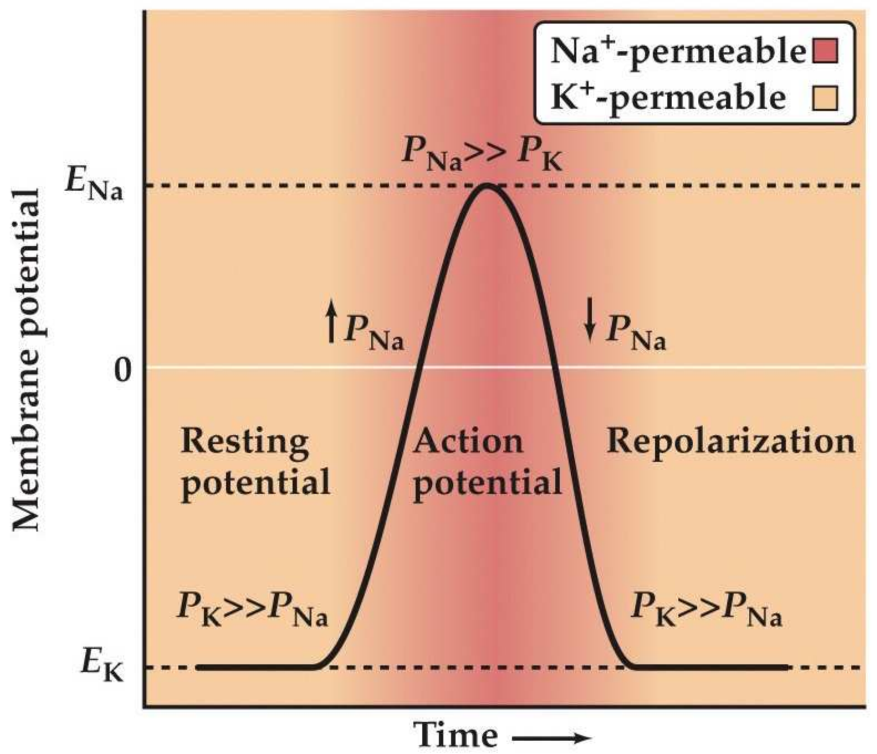
Voltage dependent membrane permeability

- Hodgkin and Huxley hypothesis– Action potential can be explained by **voltage-gated ion channels**
- Experiment– Measure ion permeability at varying membrane potentials
- Problem– Difficult to systematically vary the cell potential and also measure ion permeability
- Solution– Voltage clamping. Fix membrane potential in a cell without triggering an action potential while measuring ion permeability (~conductance)



Neuroscience 5e Fig. 2.7

Speaker notes

We learned last time that the experiments of Hodgkin, Huxley, and Katz showed that the Vm during an AP approaches ENa. And they thought that this might be due to changes in permeability for Na in the cell membrane that changes during the course of an action potential. Thus Hodgkin and Huxley hypothesized that APs can be explained by ion channels that change their permeability due to voltage— that these channels are voltage-gated.

Alan Hodgkin and Andrew Huxley began this work in the late 1930s, and quickly finished one paper before helping with the British war effort during WWII. Indeed Hodgkin said that he lost all interest in neurophysiology during those dark years as one might imagine. But as things calmed down after the war they renewed their collaboration and got back to the business of neuronal excitability.

So they needed to proved that ion permeability changes according to membrane potential but there was an issue— how to vary the membrane potential in a systematic way and also measure the ion permeabilities?

The solution was to build an electrophysiological recording apparatus with feedback circuitry such that you can fix or clamp the voltage across the cell membrane.

Action potential summary video

Neuroscience 5e Animation 2.3

Speaker notes

Summary of last time…

More V_m examples

- Given a cell with intracellular: 1 mM NaCl, 10 mM KCl; extracellular: 10 mM NaCl, 1 mM KCl
- What is the resting potential of the cell at room temperature (20ºC + 273 = 293 K) if the membrane is only permeable to K^+?
  - `(58/1)*log10(1/10) = -58 mV`
- Only permeable to Na^+?
  - `(58/1)*log10(10/1) = +58 mV`
- Only permeable to Cl^-?
  - `(58/-1)*log10(11/11) = 0 mV`
- Equally permeable to K^+ and Na^+?
  - `Pk = 0.5; Pna = 0.5; Pcl = 0; kOut = 1; kIn = 10; naOut = 10; naIn = 1; clIn = 11; clOut = 11`
  - `(58)*log10( (Pk*kOut + Pna*naOut + Pcl*clIn) / (Pk*kIn + Pna*naIn + Pcl*clOut) ) = 0 mV`

Speaker notes

1. `(58/1)*log10(1/10) = -58 mV`
2. `(58/1)*log10(10/1) = +58 mV`
3. `(58/-1)*log10(11/11) = 0 mV`
4. 0 mV:

- `Pk = 0.5; Pna = 0.5; Pcl = 0; kOut = 1; kIn = 10; naOut = 10; naIn = 1; clIn = 11; clOut = 11`
- `(58)*Math.log10( (Pk*kOut + Pna*naOut + Pcl*clIn) / (Pk*kIn + Pna*naIn + Pcl*clOut) ) = 0 mV`
- 0 mV:
- `Pk = 1; Pna = 1; Pcl = 0; kOut = 1; kIn = 10; naOut = 10; naIn = 1; clIn = 11; clOut = 11`
- `(58)*Math.log10( (Pk*kOut + Pna*naOut + Pcl*clIn) / (Pk*kIn + Pna*naIn + Pcl*clOut) )`
- -59 mV (room temp and low Pna):
- `Pk = 1; Pna = 0.001; Pcl = 0.5; kOut = 1; kIn = 10; naOut = 10; naIn = 1; clIn = 1; clOut = 11`
- `(58)*Math.log10( (Pk*kOut + Pna*naOut + Pcl*clIn) / (Pk*kIn + Pna*naIn + Pcl*clOut)`

-62 mV (body temp and low Pna):

- R = 8.3; F = 9.6e4; T = (273+37)
- `Pk = 1; Pna = 0.001; Pcl = 0.5; kOut = 1; kIn = 10; naOut = 10; naIn = 1; clIn = 1; clOut = 11`
- `((R*T)/F)*Math.log( (Pk*kOut + Pna*naOut + Pcl*clIn) / (Pk*kIn + Pna*naIn + Pcl*clOut) )`

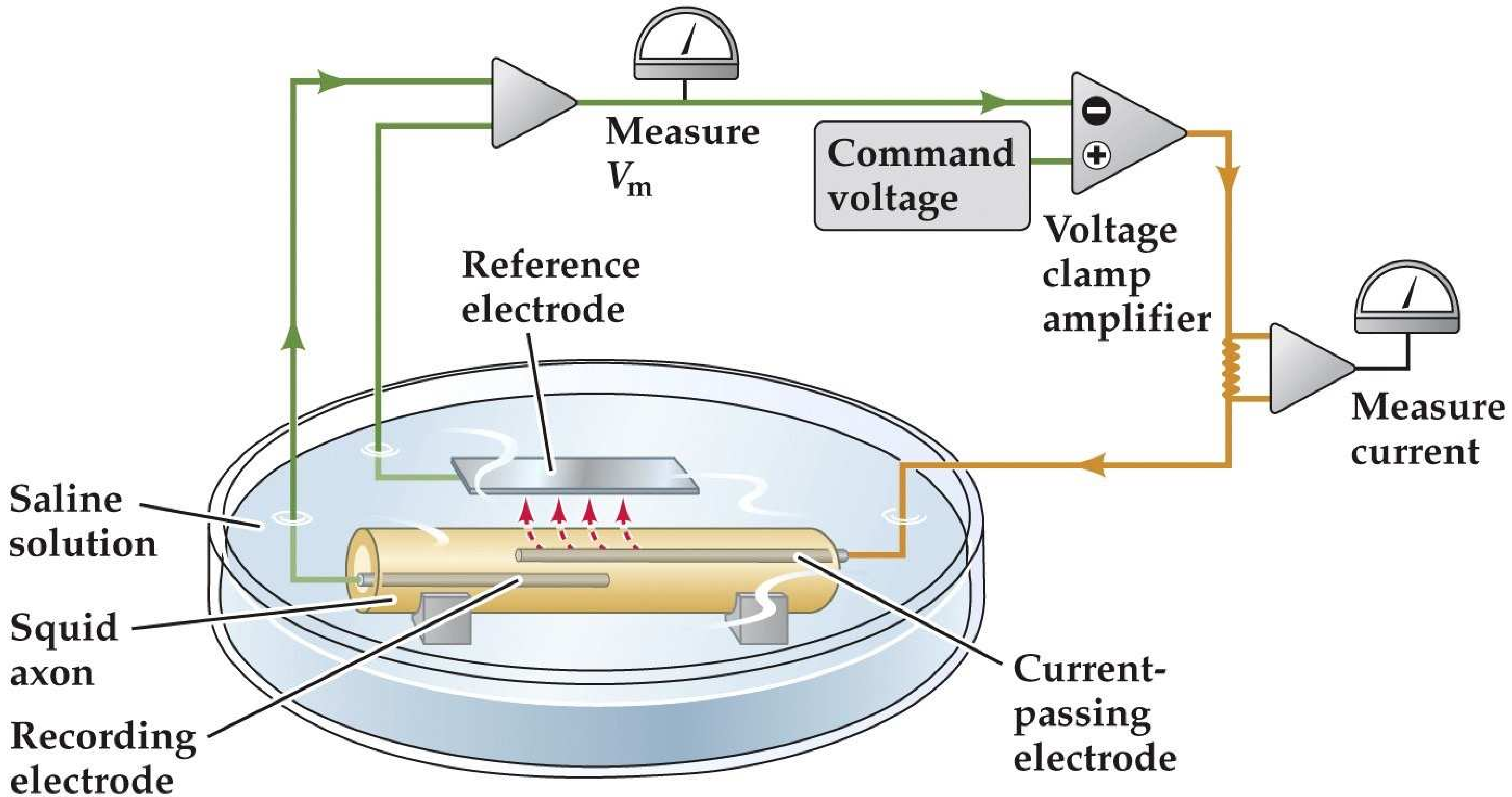
-69 mV (body temp and low Pna and physiol concentrations):

- `R = 8.3; F = 9.6e4; T = (273+37)`
- `Pk = 1; Pna = 0.05; Pcl = 0.45; kOut = 5; kIn = 140; naOut = 145; naIn = 5; clIn = 5; clOut = 110`
- `((R*T)/F)*Math.log( (Pk*kOut + Pna*naOut + Pcl*clIn) / (Pk*kIn + Pna*naIn + Pcl*clOut) )`

Calculate the total concentration of all ions for these solutions. For every one NaCl that dissolves, two ions are produced (one Na^+ and one Cl^-). Thus for 10 mmol/L NaCl outside there are (10 mmol/L)x(1 total Cl ions/NaCl) = 10mM. And for 1mM KCl outside there are (1 mmol/L)x(1 total Cl ions/KCl) = 1mM. Thus the total number of Cl^- ions per liter is 11mmol/L = 11mM

The voltage clamp technique

Voltage clamping provides a method for measuring electrical current and its direction of net flow across a cell membrane.



Neuroscience 5e/6e Box 3A

Speaker notes

This is an illustration of the voltage clamp recording method.

One internal electrode measures membrane potential and is connect to the voltage clamp amplifier.

voltage clamp amplifier compares membrane potential to the desired command potential

When Vm is different from the command potential the clamp amplifier injects current ion the axon through a second electrode. This feedback arrangement causes the membrane potential to become the same as the command potential.

The current flowing back into the axon and thus across its membrane can be measured.

This electronic feedback circuit holds the membrane potential at the desired level, even in the face of permeability changes that would normally alter the membrane potential. (such as those generated during the action potential). Most importantly, the device permits the simultaneous measure of the current needed to keep the cell at a given voltage. This current is exactly equal to the amount of current flowing across the neuronal membrane, allowing direct measurement of these membrane currents.

amplifier, electronic amplifier
: "amp"
: electronic device that can increase the power of a signal : uses energy from a power supply and controls an output signal to match input signal shape, but with greater amplitude

differential amplifier
: type of electronic amplifier : amplifies difference between two input voltages and suppresses any voltage common to the two inputs : op-amp

operational amplifier
: https://en.wikipedia.org/wiki/Operational_amplifier : "op-amp" : DC-coupled electronic amplifier
: differential input and often a single output
: can produce an output potential many thousands of times larger than the voltage difference between inputs

A. Hodgkin and A. Huxley 1952

- Do neuronal membranes have voltage-dependent permeability?
- Which ions are changing their permeability?
- Experiment– Change potential to make neuron membrane potential more negative (hyperpolarize). No currents need to be injected into cell to maintain that potential. Therefore no current is moving from inside and outside of cell
- Change potential– Depolarize cell, now see both inward and outward currents between the inside and outside of cell

Speaker notes

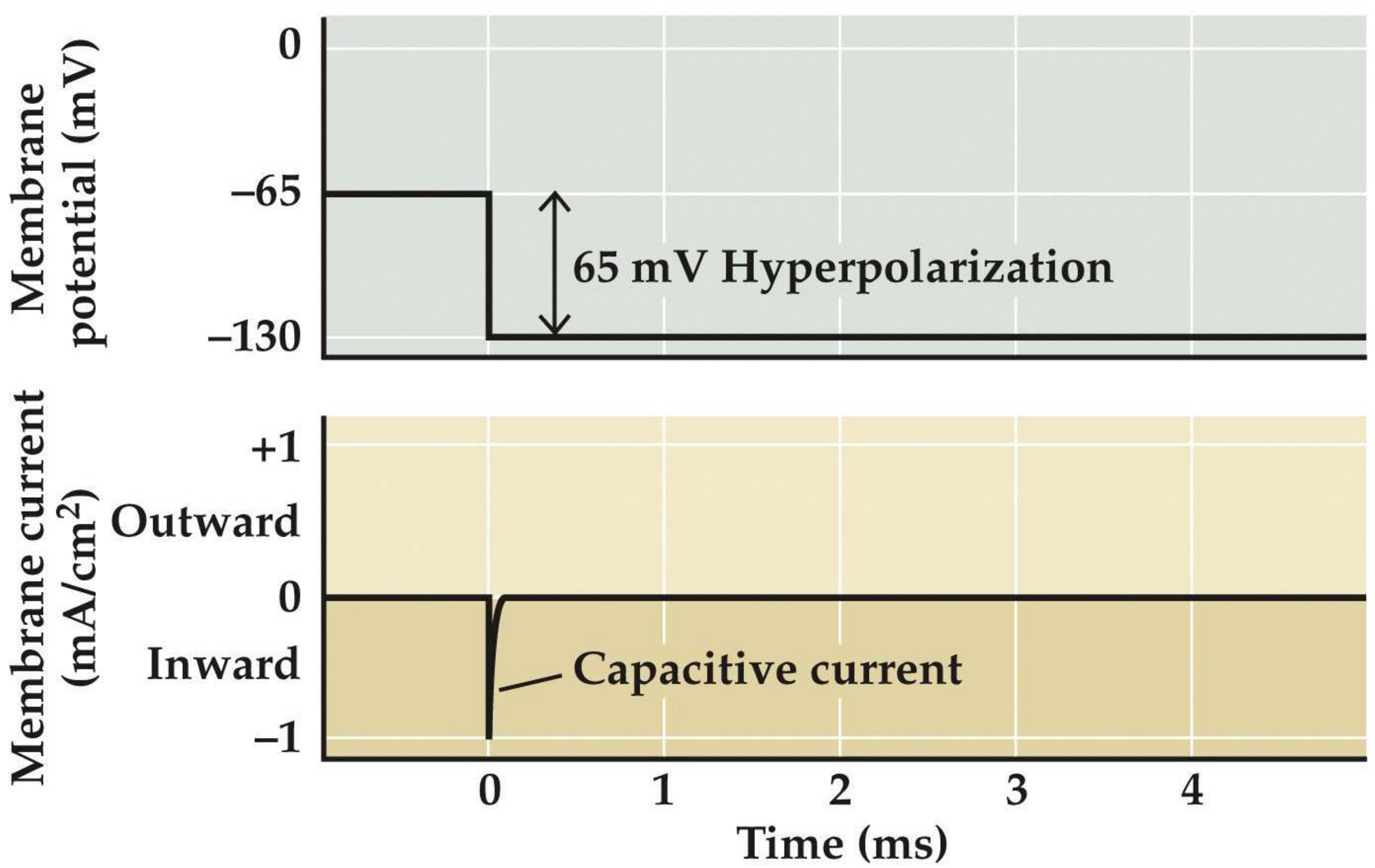
Alan Hodgkin and Andrew Huxley from the Univ of Cambridge published a series of seminal papers in 1952 that summarized their investigations using this voltage clamp method to examine voltage dependent ion flux.

They asked…

So the experiment was to hold the membrane potential at different voltages and measure charge flux into or out of the cell... —>

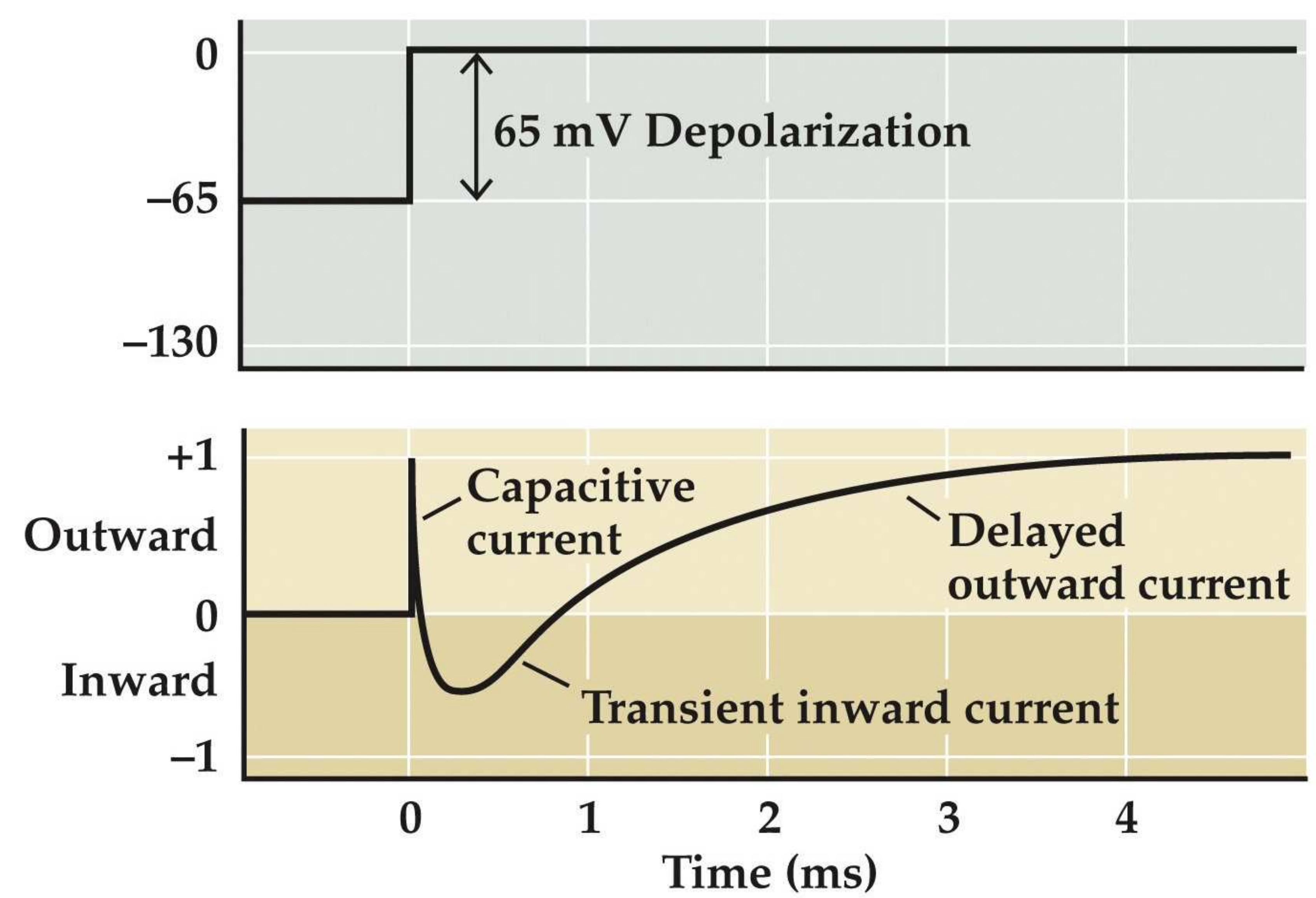
Electric current flow across a squid axon membrane during voltage clamp

negligible current (except for a capacitive transient)



Neuroscience 5e/6e fig. 3.1; from Hodgkin et al., *J. Physiol.* 1952

inward and outward currents



Neuroscience 5e/6e fig. 3.1; from Hodgkin et al., *J. Physiol.* 1952

Inward current is always downward deflection from zero in these traditional voltage clamp plots. Outward current is an upward deflection.

Speaker notes

And so here are the results from this type of voltage clamp experiment.

If you command that the cell membrane potential be hyperpolarized, you get very little or negligible current flowing across the membrane except for a very brief capacitive current that you always see in these voltage clamp experiments.

This is because the cell membrane essentially acts as a parallel RC circuit where a resistor and a capacitor are connected in parallel and to a constant current source. Ion channels are resistors, lipid bilayer with the extracellular and intracellular environments act as capacitor, storing charge in the form of ions accumulating near the surface of the membrane. When a switch is turned on in an RC circuit current flows from the battery to the capacitor until the capacitor is charged to a voltage that is same as the battery.

: capacitance of membrane: during change in applied voltage or current across membrane, positively charged ions pile on surface of one side of membrane and **electrostatically** interact with cations on the other side of membrane surface (membrane acts as thin impermeable surfaces in parallel, like a capacitor), repeling them and inducing immediate, fast capacitive current along membrane

However when Hodgkin and Huxley depolarized the membrane, a transient inward current occurs followed by a slow outward current.

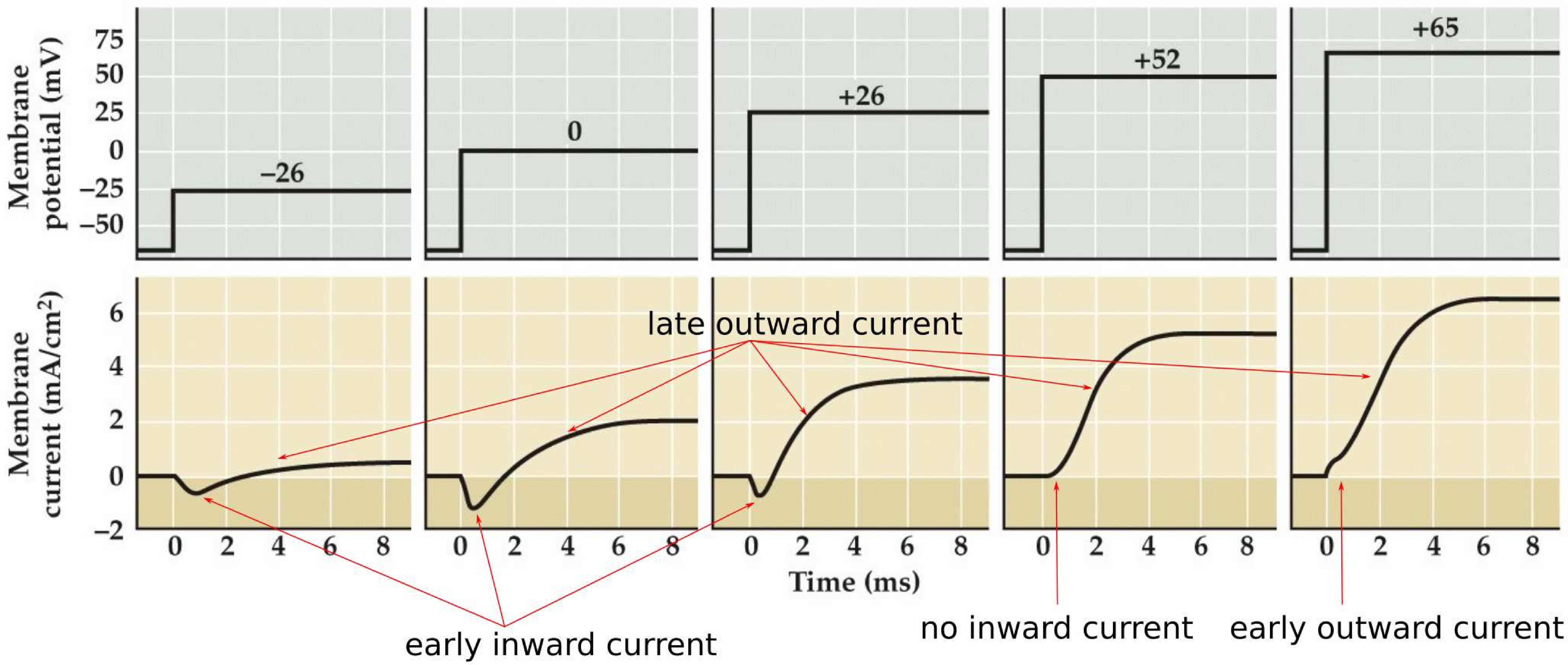
A capacitor (originally known as a condenser) is a passive two-terminal electrical component used to store electrical energy temporarily in an electric field. Consists of two parallel conductors. Lipid membrane with the inner and outer cellular environment acts as this. The membrane capacitance per unit areas is mostly constant at about 1 µF/cm2.

- When the voltage is constant, the current through the capacitative pathway is zero because the capacitor has acquired the charge Q (coulombs) according to the relationship Q=CV. C is capacitance (farads) Ic is capacitive current. Ic = C(dV/dt)
- as long as V is changing with time, there will be a current flowing towards the capacitor.
- if V is constant in time, there is no capacitive current.
- product of resistance and capacitance has the unit of time and is called the time constant. Time constant defines how quickly capacitors charge or discharge over time.

http://nerve.bsd.uchicago.edu/med98c.htm

Inward & outward currents produced at a series of clamped membrane voltages

Voltage clamp recordings from squid axon. Capacitive artifact removed for clarity.



Neuroscience 5e/6e Fig. 3.2; from Hodgkin et al., *J. Physiol.* 1952

Speaker notes

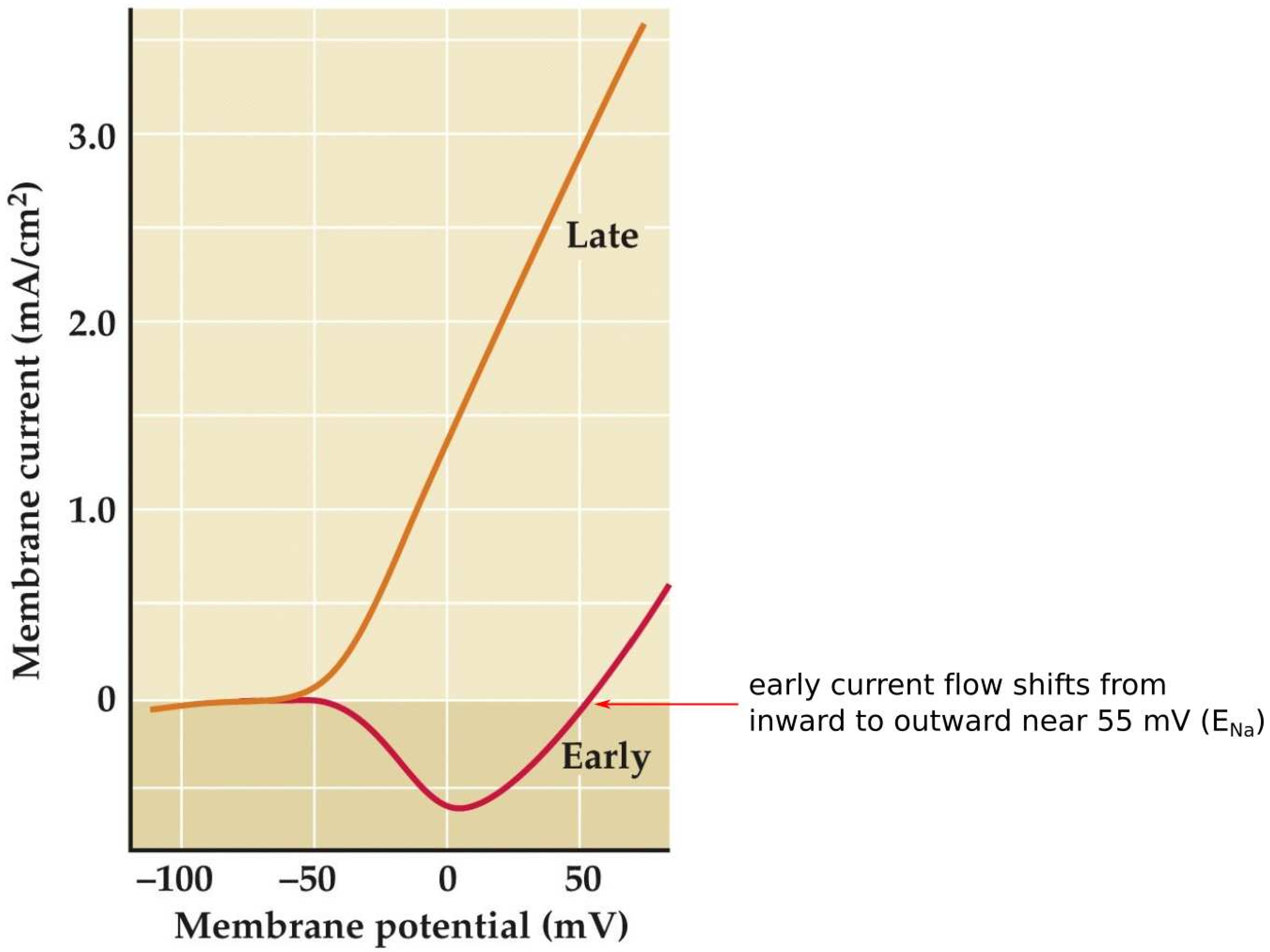
This show several different voltage steps (with the brief capacitive current omitted for clarity)

Notice a few phenonmena in this figure.

...Notice as the command voltage becomes more positive we start to approach ENa and the inward current disappears.

Relationship between current amplitude and membrane potential

External Na^+ 440 mM, internal Na^+ 50 mM, therefore Nernst says **E_{Na} = 55 mV**



Neuroscience 5e/6e Fig. 3.3; from Hodgkin et al., *J. Physiol.* 1952

Speaker notes

Don't get confused by this plot, look at the axes it is just Vm and current.

Bascially this just summarizes the peak magnitude of these these two currents at different Vm in the previous figure 3.2.

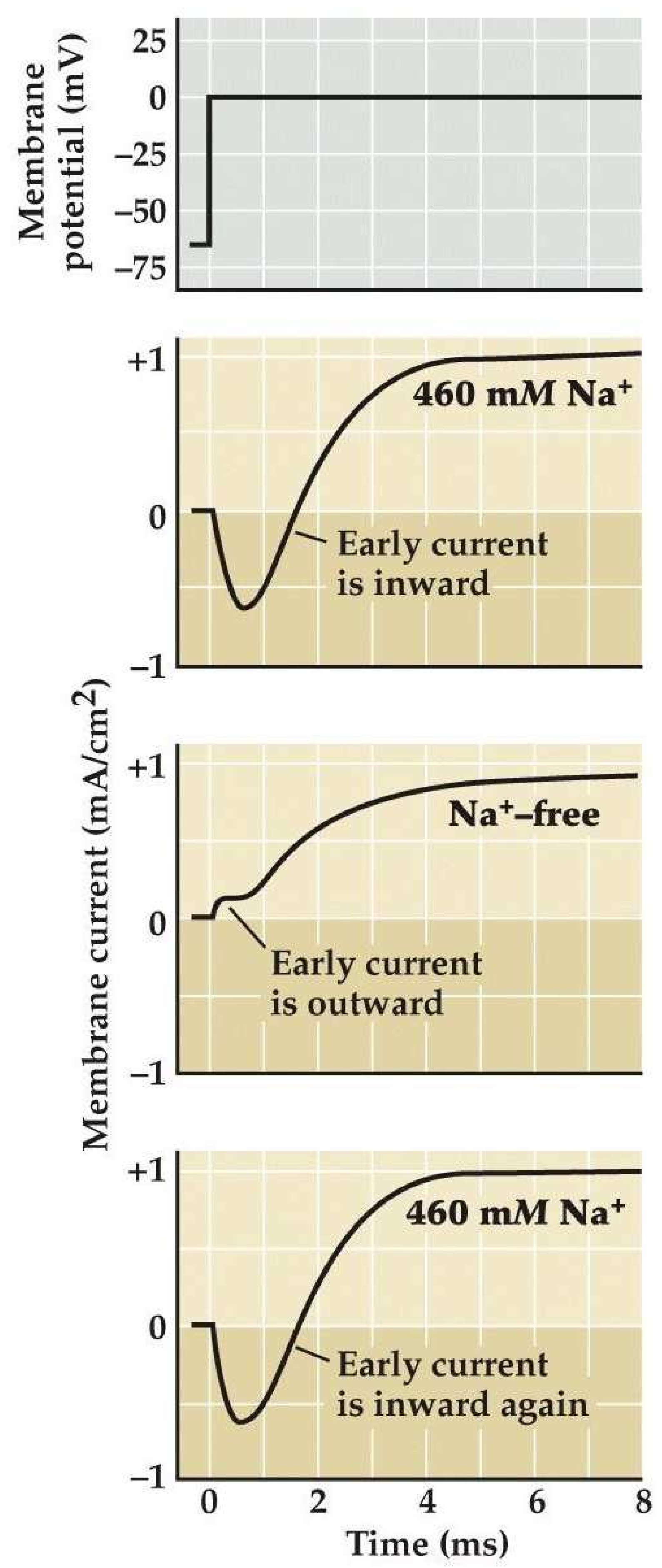
How do we prove the inward current is sodium?

- Prediction– If you could change the Na^+ concentrations in the system, for example have less sodium outside than inside (instead of the normal high outside low inside), Nernst equation would predict an early outward current instead of an early inward current
- Experiment– Change the Na^+ concentration in the bath. Normally 440 mM NaCl outside & 50 mM inside for squid axon, now make it 50 mM inside & 0 mM outside

Speaker notes

So it seems like this inward current may be carried by Na ions.

Dependence of the early inward current on sodium



Neuroscience 5e/6e Fig. 3.4; from Hodgkin and Huxley *J. Physiol.* 1952a

Squid giant axon voltage clamping

Speaker notes

> *Choline is a water-soluble nutrient. It is usually grouped within the B-complex vitamins. Choline generally refers to the various quaternary ammonium salts containing the N,N,N-trimethylethanolammonium cation. (X− on the right denotes an undefined counteranion.)*

The cation appears in the head groups of phosphatidylcholine and sphingomyelin, two classes of phospholipid that are abundant in cell membranes. Choline is the precursor molecule for the neurotransmitter acetylcholine, which is involved in many functions including memory and muscle control.

Voltage clamp method summary

Neuroscience 5e Animation 3.1

Speaker notes

No notes on this slide.

Pathways of the two currents are distinct

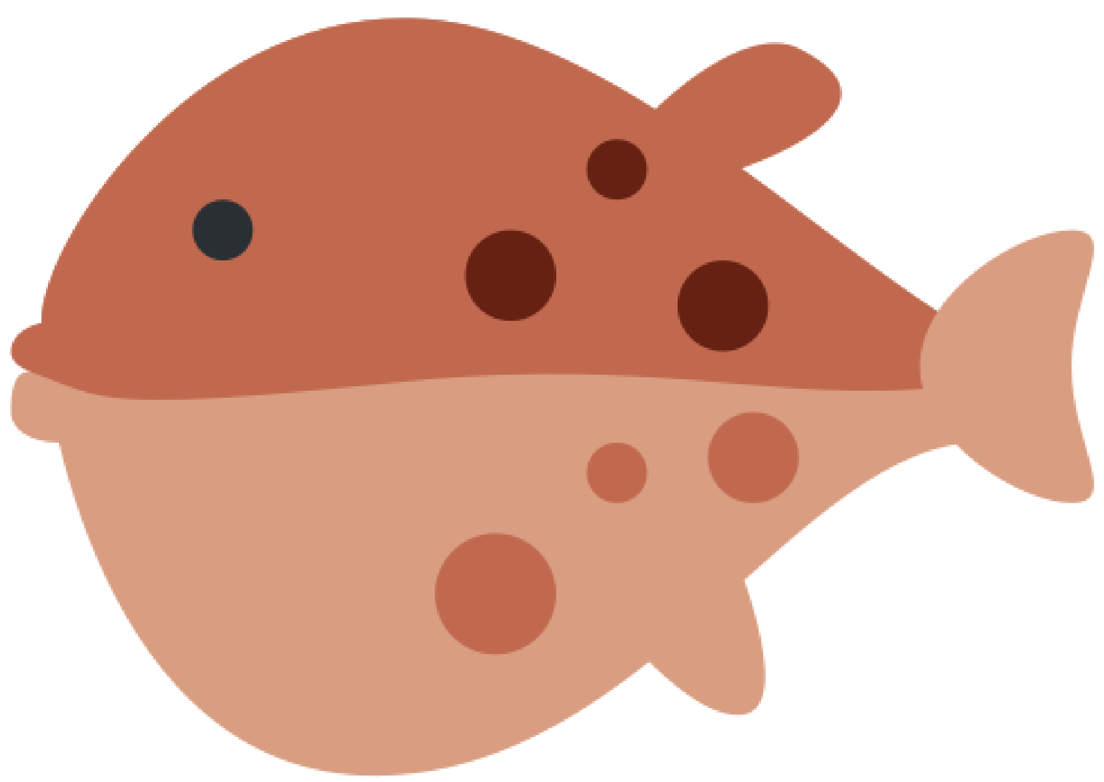
- Question– Do Na^+ and K^+ go through the same channels? Or do they have distinct channels?
  - Experiment– Add tetrodotoxin (TTX) to block inward current but not outward current
  - Experiment– Add tetraethylammonium (TEA) to block outward current but not inward current
- TTX inactivates Na^+ channels, TEA blocks K^+ channels

Speaker notes

No notes on this slide.

Neurotoxins as pharmacological tools

- Fugu (puffer fish or blow fish)
- TTX concentrated in their livers (don’t eat it)
- TTX blocks voltage-gated Na^{+} channels

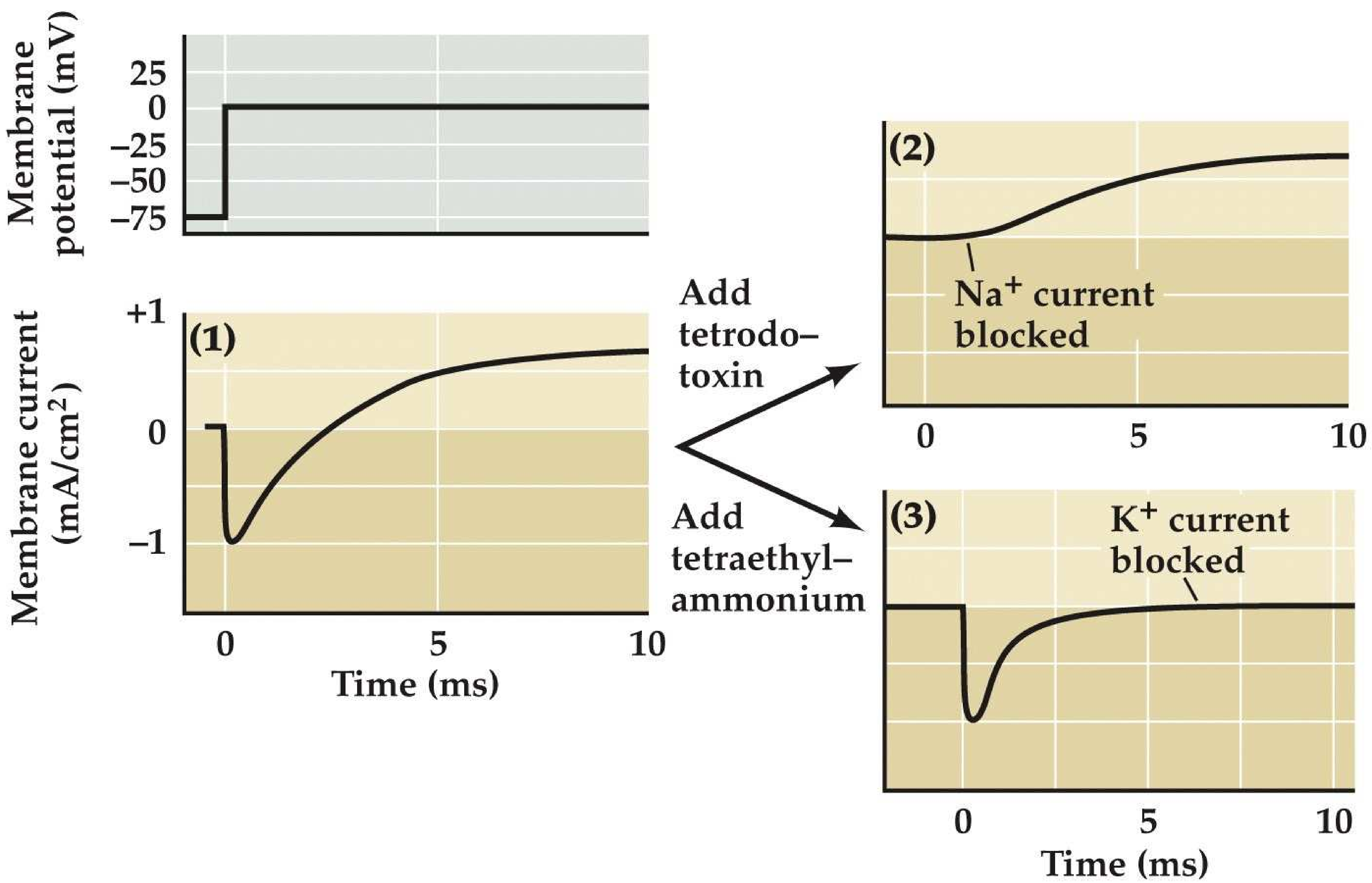


puffer fish

Speaker notes

Its mechanism of action, selective blocking of the sodium channel, was shown definitively in 1964 by Toshio Narahashi and professor John W. Moore at Duke University, using the voltage clamp technique (Narahashi et al, J Gen Physiol 1964)

Pharmacological separation of inward and outward currents into Na^+ and K^+ dependent components



Neuroscience 5e/6e Fig. 3.5; from Moore et al. *J Gen Physiol* 1967 and Armstrong and Binstock *J Gen Physiol* 1965

≡

Speaker notes

Tetramethylammonium chloride is one of the simplest quaternary ammonium salts.

https://en.wikipedia.org/wiki/Tetramethylammonium_chloride

TTX and TEA experiments from Moore 1967 J Gen Physiol; Armstrong and Binstock, 1965 J Gen Physiol

Voltage dependent membrane conductances of Na^+ and K^+

- Another way of describing permeability is using membrane conductance (*g*). Conductance (measured in siemens, *S*) is the reciprocal of resistance
  - $g = 1/R$
- Ohm's law:
  - $I = V/R$
  - $I = gV$
  - For an ion *x*,
    - I_x = ionic current flow, E_x = equilibrium potential
    - The membrane potential (V_m) minus the equilibrium potential (E_x) is the electrochemical driving force acting on an ion, thus $V = V_m - E_x$
  - $I_x = g_x V$
  - $I_x = g_x(V_m - E_x)$
- Solve for *g*:
  - $g_x = I_x/(V_m - E_x)$
- I_x determined from measurement of current changes plus or minus ion (or during pharmacological inhibition)
- E_x calculated from Nernst equation using concentrations of inside and outside ions

Speaker notes

For our purposes, we can consider conductance to be another way of describing permeability.

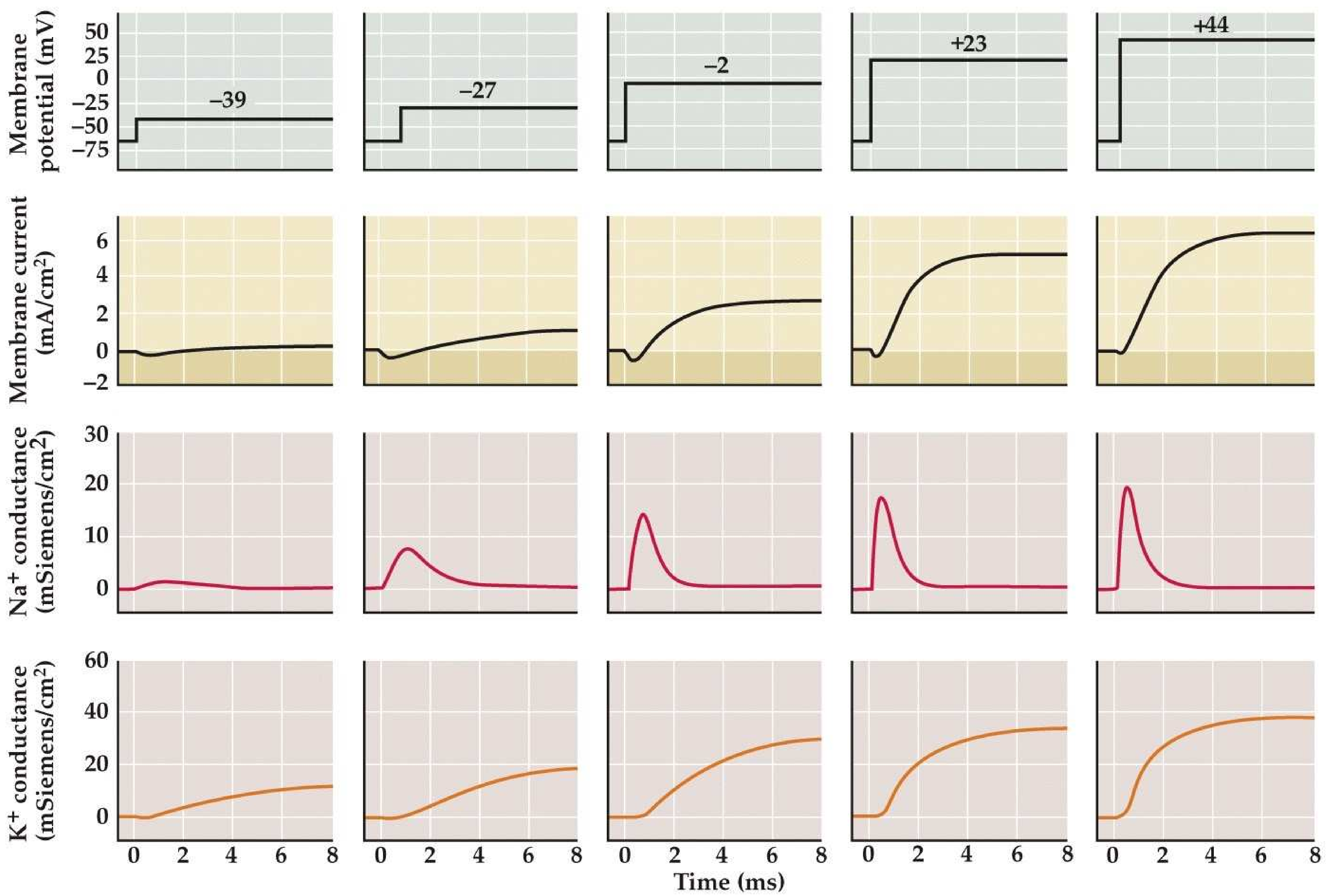
technically conductance is the degree to which an object conducts electricity, calculated as the ratio of the current that flows to the potential difference present. It deals with the movement of charge, whereas permeability refers to the ability of a specific ion to move across the cell membrane.

- http://www2.montana.edu/cftr/ion_channel_glossary.htm

Ohms law= Voltage = Current times resistance.

Can use this to calculate the dependence of Na and K conductances vs. time and membrane potential.

Membrane conductance changes are time and voltage dependent

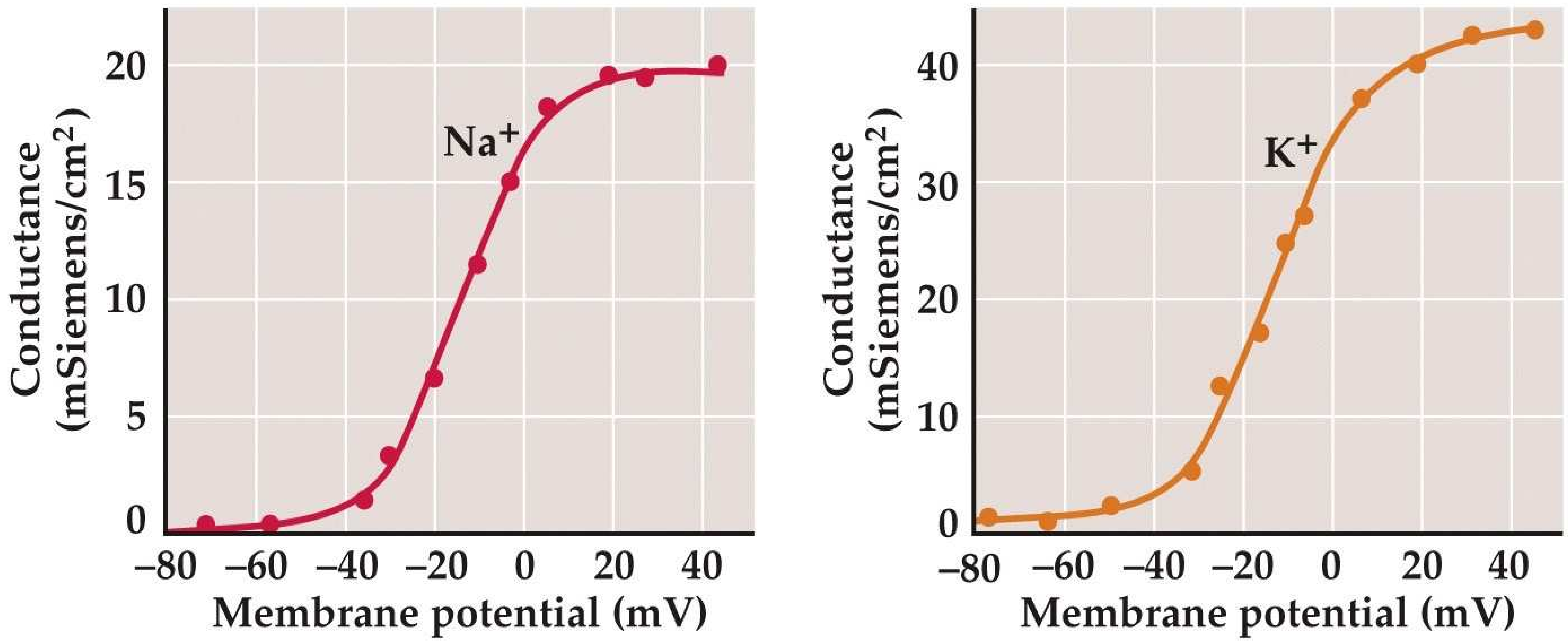


Neuroscience 5e/6e Fig. 3.6; from Hodgkin and Huxley *J Physiol* 1952b

Speaker notes

No notes on this slide.

Depolarization increases Na^+ and K^+ conductances of the squid giant axon



Neuroscience 5e/6e Fig. 3.7; from Hodgkin and Huxley *J Physiol* 1952b

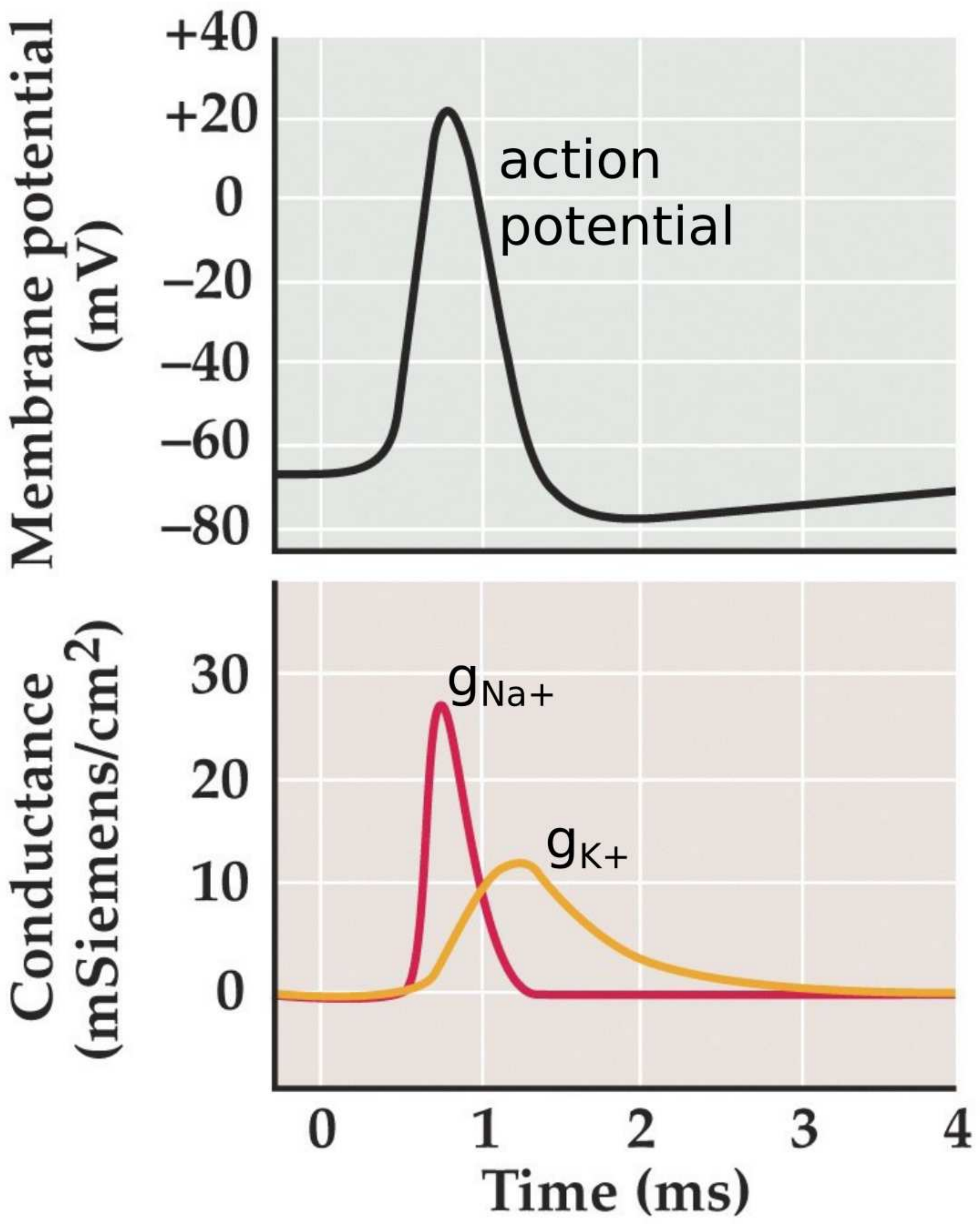
Speaker notes

Determine the peak conductance of ions at different membrane potentials.

Description of an action potential using Na^+ and K^+ conductances

- At rest (-70 mV), voltage-gated Na^+ and K^+ channels are closed. Non voltage-gated K^+ channels (K_{leak}) are open and dictate the resting potential, together with the distribution of ions across cell membranes
- A stimulus raises the membrane potential in the cell. Depolarization causes voltage-gated Na^+ channels to open, which allows Na^+ to rush in the cell which increases the membrane potential, which causes more Na^+ channels to open, which causes more Na^+ to rush in which causes higher membrane potential (a positive feedback loop). As membrane potential is approaching E_{Na}, the further depolarization causes **Na^+ channels to inactivate** which prevents more Na^+ from from flowing through these channels
- Depolarization also opens voltage gated K^+ channels, which causes K^+ to flow out, thus lowering the membrane potential

Ion conductances underlying the action potential



Neuroscience 5e/6e Fig. 3.8

Speaker notes

Summary of the conductances for Na and K during an action potential.

Based on Hodgkin and Huxley's mathematical model for the action potential (1952d).

Can see that the neuronal membrane becomes much less resistant to Na flux during the rising phase of the AP.

Can also see increases in K conductance during the AP, but this K+ conductance (underlying the outward current) are slow and sustained reaching peak permeability during the falling phase of the AP. Note when the cell is back to Vrest, the gK is still moderately high before ramping down. This is important for the refractory period.

Properties of action potentials explained

- Question– Why do APs exhibit an all-or-nothing threshold?
  - Answer– When membrane potential (V_m) is below threshold there is not enough Na^+ channels open to raise V_m high enough to open more channels. When V_m is above threshold the 'explosive' action potential cycle is activated.
- Question– Why to APs exhibit an undershoot?
  - Answer– During the AP voltage-gated K^+ conductance slowly increases (delayed activation of voltage-gated K^+ channels) and during the falling phase these K^+ channels are still open and active whereas voltage-gated Na^+ channels are inactivated… as V_m approaches E_k there is briefly more K^+ flowing out than at rest and the hyperpolarization inactivates voltage-gated K^+ channels. K^+ leak channels and ion transporters bring back cell to resting potential.

Speaker notes

The threshold is a point of criticality in the system like trying to balance on a knifes edge. Just imagine any self-organized phenomena in nature: a snow field suddenly turning into an avalanche, liquid water turning into gas or solid forms, videos of cat memes suddenly going viral. The point at which the states of these systems veer on the edge of more or less order (or less or more disorder) is the point of criticality also known to physicists as a phase transition.

Properties of action potentials explained

- Action potential propagation and directionality?
- Refractory periods?
- What does myelin do?

Speaker notes

Next we will look at the following properties of APs such as:

Action potential propagation

- Charge flowing in through Na^+ channels can diffuse inside the axon. This passive current cannot diffuse very far because of current leakage. Potentials below threshold taper out fast (like passive conduction of subthreshold depolarizations).
- Potentials above threshold cause increased depolarization (due to more Na^+ channels open). Now there is enough current to diffuse laterally and still be above threshold for a new set of Na^+ channels.

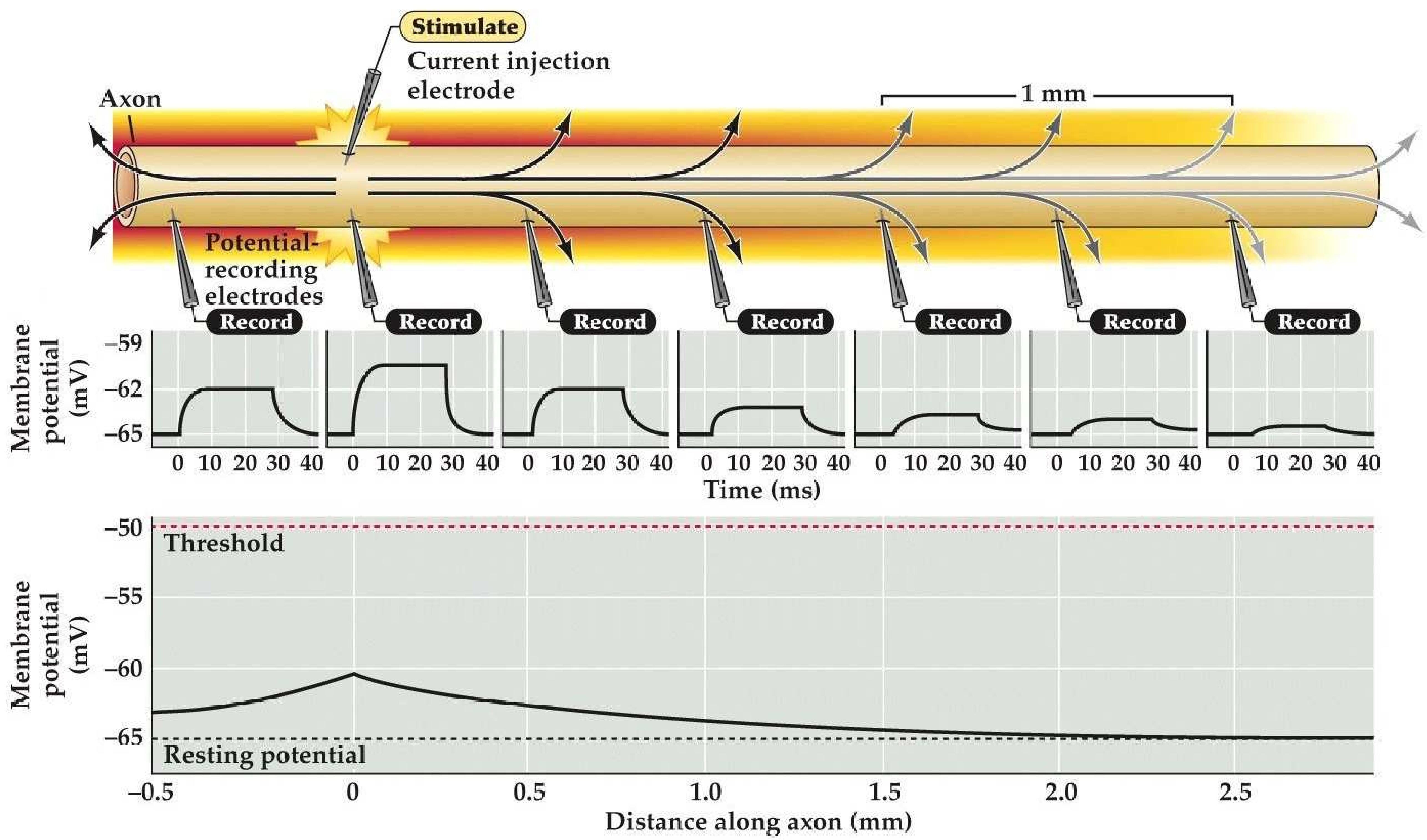
Speaker notes

First let’s talk about AP propagation.

During an action potential, inward current through Na^+ channels

Passive current flow in an axon

subthreshold changes decay rapidly



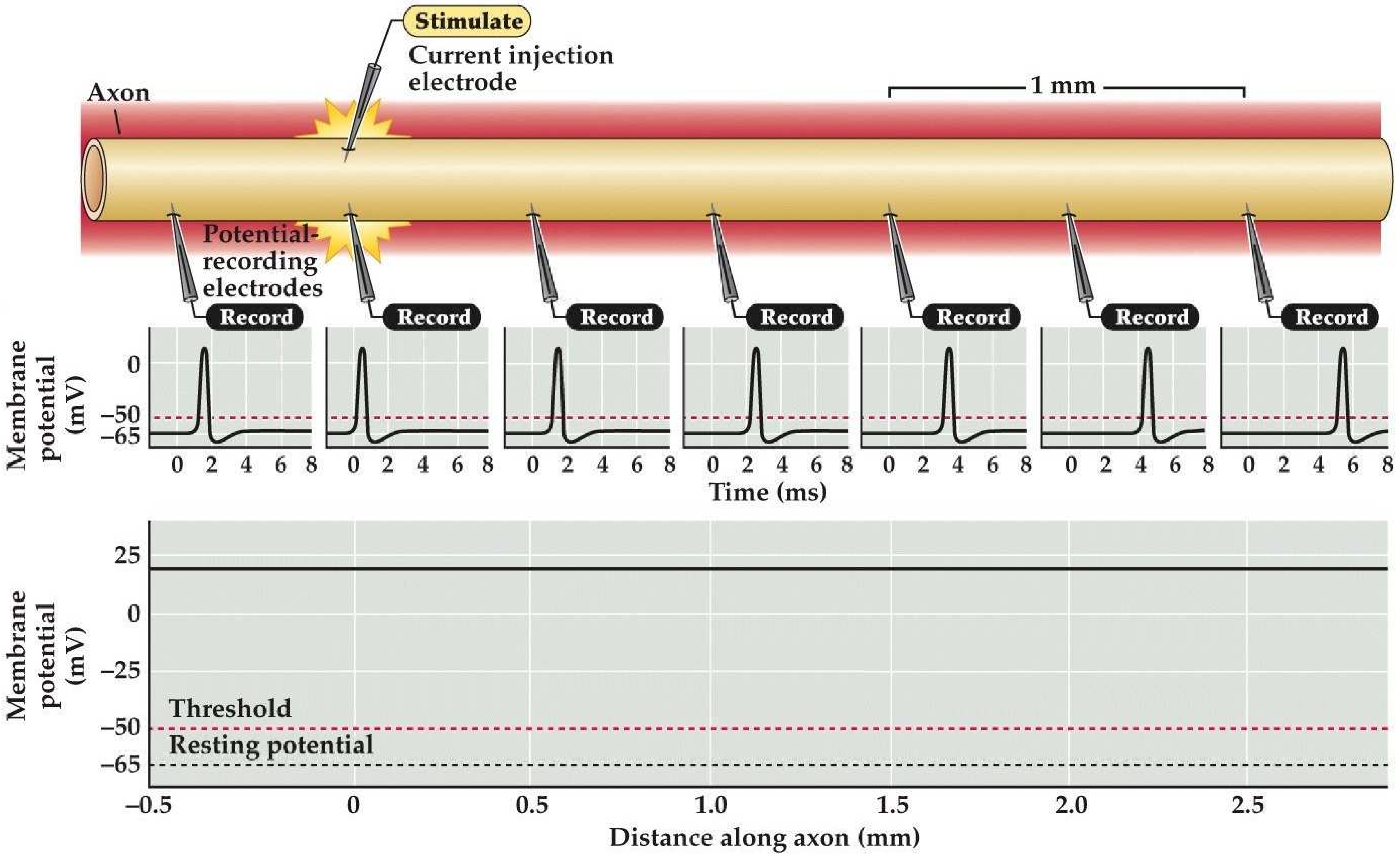
Neuroscience 5e/6e Fig. 2.3

Speaker notes

bottom graph shows the peak Vm

Propagation of an action potential

suprathreshold depolarizations propagate down the axon and don't decay



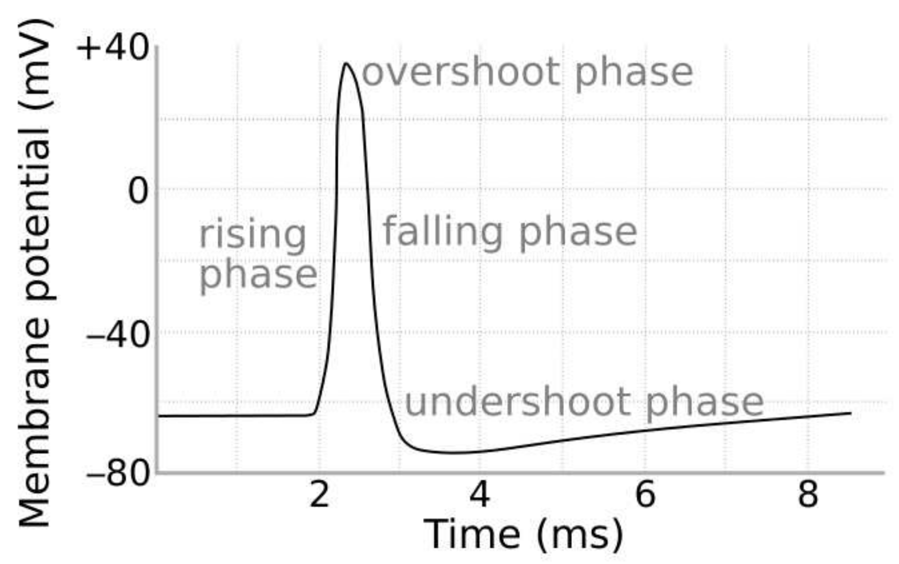
Neuroscience 5e/6e Fig. 2.3

Speaker notes

bottom graph shows the peak Vm

Why is there a refractory period?

- Remember during the falling to undershoot phase of an action potential K^+ channels are still open but Na^+ are channels inactivated (decreased g_{Na}), leading to temporary hyperpolarization more negative than the resting membrane potential
- Therefore (1) inactivation of Na^+ channels and (2) slow K^+ channel kinetics are responsible for the refractory period
- This makes it harder to initiate a new AP either from a new stimulus or for an AP to propagate backwards
- Different axons will have different refractory periods (and thus different maximal firing rates) depending on the particular subtypes of Na^+ and K^+ channels they express



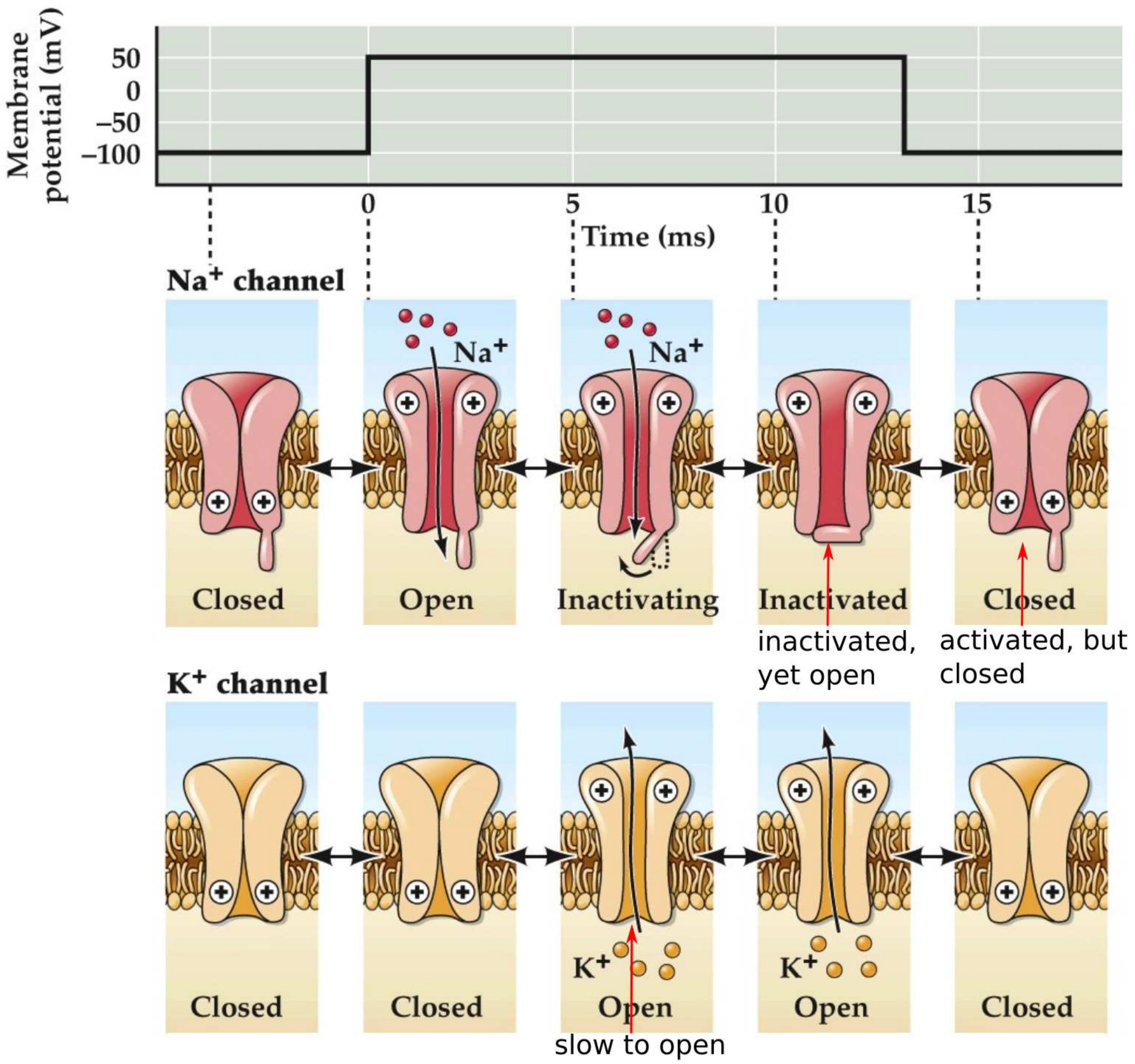
Speaker notes

Might here in other classes, especially human physiology about absolute and relative refractory periods.

Refractory period we've been discussing here is mostly the absolute refractory period-- due to Na+ channel inactivation.

Relative refractory period is the transient hyperpolarization during the undershoot when potassium conductance is still greater than normal-- when K+ conductance returns to resting value the relative refractory period is over. Until this time, a greater secondary depolarizing stimulus will be required to reach AP threshold.

Voltage-gated channel states during an action potential



Neuroscience 5e fig. 4.3

Speaker notes

No notes on this slide.

What does myelin do?

- Rate of action potential formation limits the flow of information
- How to speed up AP conduction?
  - Increase the diameter of the axon– bigger axon diameters have less resistance (decreased resistance to passive current flow)
  - Myelin **insulates the axon**, reducing current leak. Example AP conduction velocities for axons: unmyelinated 0.5–10 m/s, myelinated 150 m/s

Speaker notes

No notes on this slide.

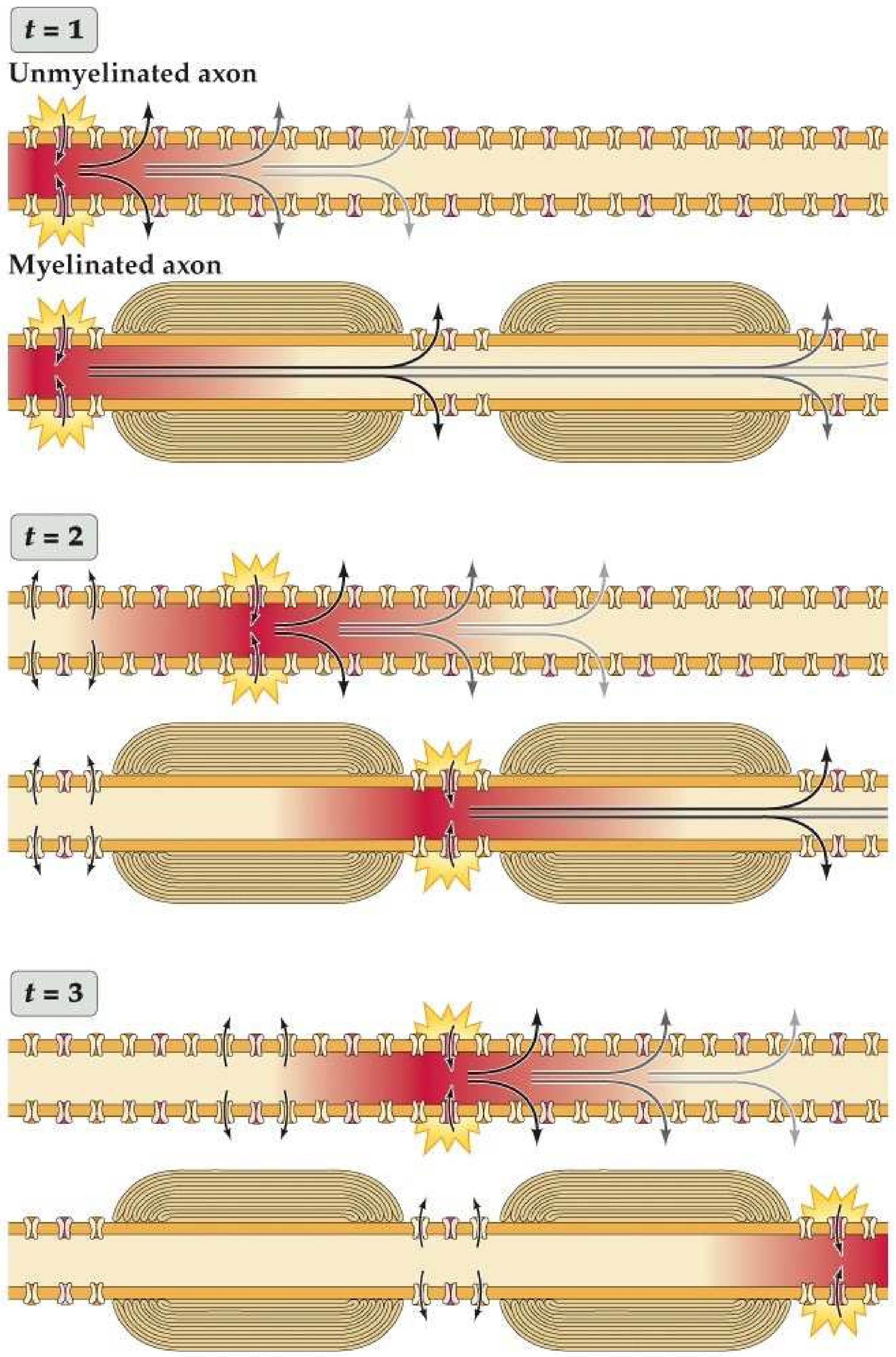
Nodes of Ranvier

- Can’t insulate the whole axon because transmembrane current flow is required to generate the action potential
- Current from one action potential flows passively to next node where a new action potential is made
- Action potentials have saltatory conduction– meaning from node to node

Speaker notes

saltatory action potential condution along a myelinated axon

Speed of action potential conduction in unmyelinated versus myelinated axons



Neuroscience 5e Fig. 3.12

Speaker notes

figure comparing action potential propagation speed in an unmyelinated and myelinated axon.

action potential genaration occurs only at specific points, the nodes of Ranvier, along the myelinated axon

Multiple sclerosis

- Disease caused by myelination defects and loss of neurons
- Seems like an autoimmune disease
- 1/750 of population in US get multiple sclerosis (MS)
- 1/40 risk if a parent has it
- 1/3 if an identical twin gets it
- Genetic and environmental risk factors

Speaker notes

onset between ages 20-40.

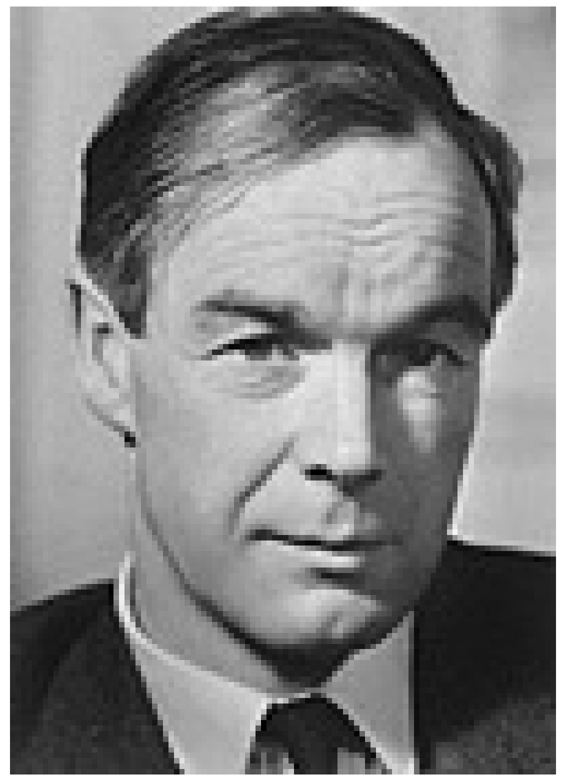
blindness, motor weakness, paralysis.

ultimate cause of MS remains unclear. Immune system contributes to damage and is key component. Immune cells in CSF and injection of myelin in animals can cause EAE.

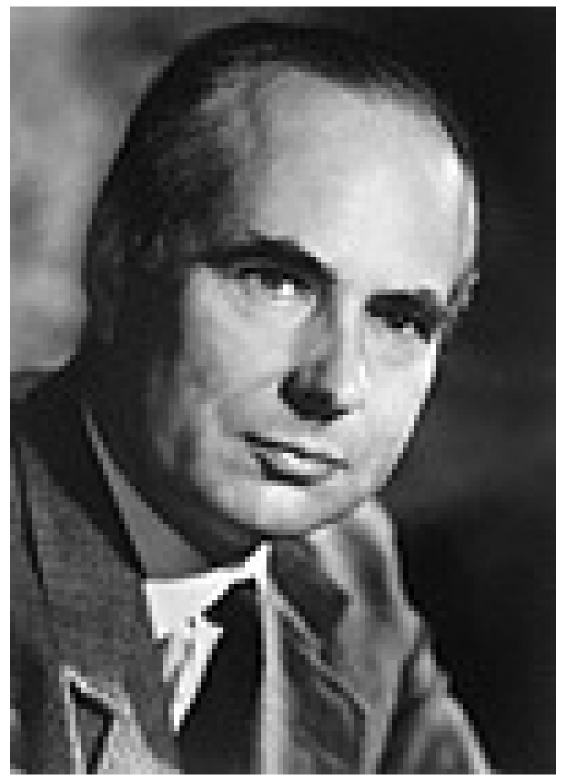
- women to men ratio 3/2
- Genetic component is likely the effect of multiple genes

The Nobel Prize in Physiology or Medicine (1963)

> *"for their discoveries concerning the ionic mechanisms involved in excitation and inhibition in the peripheral and central portions of the nerve cell membrane"*



Alan Lloyd Hodgkin

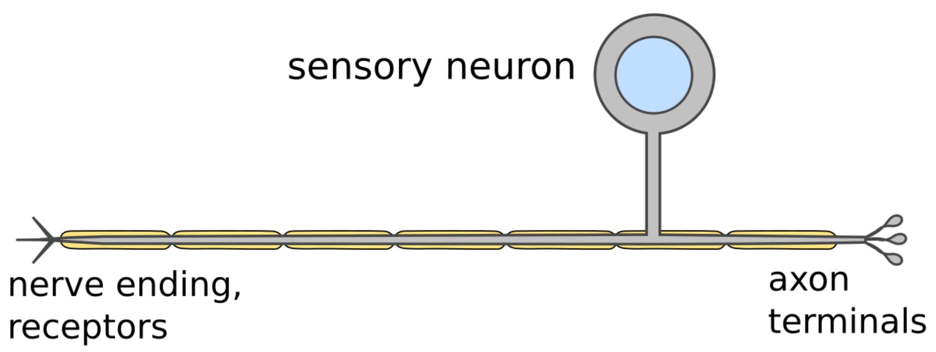


Andrew Fielding Huxley

Speaker notes

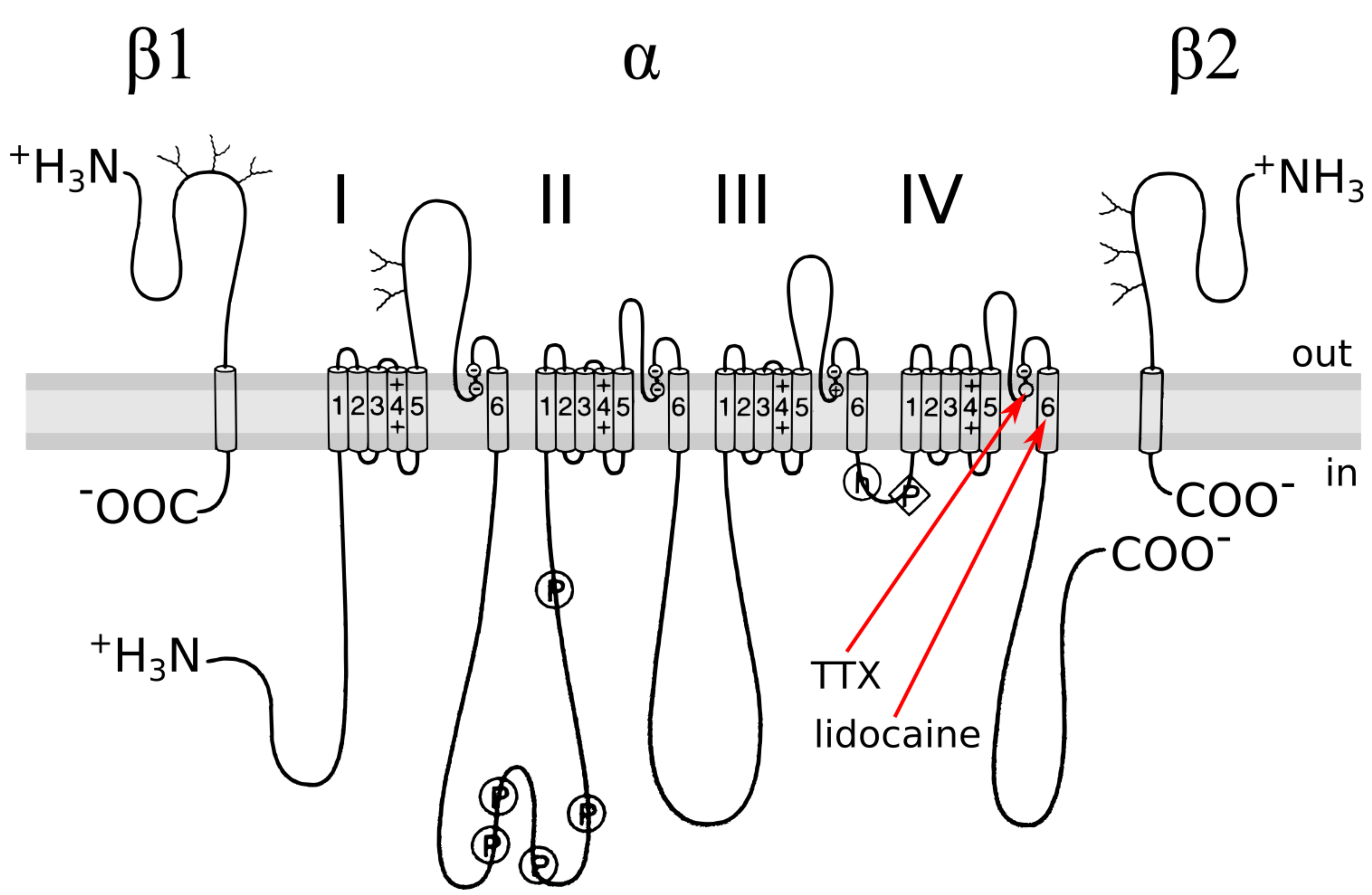
No notes on this slide.

Painless dentistry



- Lidocaine blocks some types of Na^+ channels
- Blocks action potentials in sensory axons
- Pain signals do not reach the brain

voltage-gated sodium channel and drug binding sites



Adapted from Basic Neurochemistry 6e Fig. 6.6

Speaker notes

No notes on this slide.