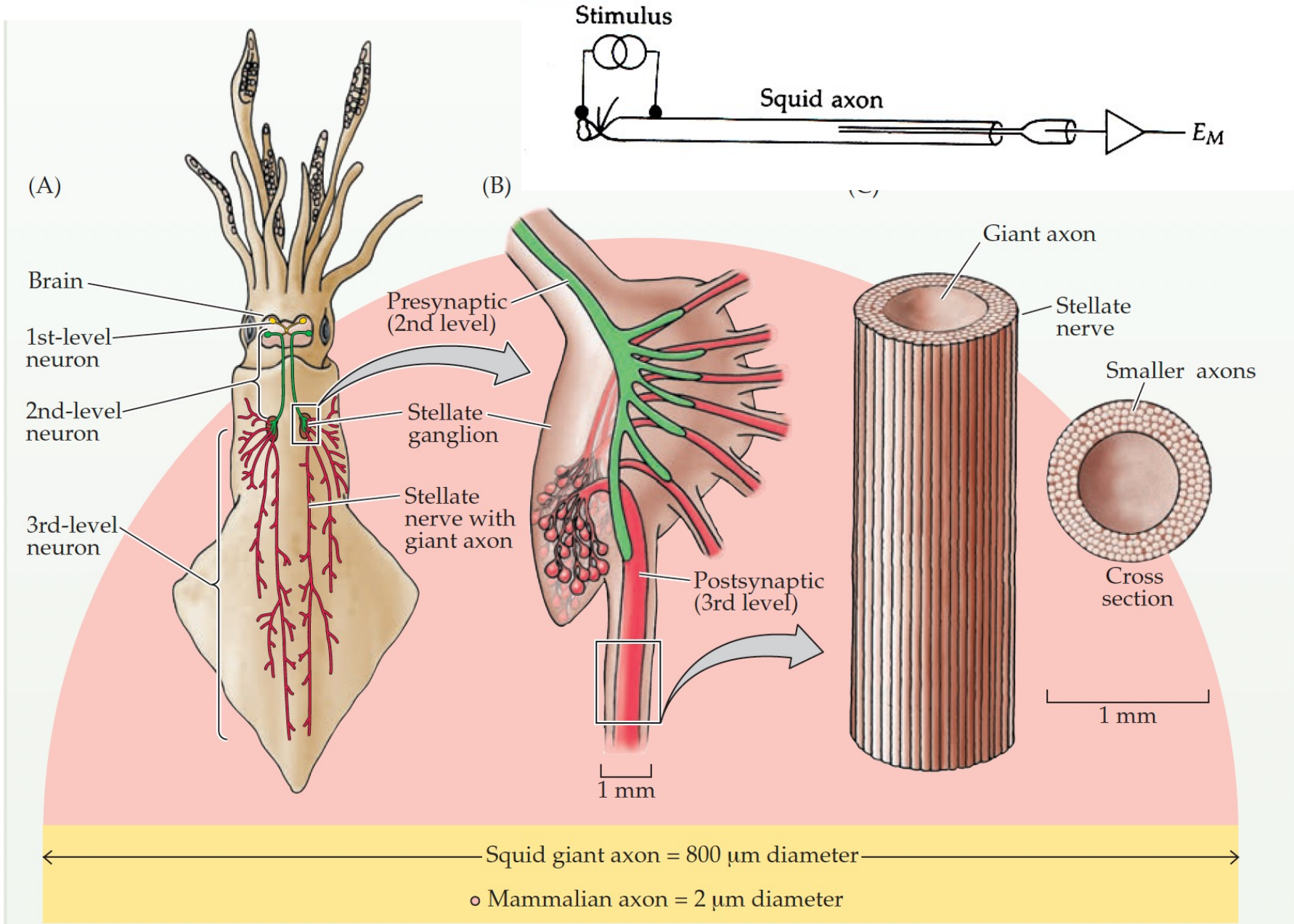
- Intracellular recording of V_m
- An example of "current clamp"



Hille, Figure 2.4

Purves, Box 2A

Insights drawn from the squid's 'giant axons'



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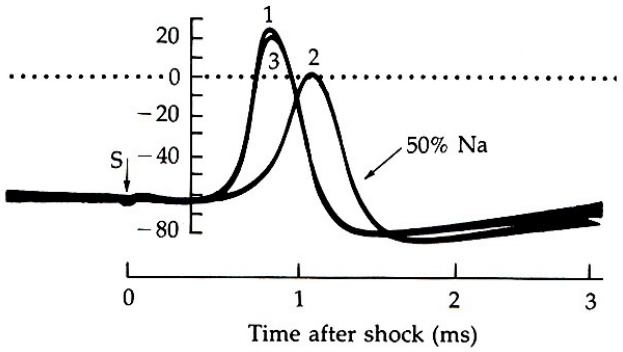
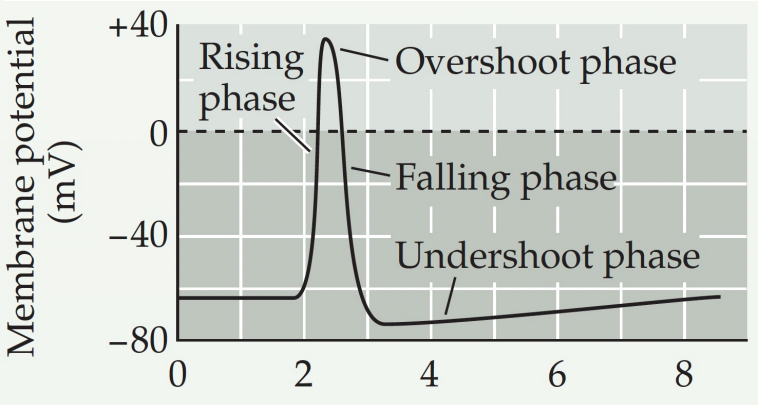


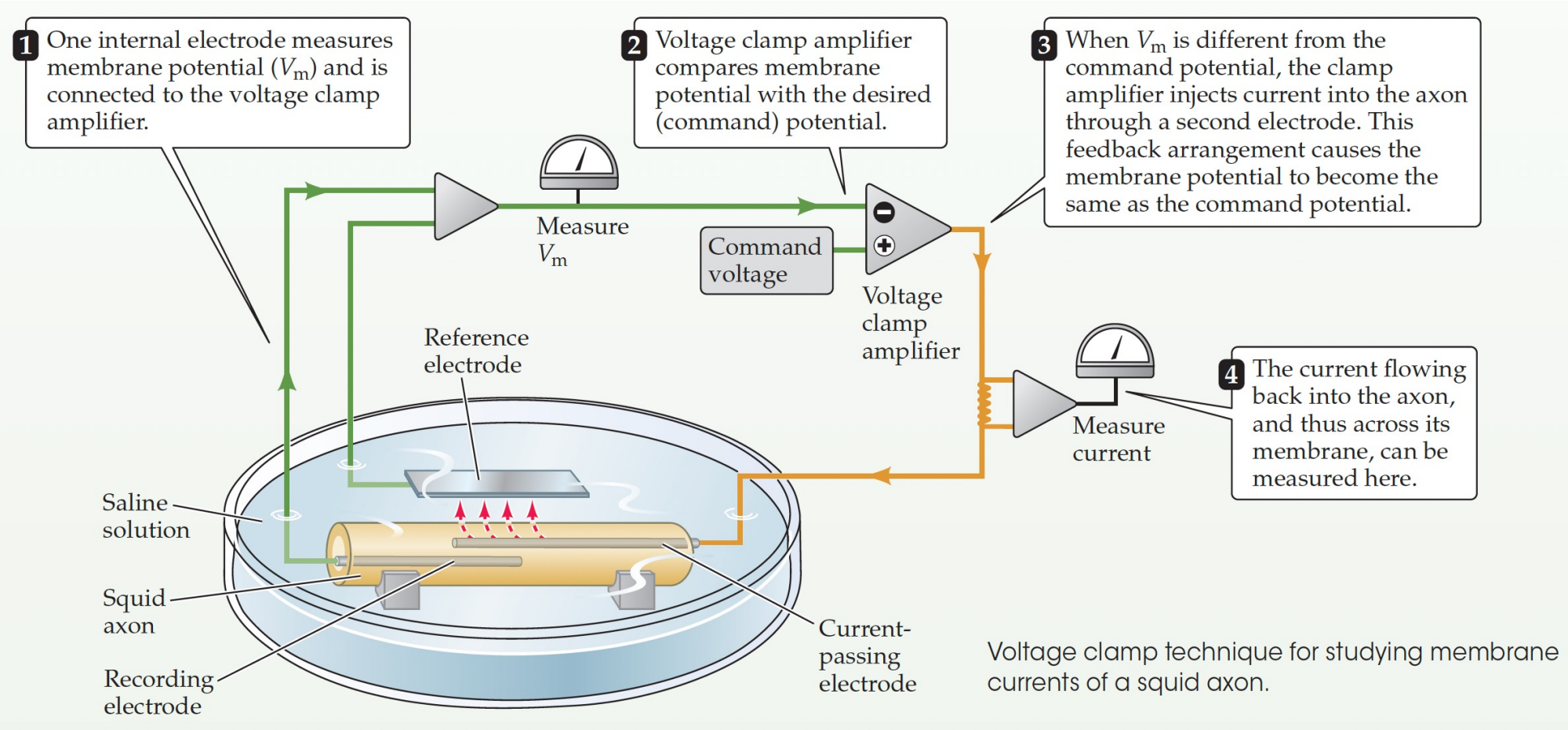
Hille, Figure 2.4



Purves, Box 2A

Purves, Box 2B

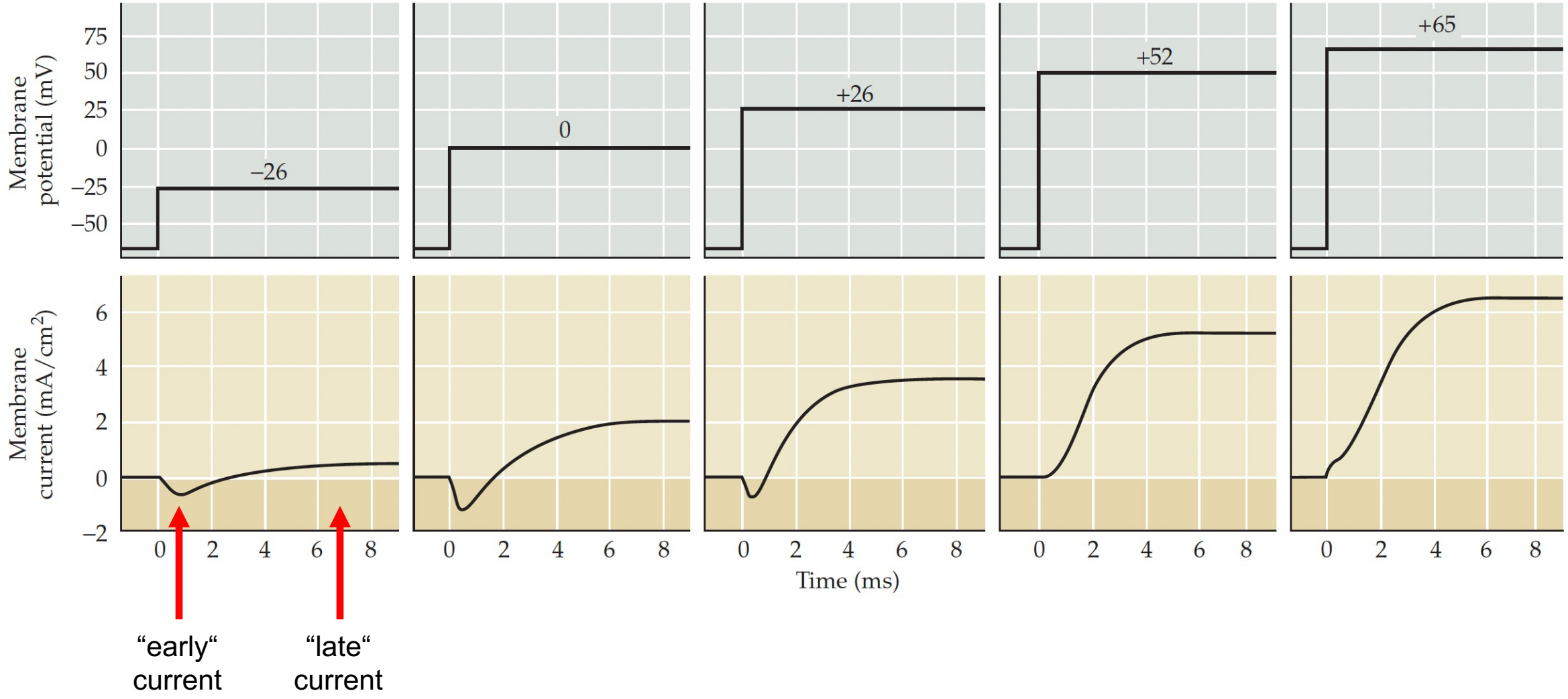
How do we uncover the currents giving rise to the “action potential“?



- Measurement of *membrane potential* , V_m, with an intracellular electrode
- A second electrode “injects“ current by a feedback amplifier <u>to keep V_m constant</u>
- Voltage clamp offers control over a key variable (V_m) that determines channel gating

Purves, Box 3A

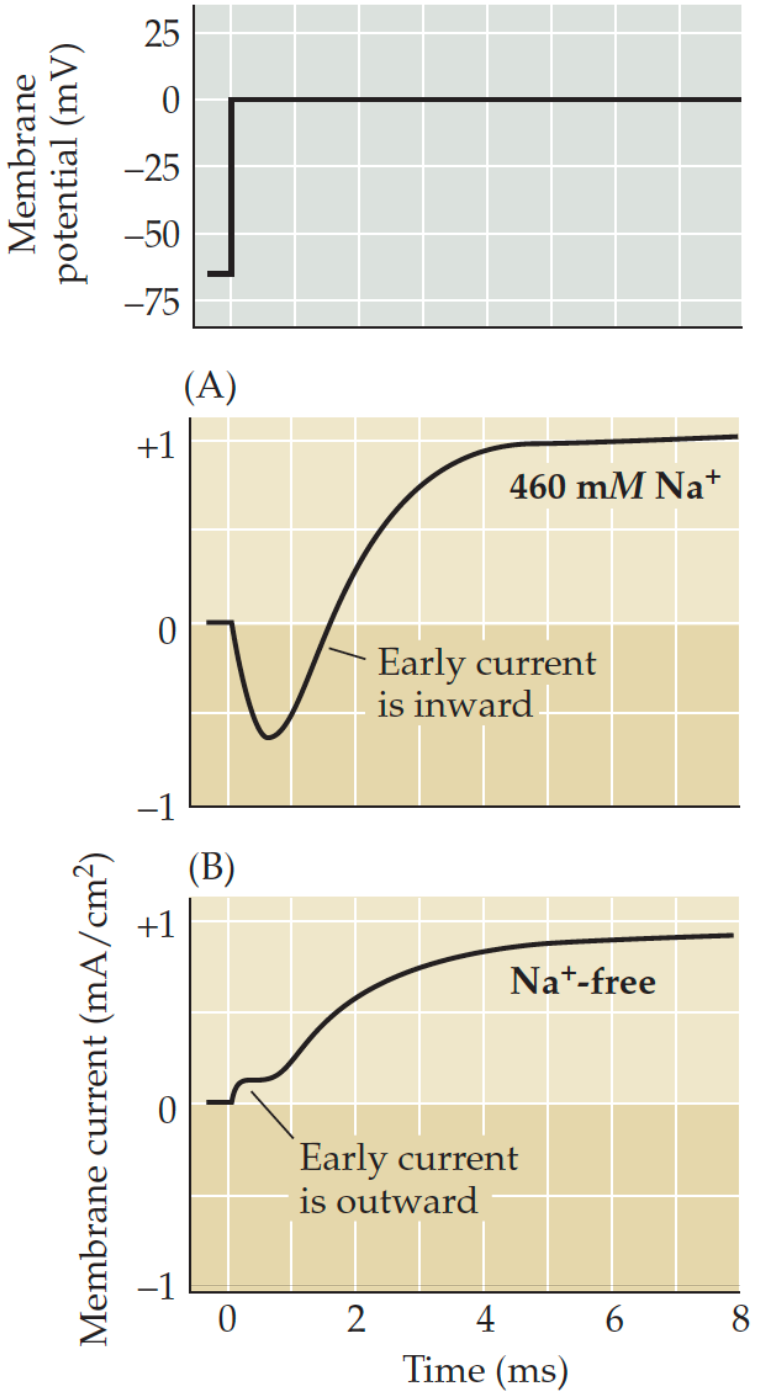
Currents measured with voltage-clamp in the squid giant axon



Recall that current drawn downward is called "inward" current and would depolarize the cell

Purves, Figure 3.2

The early current requires extracellular Na^+

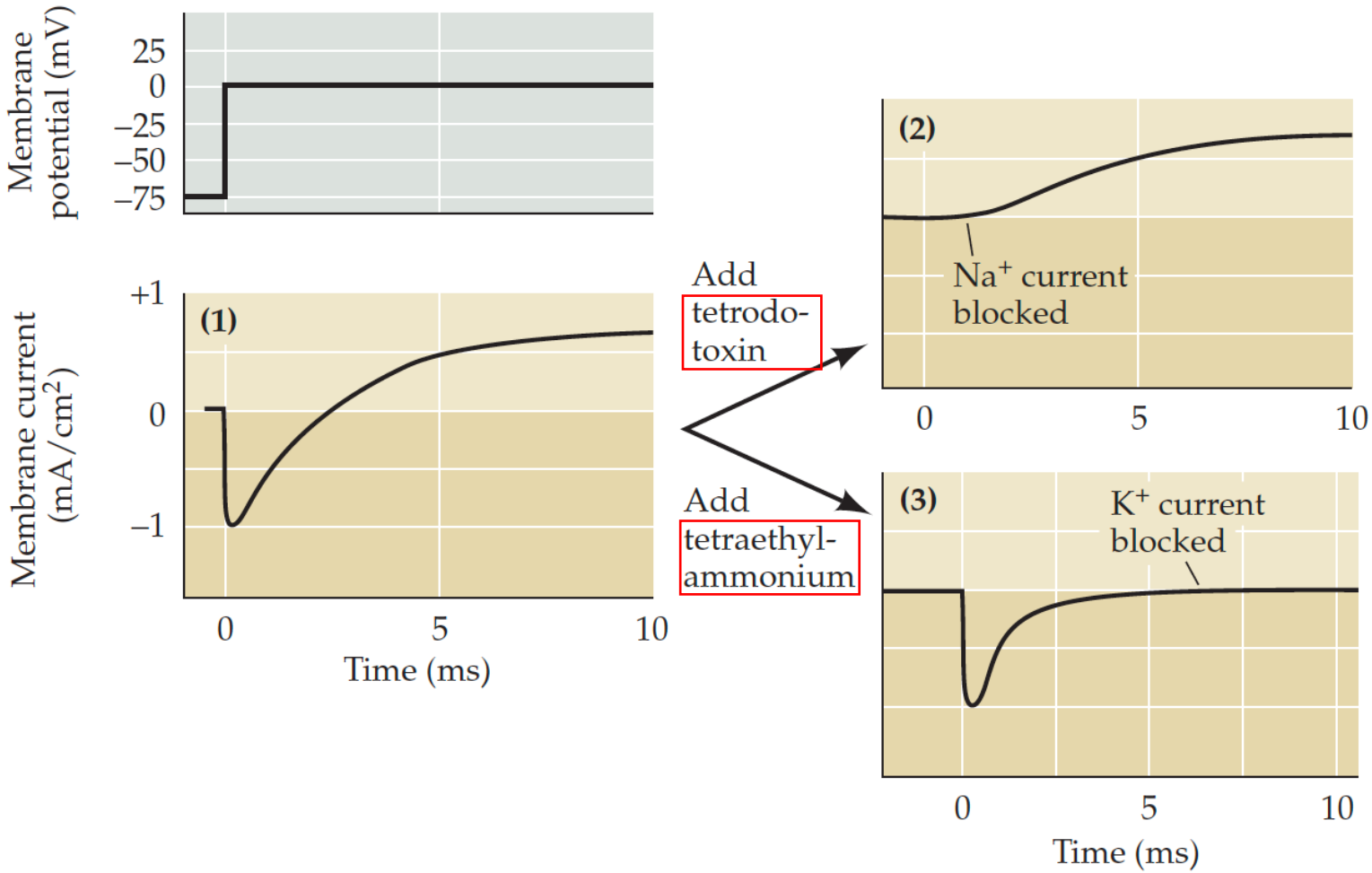


Note: 460 mM Na^+ is because the squid is a seawater organism. There is higher [Na^+] in the extracellular fluids of these animals.

- The early current must be a movement of Na^+ ions from outside to inside (i.e., inward current)

Purves, Figure 3.4

One can independently block either the early or the late current



- Hodgkin-Huxley proposed that separate conductances for Na^+ ions and K^+ ions are activated during depolarization (there is also a leak conductance)

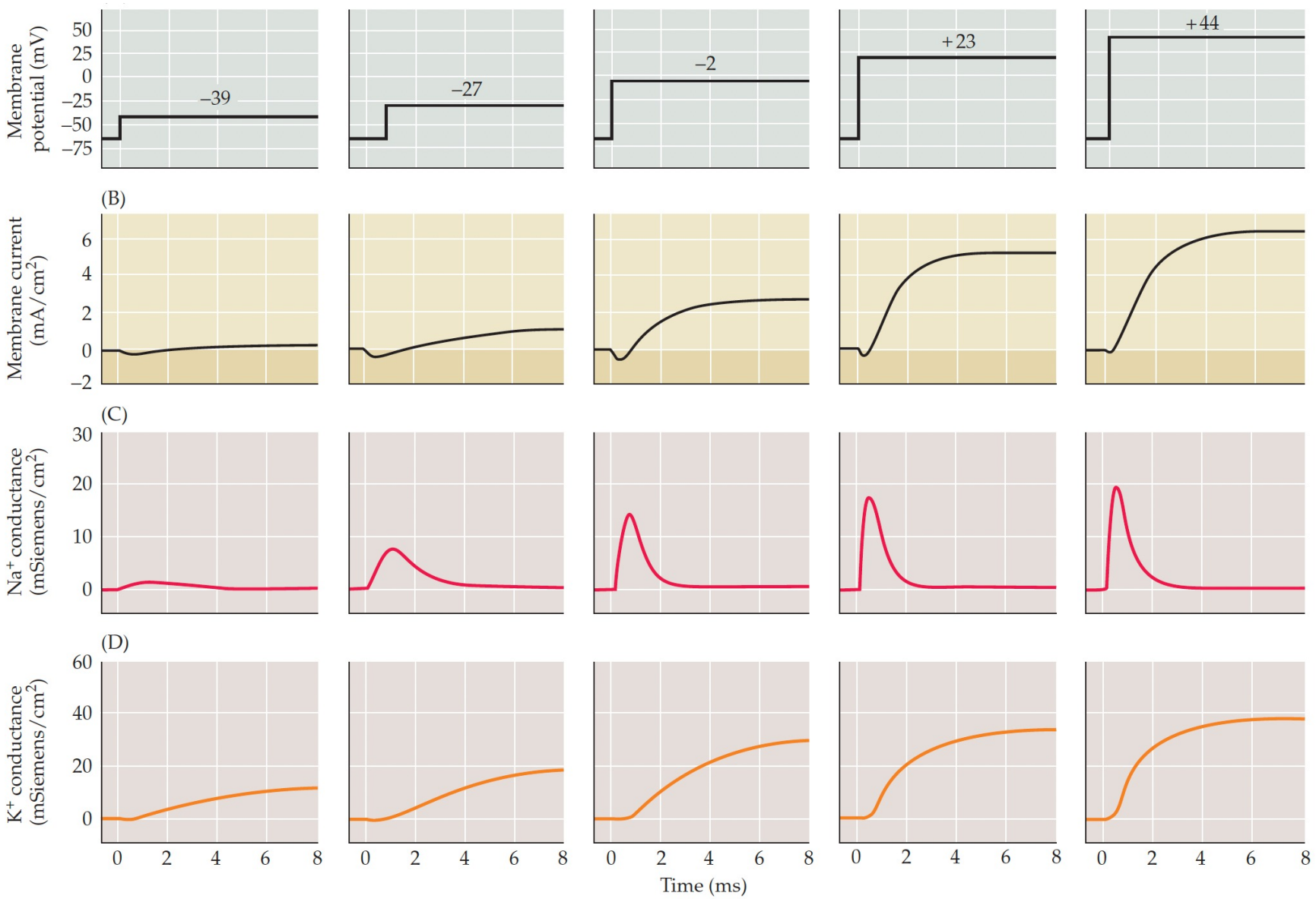
Purves, Figure 3.5

Action potential Na^+ and K^+ conductances depend on voltage and time

Holding potential

Na^+

K^+



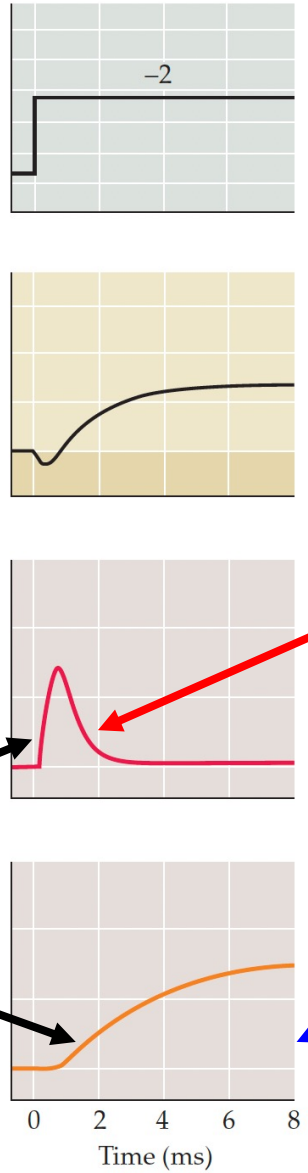
Purves, Figure 3.6

Three important concepts for voltage-dependent ion channel gating

Holding potential

Na^+

K^+



1. **Activation**
There is an increase
in conductance (channels open),
usually upon depolarization

2. **Inactivation**
There is a decrease in conductance
(channels close), despite ongoing depolarization

ONLY in Na^+ channels

3. **Deactivation**
Channels close when the voltage step
ends (not visible in this schema)

Purves, Figure 3.6

Activation – The time-dependent **opening** of ion channels in response to a stimulus, typically membrane depolarization (*or agonist pulse in ligand-gated ion channels*)

Inactivation - The time-dependent **closing** of ion channels *despite maintained depolarization* (V_m step for voltage-gated channels)

Deactivation - channel **closes** upon repolarization (not visible for I_{Na} because most channels are already inactivated)

Aside - 1952, a remarkable year for Hodgkin & Huxley

Experimental

Measurement of current-voltage relations in the membrane of the giant axon of Loligo.
HODGKIN AL, HUXLEY AF, KATZ B. *J Physiol*. 1952 Apr;116(4): **pp. 424-448**.

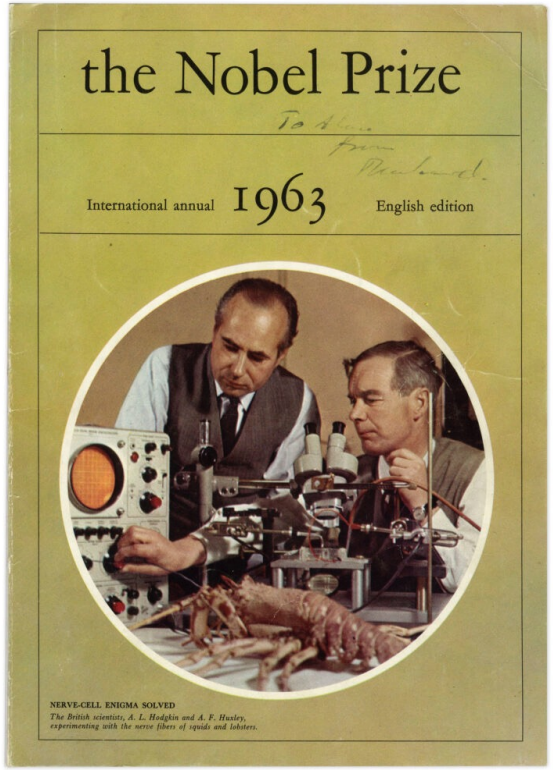
Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Apr;116(4): **pp. 449-472**.

The components of membrane conductance in the giant axon of Loligo.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Apr;116(4): **pp. 473-496**.

The dual effect of membrane potential on sodium conductance in the giant axon of Loligo.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Apr;116(4): **pp. 497-506**.

Mathematical modeling study

A quantitative description of membrane current and its application to conduction and excitation in nerve.
HODGKIN AL, HUXLEY AF. *J Physiol*. 1952 Aug;117(4): **pp. 500-544**.

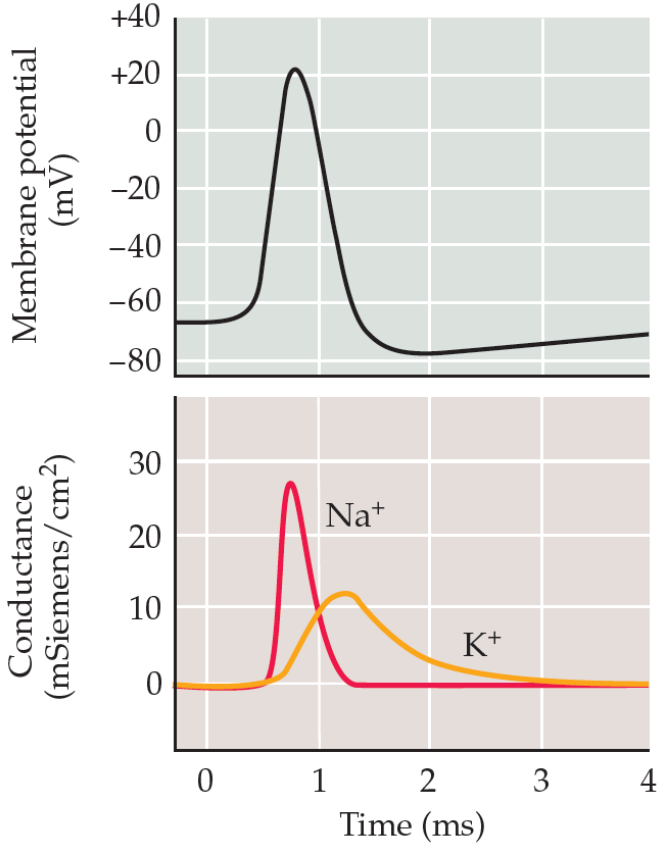


Hodgkin & Huxley

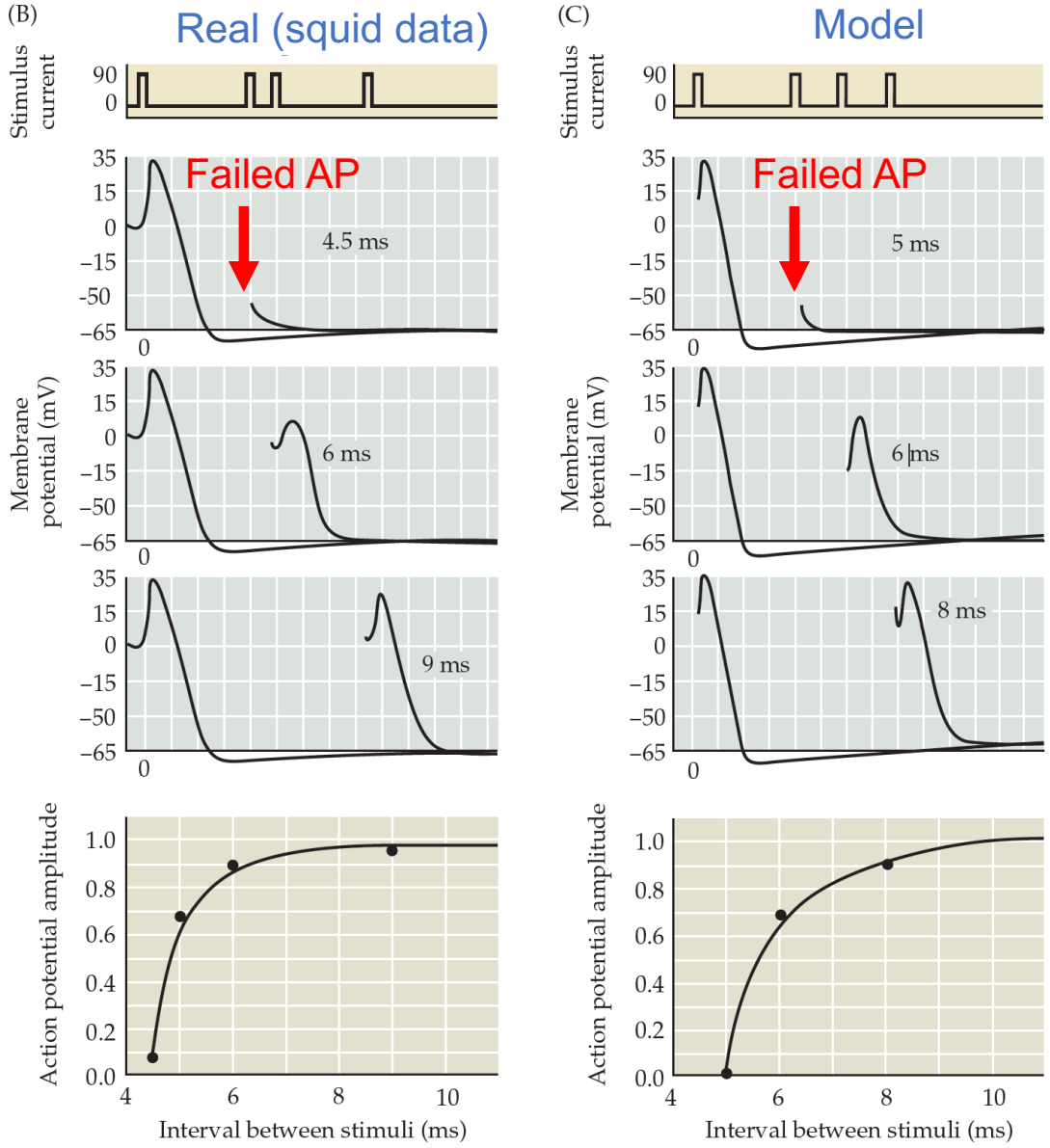
"This article concludes a series of papers concerned with the flow of electric current through the surface membrane of a giant nerve fibre. Its general object is to discuss the results of the preceding papers (Part I), to put them into mathematical form (Part II) and to show that they will account for conduction and excitation in quantitative terms (Part III)."

Hodgkin's Nobel Prize lecture: https://www.nobelprize.org/prizes/medicine/1963/hodgkin/lecture/

Mathematical reconstruction of the action potential

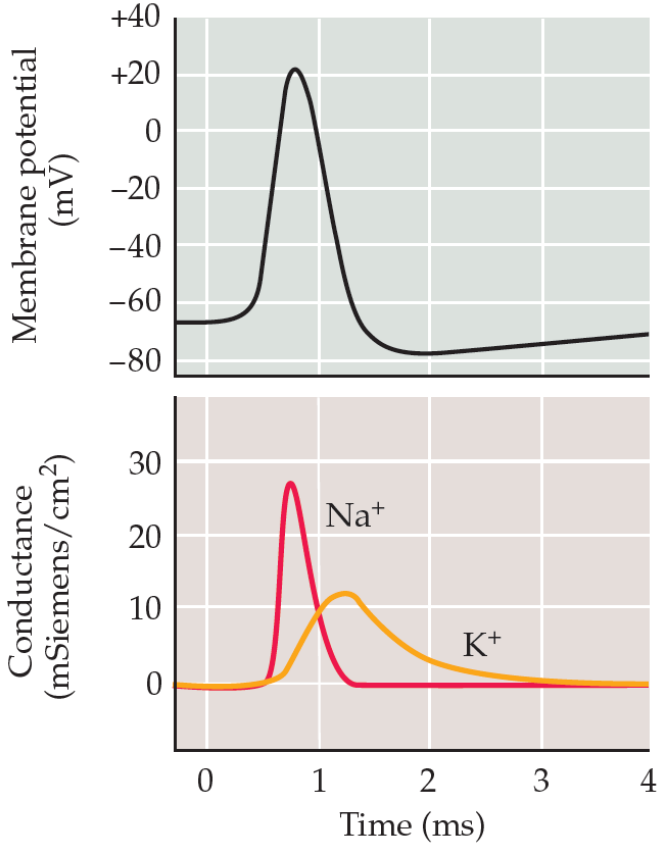


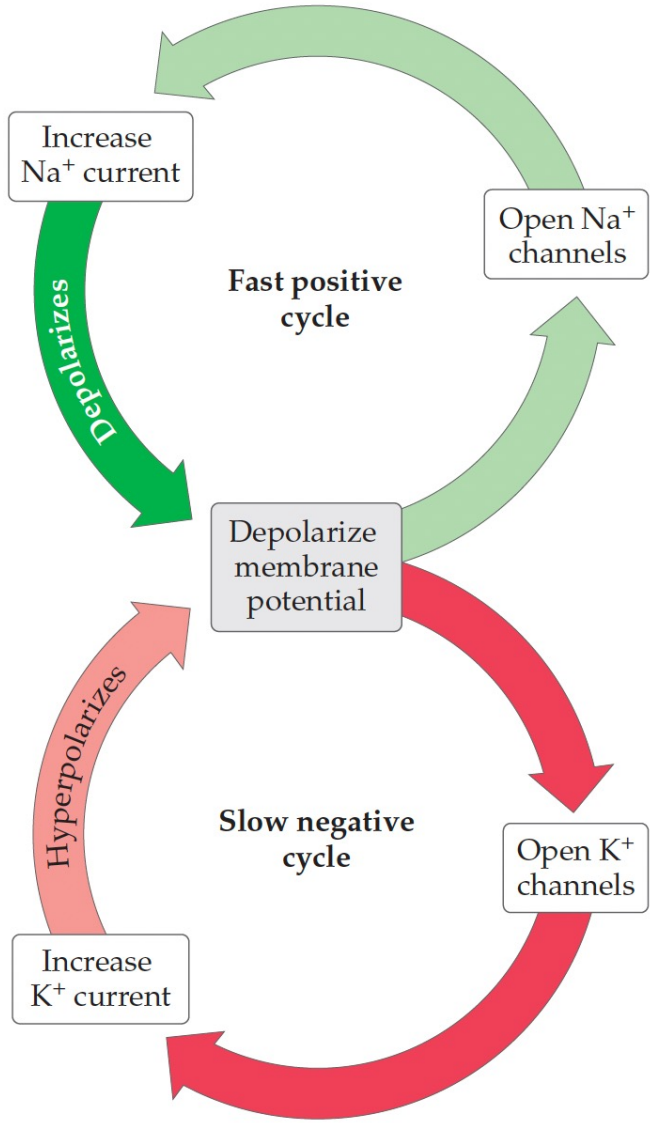
In 1952, H&H did not know yet about "ion channels" or other transmembrane proteins. They concluded that there are separate membrane conductances for Na^+ and K^+ and predicted these might be ion channels



Purves, Figure 3.8

The action potential includes positive and negative feedback loops

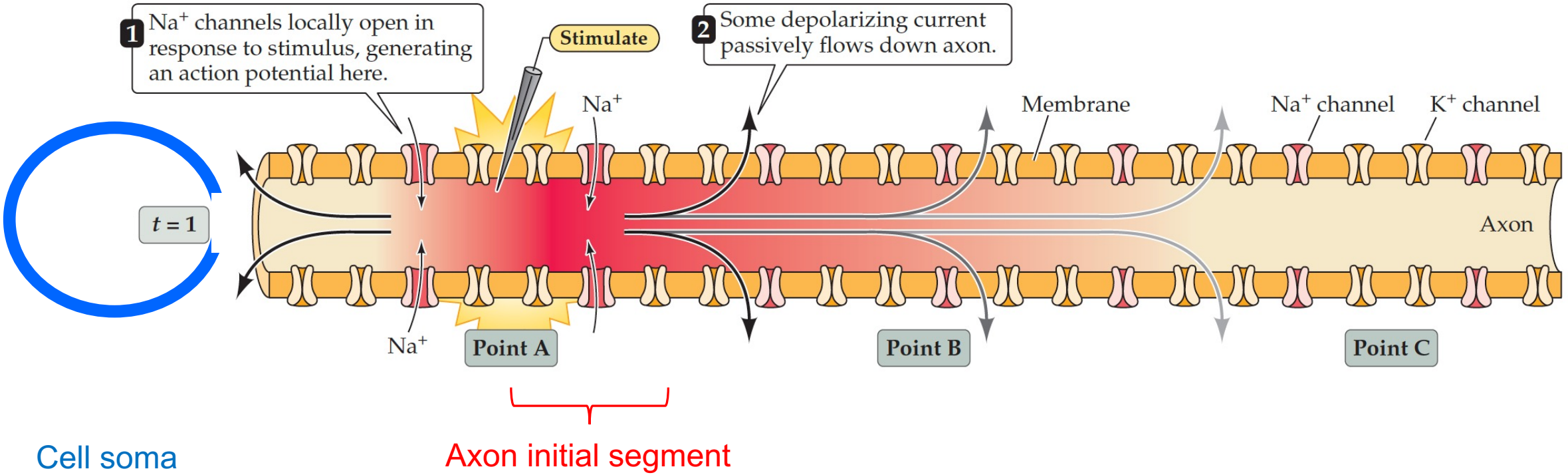




Na^+ currents are ***positive*** feedback
K^+ currents are ***negative*** feedback

Purves, Figure 3.9

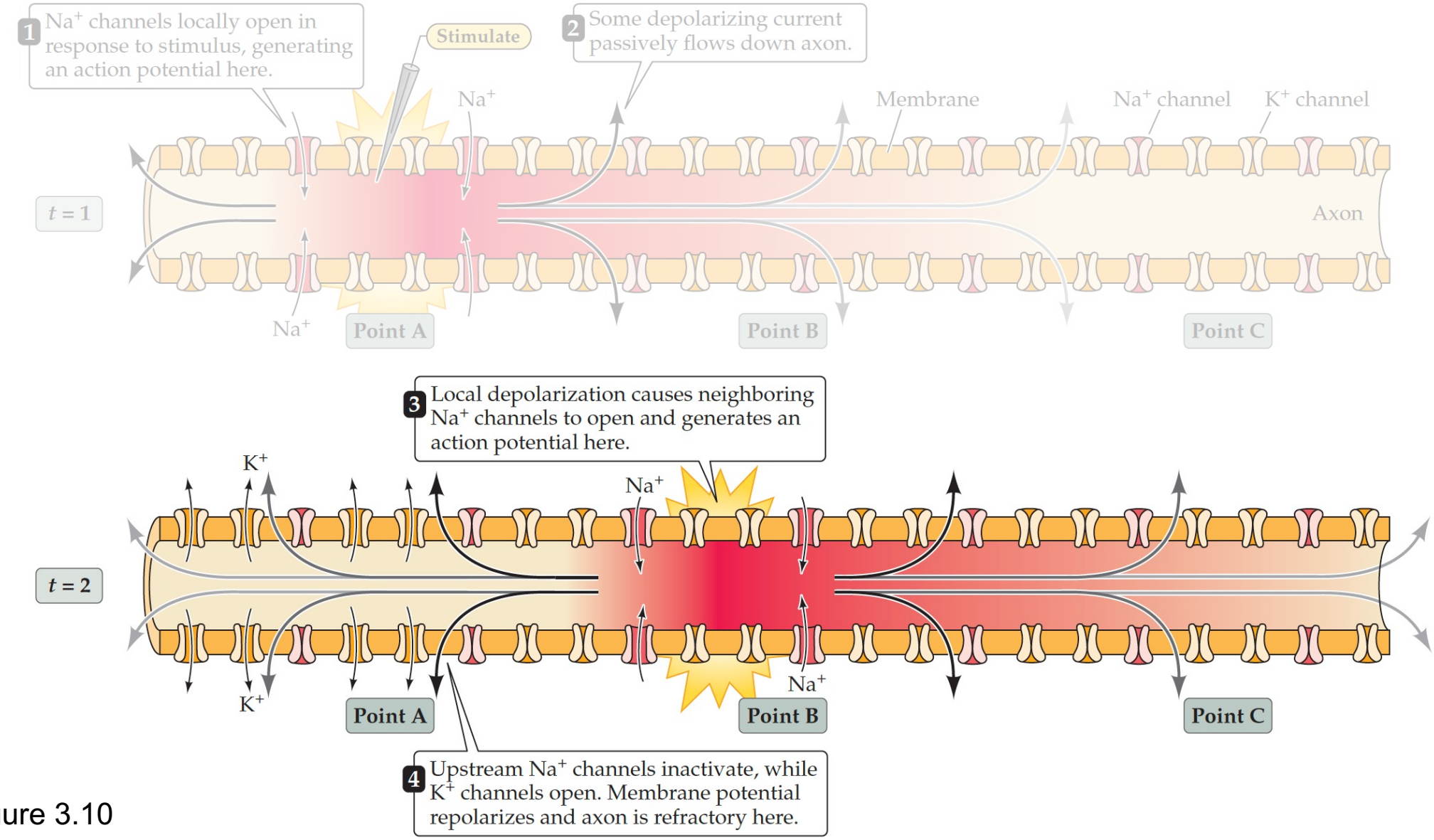
Action potentials are generated at the axon initial segment (near the soma) when the threshold V_m is reached



"*Stimulate*" refers to a strong EPSP / membrane depolarization at the soma/dendrites, reaching the **threshold V_m of** ~ -50 mV

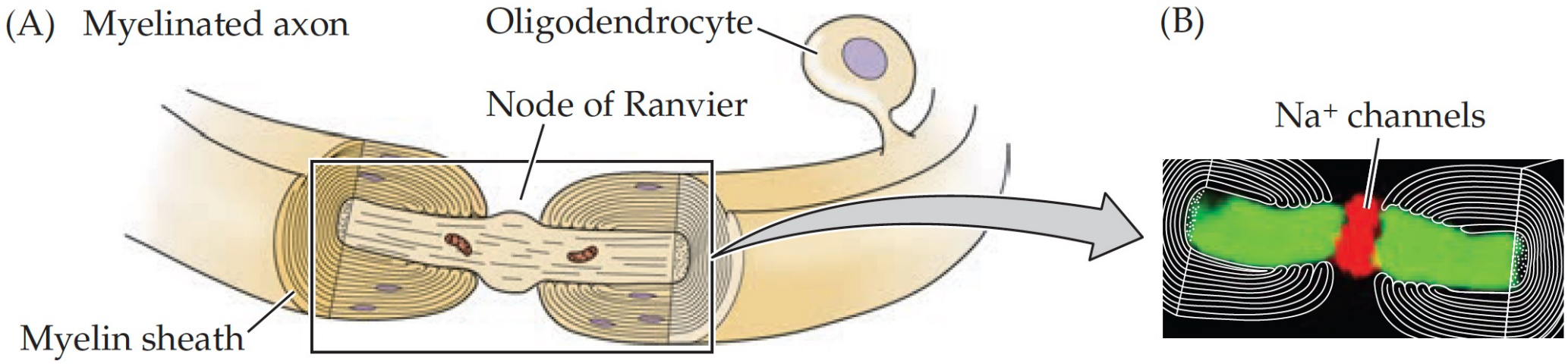
Purves, Figure 3.10

Action potentials propagate by *passive flow* and *asymmetric Na^+ inactivation*



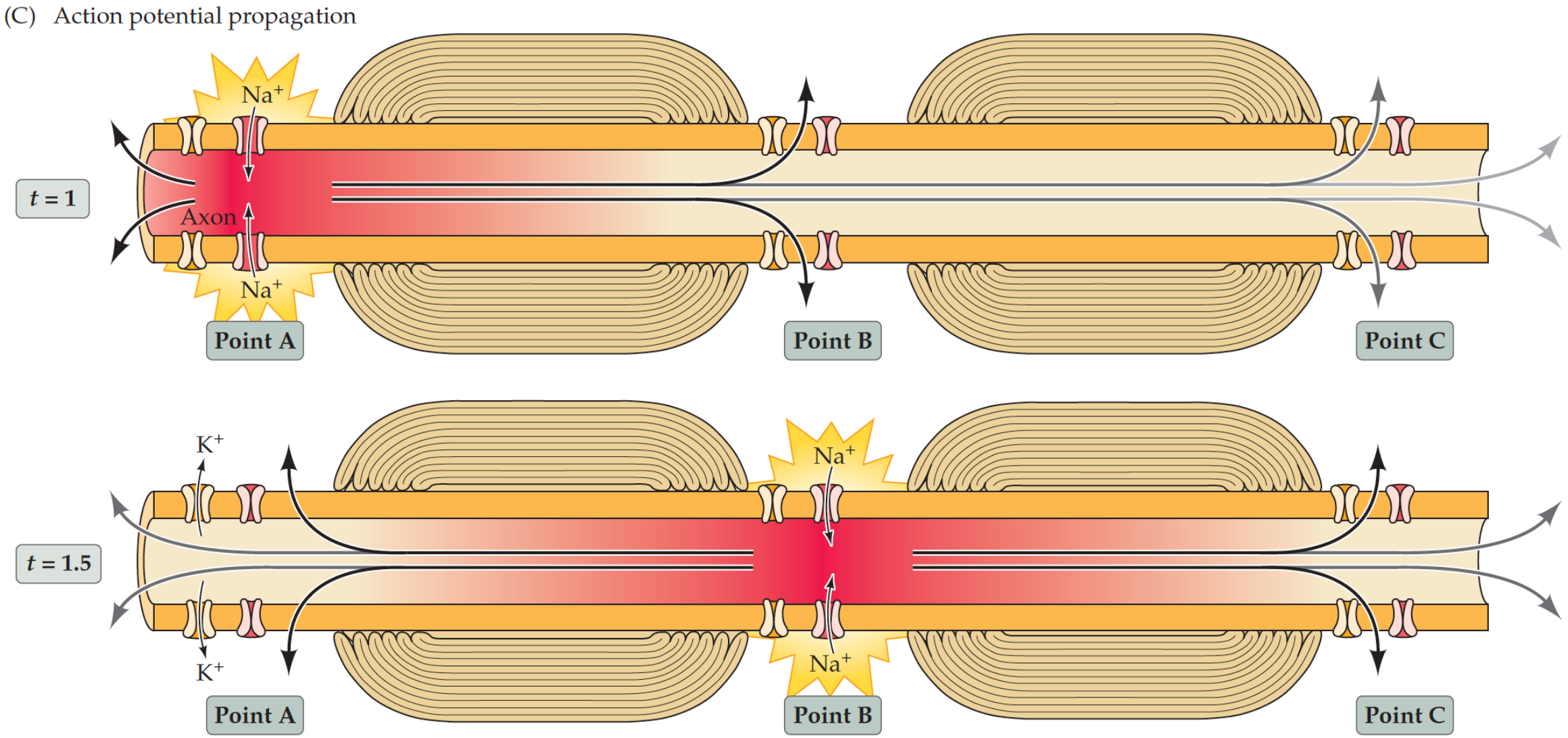
Purves, Figure 3.10

Saltatory ("jumping") conduction of action potentials in myelinated axons



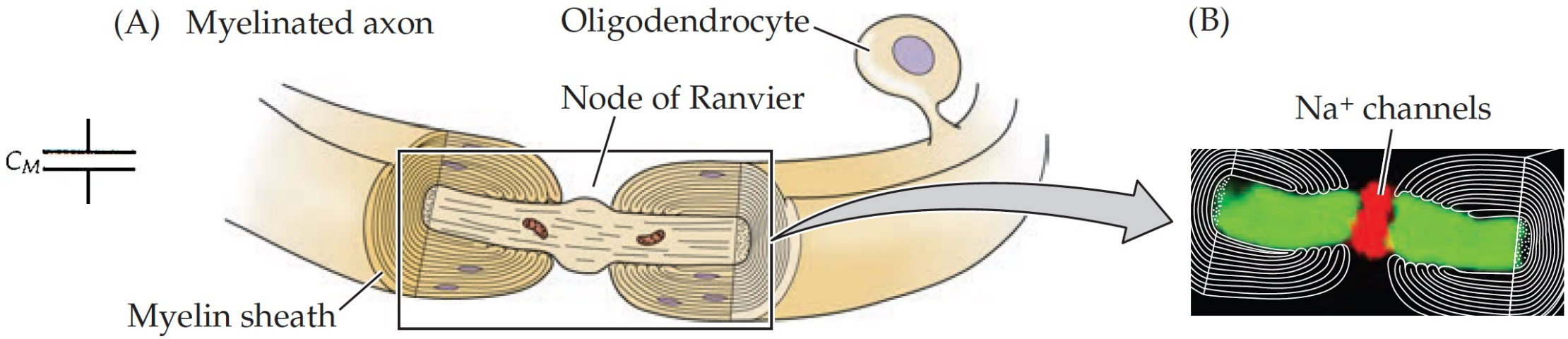
Purves, Figure 3.11

Saltatory ("jumping") conduction of action potentials in myelinated axons



Purves, Figure 3.11

Action potential conduction is faster in myelinated axons



Larger distance **d** through membrane in myelinated region:
Causes a ↓ C_m (see eqn)

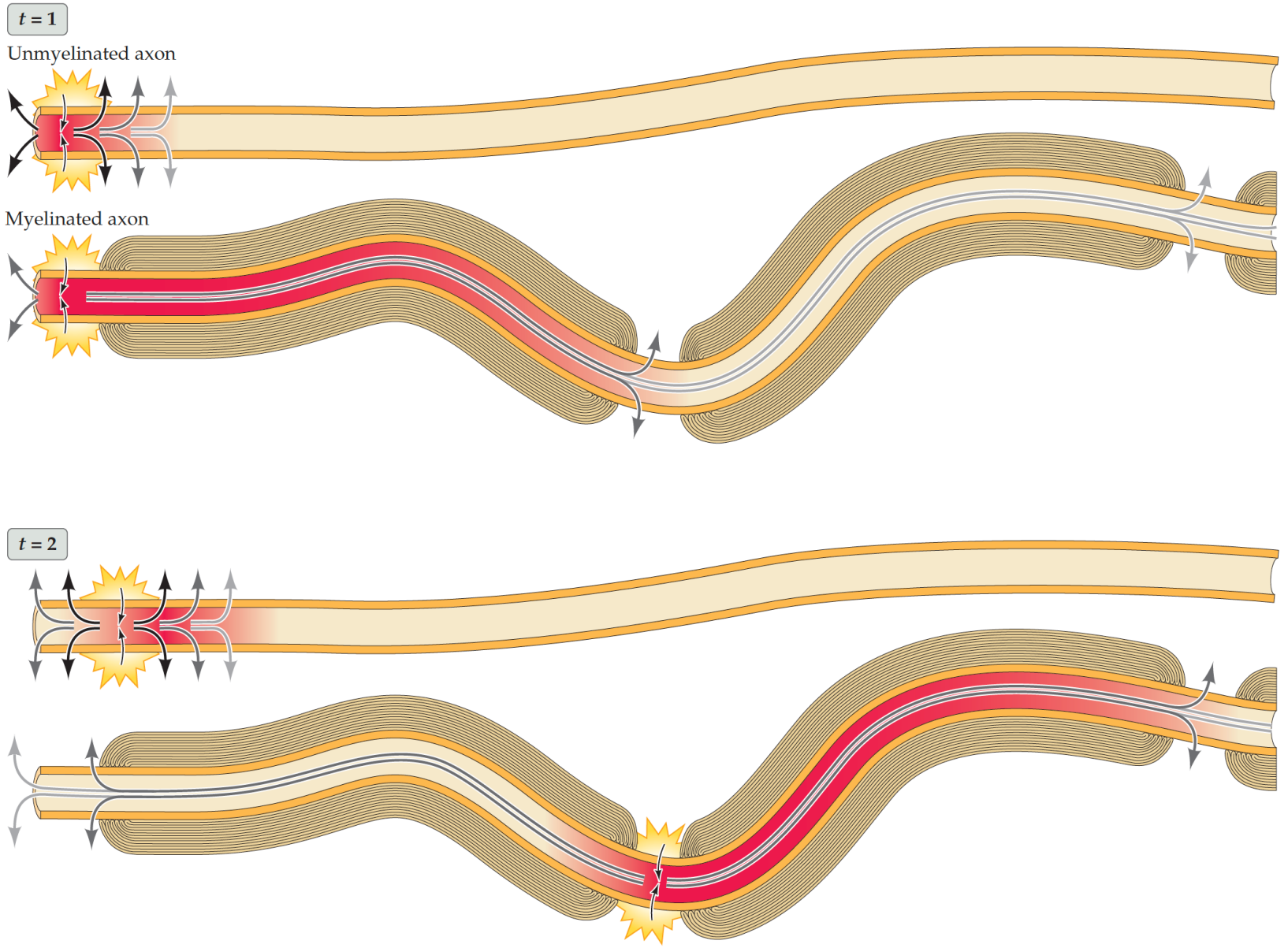
$$C = \frac{\varepsilon \cdot \varepsilon_0 \cdot A}{d}$$

Few ion channels in myelinated region:
Causes a ↑ R_m

Less ***loss*** by capacitative and resistive currents along the axons leads to ***faster*** conduction!

Purves, Figure 3.11

Action potential conduction is faster in myelinated axons



Purves, Figure 3.12

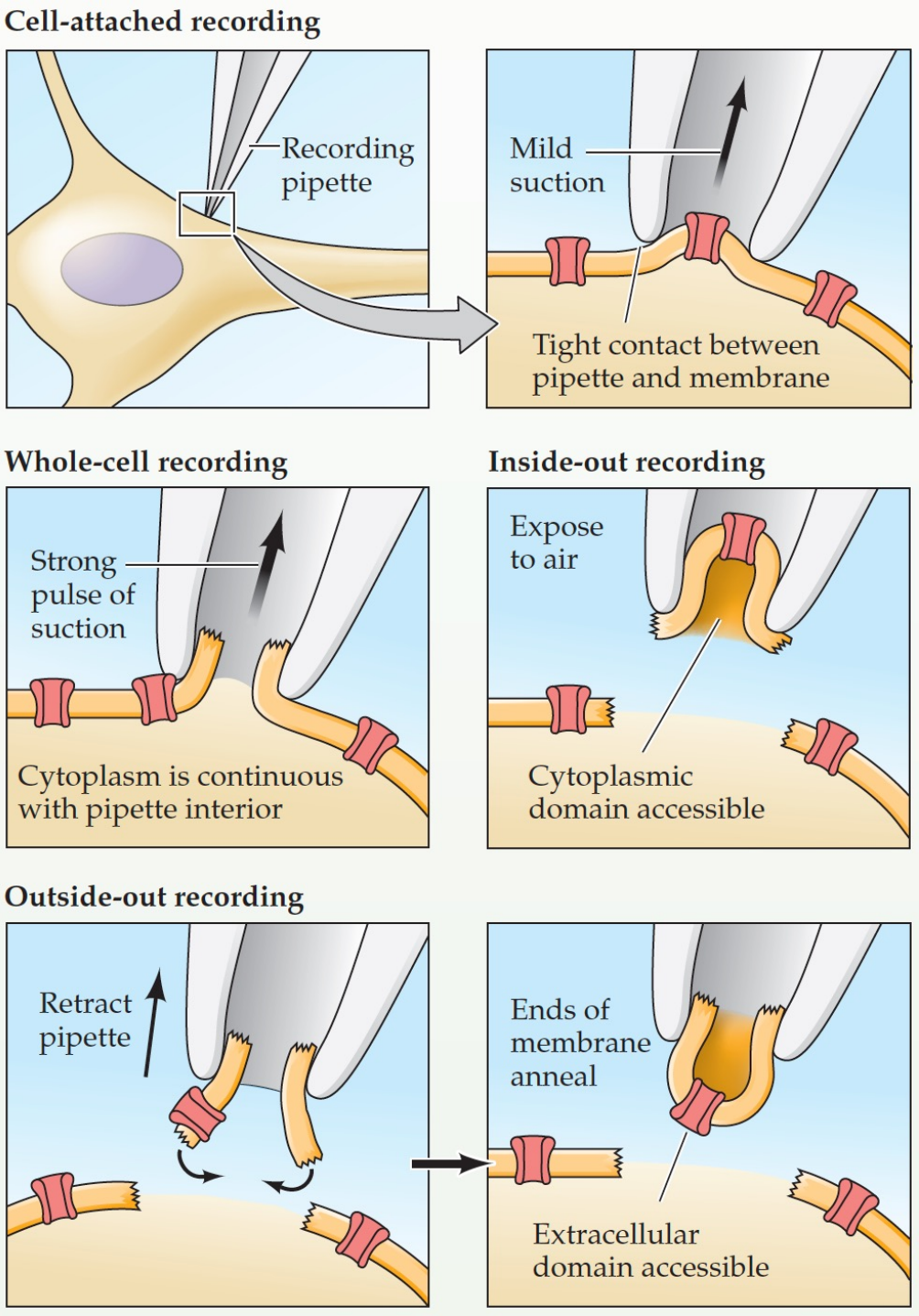
We now know that Na^+ and K^+ currents are mediated by separate ion channels.

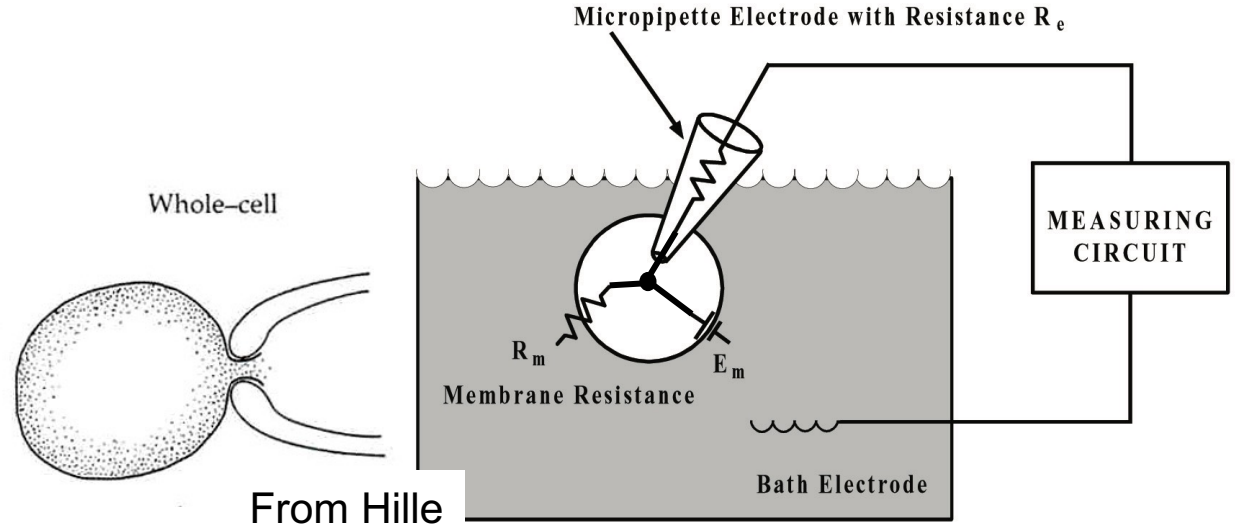
Goal: How can we learn more about the properties of these ion channels?

One approach: Single-channel “patch-clamp” recordings (early 1980's)

Multiple configurations of patch-clamp recordings

- **Cell-attached** recordings: high resistance contact
- **Whole-cell** recordings: break-in to cell interior with patch pipette filled with “intracellular solution“
- **Inside-out** recordings: channel interior becomes accessible
- **Outside-out** recordings: channel exterior becomes accessible to e.g., test channel ligands



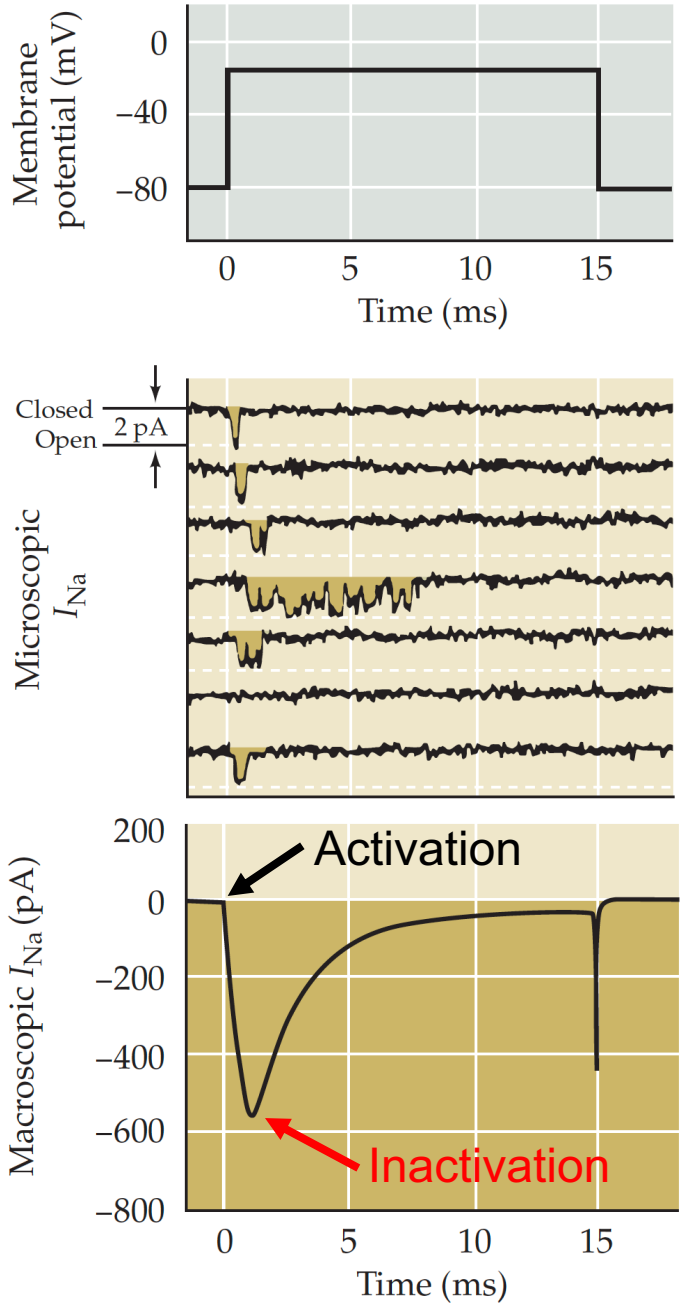


From Hille

Purves, Box 4A

From single-channels to macroscopic currents: voltage-gated Na^+ channels

Single voltage-gated **Na^+-channels** in a membrane "patch" give early **inward** depolarizing currents



Note that single-channel gating is *stochastic* despite repeated identical voltage-steps

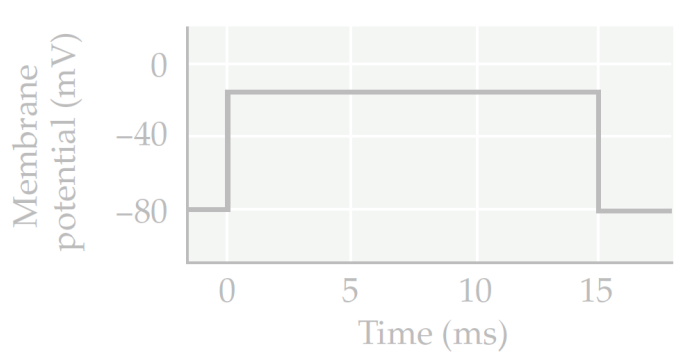
Total "ensemble" current given by:

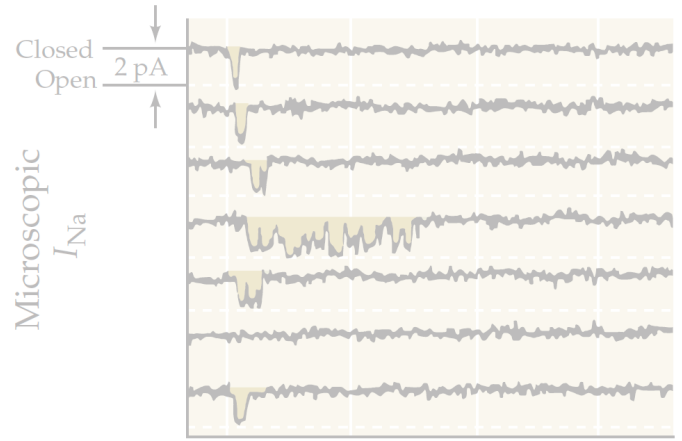
$$I_{total} = i_{single\ channel} \times N_{channels} \times p_{open}$$

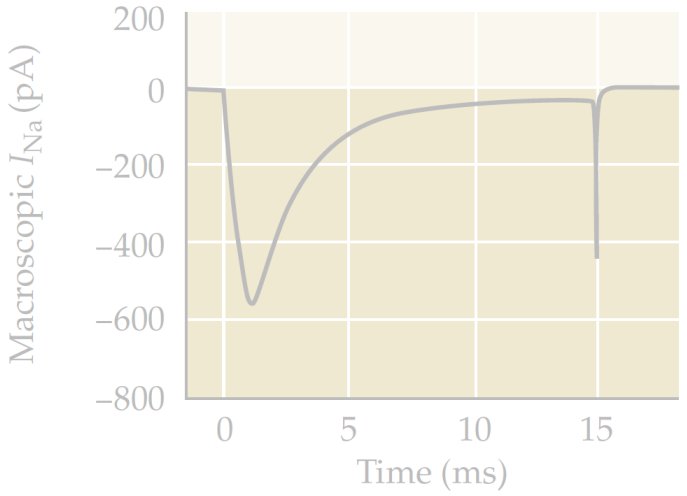
Purves, Figure 4.1

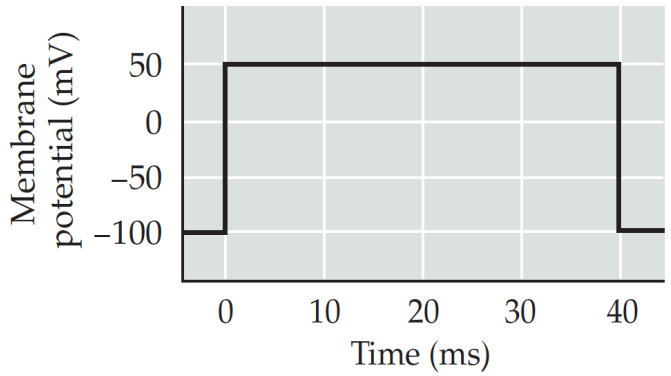
From single-channels to macroscopic currents: voltage-gated K^+ channels

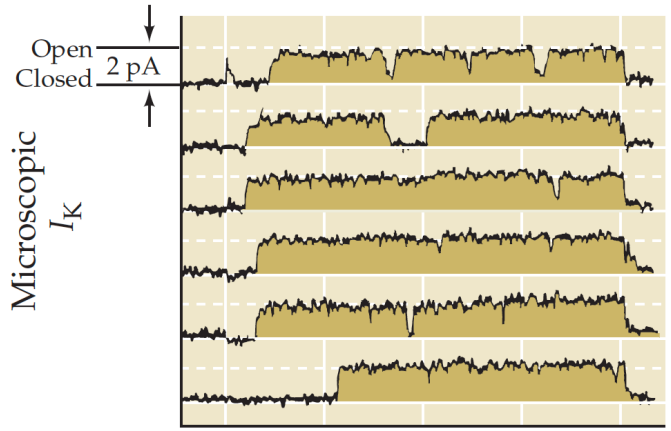
Single voltage-gated **Na^+-channels** in a membrane "patch" give early **inward** depolarizing currents



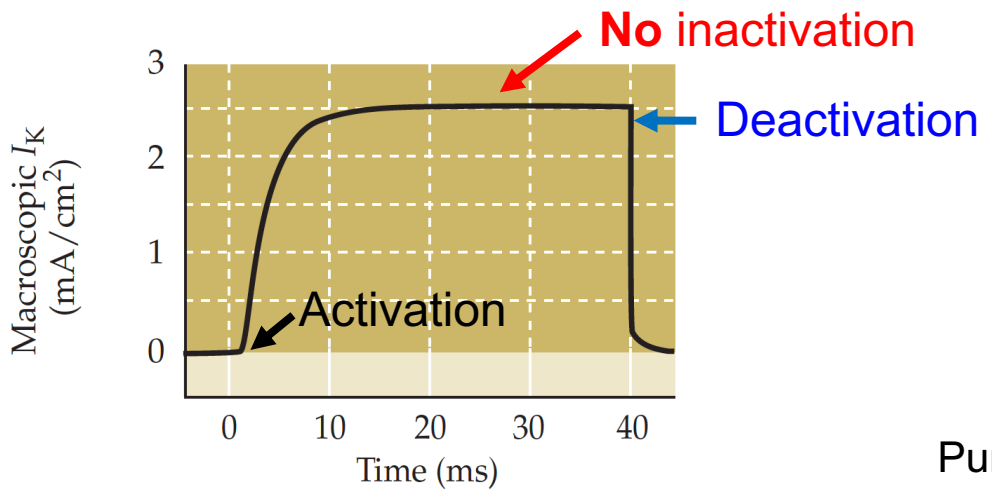






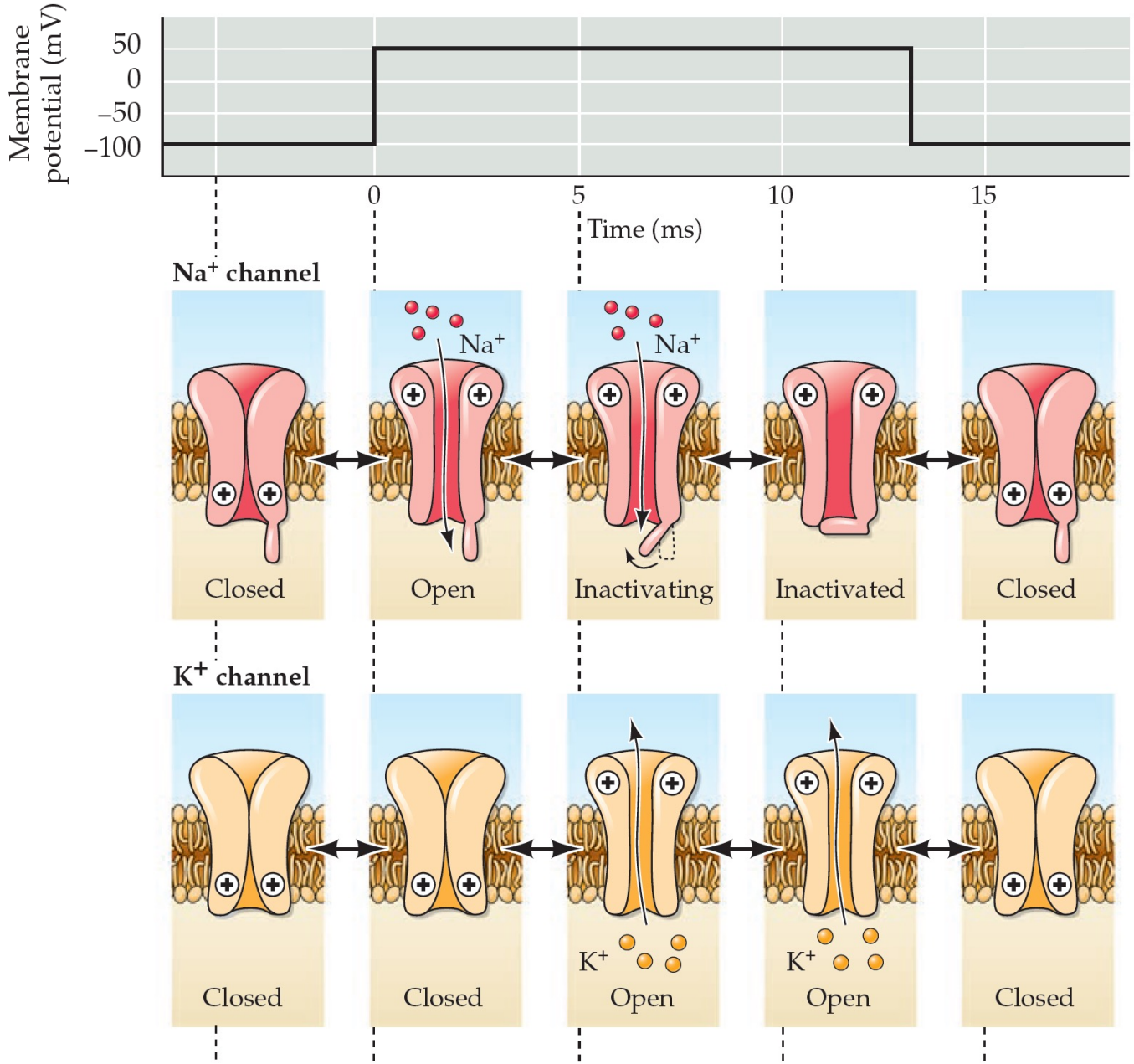


Single voltage-gated **K^+-channels** in a membrane "patch" give more slowly activating, **outward** hyperpolarizing currents



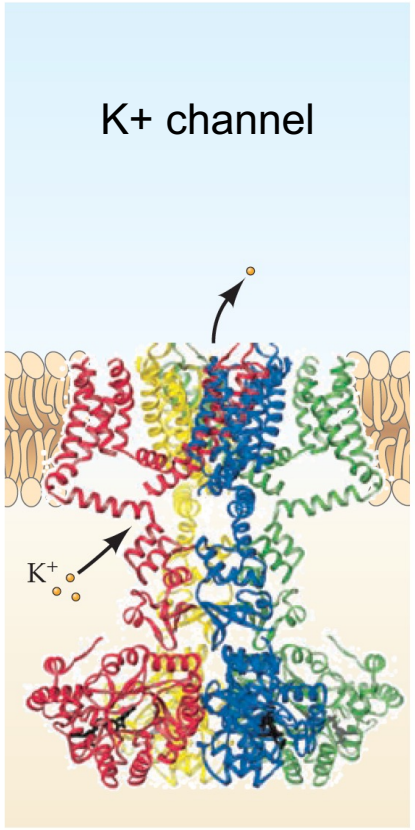
Purves, Figure 4.2

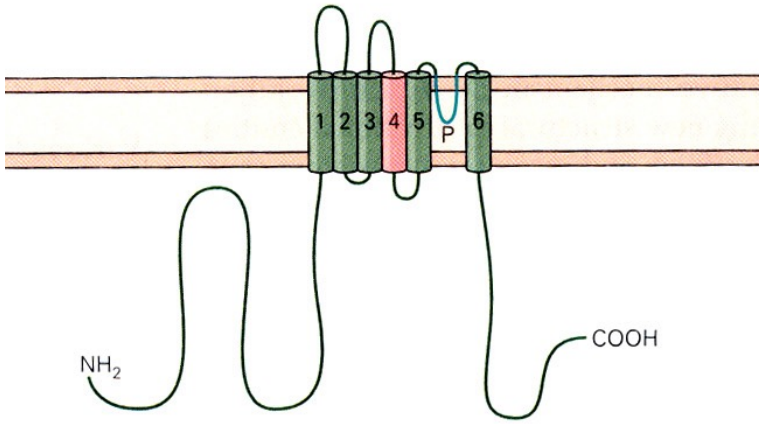
Schema of voltage gated ion channels

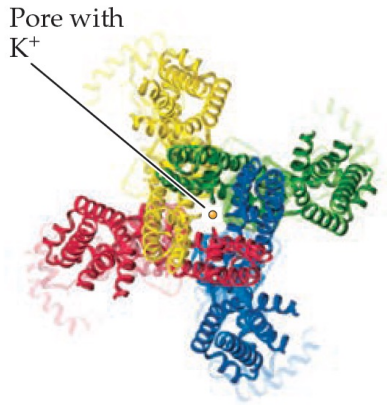


Purves, Figure 4.3

Crystallography structures of voltage-gated K+ ion channels





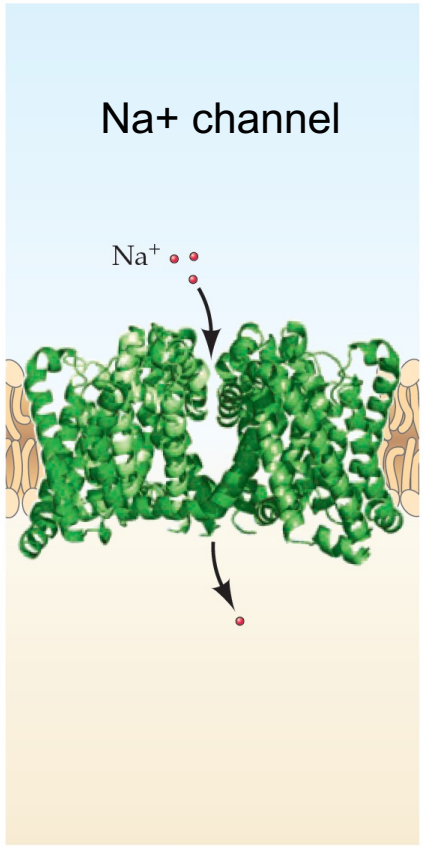


- N-terminal and C-terminals intracellular
- 6 membrane-spanning domains (S1-S6)
- S4 contains positively-charged residues (arginines, lysines) acting as a voltage-sensor for gating
- K^+-channels are tetramers (4 subunits form a channel)
- Accessory β-subunits

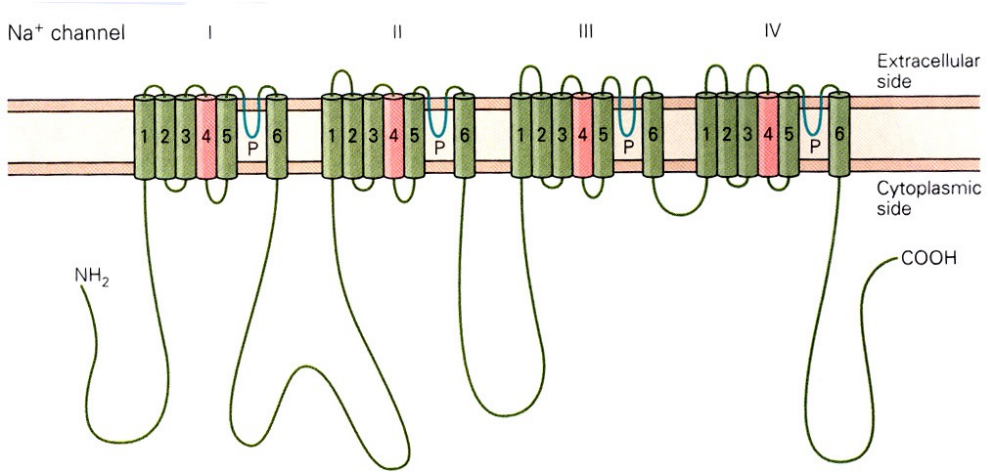
Purves, Figure 4.6

Kandel, Figure 7-14

Crystallography structures of voltage-gated Na+ ion channels

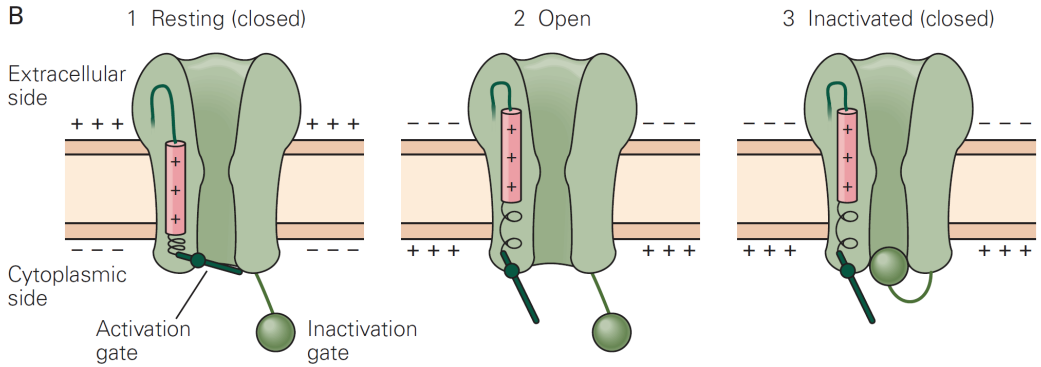


Purves, Figure 4.6



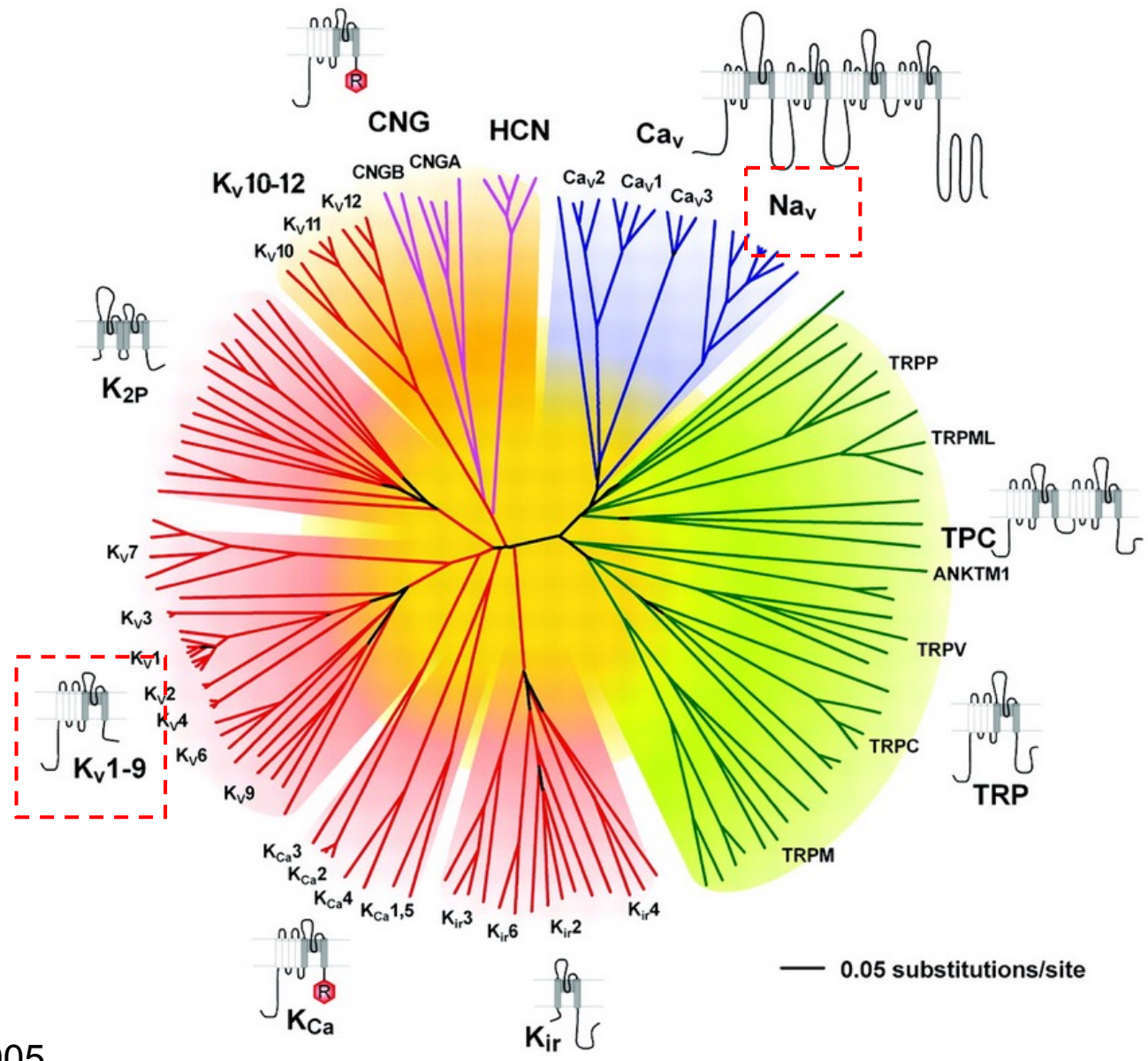
Kandel, Figure 7-14

- 4 "repeats" of K^+ channel-like structure
- Accessory β-subunits
- Separate activation and inactivation gates:



Kandel, Figure 7-13

A large number of genes code for voltage-gated and related ion channels



Na_v - voltage-gated Na^+ channels

K_v - voltage-gated K^+ channels

Ca_v - voltage-gated Ca^{2+} channels

TRP - "transient receptor potential" channels (touch, nociception)

CNG - cyclic nucleotide gated channels (olfaction, vision)

K_{Ca} - Ca^{2+} activated K channels

… and more …

Yu & Catterall 2005

Summary: Important concepts and keywords

- Action potential
- Measurement techniques:
  - 2-electrode voltage clamp
  - patch-clamp techniques
- Definition of current clamp and voltage clamp measurements
- Voltage-gated membrane currents, early & late currents, and their ionic basis (Na^+ and K^+)
- Measurements and consequences of single Na^+ and single K^+ channel conductances (different proteins)
- Sequence of events during the action potential
- Voltage-gated ion channels:
  - Principal topology of Na^+ and K^+ channels