

Ionic Basis of Action Potentials

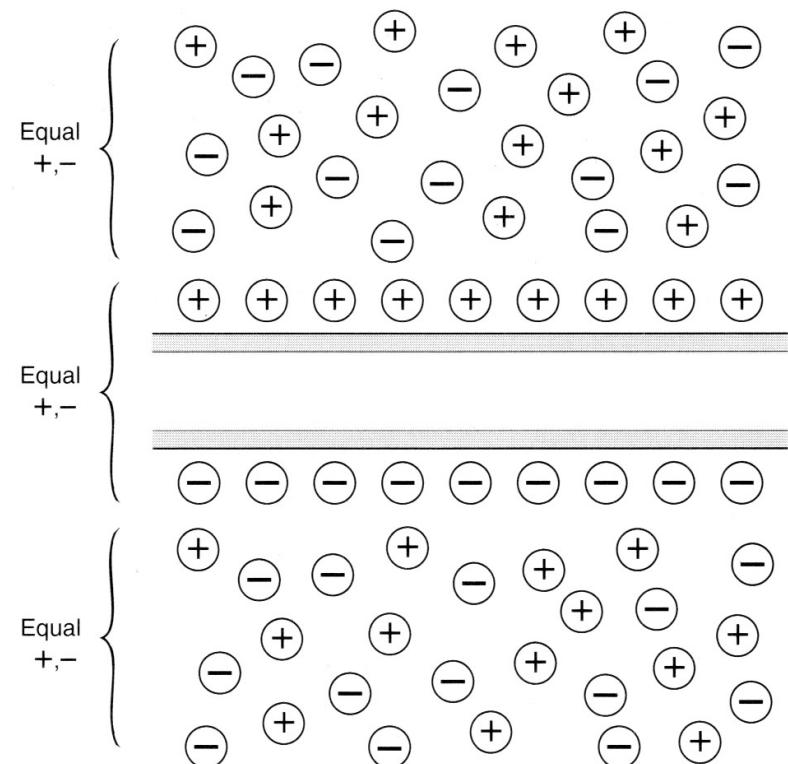
Review: Ionic Basis of the resting potential

- Neurons (and other excitable cells) express an electrical potential (stored charge) across their plasma membrane
 - Inside has a net negative charge compared with outside
- This electrical potential is due to:
 - (1) differences in concentration of ions between the intracellular and extracellular fluid
 - Inside: High K⁺, low Na⁺ & Cl⁻; outside: high Na⁺ & Cl⁻ low K⁺
 - (2) selective permeability of ions across the plasma membrane
 - High permeability to K⁺, low permeability to Na⁺
- Ion gradients are established and maintained by ion pumps (Na⁺/K⁺ ATPase)

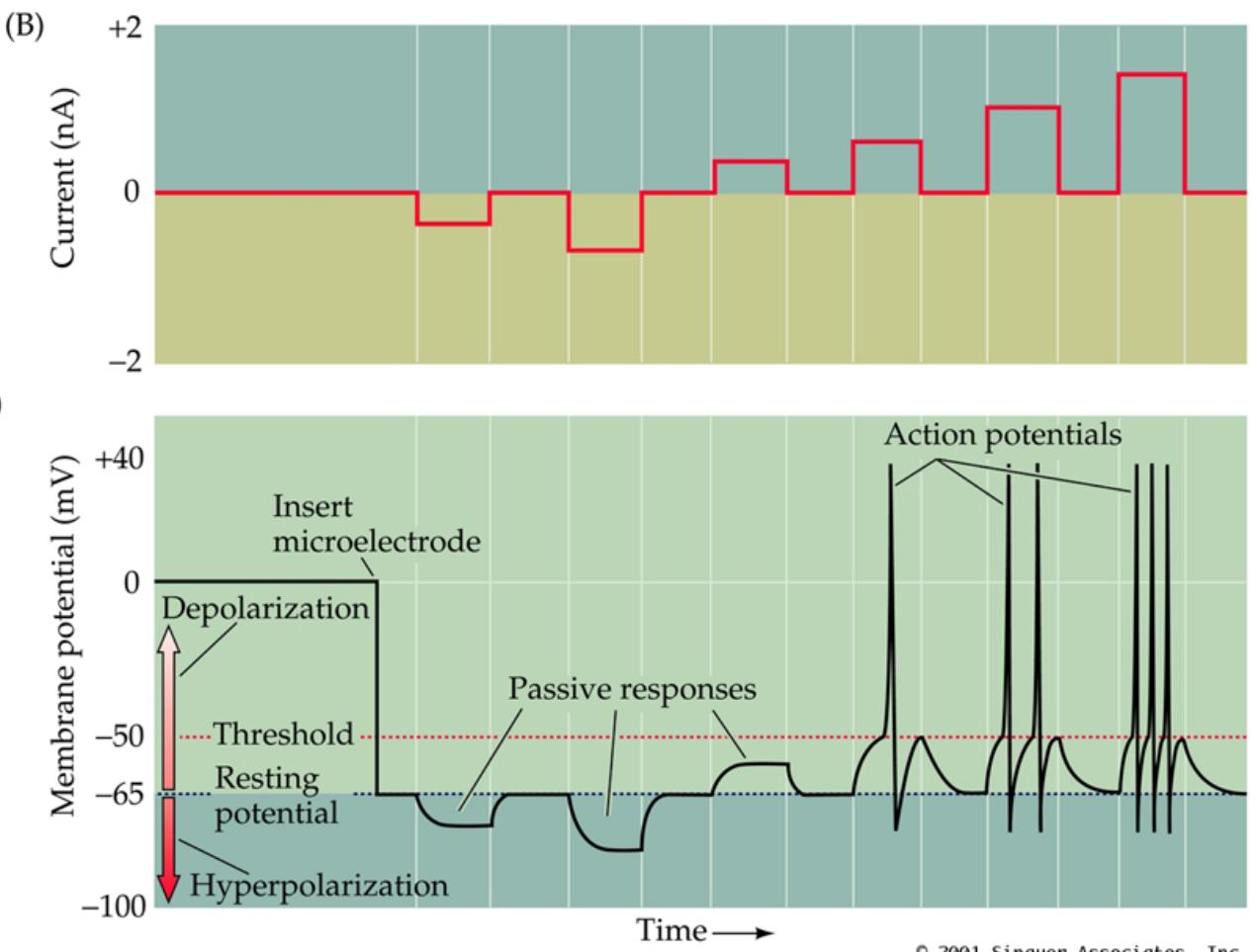
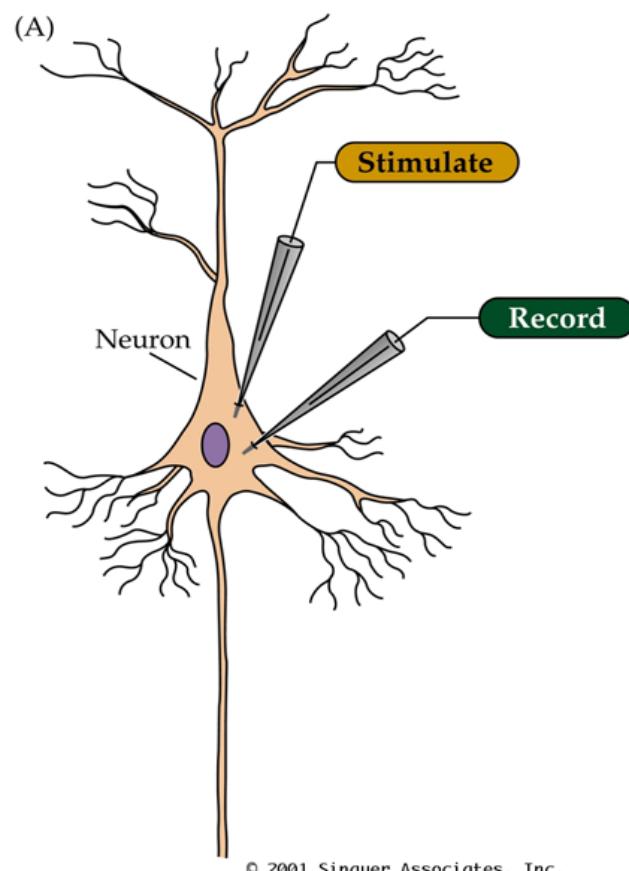
Only a small number of unpaired charges are needed to establish the resting potential

A -70 mV resting potential difference only requires $\sim 1 \text{ pM/cm}^2$ of unpaired negative charges separated from an equal number of positively charged ions.

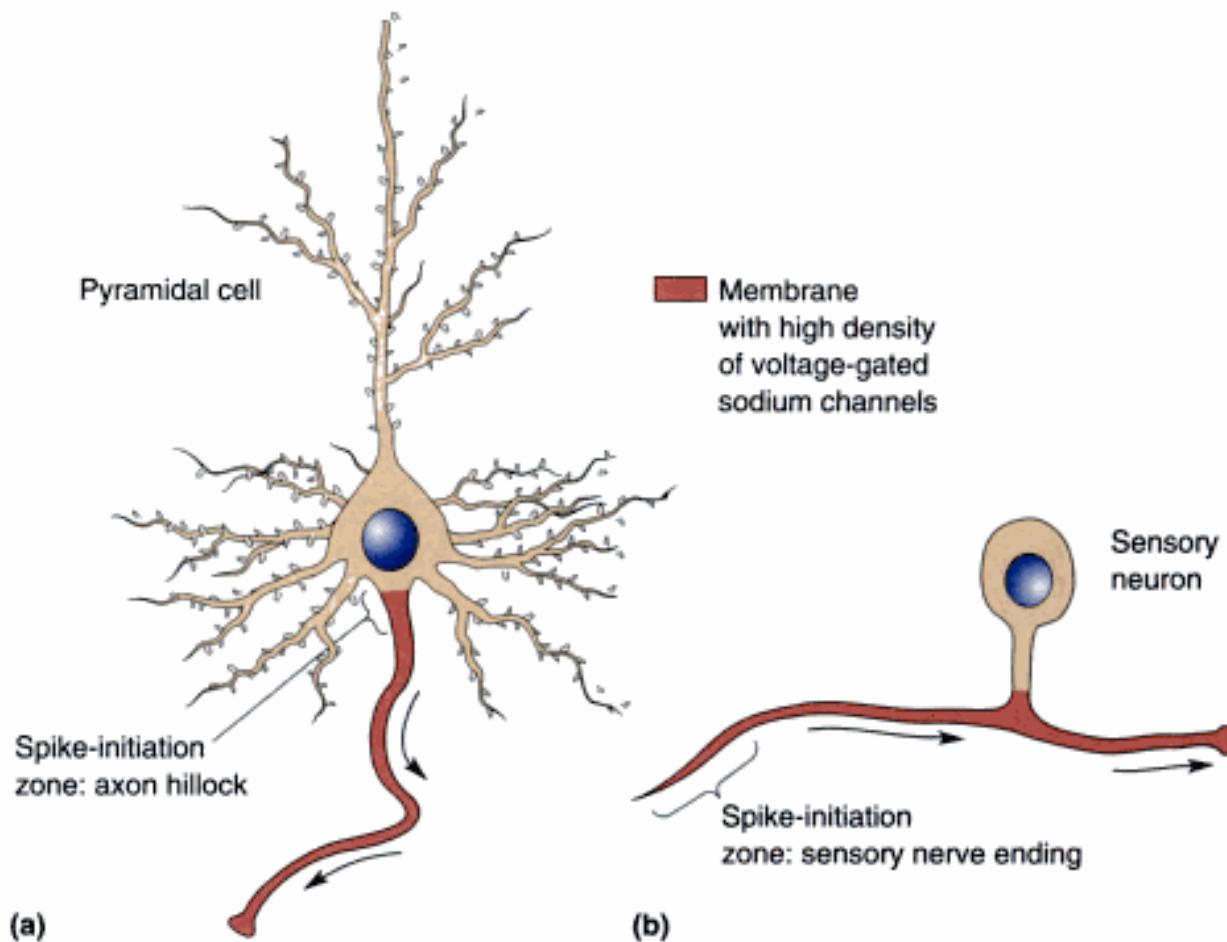
This represents only $\sim 0.0001\%$ of all ions in the cell.



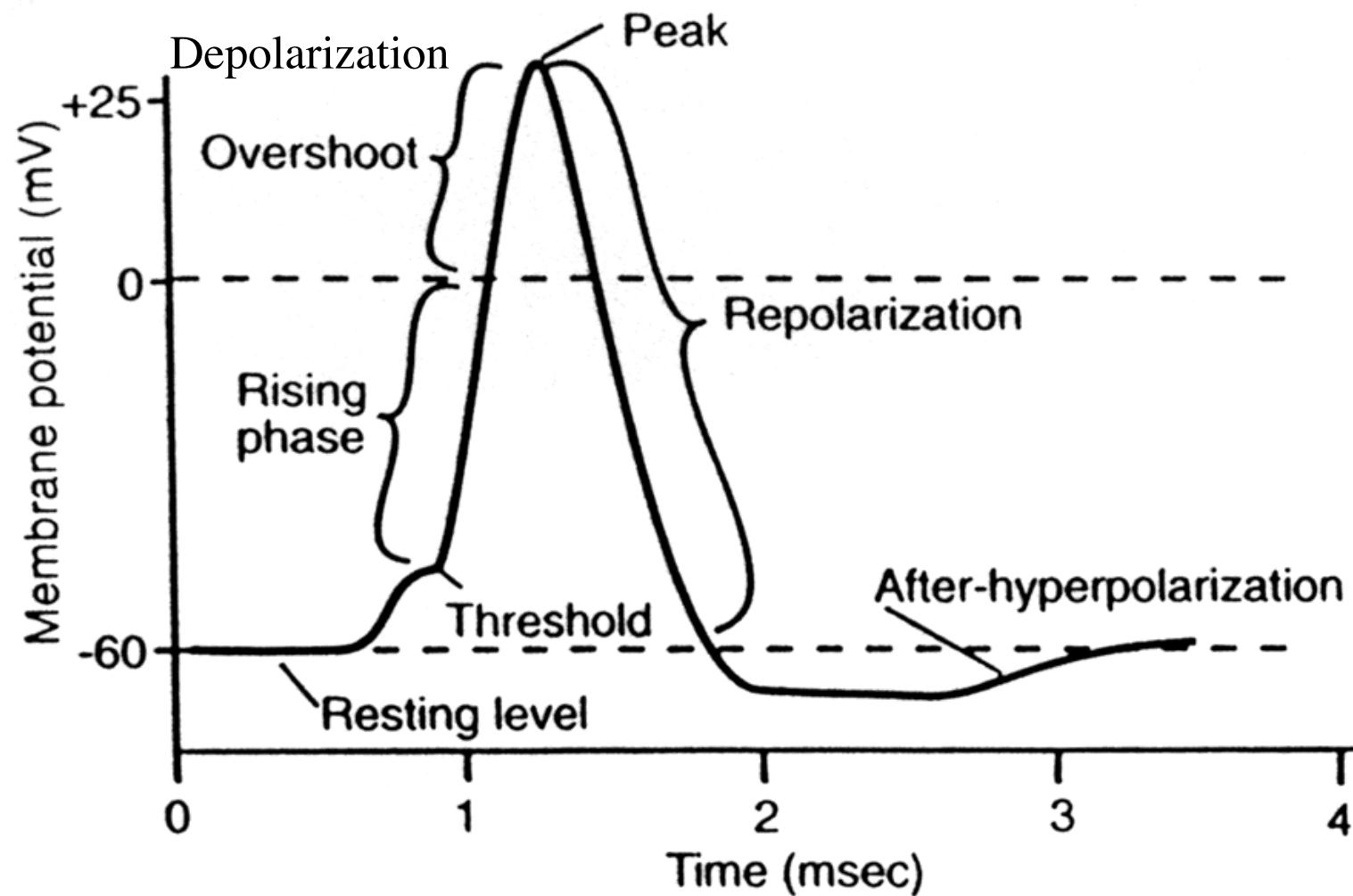
Action Potentials



Where is the action potential initiated?



Components of the action potential



Action potentials – a history

- Jan Swammerdam (1660s) – developed frog nerve-muscle preparation
- Hermann von Helmholtz (1850-52) – measurement of impulse propagation in frog nerve (25-40 m/s). Slow speed suggested that signal was more complex than passive spread of current. Also noted that speed was temperature dependent.
- Sidney Ringer (1881-1887) – Importance of Na^+ and K^+ ions in muscle contraction
- Walther Nernst (1888) – electrical potentials can develop from diffusion of ions in solution.

Julius Bernstein (1860s – 1900)

1868 - First recording of an action potential. Estimated that at rest, the cut end of a nerve is ~ 60 mV more hyperpolarized than surface. The AP was characterized as a loss of this negative potential.

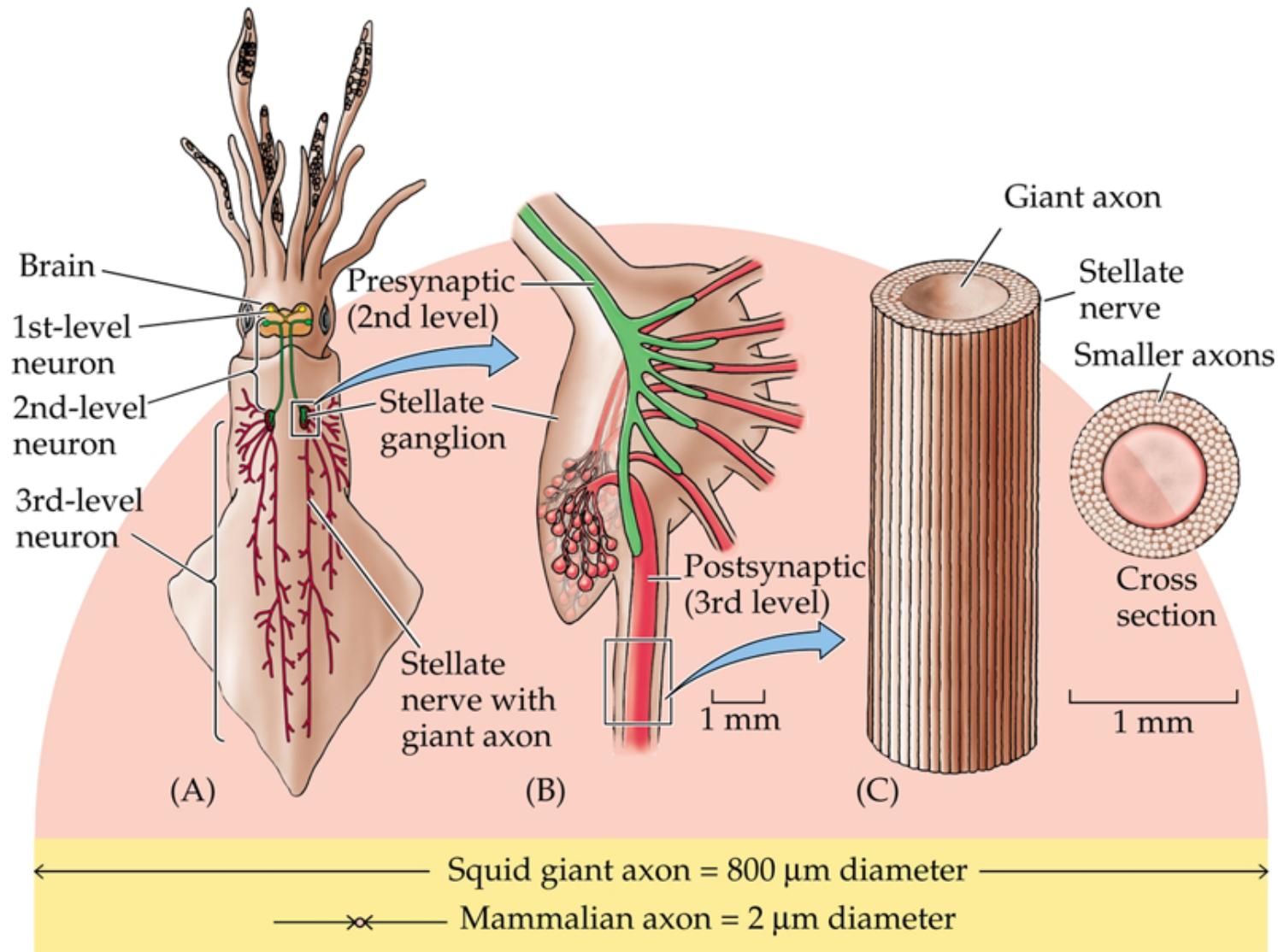
1896 – Proposed that resting potential is due to selective permeability of K⁺ ions.

“Membrane breakdown” hypothesis - during an action potential, the membrane transiently loses its selective permeability.

Action potentials – a history

- Charles Overton (1902) – Extracellular Na^+ ions are necessary for the loss of negative potential recorded by Bernstein. Proposed that action potential is due to K^+ and Na^+ exchange across the membrane. Also proposed that membrane composed of lipids.
- Cole and Curtis (1923) - Cells have electrically conductive cytoplasm surrounded by lipid membrane with a capacitance of $\sim 1\mu\text{F}/\text{cm}^2$
- John Z. Young (1936) – Description of the squid giant axon

The squid giant axon allowed neuroscientists to study the mechanisms of action potential generation and propagation

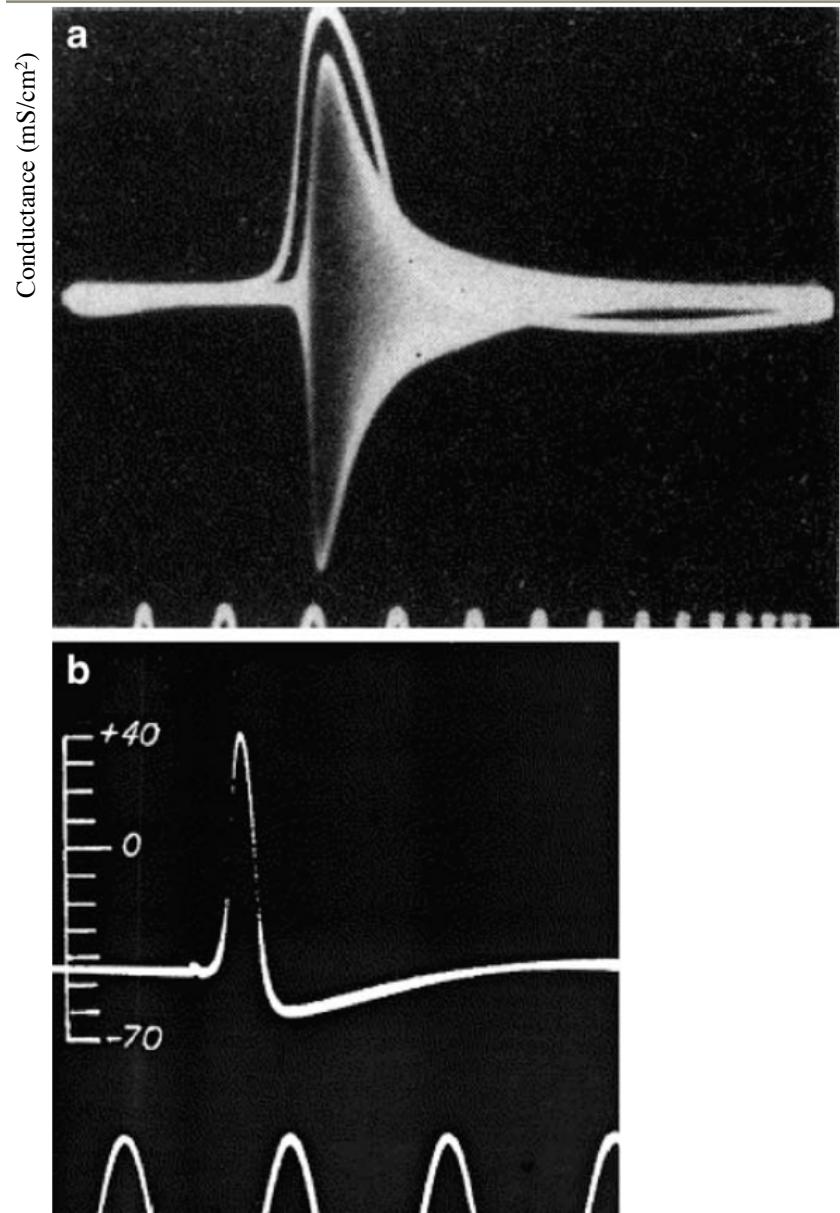


Cole & Curtis / Hodgkin & Huxley

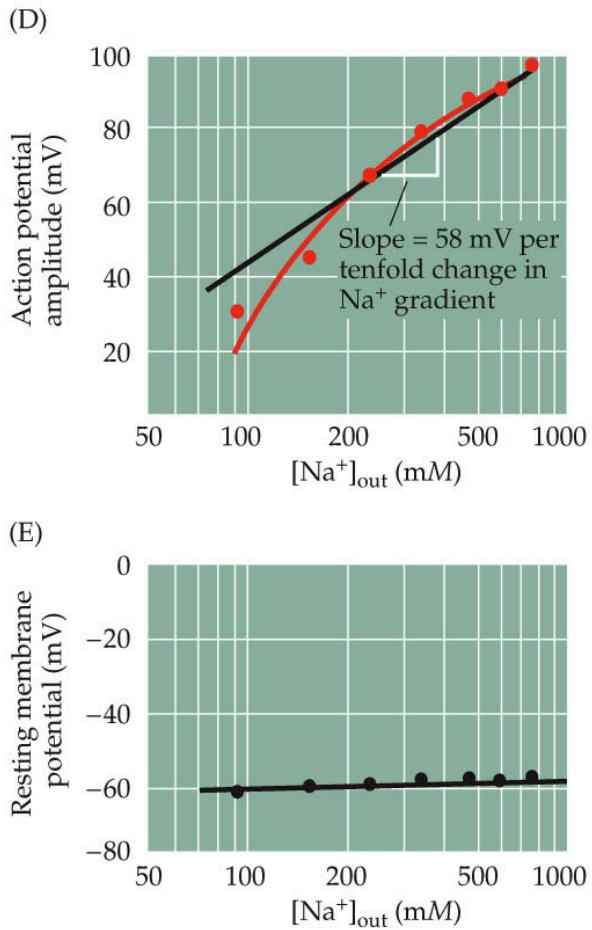
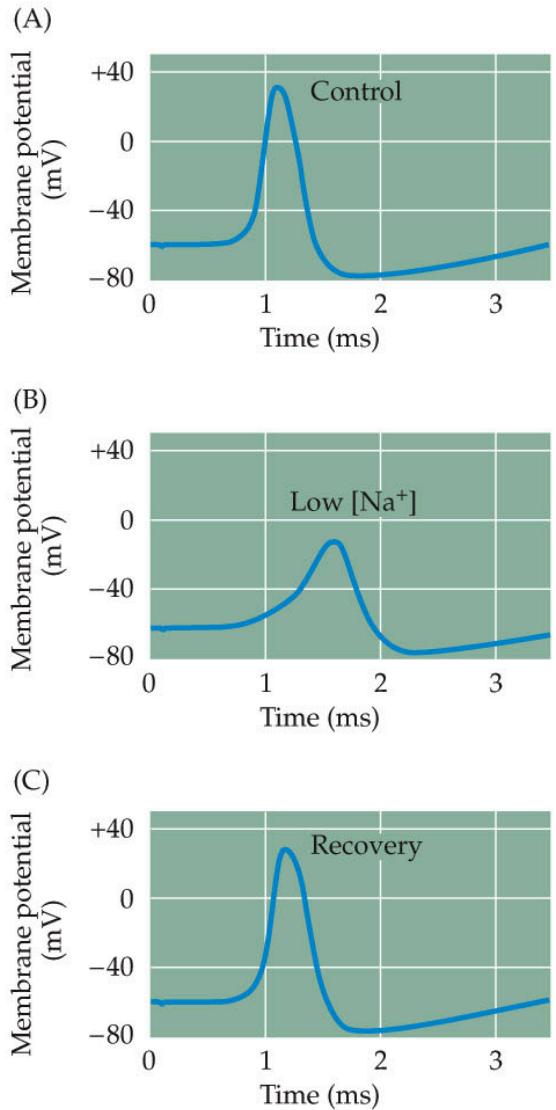
Cole & Curtis (1939) – Demonstrated that conductance of membrane increases during action potential.

Hodgkin & Huxley; Cole & Curtis (1939) – First intracellular recording of an action potential in the squid giant axon.

The voltage overshoot during an AP disproved Bernstein's "membrane breakdown hypothesis" and suggested that Na^+ influx may be responsible for depolarizing phase of an AP

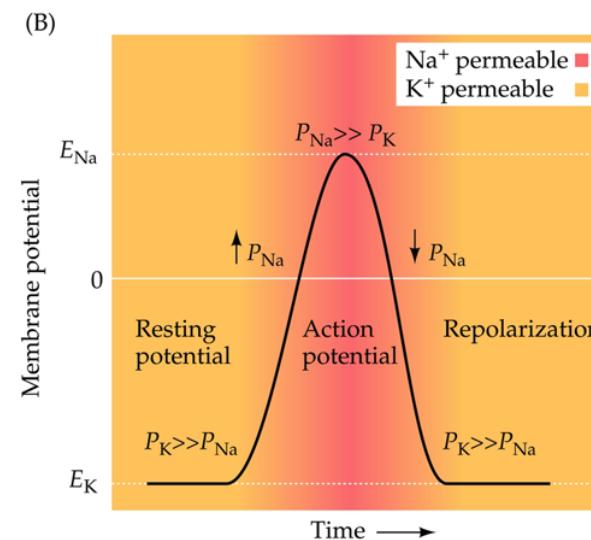
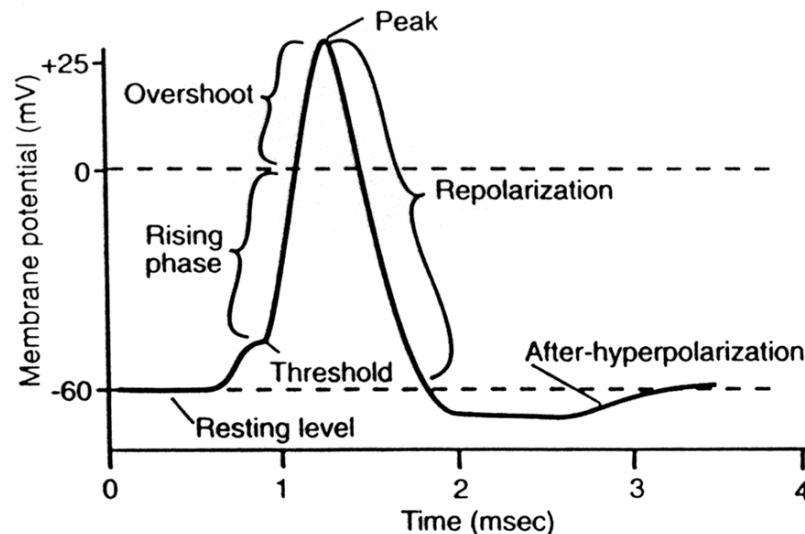


Na^+ ions and the action potential



- Changing external $[\text{Na}^+]$ does not produce a large change in the resting potential
- Changing external $[\text{Na}^+]$ does have a significant influence on the amplitude and rise time of an action potential
- The rate of repolarization is too fast to be explained by just the re-establishment of the resting membrane potential by “leak” type K^+ channels suggesting a more active process

Changes in ion permeability are voltage dependent



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Voltage dependence of threshold suggests that change in Na permeability is voltage dependent. **So if permeability changes with voltage, how to study it?**

Ohm's law – the current (I) that flows is proportional to the conductance (g) and the driving force (voltage - V)

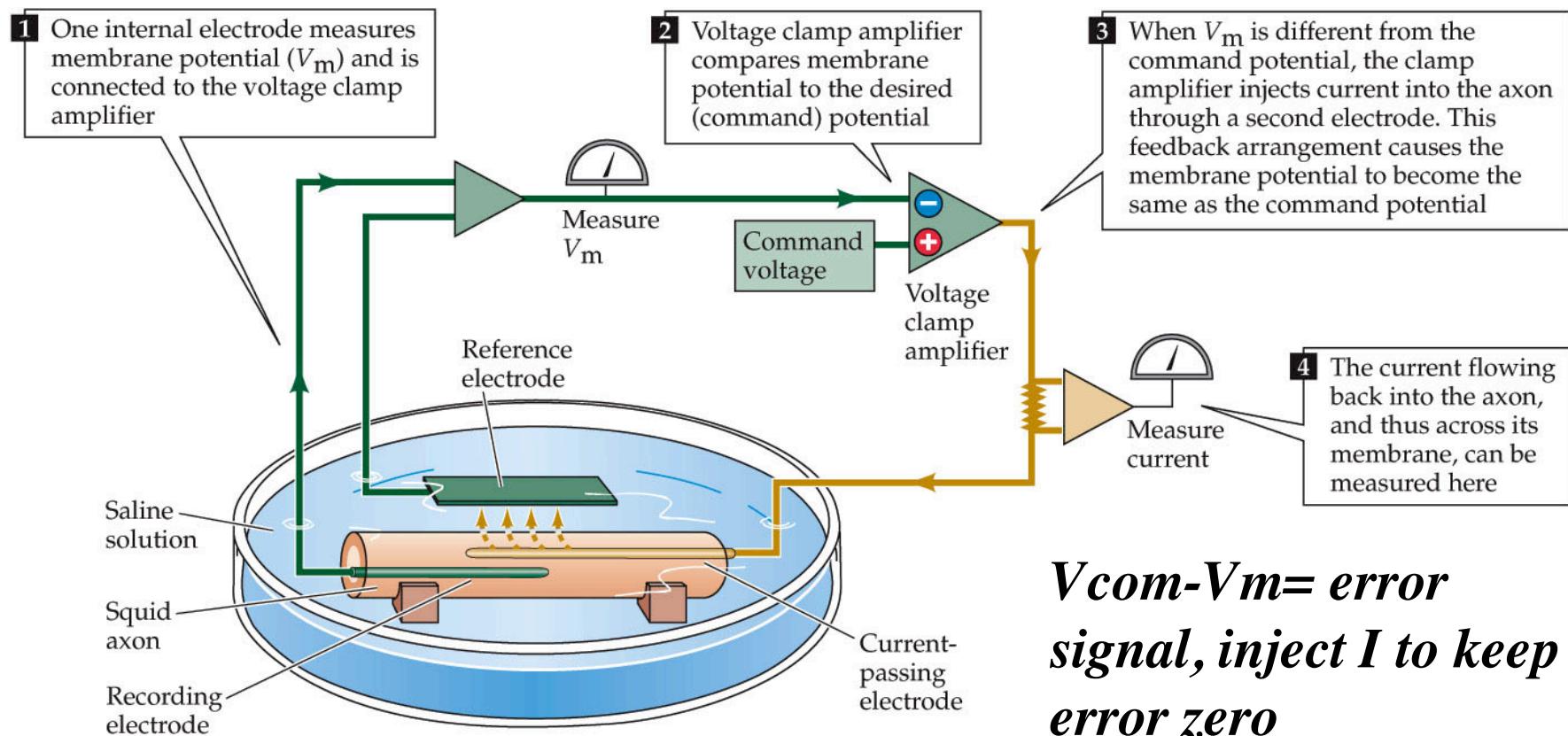
$$I = gV$$

For the current carried by a single ion species, this equation can be re-written as:

$$I_{\text{ion}} = g_{\text{ion}} (V_m - E_{\text{ion}}) \leftarrow \text{how far away is } V_m \text{ from equilibrium potential (}E_{\text{ion}}\text{)}$$

So since we know E_{ion} (Nernst Eq.), if we vary V_m and measure I_{ion} we can determine g_{ion}

The voltage clamp technique allows for the study of current flow across the membrane



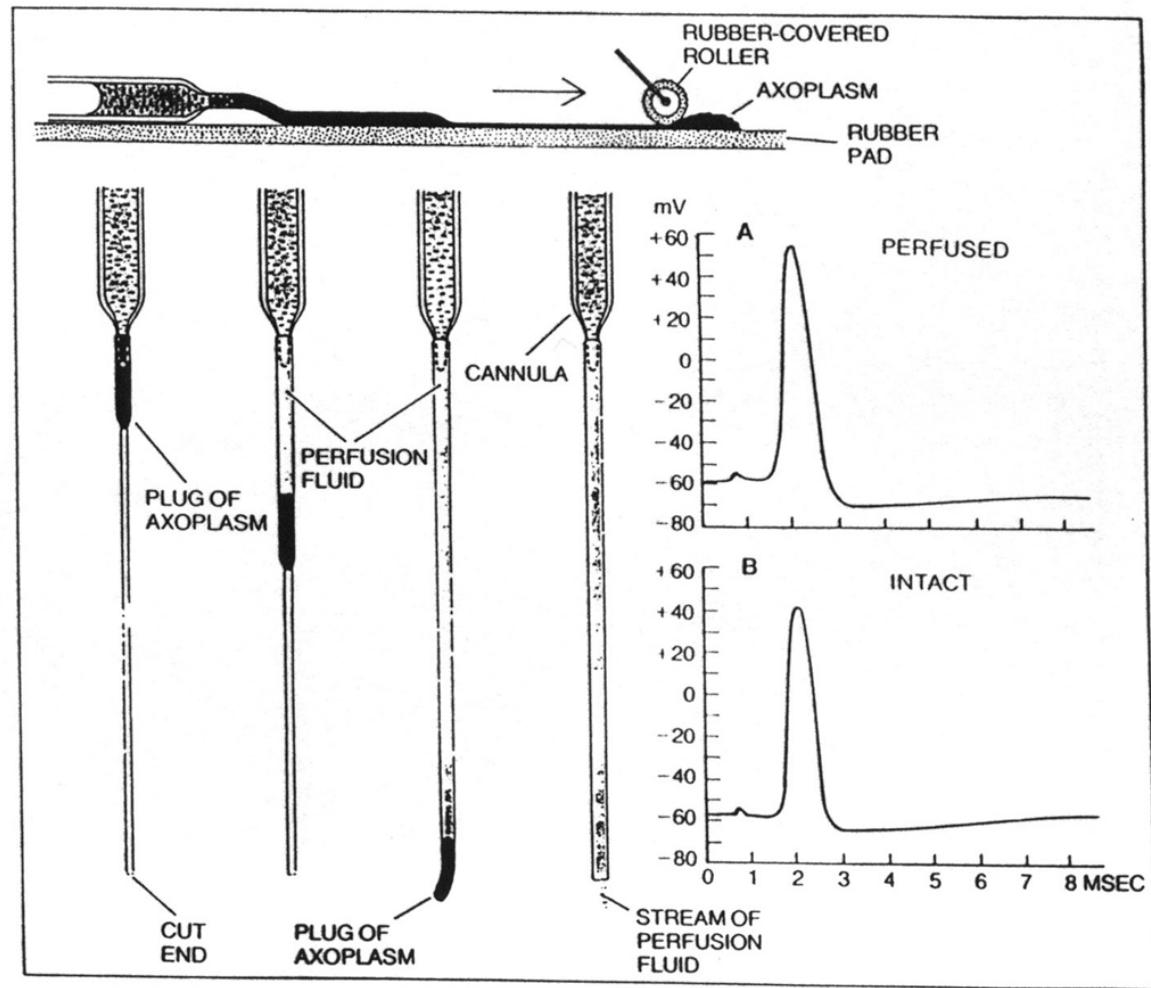
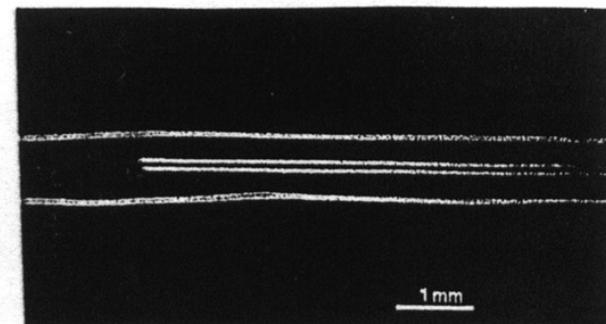
$V_{com} - V_m = \text{error}$
signal, inject I to keep
error zero

Developed independently in 1949 by Cole & Marmont.
Technique formed the basis for a series of papers by Hodgkin & Huxley in early 1950s that earned them the Nobel Prize in 1963

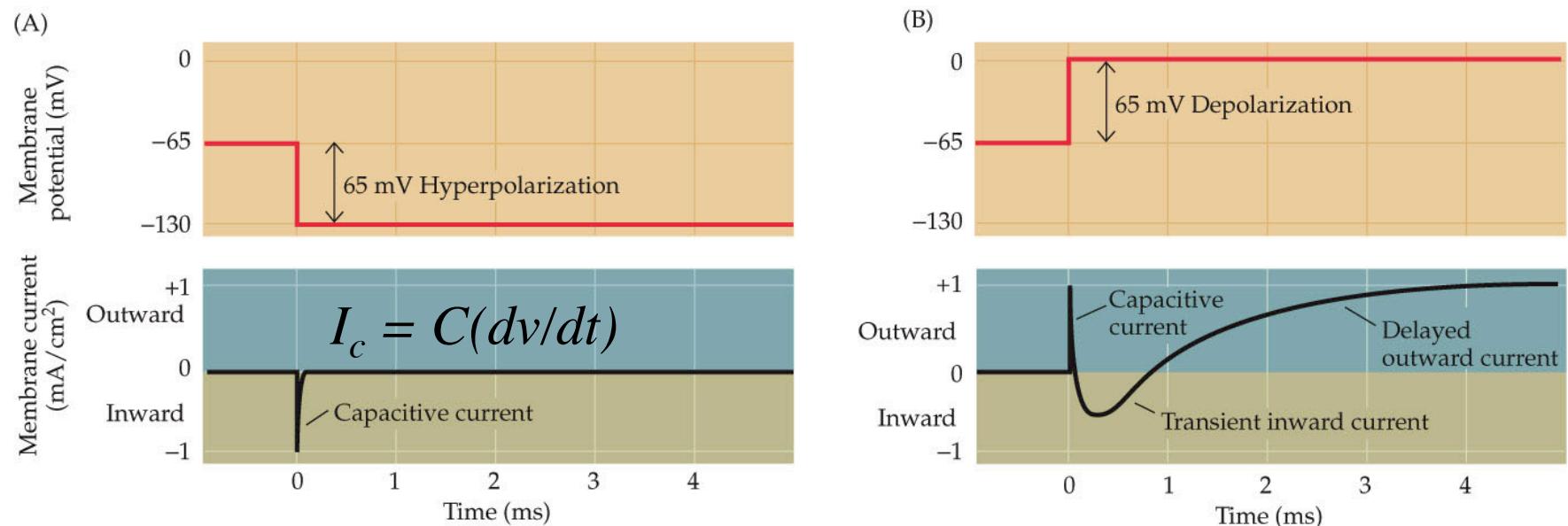
Giant Squid axons allowed us to analyze APs

Showed that ion concentration differences & semipermeable membrane are sufficient to produce an action potential

First axon from which we could measure internal voltage directly



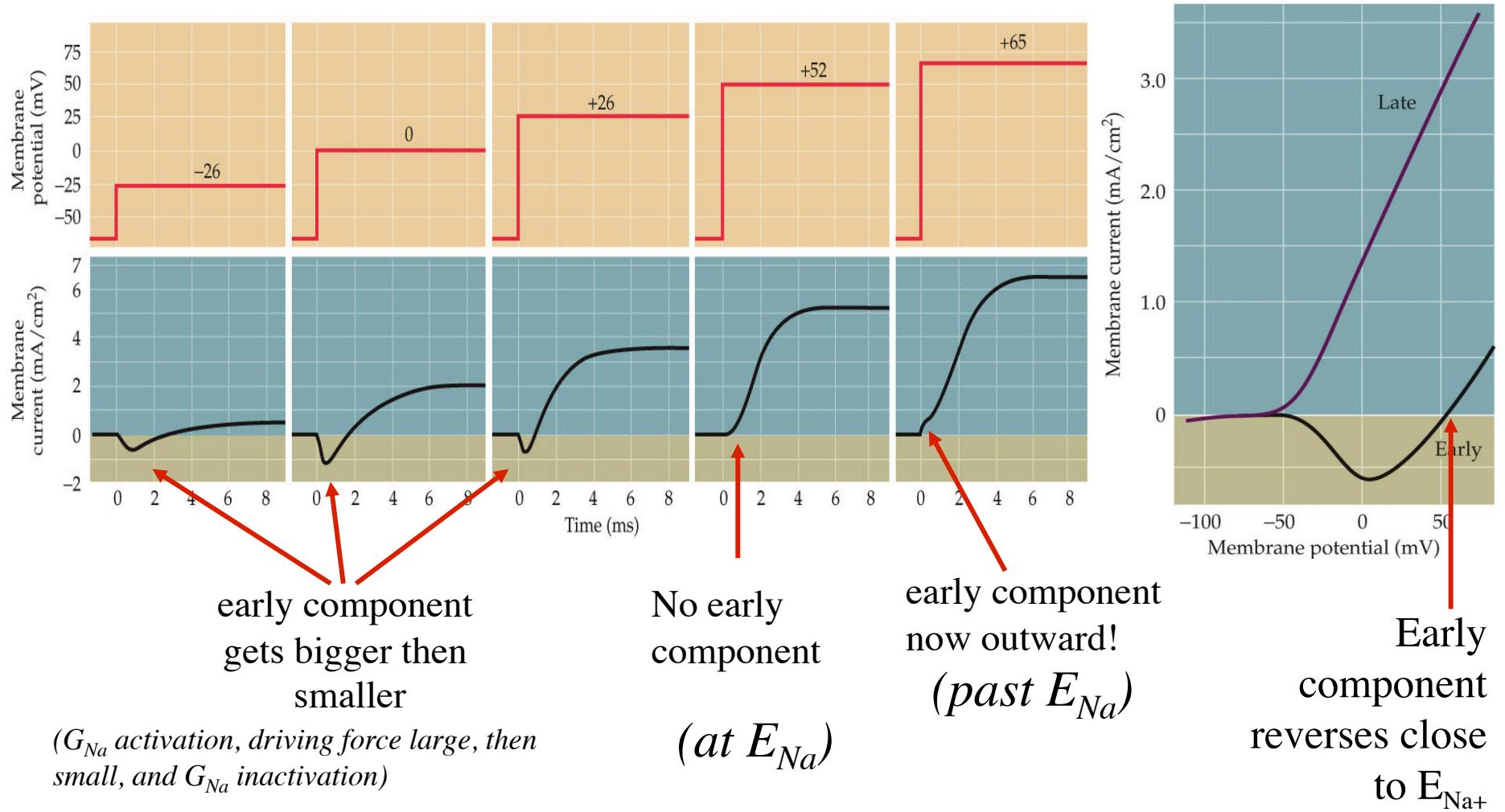
Depolarization evokes a biphasic flow of current in the squid giant axon (change over time)



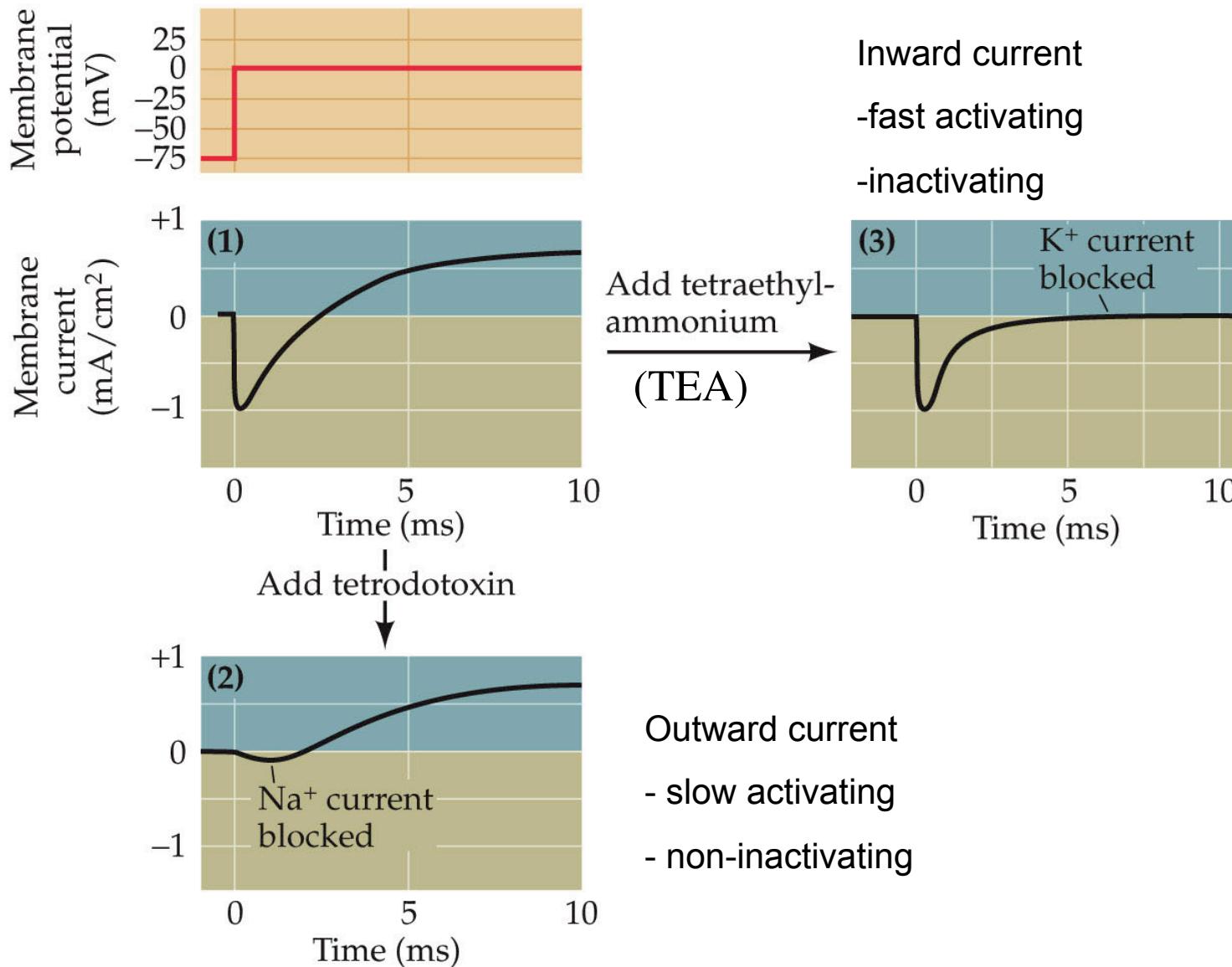
Inward current – increase in + charge moving into the cell (or – charge moving out of the cell)

Outward current – increase in + charge moving out of the cell (or – charge moving into the cell)

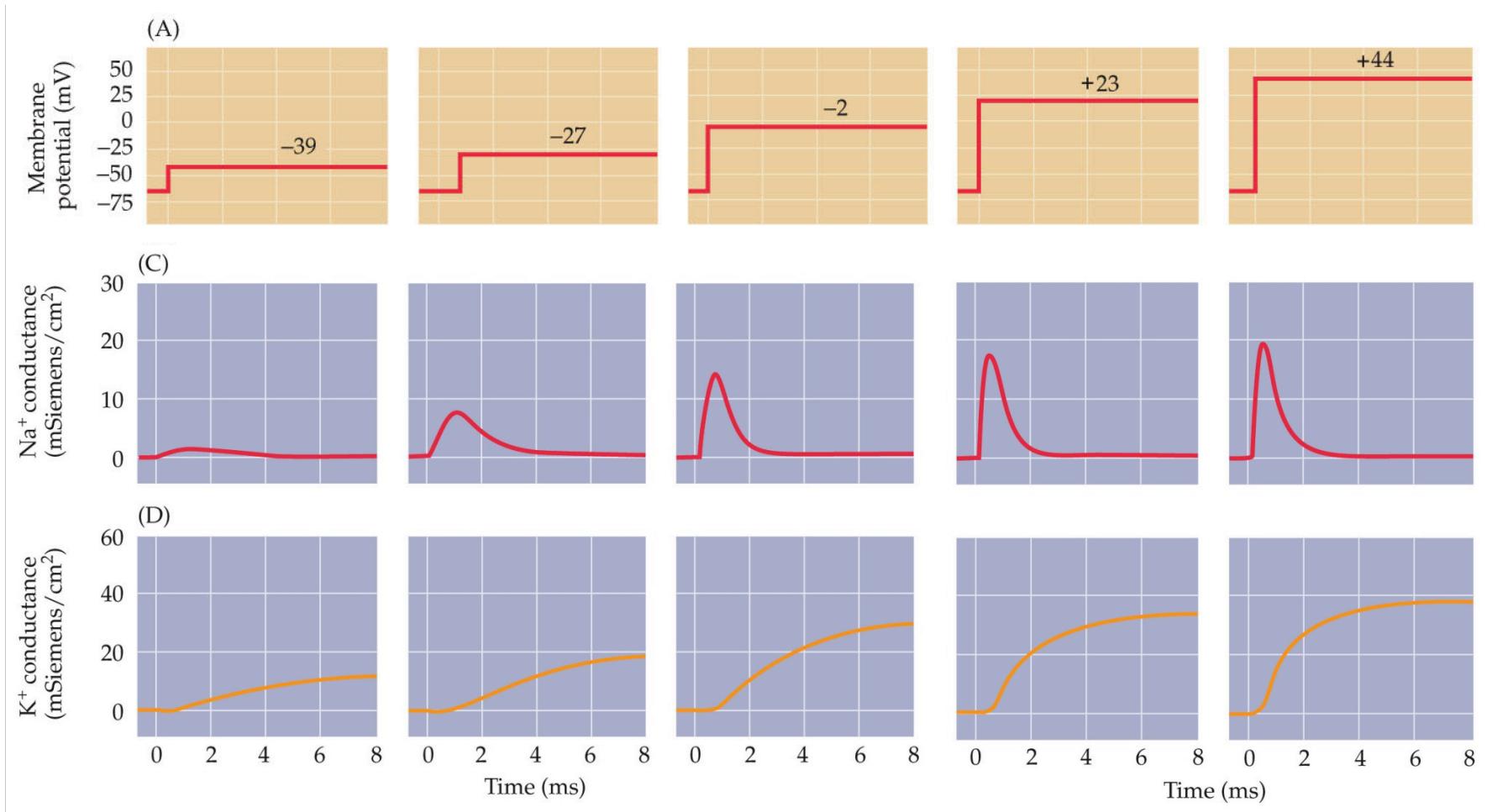
Varying the membrane potential has different effects on the inward and outward current



Pharmacological isolation of inward and outward currents

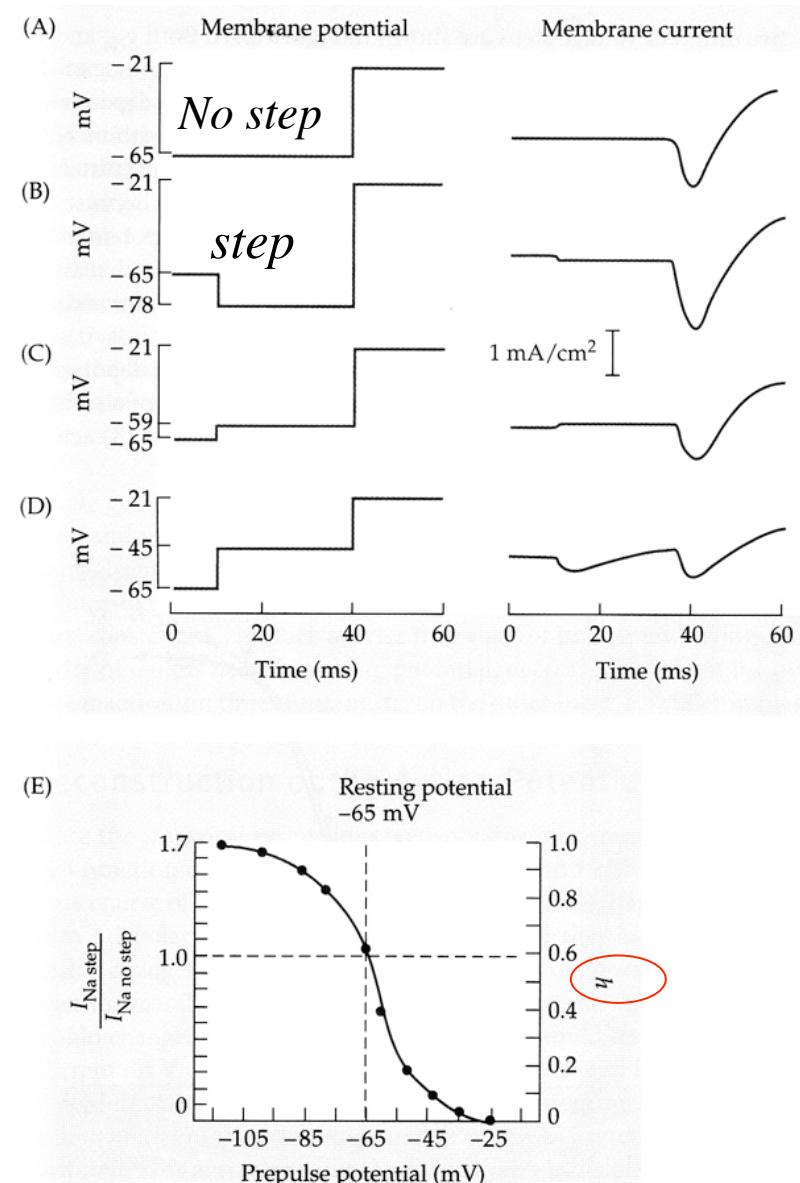


Voltage dependence of Na^+ and K^+ conductance



Sodium currents show voltage dependent inactivation

- Subthreshold depolarization or “conditioning” (below AP threshold) is sufficient to inactivate a % of total Na^+ current
- Na^+ activation and inactivation processes are separate (Na^+ current does not need to activate before inactivating) – different activation and inactivation “particles” on v-gated ion channel.

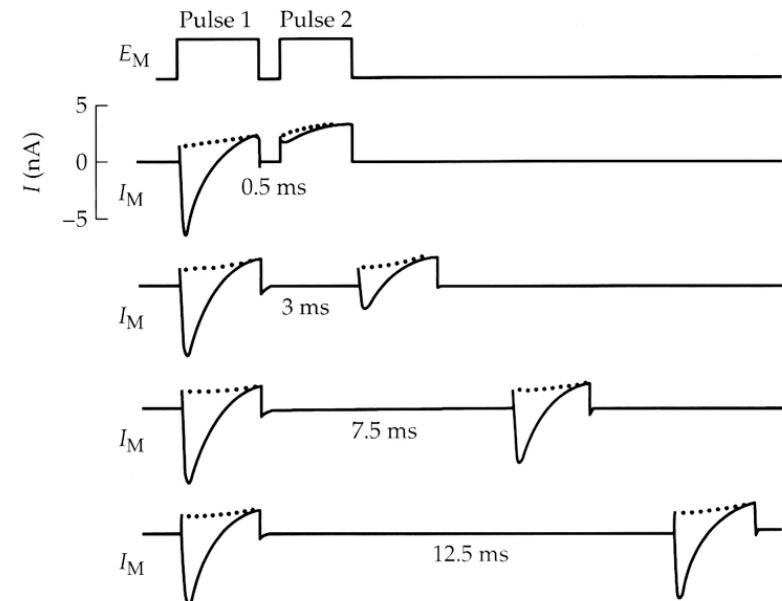


Recovery of Na^+ current inactivation

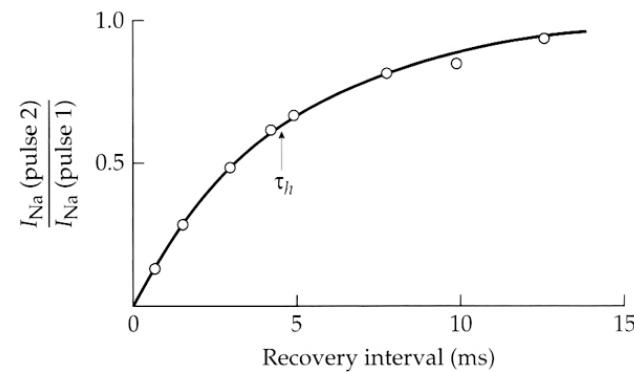
Following inactivation of the Na^+ current by depolarization, recovery takes several milliseconds at resting potential

Hyperpolarization of the membrane speeds Na^+ current recovery

(A) TWO-PULSE EXPERIMENT

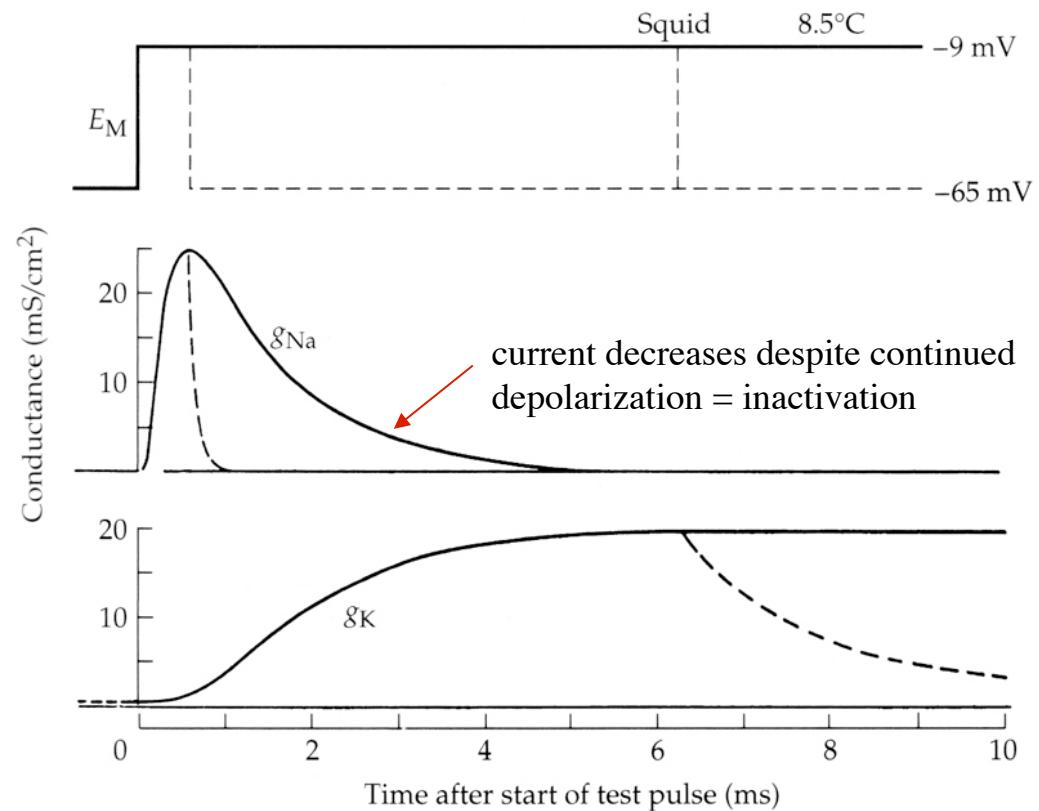


(B) RECOVERY CURVE



Na^+ and K^+ currents have different activation kinetics

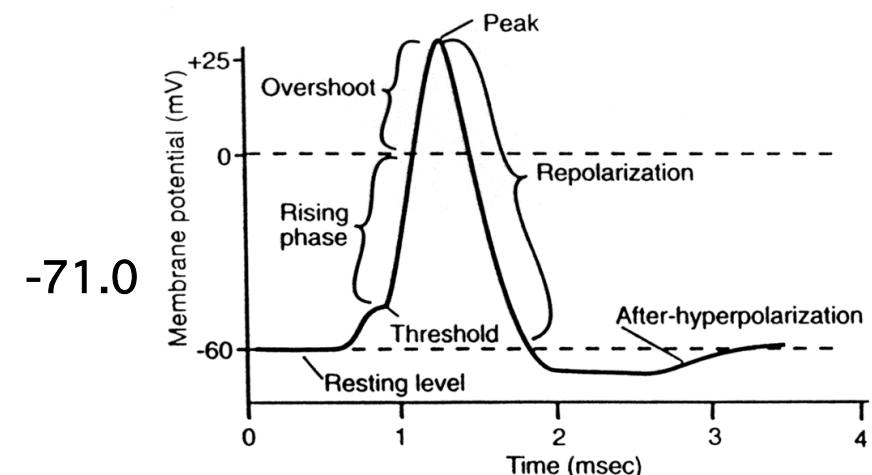
- Na^+ currents activate and inactivate quickly
- K^+ currents activate with much slower kinetics
- K^+ currents in squid are non-inactivating but in some mammalian cells, inactivation is present



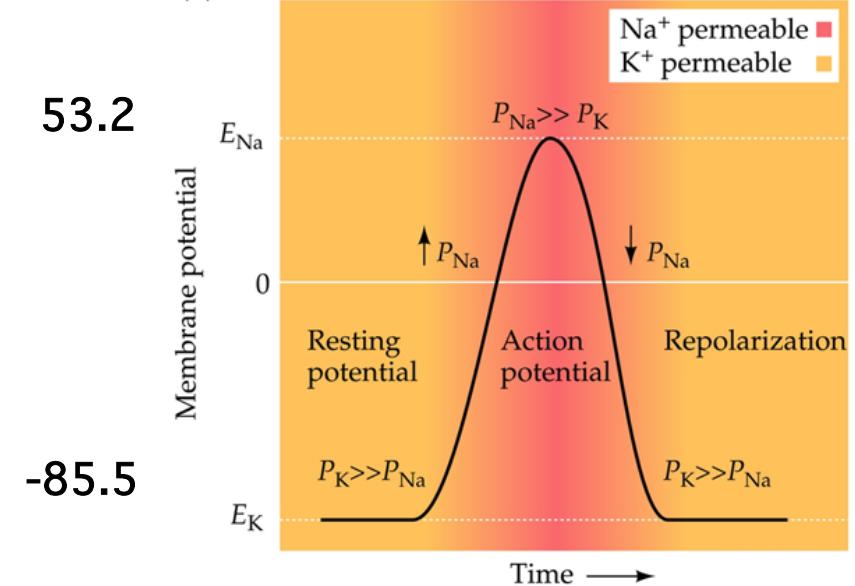
Changes in permeability change membrane potential

| ion | Cin | Cout | Eion | rel. perm |
|-----------------------|-----|------|-------|-----------|
| Resting Potential | | | | |
| Na+ | 12 | 145 | +66.0 | 0.04 |
| K+ | 150 | 4.5 | -92.9 | 1 |
| Cl- | 8.5 | 125 | -71.2 | 0.45 |
| Peak Action Potential | | | | |
| Na+ | 12 | 145 | +66.0 | 20 |
| K+ | 150 | 4.5 | -92.9 | 1 |
| Cl- | 8.5 | 125 | -71.2 | 0.45 |
| End Action Potential | | | | |
| Na+ | 12 | 145 | +66.0 | 0.02 |
| K+ | 150 | 4.5 | -92.9 | 2 |
| Cl- | 8.5 | 125 | -71.2 | 0.45 |

Ememb

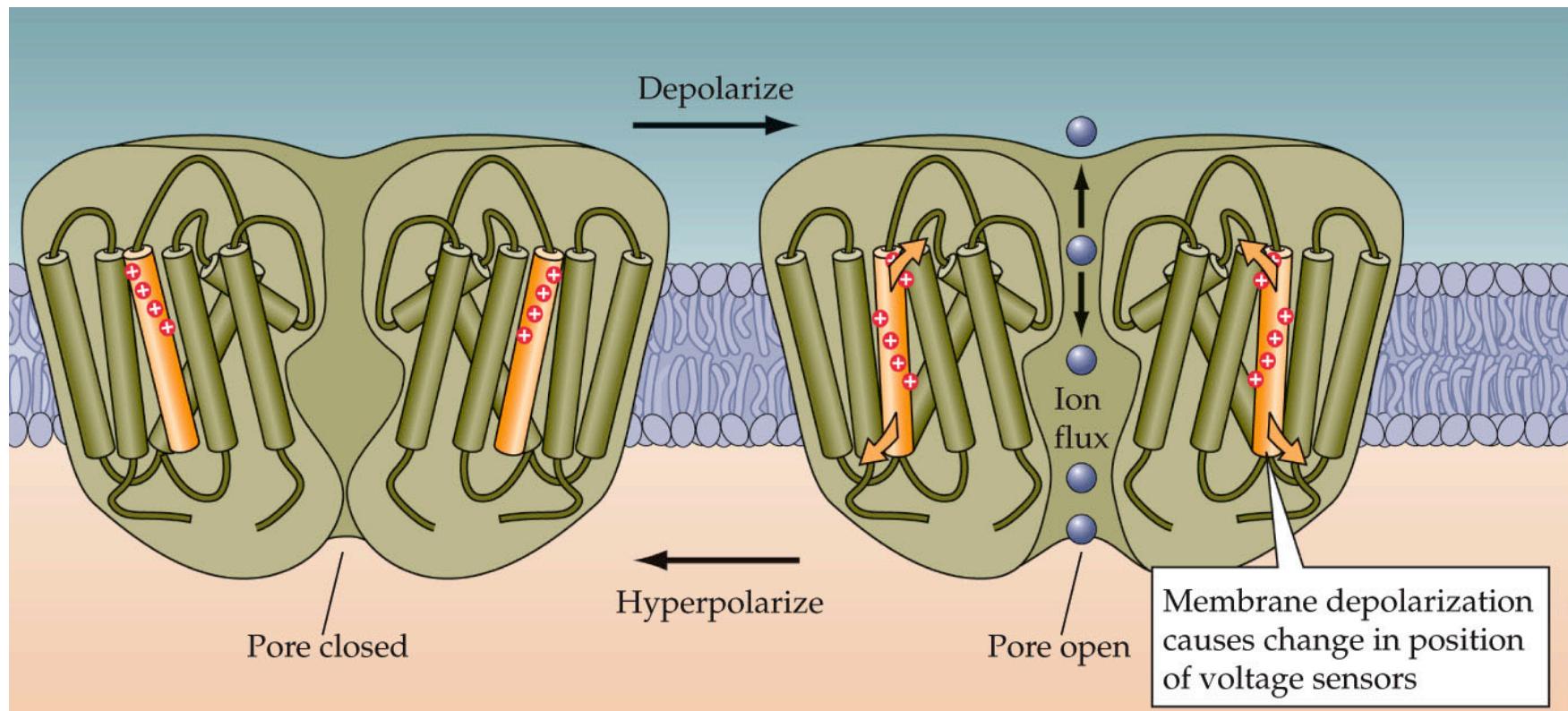


(B)

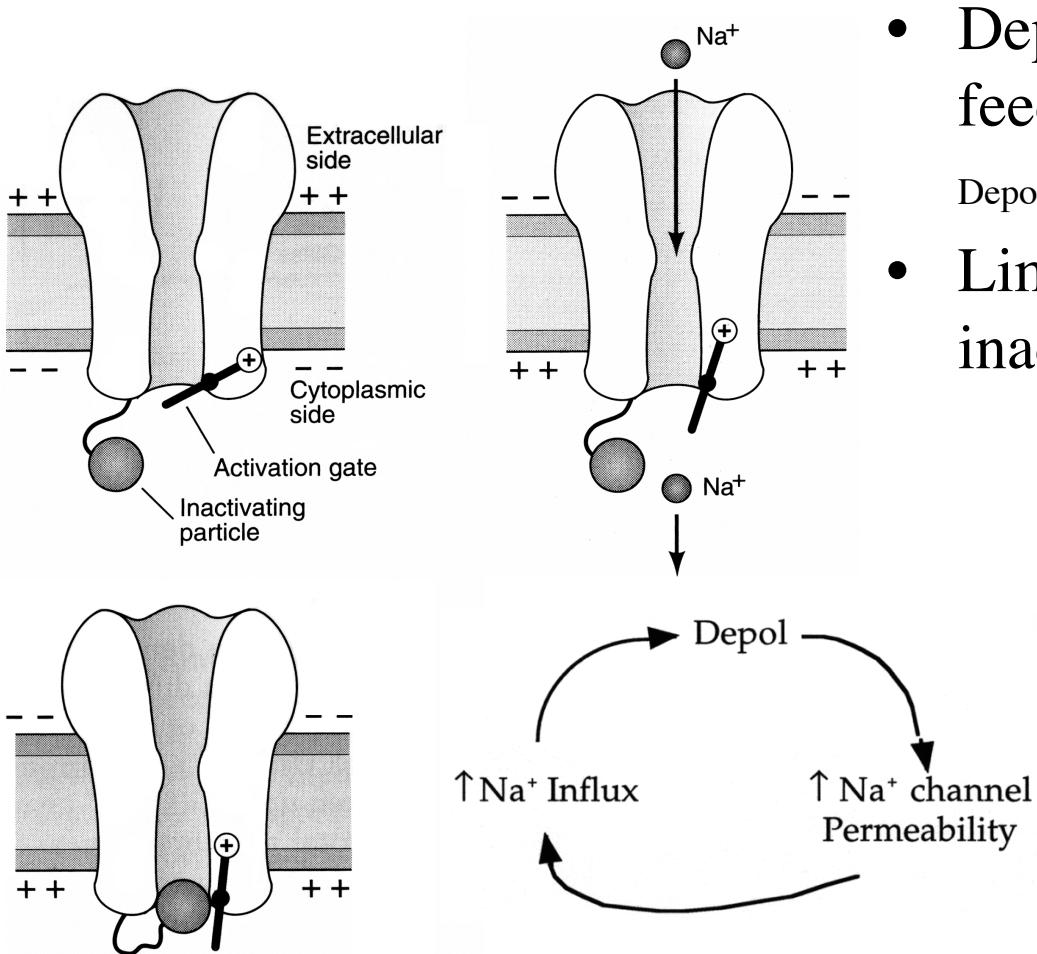


Voltage Gated channels

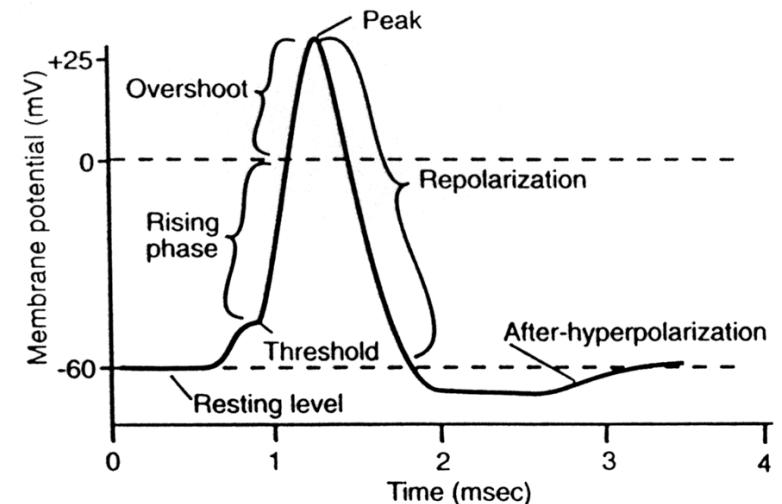
- Changes in membrane permeability for K^+ and Na^+ ions is due to the opening and closing of voltage gated ion channels which allow these ions to pass across the membrane
- Open or close in response to changes in membrane potential



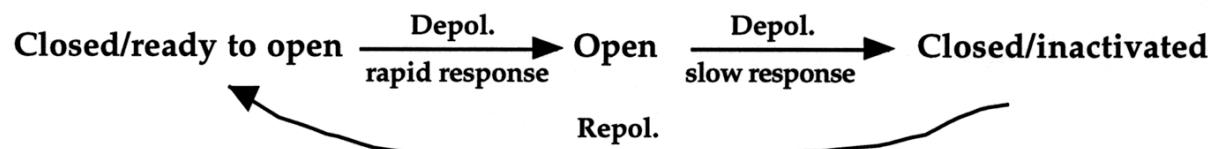
Behavior of V-gated Na⁺ channel



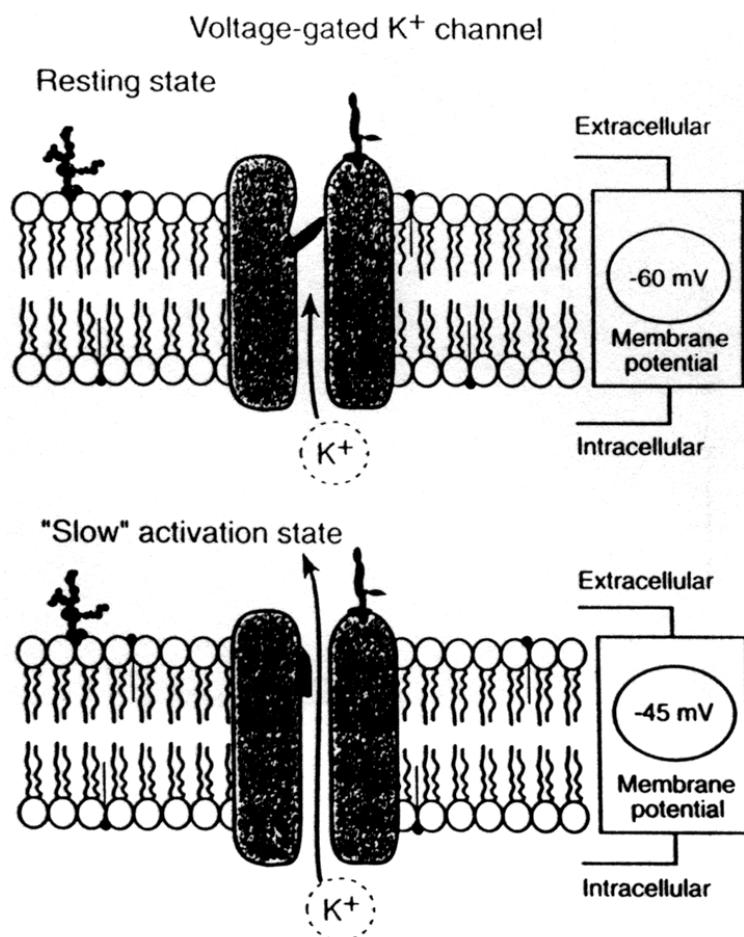
- Depolarization creates a positive feedback process
Depolarization → Na⁺ influx → depolarization, etc
- Limited to a short period by inactivation



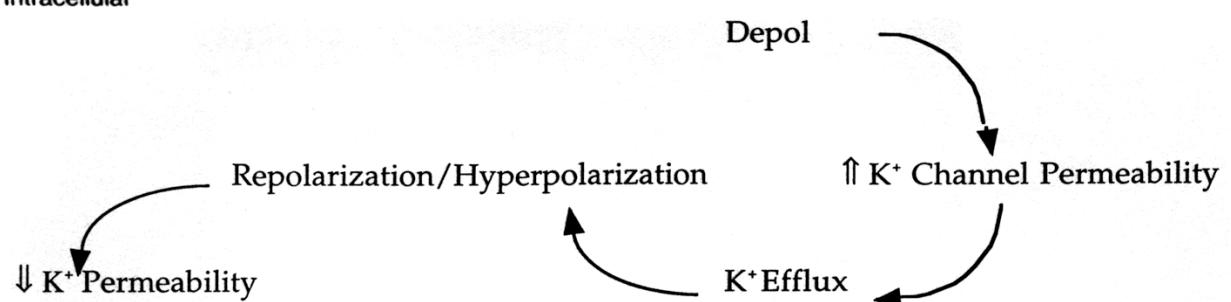
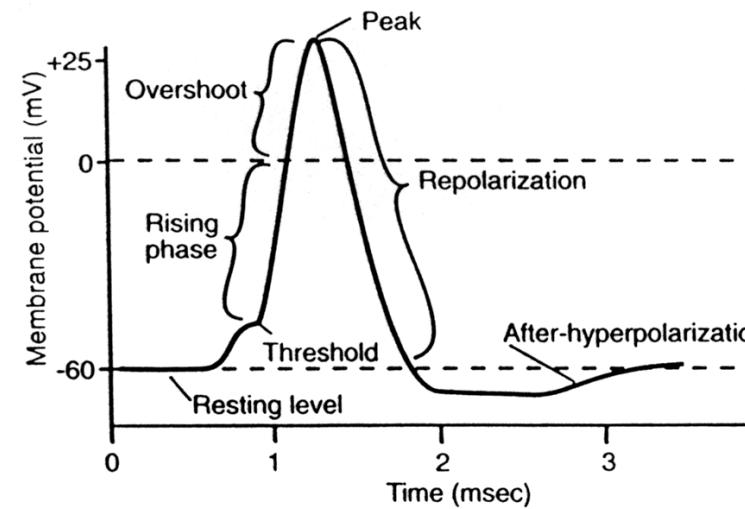
Cycle of Na⁺ channel states:



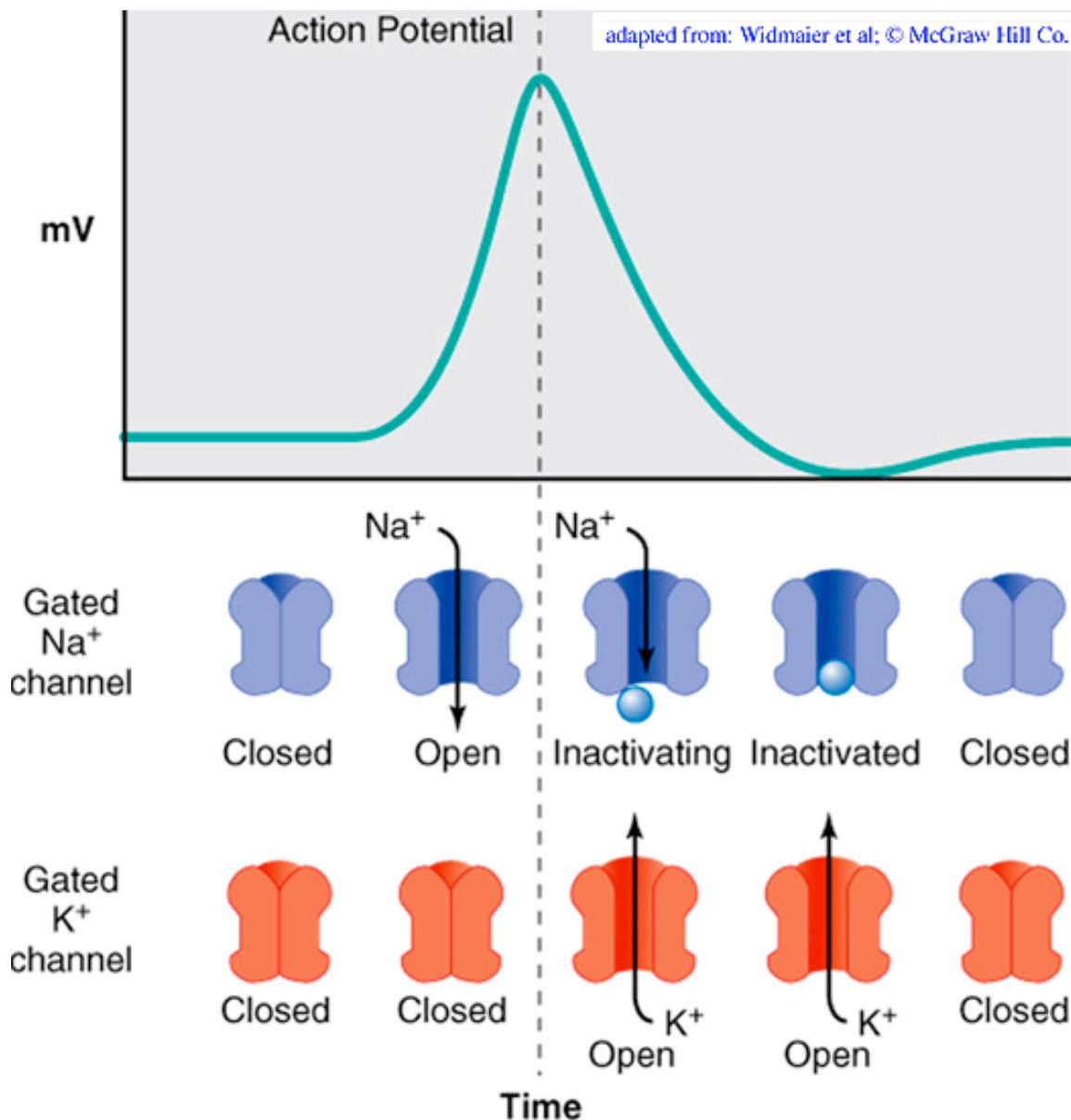
Behavior of V-gated K⁺ channel



- V-gated K⁺ channels have slower kinetics than v-gated Na⁺ channels
- Opening of K⁺ channel is self-limiting (negative feedback-decrease driving force)
Depolarization → K⁺ efflux → repolarization



Channel responses



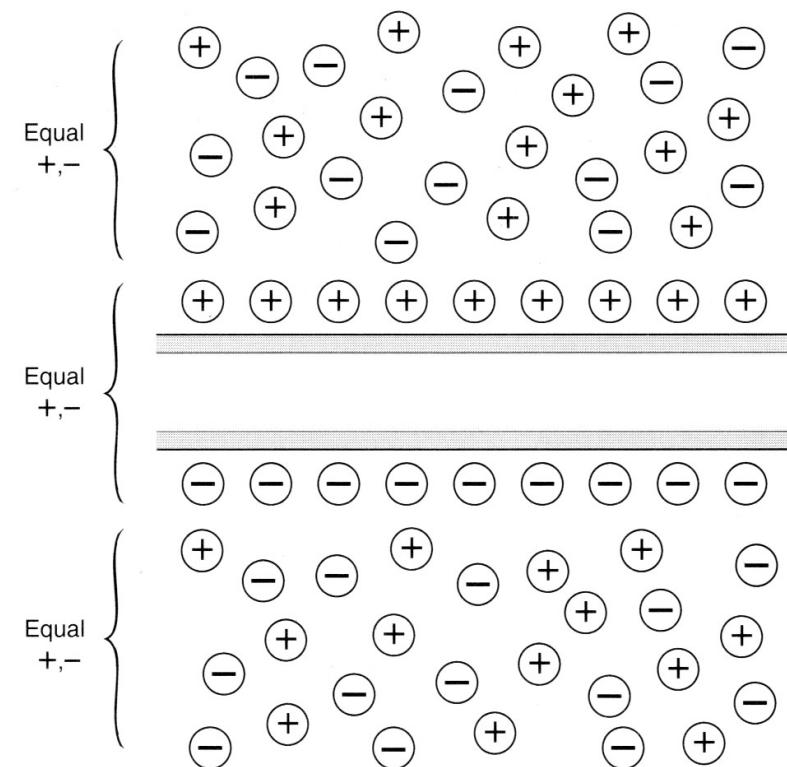
- **Na⁺ channel:**
 - Fast response
 - Two gates
 - Rapid opening followed by slower closing (inactivation)
 - Results in rapid influx of Na⁺
- **K⁺ channel**
 - Slow opening & closing
 - One gate
 - Results in delayed efflux in K⁺

Action potentials only require movement of (relatively) small numbers of ions

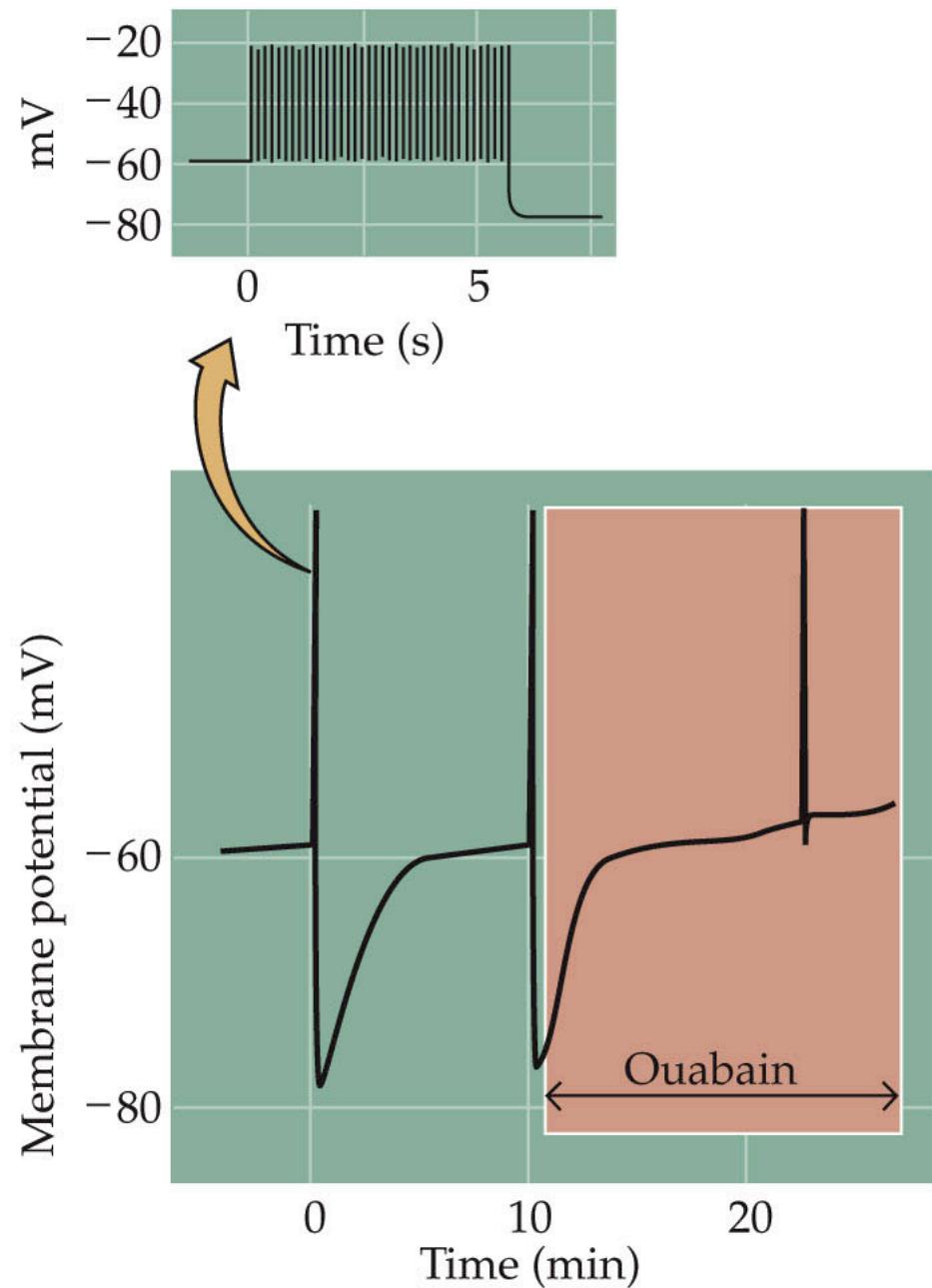
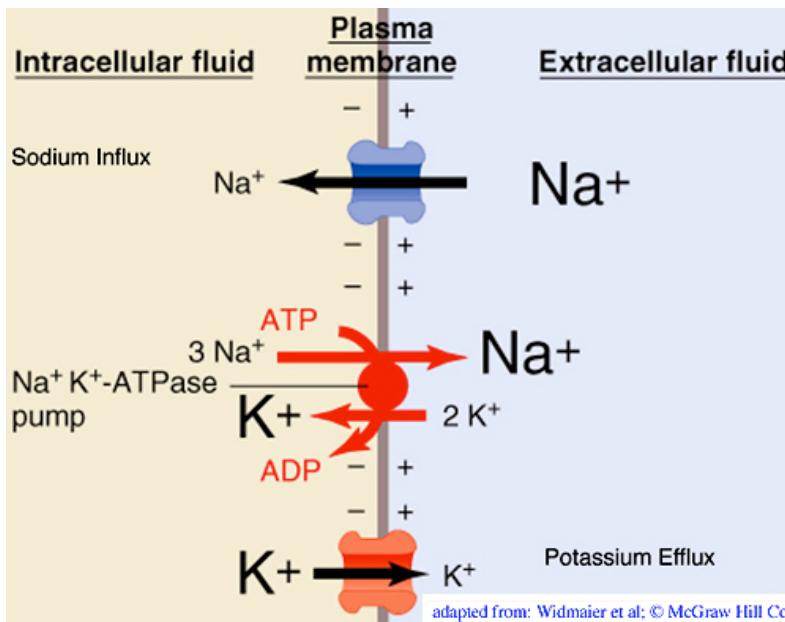
At rest (~ -70 mV), ~ 1 pM/cm² of + charged ions are separated from an equal number of - charged ions.

During an action potential, $\sim 3-4$ pM/cm² of Na⁺ ions cross the membrane

In a squid giant axon, this represents an increase of 0.00003% of the total intracellular Na⁺ concentration per AP

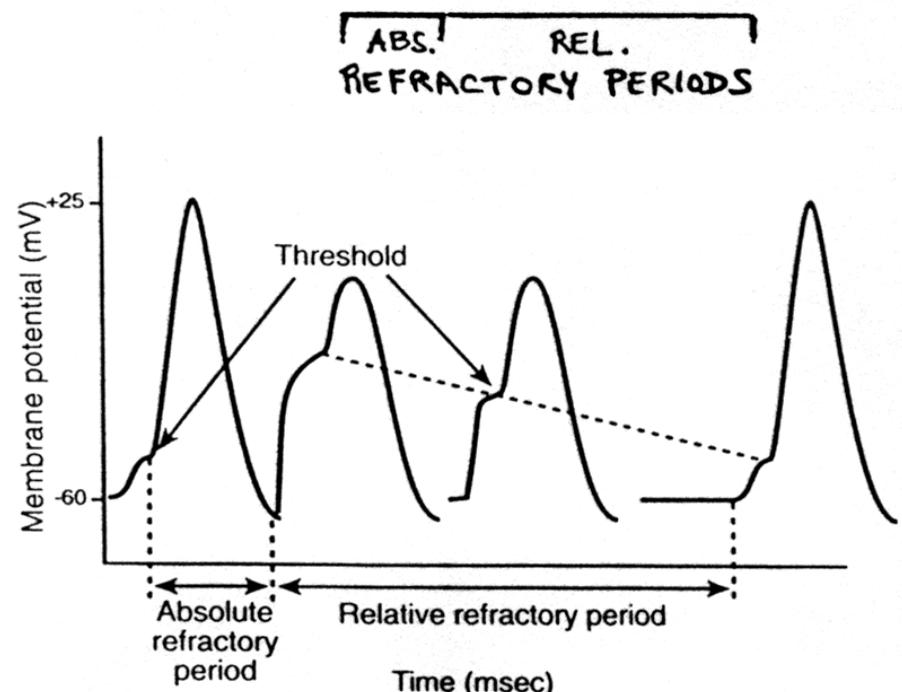
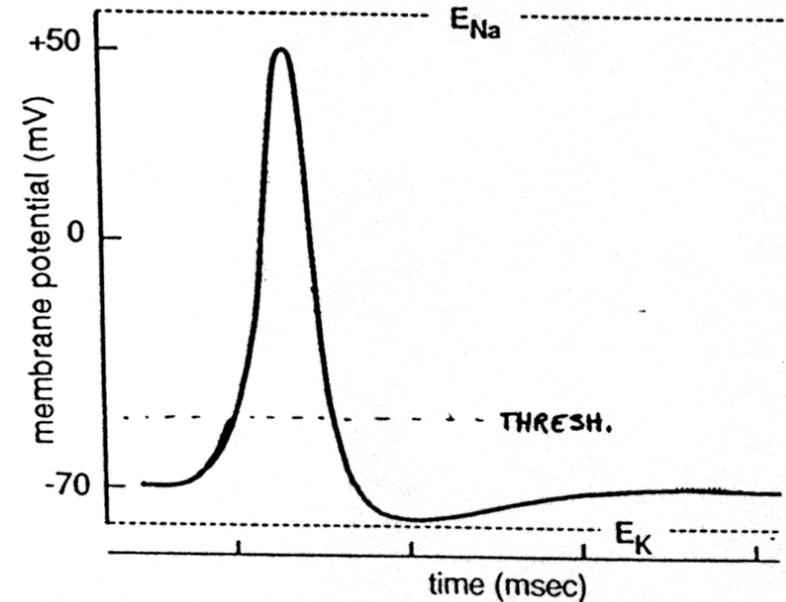


The Na^+/K^+ ATPase does not participate in repolarization following a single AP, but can influence membrane potential during repetitive stimulation

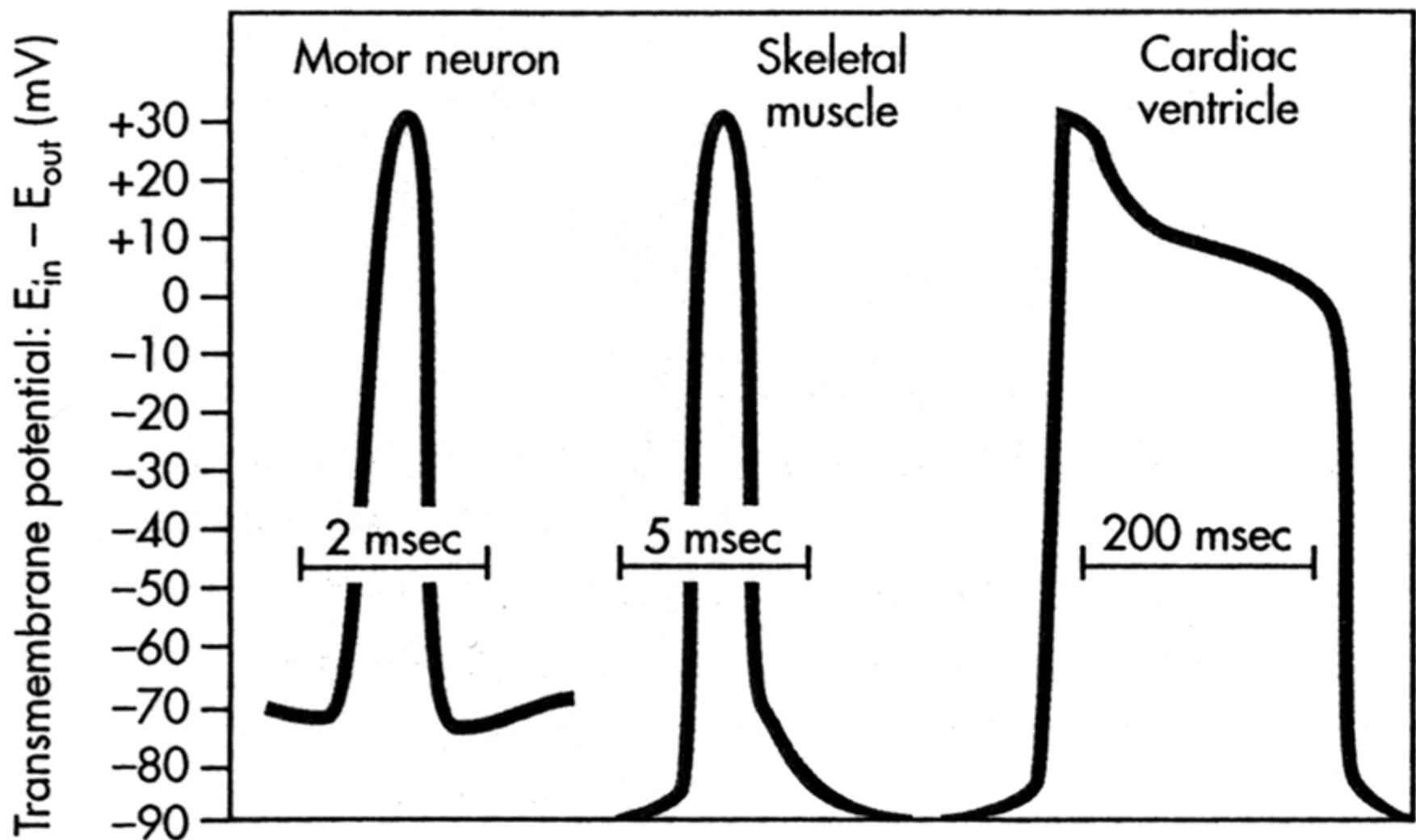


Refractory periods

- After one action potential it is difficult or impossible to initiate a second action potential
 - Due to:
 - Na^+ inactivation
 - Increased K^+ permeability
 - Absolute followed by Relative
 - Absolute: no AP by any stimulus
 - Relative: AP only by larger stimulus
- Implies that threshold for evoking a spike changes*



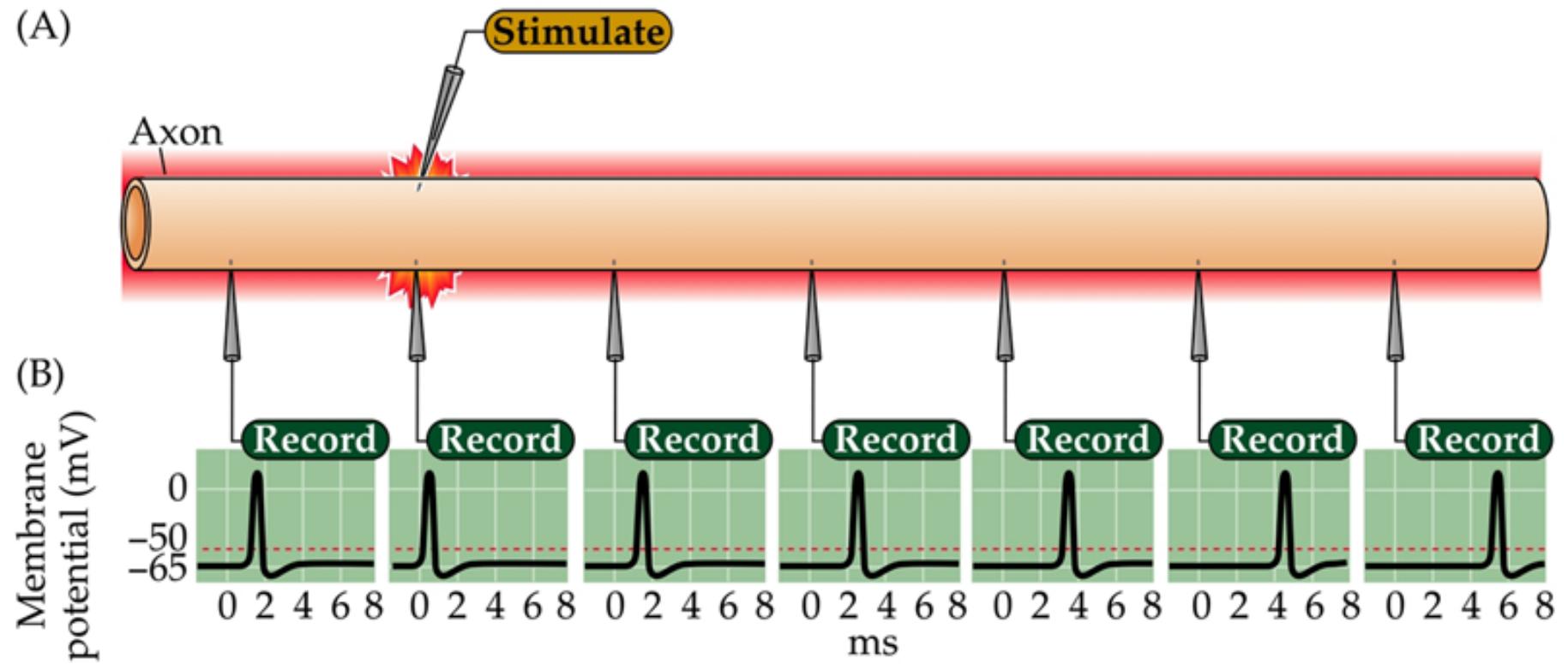
Action potentials differ in different cells



- Action Potential shapes differ due to different channel properties

Action potentials

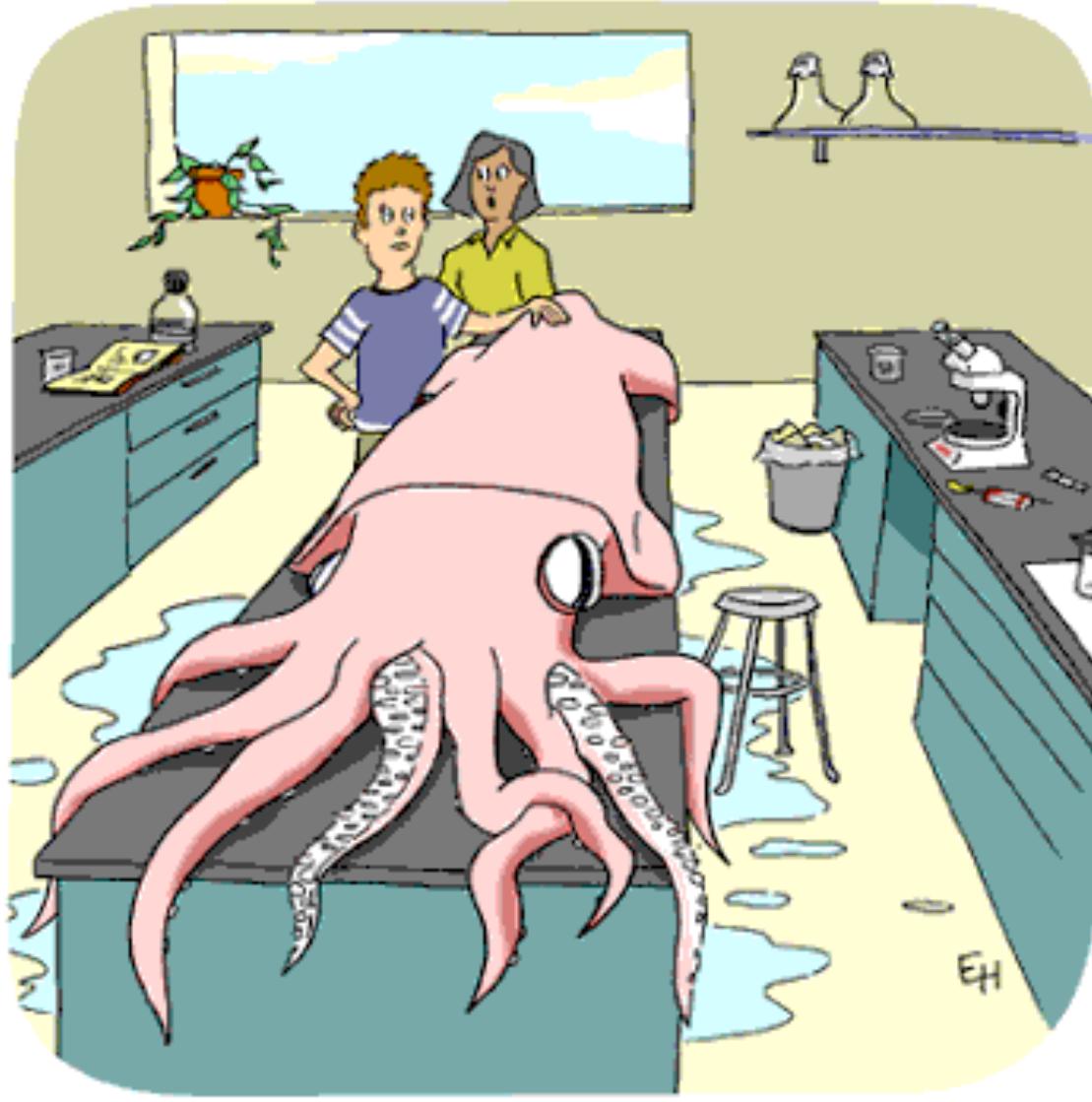
- Propagate at constant velocity; and have constant amplitude



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Action Potential Characteristics

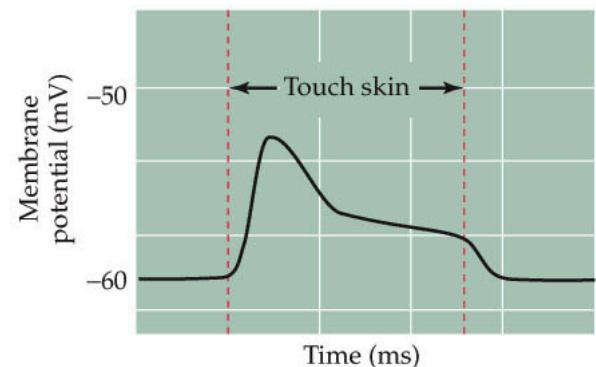
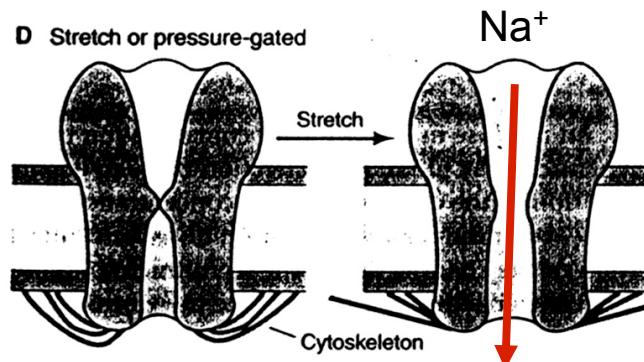
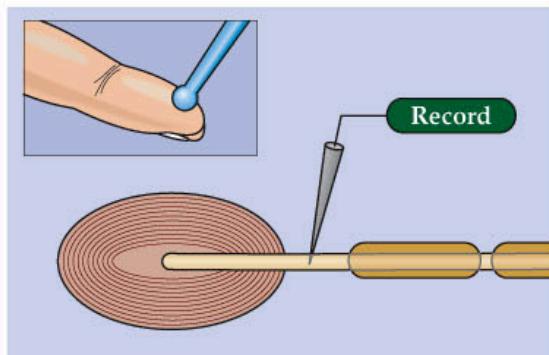
- 1) **Threshold** – an action potential is triggered when a stimulus causes the membrane potential of a cell to reach this threshold.
- 2) **All or none behavior** – each action potential produced by a cell is roughly the same size and shape. Larger stimuli do not produce a larger or longer action potentials (although they may trigger more than one).
- 3) **Overshoot** – during an action potential, the membrane potential briefly crosses zero and peaks $\sim +40\text{-}50\text{ mV}$.
- 4) **Speed** – the sequence of depolarization and hyperpolarization only lasts a few milliseconds.
- 5) **Refractoriness** – following an action potential, there is a period of time during which a cell cannot fire another action potential.
- 6) **Propagation without decrement** – action potentials are able to propagate along nerve fibers without any reduction in amplitude. The speed of propagation is also constant.



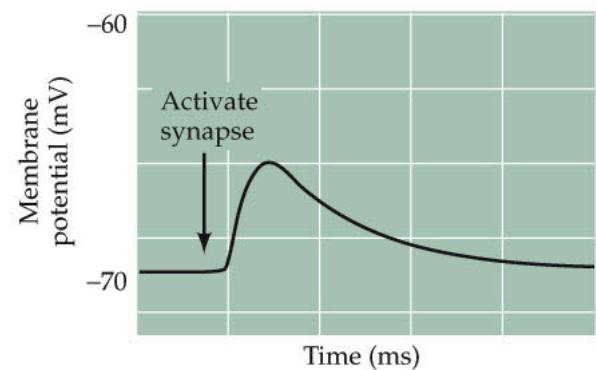
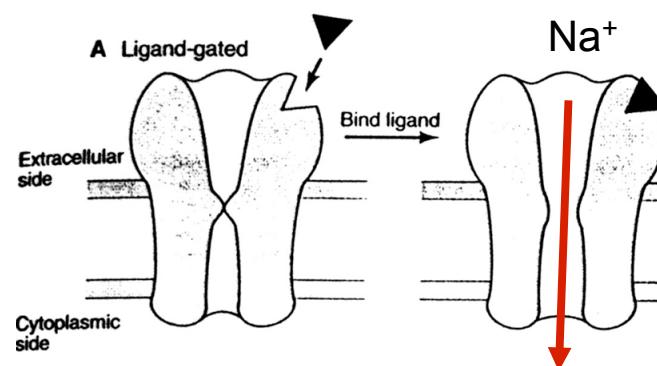
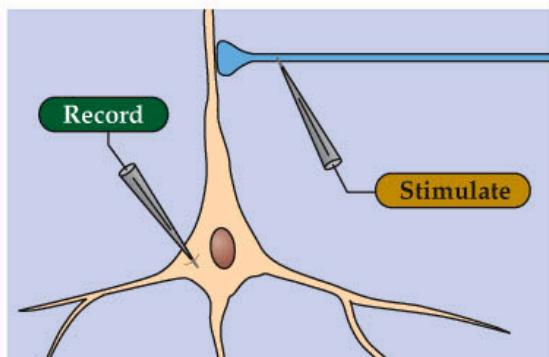
I appreciate the effort, but next time remember
we work on the *squid* giant axon,
not the *giant squid* axon.

Examples of stimuli that affect membrane potential

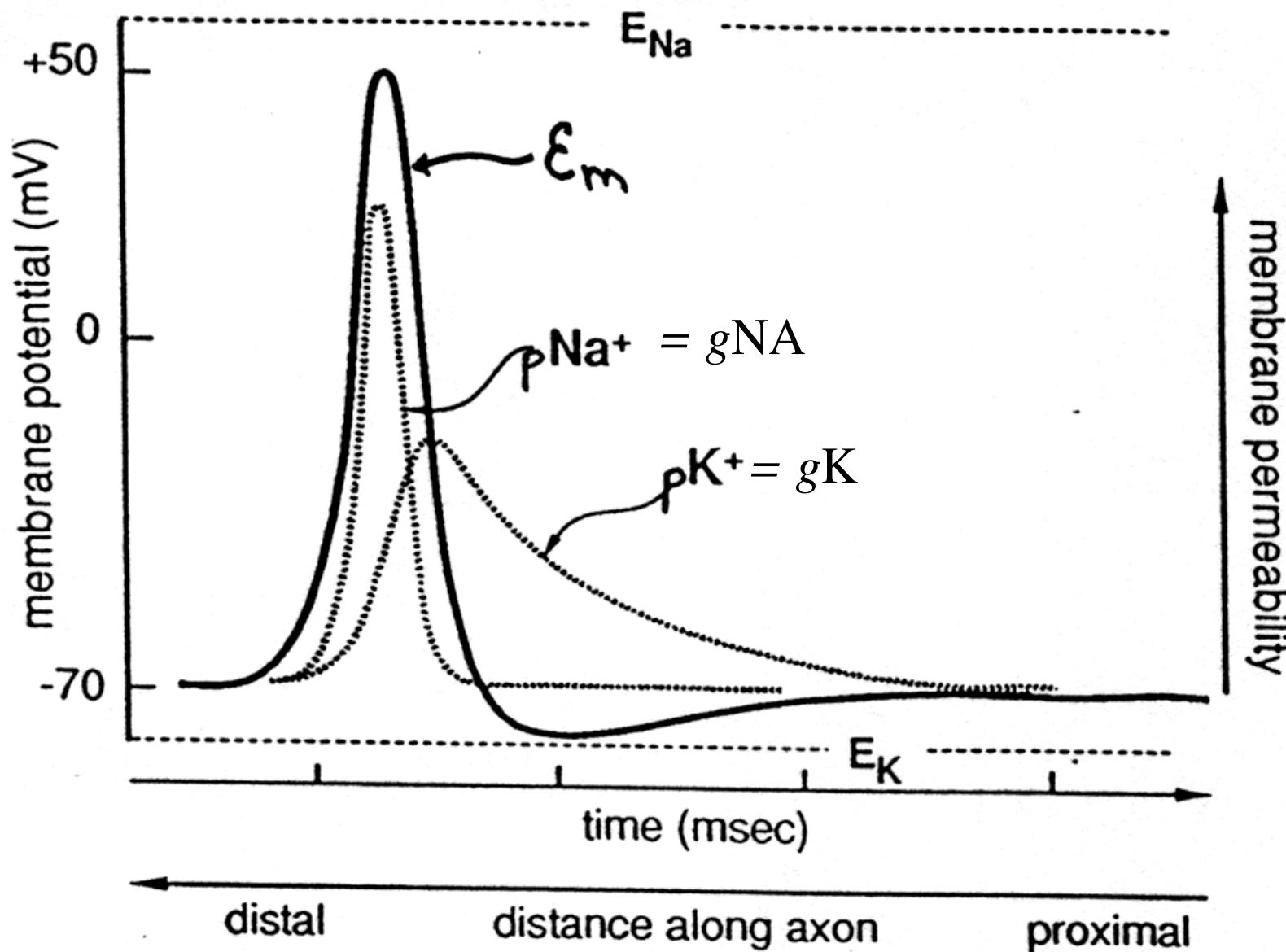
(A) Receptor potential



(B) Synaptic potential



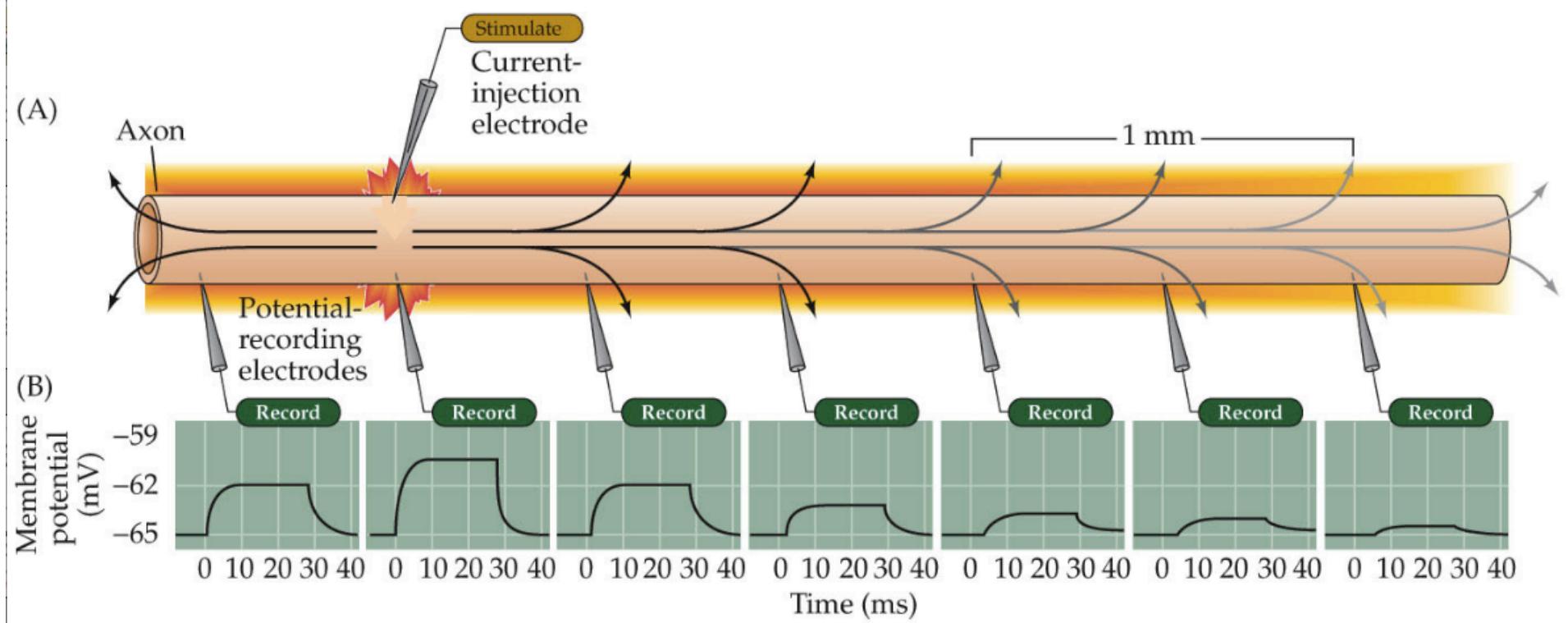
Membrane permeabilities during an AP



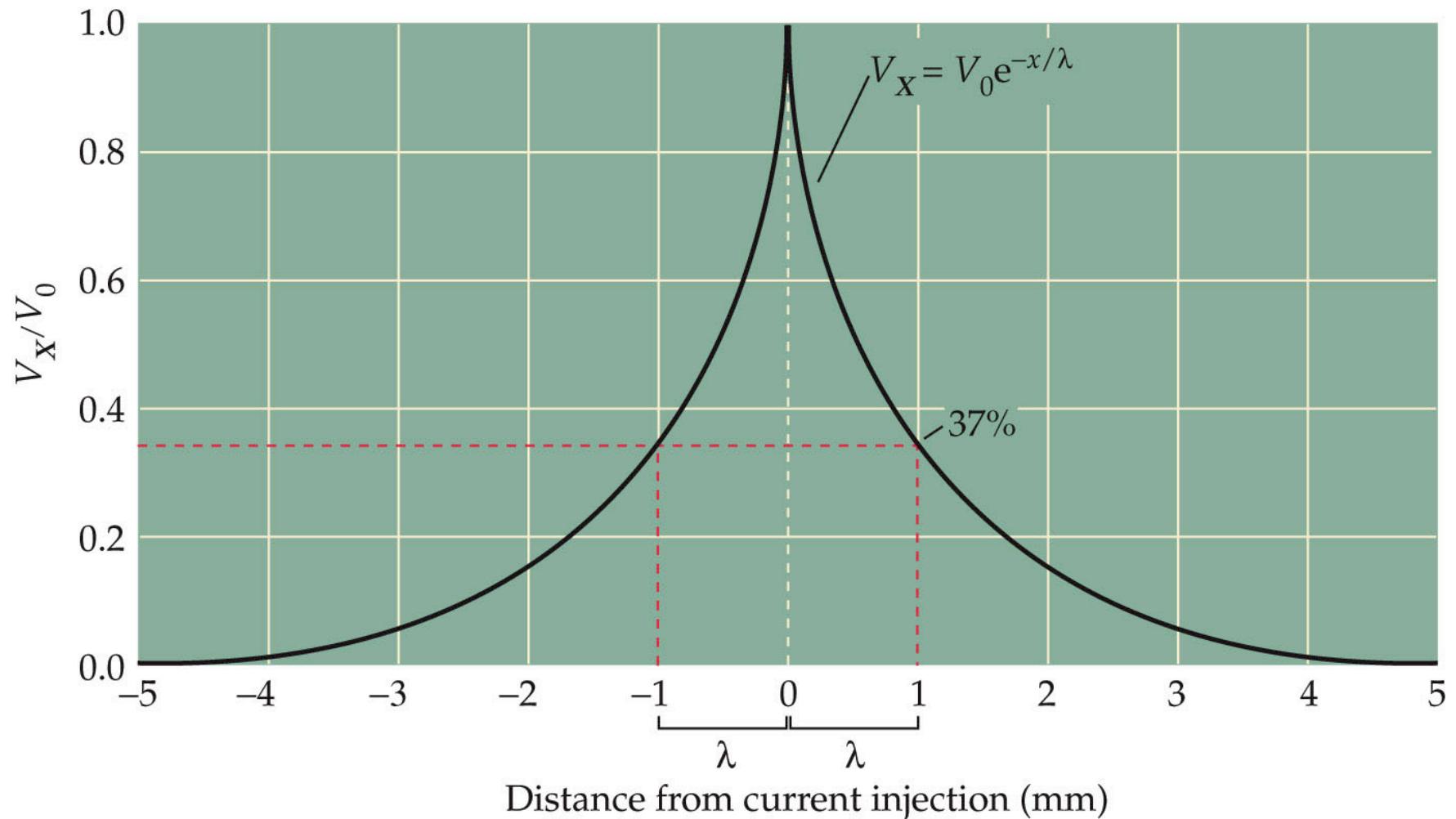
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Passive membrane properties, signal decay in leaky membrane (imperfect conductor)



Passive membrane properties (length or space constant)



Length constant in action

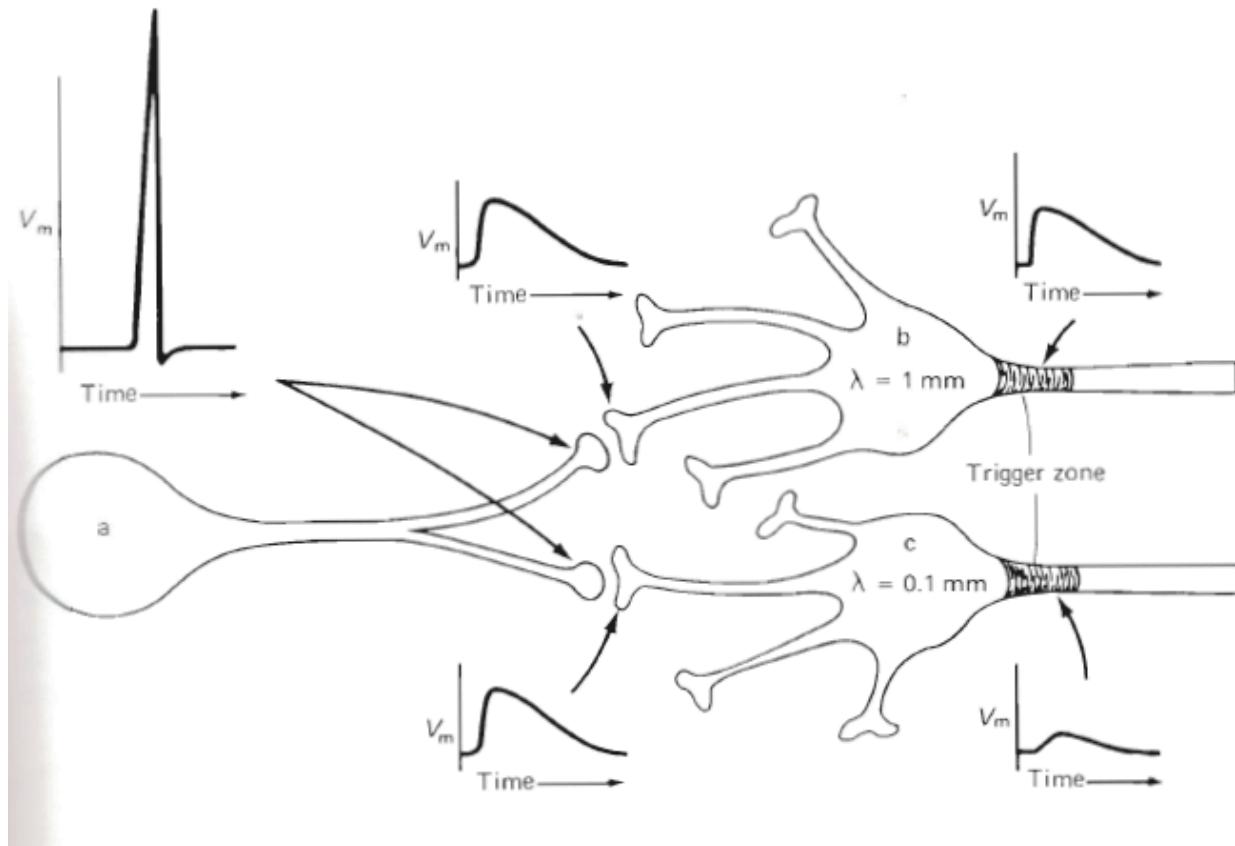
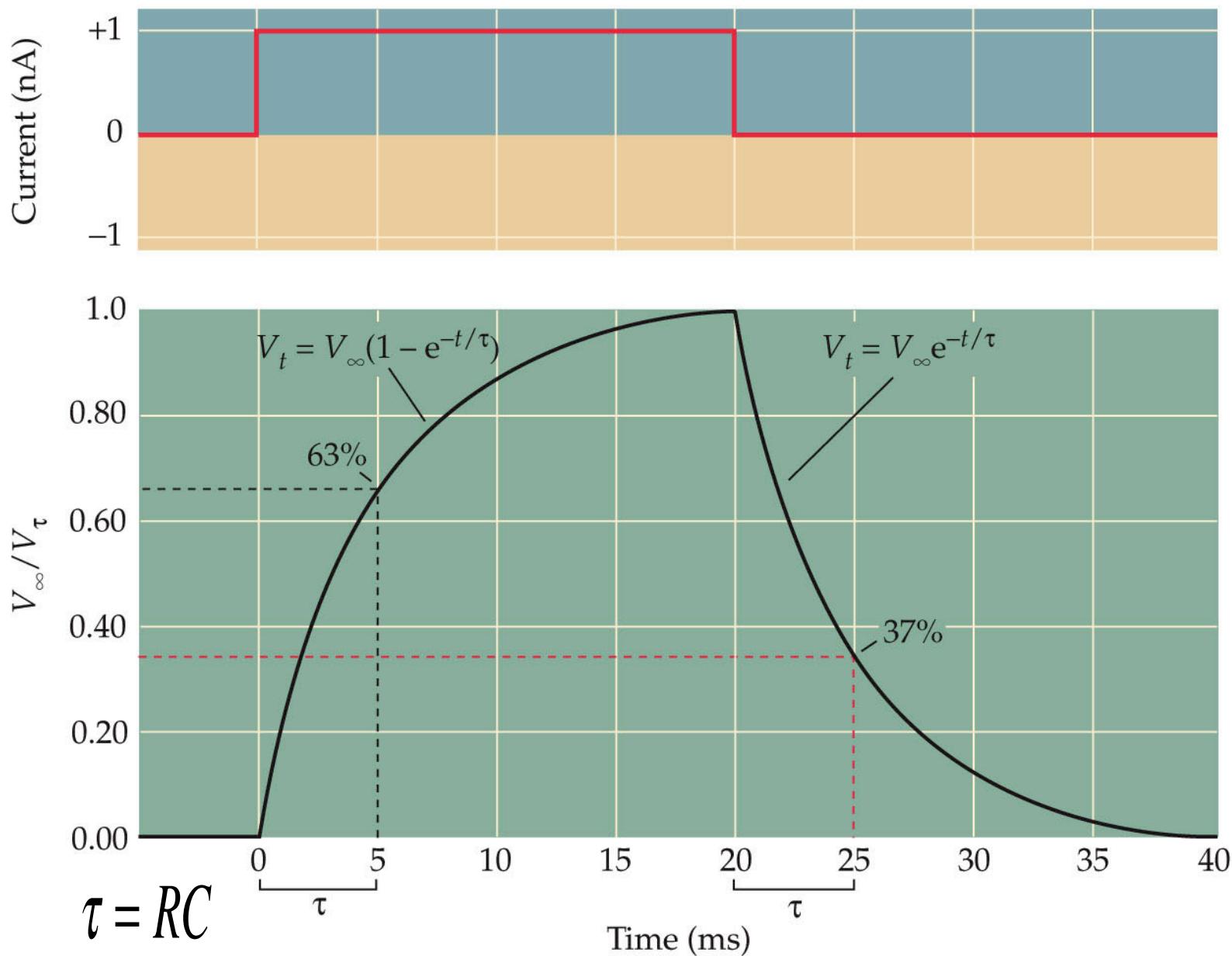
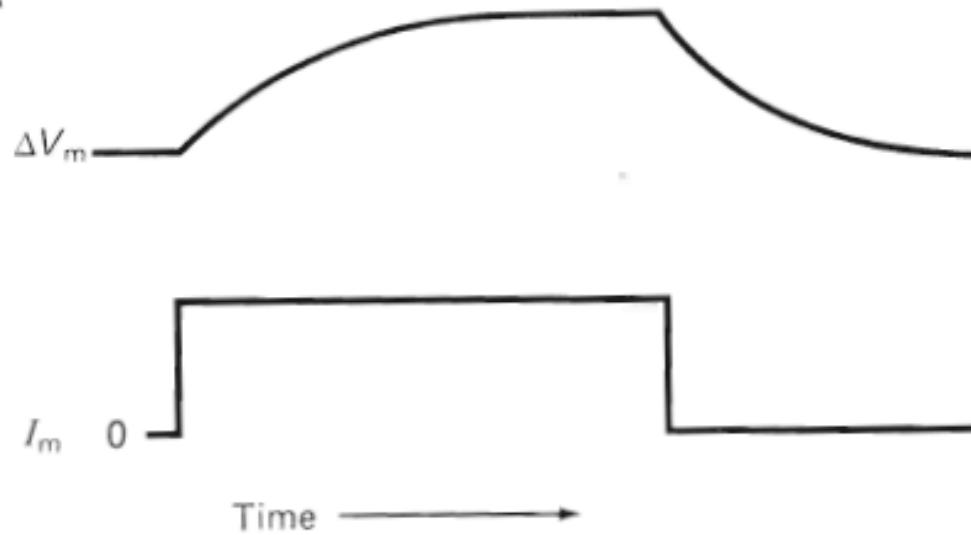
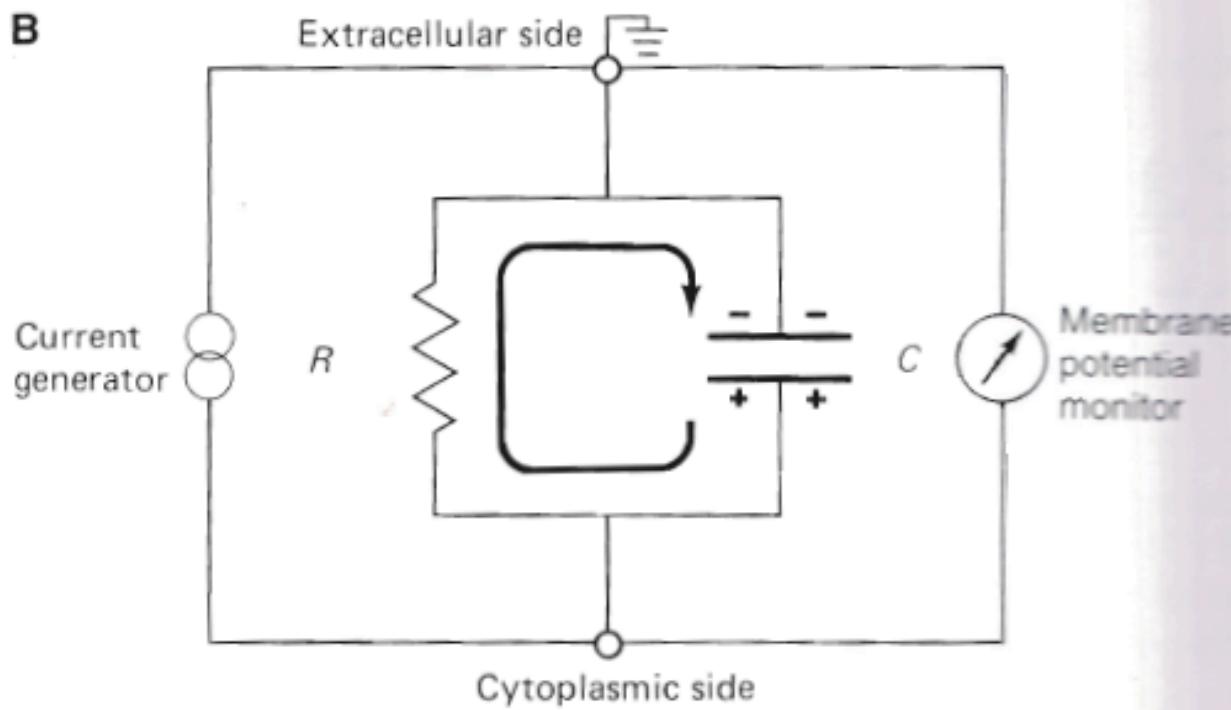


FIGURE 7–7

The length constant (λ) affects the efficiency of electrotonic conduction of synaptic potentials. An action potential in cell a elicits synaptic potentials in cells b and c. The two synaptic potentials are equal in amplitude at their sites of initiation and travel the same distance in both cells b and c. However, the amplitude of the synaptic potential that arrives at the trigger zone in cell b is much larger than in c because the length constant of the dendrites of b is much greater [1 mm] than that of cell c (0.1 mm).

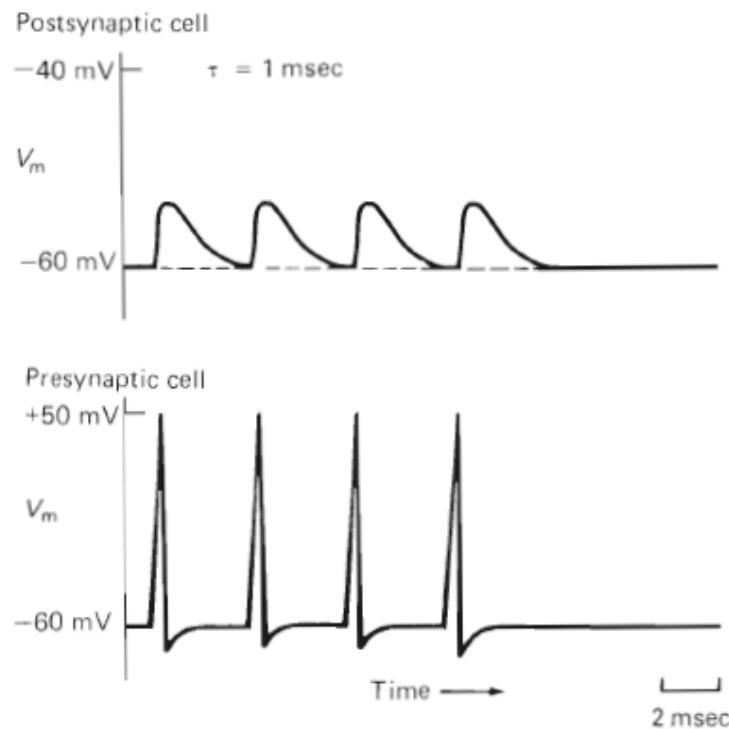
Passive membrane properties, Time Constant (τ)



A**B**

Time constant in action

A



B

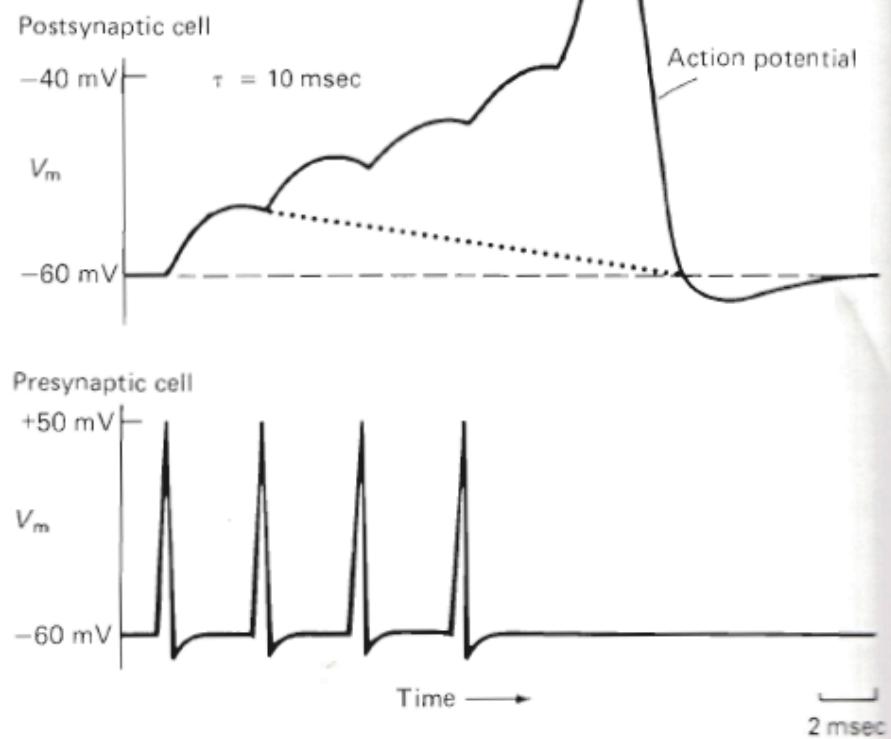
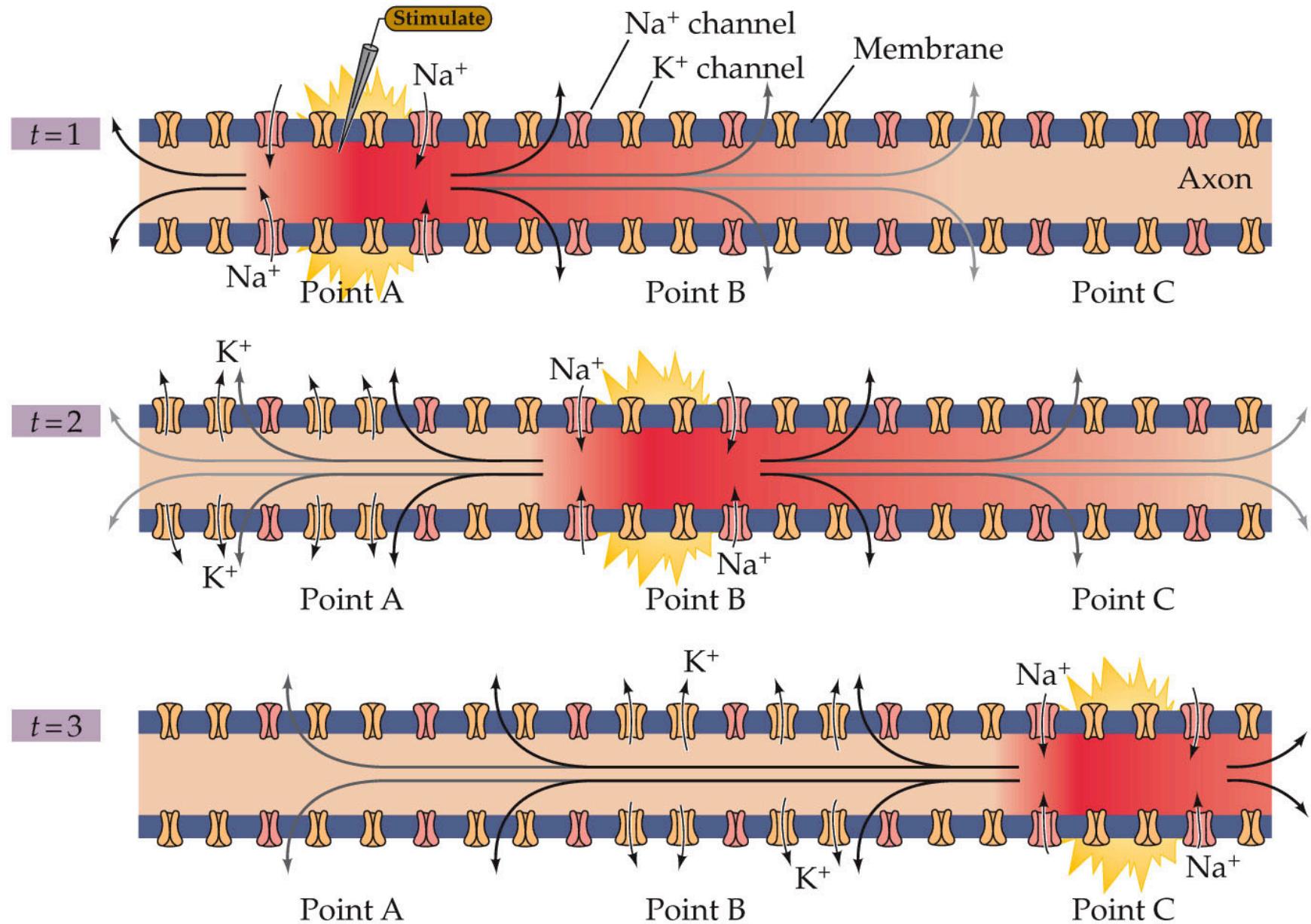


FIGURE 7-4

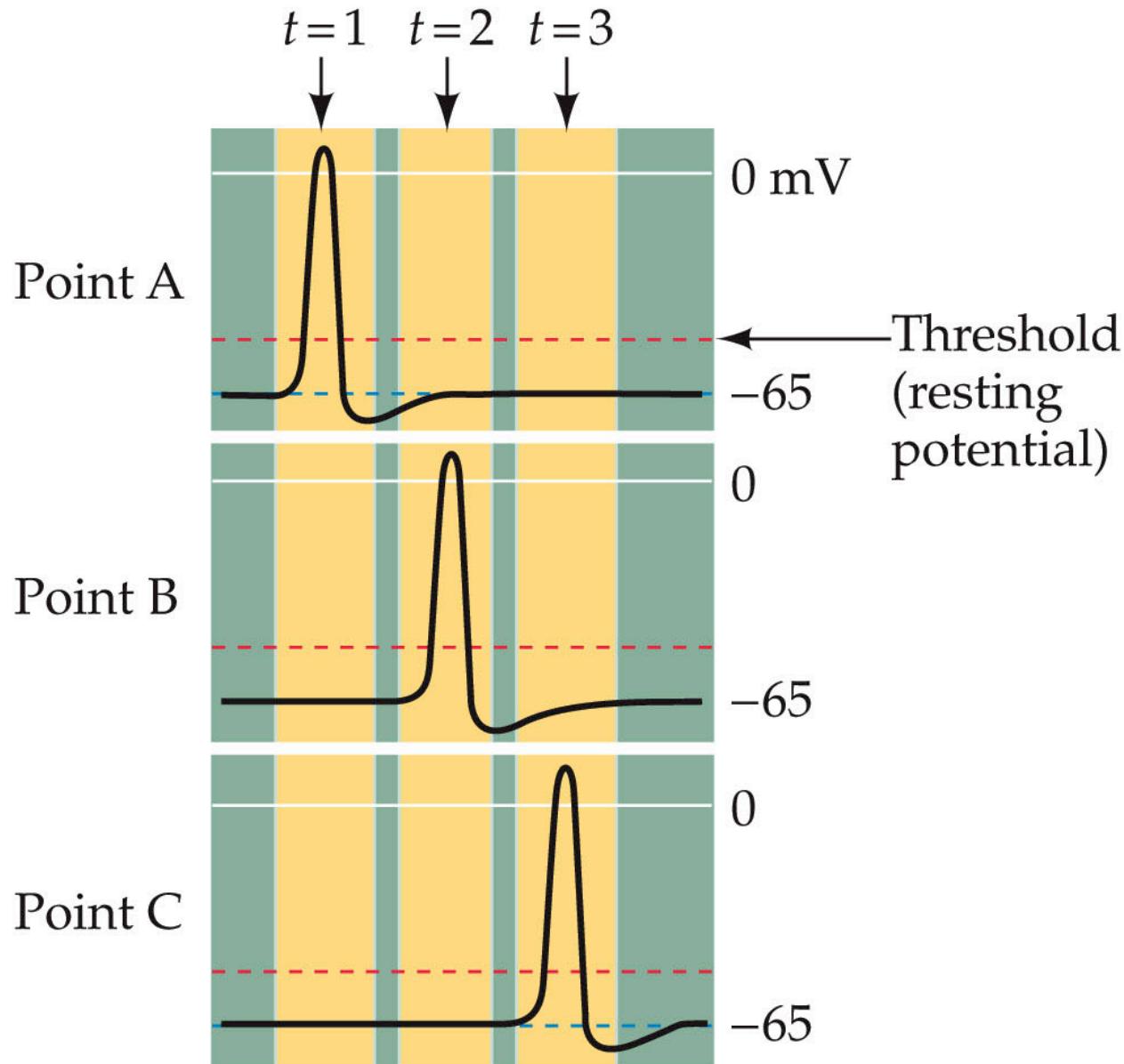
When the time course of individual postsynaptic potentials is longer than the interval between spikes in the presynaptic cell, the postsynaptic potentials overlap and their temporal summation can drive the membrane potential to the threshold for an action potential. The larger the membrane time constant (τ) of the postsynaptic cell, the longer the postsynaptic potential lasts

and the greater the extent of temporal summation. Here the consequences of different time constants in two postsynaptic cells are compared. In A the time constant is 1 ms; in B it is 10 ms. The **dotted line** shows the extrapolated falling phase of an individual excitatory postsynaptic potential.

Along length of axon, does the AP go in 1 direction or both?



NEUROSCIENCE, Fourth Edition, Figure 3.12 (Part 1)

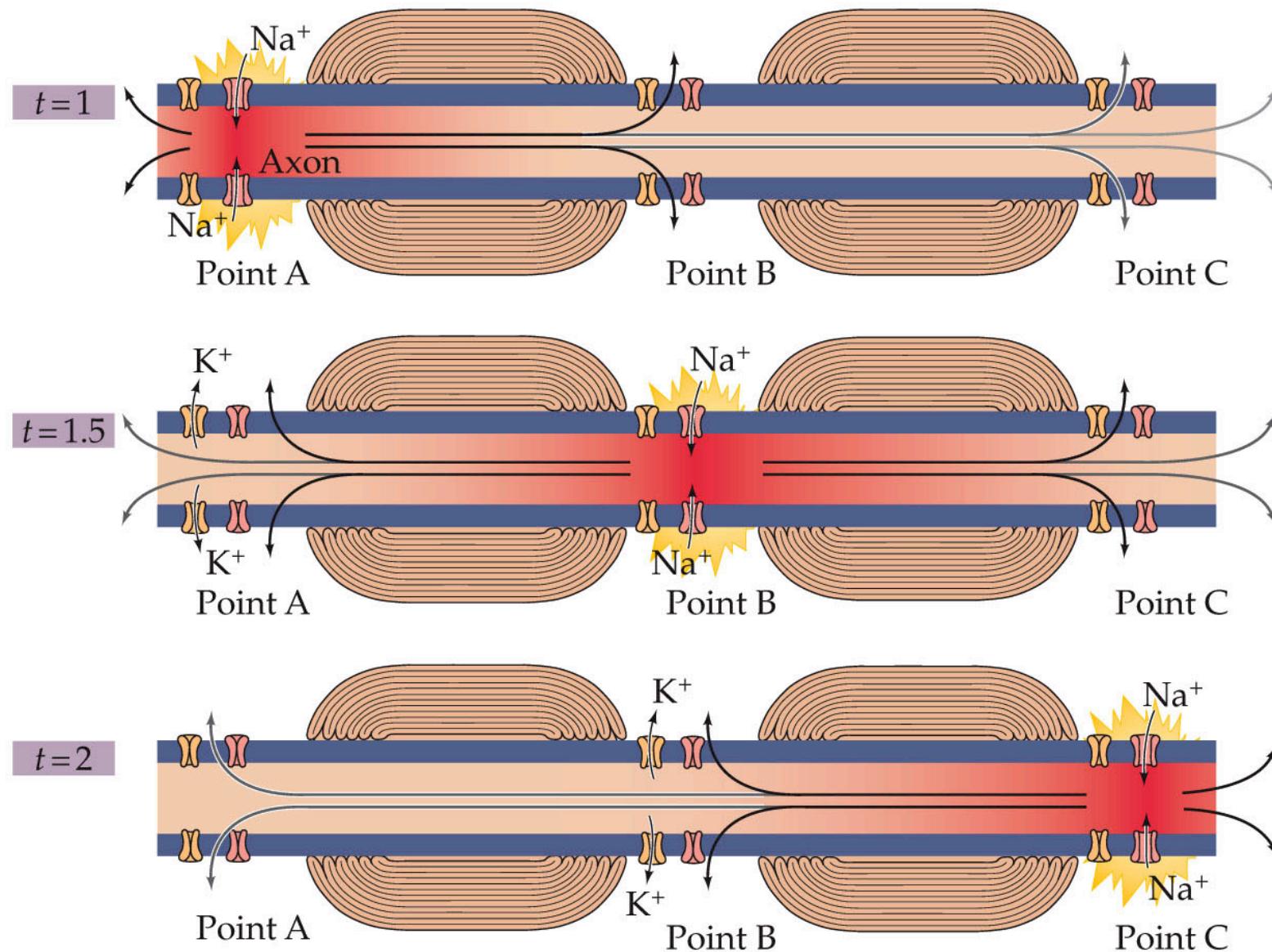


NEUROSCIENCE, Fourth Edition, Figure 3.12 (Part 2)

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Myelin, Nodes of Ranvier & saltatory conduction

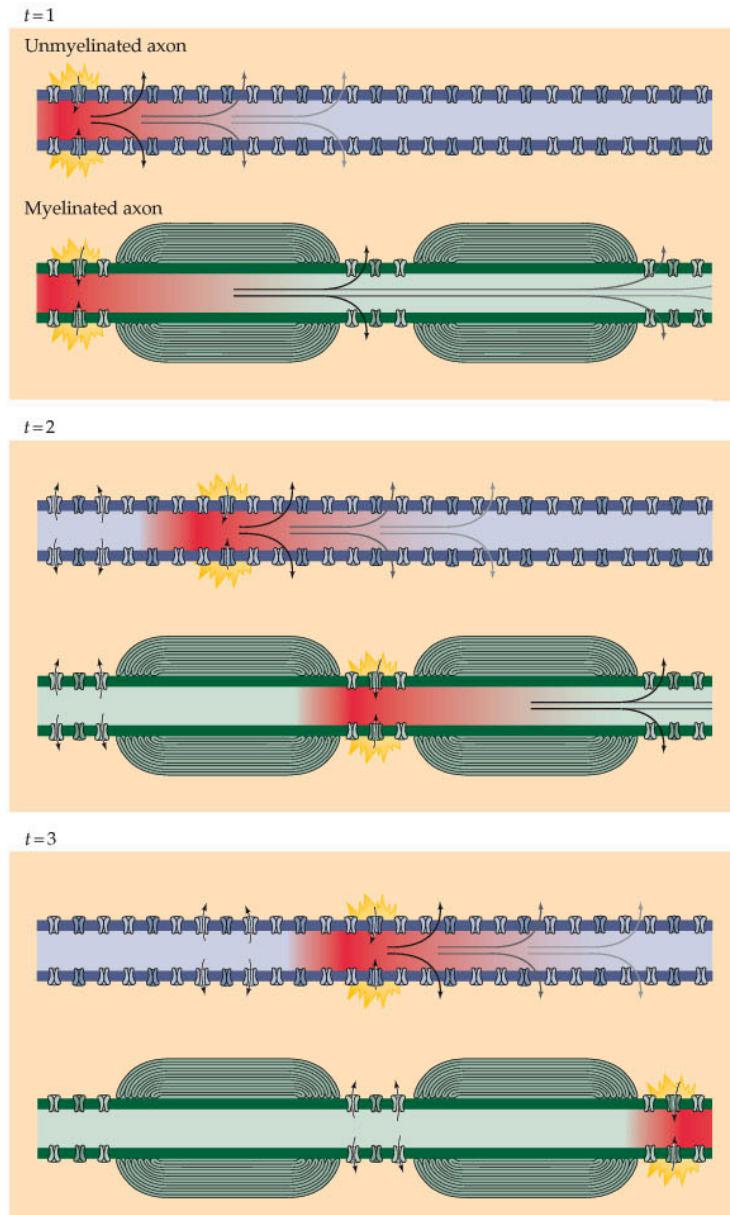
(C) Action potential propagation



“Charge transfer” is very fast if membrane is not leaky

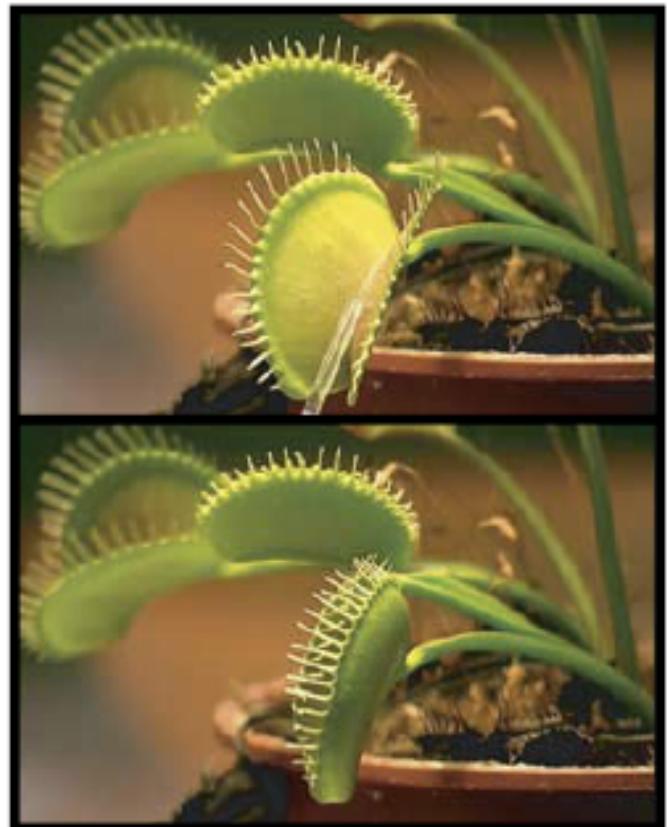
Unmyelinated axon: conduction velocity = 0.5-10 m/s

Myelinated axon: CV up to 150 m/s



NEUROSCIENCE, Fourth Edition, Figure 13.14

Charge transfer does not mean diffusion of ions from point A to B



NATURE | VOL 433 | 27 JANUARY 2005 |

How the Venus flytrap snaps

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The rapid closure of the Venus flytrap (*Dionaea muscipula*) leaf in about 100 ms is one of the fastest movements in the plant kingdom. This led Darwin to describe the plant as “one of the most wonderful in the world”¹. The trap closure is initiated by the mechanical stimulation of trigger hairs. Previous studies^{2–7} have focused on the biochemical response of the trigger hairs to stimuli and quantified the propagation of action potentials in the leaves. Here we complement these studies by considering the post-stimulation mechanical aspects of Venus flytrap closure. Using high-speed video imaging, non-invasive microscopy techniques and a simple theoretical model, we show that the fast closure of the trap results from a snap-buckling instability, the onset of which is controlled actively by the plant. Our study identifies an ingenious solution to scaling up movements in non-

The action potential of *Dionaea muscipula* Ellis*

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Abstract. The intention of this investigation was to acquire more concise information about the nature of the action potential of *Dionaea muscipula* Ellis and the different types of cells generating and conducting it. It is shown by microelectrode measurements that, besides the sensory cells, all the major tissues of the trap lobes are excitable, firing action potentials with pronounced after-hyperpolarizations. The action potentials are strictly dependent on Ca^{2+} . Their peak depolarizations are shifted 25–27 mV in a positive direction after a tenfold increase in external Ca^{2+} concentration. Perfusions with 1 mM ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) or 1 mM LaCl_3 completely inhibit excitability. Magnesium ions only slightly affect the peak depolarizations but considerably prolong action potentials. Sodium azide and 2,4-dinitrophenol also abolish excitation, probably by reducing the intracellular ATP concentration. Furthermore, it is tested whether the sensory cells can be distinguished from the other cells of the trap by their electrical behaviour. The resting potentials of sensory cells (-161 ± 7 mV) and mesophyll cells (-155 ± 8 mV) are of the same magnitude. Changes in external ion concentrations affect resting and action potentials in both cell types in a similar way. Additional freeze-fracture studies of both cell types reveal similar numbers and distributions of intra-

membrane particles on the fracture faces of the plasma membrane, which is most likely the mechanosensor. These findings stress the view that the high mechanosensitivity of the sensory hair results from its anatomy and not from a specialized perception mechanism. It is proposed that trap closure is triggered by a rise in the cytoplasmic concentration of Ca^{2+} or a Ca^{2+} -activated regulatory complex, which must exceed a threshold concentration. Since the Ca^{2+} influx during a single action potential does not suffice to reach this threshold, at least two stimulations of the trap are necessary to elicit movement.

Key words: Action potential – *Dionaea* – Plasma membrane (freeze etching) – Resting potential – Sensory cell.

Introduction

The leaves of *Dionaea muscipula* Ellis, commonly called Venus's flytrap, are modified into strong fast-closing traps which catch and digest small animals like spiders and insects. Six trigger hairs protruding from the upper leaf epidermis act as mechanosensors. Bending a hair leads to large deformations of sensory cells in the joint region at the base of the hair (Haberlandt 1906), thereby elicit-

A silicon neuron

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By combining neurophysiological principles with silicon engineering, we have produced an analog integrated circuit with the functional characteristics of real nerve cells. Because the physics underlying the conductivity of silicon devices and biological membranes is similar, the 'silicon neuron' is able to emulate efficiently the ion currents that cause nerve impulses and control the dynamics of their discharge. It operates in real-time and consumes little power, and many 'neurons' can be fabricated on a single silicon chip. The silicon neuron represents a step towards constructing artificial nervous systems that use more realistic principles of neural computation than do existing electronic neural networks.

The electrical properties of nerve cells enable brain circuits to perform the prodigious feats of computation that make intelligible the torrent of sensory information confronting us each second. The electrical behaviour of each neuron is determined by combinations of voltage-, ion- and neurotransmitter-sensitive conductances, which control currents of various ions across the membrane. These ion currents determine the voltage of the cell membrane and hence the electrical properties of the nerve cell. Here we use combinations of complementary metal-oxide-semiconductor (CMOS) circuits, fabricated in very-large-scale integrated (VLSI) technology, to represent the different ion

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towards its maximum value. The activation current controls the ‘conductance’ transistor. This transistor regulates the flow of ‘ion’ current down the gradient between the ‘membrane’ voltage and the ‘equilibrium potential’ of the ion. The conductance transistors are fabricated with dimensions that make their behaviour more ohmic than standard CMOS transistors. The ohmic behaviour is not ideal, however, and this causes the small deviation between real and analogue performance noted in Fig. 1c. The time dependence of the analogue conductances is achieved by using follower-integrators with variable time constants to act as low-pass filters for the membrane voltage (Fig.

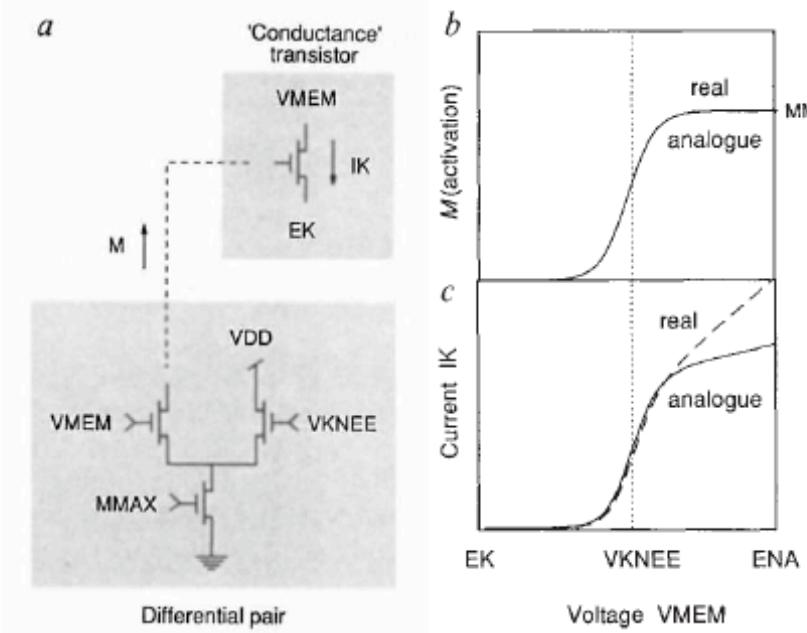


FIG. 1 A CMOS circuit and the biological voltage-dependent ion current it represents. Acronyms are given in capitals to emphasize that they are analogue signal names and to avoid confusion with equivalent names in the biological literature, which are usually given as subscripts; where necessary, analogue concepts appear in quotes to avoid ambiguity. *a*, Basic circuit used in the silicon neuron to emulate ion currents. The circuit consists of an ‘activation’ and a ‘knee’ transistor which form a differential pair, their associated bias transistor, and a ‘conductance’ transistor. The gate voltages of the activation and knee transistors are controlled by the ‘membrane’

