Ion channels underlie action potentials

- Predictions about the nature of ion channels from Hodgkin and Huxley:
  - Because conductances are large, channels must be able to pass ions at high rate
  - Channels must be gated by the membrane potential
  - Different channels for Na^+ and K^+
- Problem– Voltage clamping cannot look at individual channels...it's measuring the aggregate current flowing through a whole bunch of channels at once. What does an individual channel look like? How does it work?
  - Solution– Patch Clamping

2021-10-07T14:03:23-07:00

Speaker notes

Today we will take a closer look at the nature of **ion channels** and how they are able to exhibit their remarkable properties that enable action potentials and all forms of electrical signaling in the nervous system.

Now we know from our previous classes covering the work by Hodgkin-Huxley, that there are some predictions we make concerning the nature of ion channels..

Question

During the rising phase of the action potential:

- a. All sodium channels are closed
- b. Some of the sodium channels are closed
- c. All potassium channels are open
- d. All sodium channels are open
- e. The membrane potential is hyperpolarizing

Speaker notes

No notes on this slide.

Patch clamp recordings

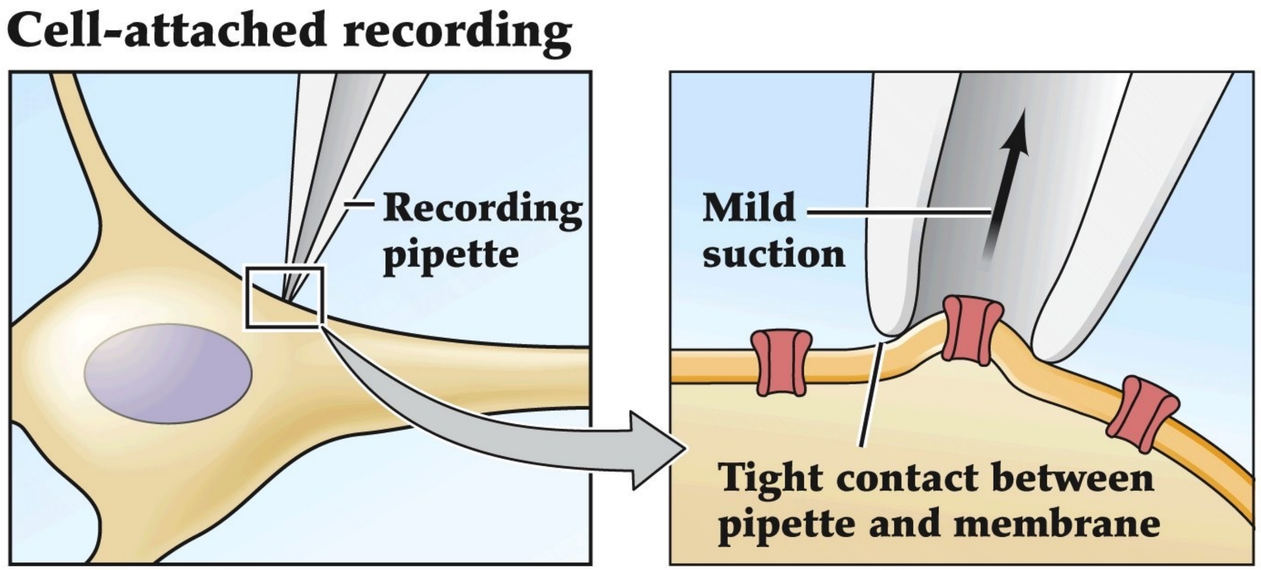
- Allows one to look at currents flowing through a single channel
- Pipette with small opening makes a tight seal with the membrane
- Currents are amplified and measured
- Can be adapted to do whole cell recordings, inside out recordings or outside out recordings

Speaker notes

Remember voltage-clamp recordings that we've been talking about before. Patch-clamping is an adaptation of the voltage-clamp method that allows you to assess the currents flowing across very small patches of lipid membrane. So if you have one or few ion channels in that small patch of cell membrane you can study the microscopic functional properties of those individual channels.

The patch clamp method

Can measure ion flow through a single channel.



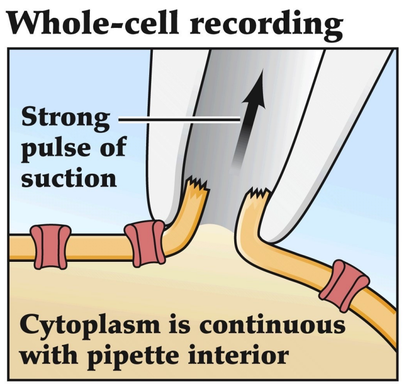
Neuroscience 5e Box 4A

Speaker notes

No notes on this slide.

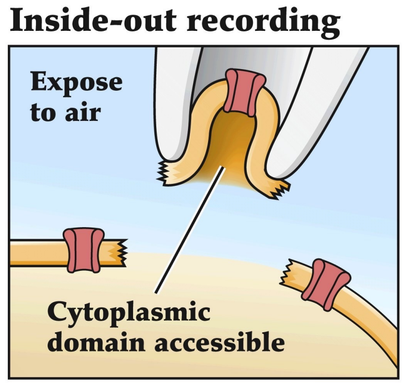
The patch clamp method

Neuroscience 5e Box 4A



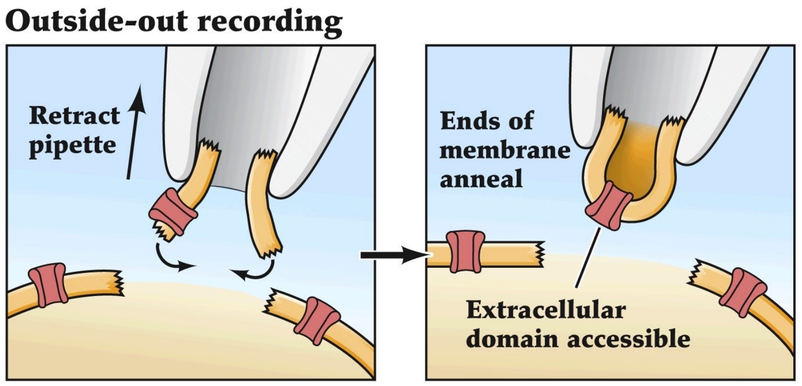
Can measure potentials and currents from entire cell and introduce things into the cytoplasm

Neuroscience 5e Box 4A



Makes it easy to introduce things to the cytoplasmic side of the channel

Neuroscience 5e Box 4A



Makes it easy to introduce things to the extracellular side of the channel

Speaker notes

No notes on this slide.

The Nobel Prize in Physiology or Medicine (1991)

> *"for their discoveries concerning the the function of single ion channels in cells"*

Erwin Neher

Bert Sakmann

Speaker notes

Patch clamp established by E. Neher adn B. Sakmann at Max Planck Institute in Germany 1976.

Patch clamping Na^+ channels

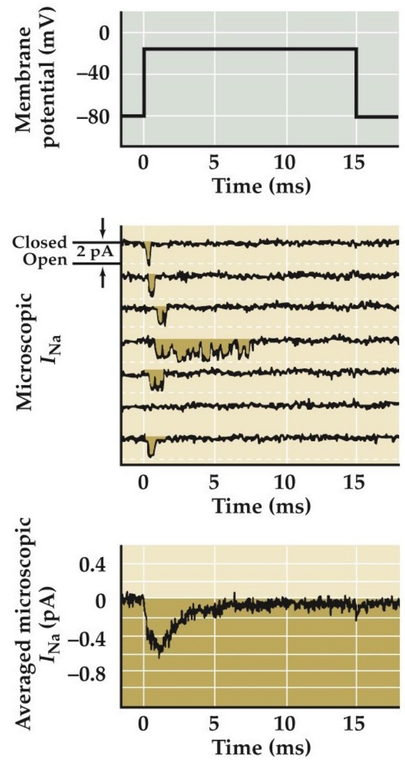
- Block K^+ channels with Cs^+ or with tetraethylammonium (TEA)
- Brief depolarizations cause small inward currents that disappear right away
- Each inward current is the opening of one Na^+ channel
- About 1-2 pA of current ≈ thousands of ions/ms
- Stochastic opening, biased at the beginning of a pulse
- Probability of opening varies with membrane potential
- If you remove Na^+ from medium, do not see currents
- Tetrodotoxin (TTX) blocks currents

Speaker notes

TEA
: tetraethylammonium
: quaternary ammonium cation
: blocks voltage gated K+ channels

Measurements of ionic currents flowing through single Na^+ channels

- Small (picoampere) inward currents
- Unitary amplitudes
- Open at beginning of pulse
- Inactivate quickly



Neuroscience 5e Fig. 4.1

Speaker notes

Patch a piece of membrane and block K currents. Do a bunch of short recordings while clamping the membrane at depolarized potential. e.g. here is 7 experimental trials. **Notice the amplitude is discrete**— it is unitary. If you were recording from lots of these single channels simultaneously or added together all the recordings from one channel you'd -->

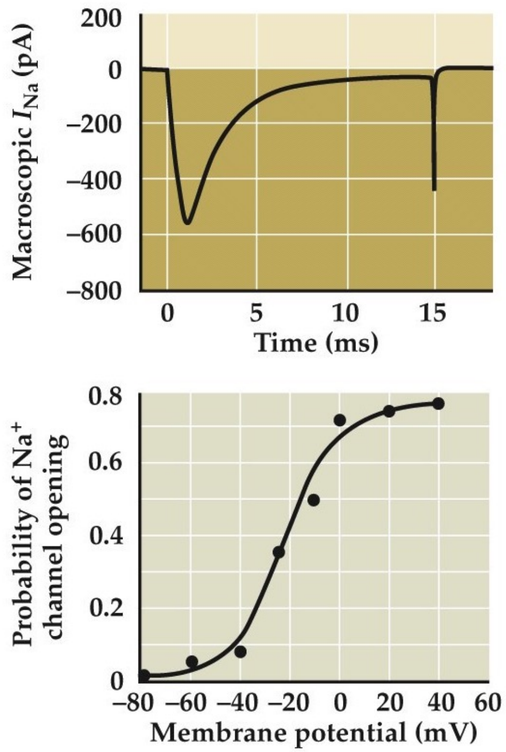
Transient channel opening in Na^+ channels (inward current).

This research is from Bezanilla and Correa 1995, Vandenburg and Bezanilla 1991, Correa and Bezanilla 1994

unitary (wn, adj)
: one, unitary -- (having the indivisible character of a unit; "a unitary action"; "spoke with one voice")

Measurements of ionic currents flowing through single Na^+ channels

- **Summed current from many single channels looks like macroscopic currents seen in voltage clamping**
- Probability of opening increases as a function of membrane potential



Neuroscience 5e Fig. 4.1

Speaker notes

Average the microscopic currents together and you get something very similar to this macroscopic voltage-clamp current shown at the top.

Notice that even at -20 to -10mV when you expect an action potential to be well into its rising phase above threshold, the probability of sodium channel opening is just 40-50% (and never reaches 100%).

Bezanilla and Correa 1995, Vandenburg and Bezanilla 1991, Correa and Bezanilla 1994

Patch clamping K^+ channels

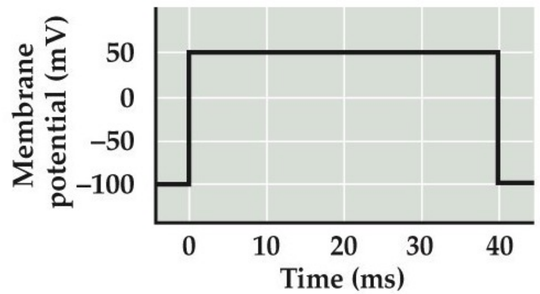
- Add tetrodotoxin (TTX) to block Na^+ channels
- Depolarization pulses cause outward currents
- Once a channel opens (usually with a delay) it remains open for the duration of the pulse
- The probability of channel opening depends on the membrane potential
- Sensitive to TEA

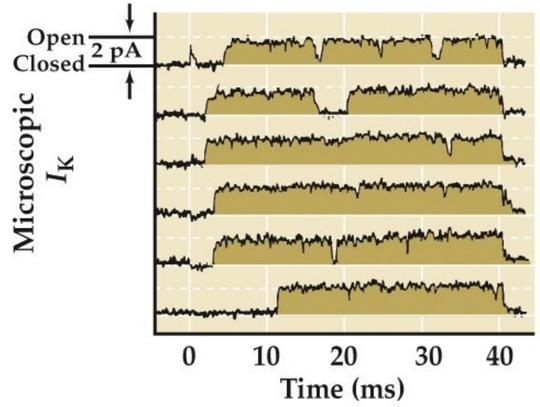
Speaker notes

Now let's examine patch clamp experimental data from K+ channels.

Measurements of ionic currents flowing through single K^+ channels

- Early delay in opening
- Once open stay open





Neuroscience 5e Fig. 4.2

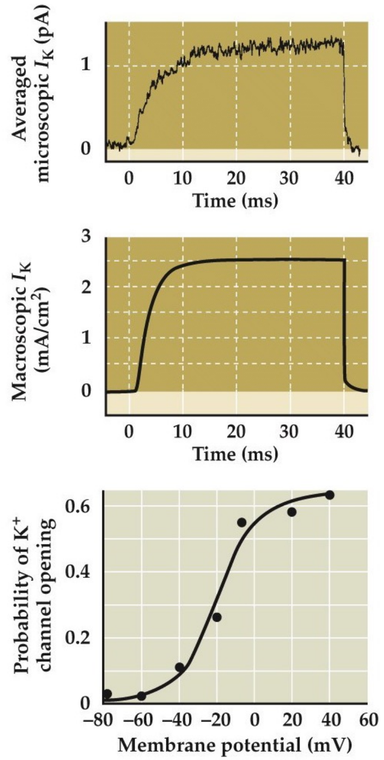
Speaker notes

Sustained channel opening in K^+ channels (outward current).

This research is from Augustine and Bezanilla, Hille 2001; Augustine and Bezanilla 1990; Perozo et al 1991

Measurements of ionic currents flowing through single K^+ channels

- **Summed current from many single channels looks like macroscopic currents seen in voltage clamping**
- Probability of opening increases as a function of membrane potential



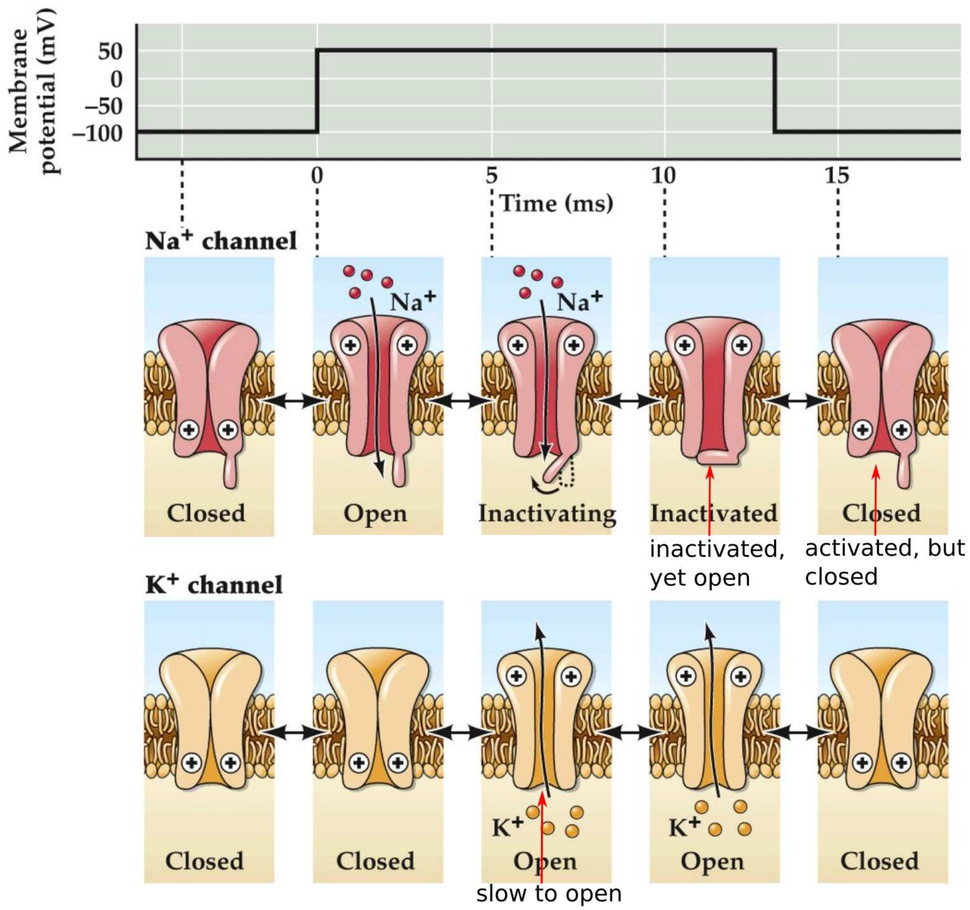
Neuroscience 5e/6e Fig. 4.2

Speaker notes

Sum a bunch of these microscopic channel currents and you get this top curve and which looks very similar to the macroscopic current curve as we've seen previously.

This research is from from Augustine and Bezanilla, Hille 2001; Augustine and Bezanilla 1990; Perozo et al 1991

Functional states of voltage-gated Na^+ and K^+ channels



Neuroscience 5e/6e Fig. 4.3

Speaker notes

Remember this figure from last time, shown here is a model of the functional states for these channels. Notice a few states for Na vs two for K.

Conclusions from patch clamp experiments

- Allowed the direct observation of ionic currents flowing through single ion channels
- Both Na^+ and K^+ channels are voltage gated
- Thus there must be a voltage sensor in these channels
- Depolarization inactivates Na^+ channels but not K^+ channels

Speaker notes

No notes on this slide.

Patch clamp method summary video

Neuroscience 5e Animation 4.1

Speaker notes

No notes on this slide.

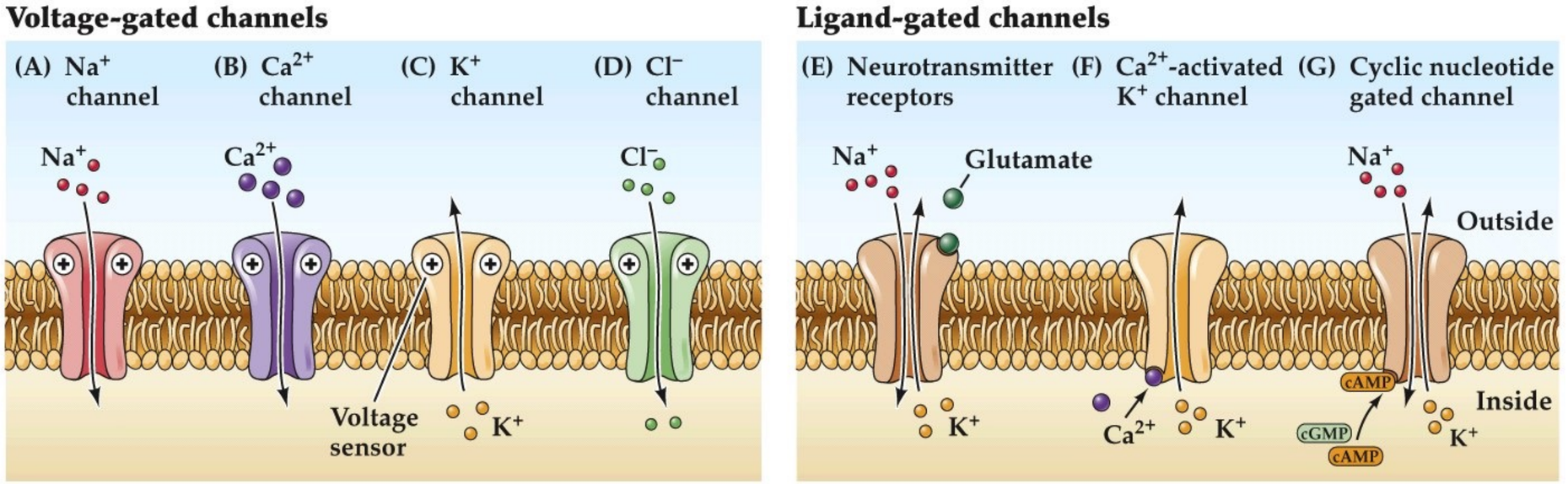
Many genes encode ion channels

- There are hundreds of genes encoding ion channels (e.g. about 100 K^+ channel genes)
- They have common properties (similarities in amino acid sequence and protein topology)
- They also have variations (differences in ion selectivity, how they are gated, inactivation mechanisms)

Speaker notes

Now everything going on in our nervous systems depends on the function of ion channels. And there are lots of them.

Different ways to gate ion channels



Neuroscience 5e Fig. 4.4

Speaker notes

Different classes of gated ion channels.

voltage gated ion channels, such as we've been discussing over the last couple classes.

ligand gated channels such as those that bind neurotransmitters, will talk about more later and next class.

others are ligand gated channels sensitive to chemical signals arising in the cytoplasm of neurons such second messengers like Ca^{2+}, cyclic nucleotide cAMP and cGMP.

Lots of variation among ion channels and their properties

- Voltage gated– Na^+, K^+, Cl^-, and Ca^{2+} channels
- Approximately 10 different genes for Na^+ channels, 16 Ca^{2+}, 3–5 Cl^- and 100 K^+ channels
- Different genes may give rise to channels with different properties– e.g. different inactivation times, probability of opening at a given voltages, gating mechanisms
- Can also be multiple splice variants of the same gene
- Creates huge diversity of channels
- How to characterize all these channels?

Speaker notes

No notes on this slide.

Xenopus oocytes

- Large (1 mm in diameter) cell that contains lots of protein synthesis machinery
- Can inject RNA into it and it will express protein encoded by RNA
- Works great for expressing a gene of interest (ion channels!). Can voltage clamp and determine properties of a given channel
- Can make specific mutations in genes and see what happens to function of protein

Speaker notes

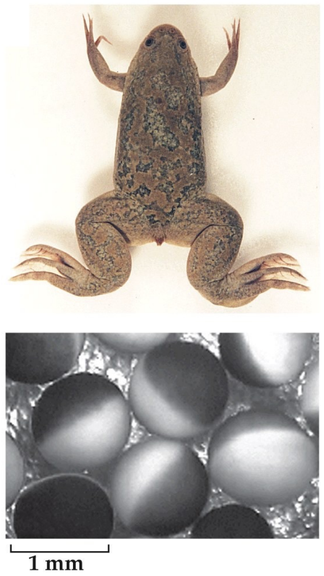
If you have a gene for a channel, how do you determine its properties?

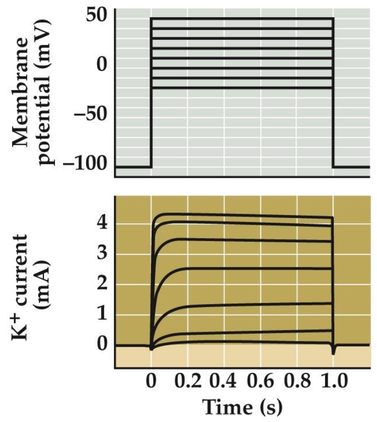
frog germ cells

- Need an experimental system where you can express gene of interest functionally and away from other channels
- Xenopus oocytes have been a historical way to do this

Xenopus oocytes for ion channel physiology studies

Inject ion channel mRNA into oocyte ⟶ oocyte makes protein ⟶ patch clamp recordings



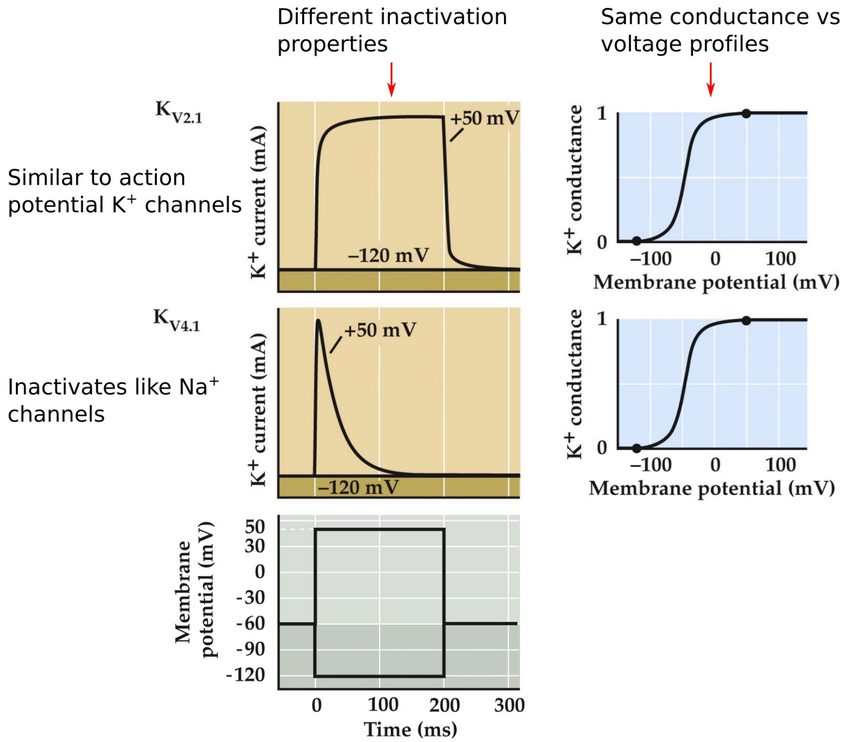


Neuroscience 5e Box 4C

Speaker notes

shows voltage clamp experiment results after expression of a K channel in an oocyte.

Different K^+ channels can have diverse properties



Neuroscience 5e Fig. 4.5

Speaker notes

- Kv2.1 show little inactivation and are closely related to the delayed rectifier K channels involved in AP repolarization

from channelpedia:

> *Kv2.1 is widely expressed in brain and composes the majority of delayed rectifier K+ current in pyramidal neurons in cortex and hippocampus and is also widely expressed in interneurons. Dynamic modulation of Kv2.1 localization and function by a mechanism involving activity dependent Kv2.1 dephosphorylation dramatically impacts intrinsic excitability of neurons.*

- Kv4.1 channels inactivate during a depolarization.

> *a voltage-activated A-type potassium ion channel and is prominent in the repolarization phase of the action potential. This gene is expressed at moderate levels in all tissues analyzed, with lower levels in skeletal muscle.*

- inward rectifier K channels allow more K current to flow at hyperpolarized potentials than at depolarized potentials
- human Ether-à-go-go-Related Gene), best known for its contribution to the electrical activity of the heart that coordinates the heart's beating, mediates the repolarizing IKr current in the cardiac action potential).
- HERG channels inactivate so rapidly that current flows only when inactivation is rapidly removed at end of a depolarization

inward rectifier K channels allow more K current to flow at hyperpolarized potentials than at depolarized potentials

Ca activated K channels open in response to intracellular Ca ions

2-P K channels ("two-pore", or KCNK gene family, 50+ genes?) can respond to other signals (e.g. pH changes for the TASK (KCNK3 and KCNK9) channel subtypes) rather than changes in membrane potential and are important in regulating the ongoing membrate potential of neurons at "rest", playing a role in the historically termed "K_{leak}" current.

https://www.nature.com/articles/35058574

Molecular structures of ion channels

- Multiple membrane spanning domains
- K^+ channels– 4 subunits come together, (each with 6 transmembrane helices)
- Na^+ channels– 1 protein with 24 transmembrane helices
- Center has an opening that makes a pore for the ion to flow through
- Contains selectivity filter
- Voltage-gated ion channels also contain a voltage sensitive transmembrane domain

Speaker notes

We've learned from biophysical structure studies that in general ion channels have 24 transmembrane peptide domains with…

We can also guess a few characteristics of their structure from the classic voltage clamp and patch clamp studies we've discussed over the past couple classes…

X-ray crystallography
: tool for identifying the atomic and molecular structure of a crystal
: crystalline atoms cause high energy (high frequency/short wavelength) electromagnetic waves (X-rays) to scatter in different directions
: measure intensities and angles of the diffracted beams and compute a 3D model of the electron density in a crystal
: information on mean atomic positions, type of chemical bonds, and more can be extracted

Structure of the bacterial K^+ channel

- Bacteria have K^+ channels that are very similar in structure to mammalian K^+ channels. Main difference is that they are not gated by voltage
- Could be crystallized in the bacterial membrane
- 3D structure tells us a lot about function
- Roderick Mackinnon Nobel Prize in Chemistry 2003

> *"for structural and mechanistic studies of ion channels"*

Speaker notes

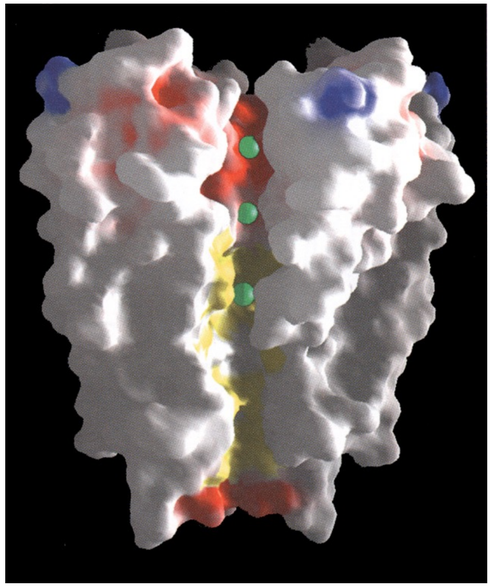
prokaryotic

eukaryotic

Structure of the bacterial K^+ channel

A space-filling model of the KcsA channel, showing the pore. Ions (green balls) tend to occupy three sites in the channel, two in the selectivity filter and one in a pool of water in the center of the channel.

red (–) charge; blue (+) charge; yellow hydrophobic



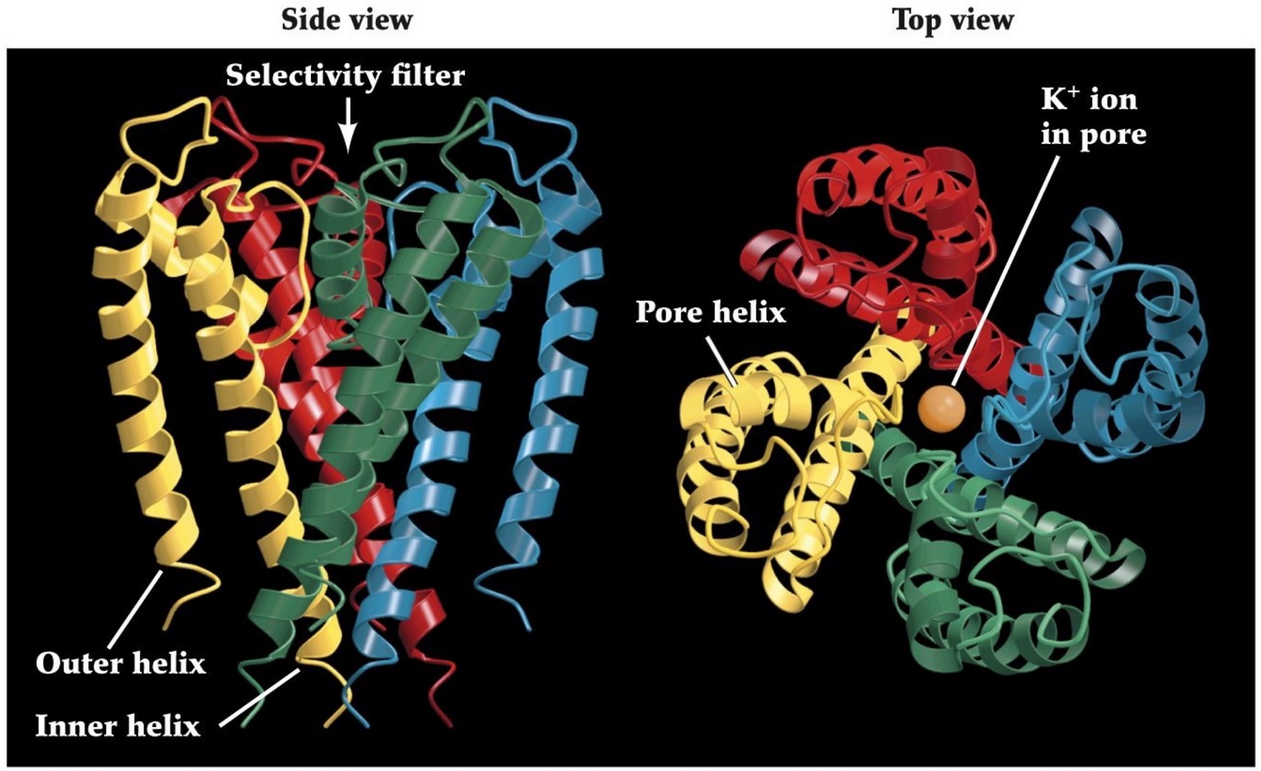
Doyle et al, Science 280:69, 1998

Speaker notes

(Doyle et al, Science 280:69, 1998)

Structure of the bacterial K^+ channel

Each subunit has 2 transmembrane domains, 4 subunits make a channel

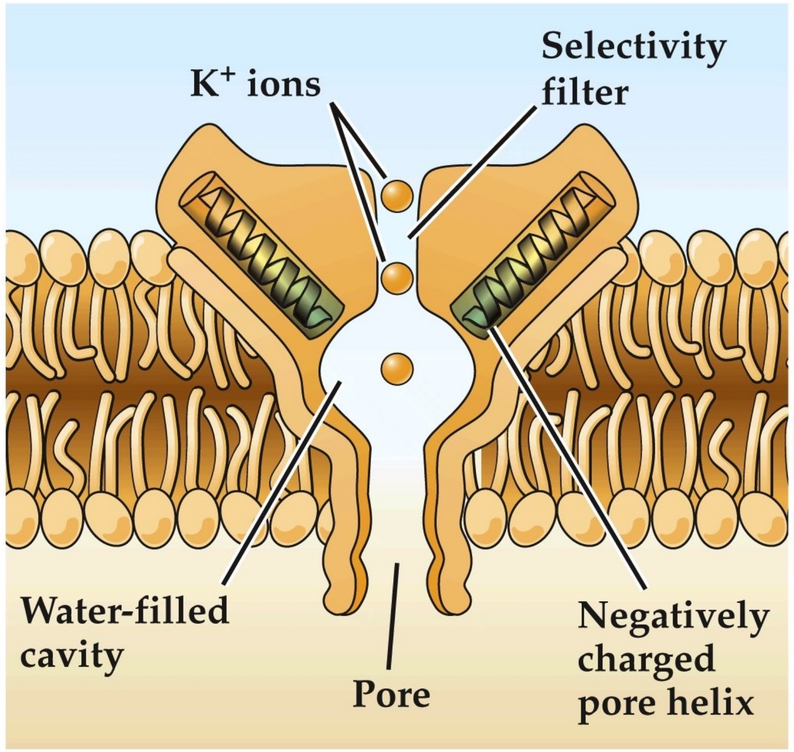


Neuroscience 5e Fig. 4.7

Speaker notes

(Doyle et al, Science 280:69, 1998)

Structure of a bacterial K^+ channel determined by crystallography



Neuroscience 5e Fig. 4.7

Speaker notes

Simplified model of bacterial K channel, showing you the pore and selectivity filter.

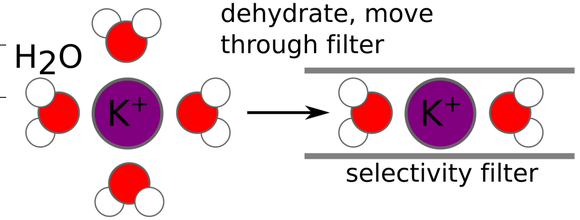
helical domains of channel point negative charges towards cavity allowing K ions to become dehydrated and then push through selectivity filter through electrostatic repulsion.

outside inside helps dehydrate K^+ ions

Selectivity filter of the K^+ channel

- Up to 4-6 water molecules form hydration shells around both Na^+ and K^+ ions
- Ions move with their hydration shells
- To pass through the potassium channel, an ion must remove most of its surrounding water molecules (dehydrated)
- K^+ is dehydrated by the K^+ channel selectivity filter (leaving just two water molecules– one at front and one at back)
- Na^+ has a more stable water shell, binding H_2O more strongly and thus has a larger effective diameter— would require more dehydration energy than K channel pore region can provide

ion	ion diameter (nm) free	ion diameter hydrated
Na	0.19	0.52
K	0.27	0.46



JA, CC0

Speaker notes

remember water is a polar molecule. Has a net dipole moment of opposing charges in the hydrogen-oxygen bonds.

Larger cations cannot traverse the pore region, smaller cations like Na cannot enter the pore because the walls are just too far apart to stabilize a dehydrated Na ion long enough to pass through.

Na is the most hydrated ion with 4 to 6 water molecules in the first shell. Binds water strongly, making a stable hydration shell and moving together with the cation. Any sodium movement is followed by H2O movement (water retention, excretion).

Potassium ion is larger, having 8 more electrons shielding positively charged nucleus, thus K^+ makes transient associations with water rather than a discrete hydration layer. Helps explain higher permeability across cell membrane for K^+.

ion | ion diameter (nm) free | ion diameter hydrated

--- | ---------- | ----------

Na | 0.19 | 0.52

K | 0.27 | 0.46

a quote from http://web-books.com/MoBio/Memory/Channel.htm:

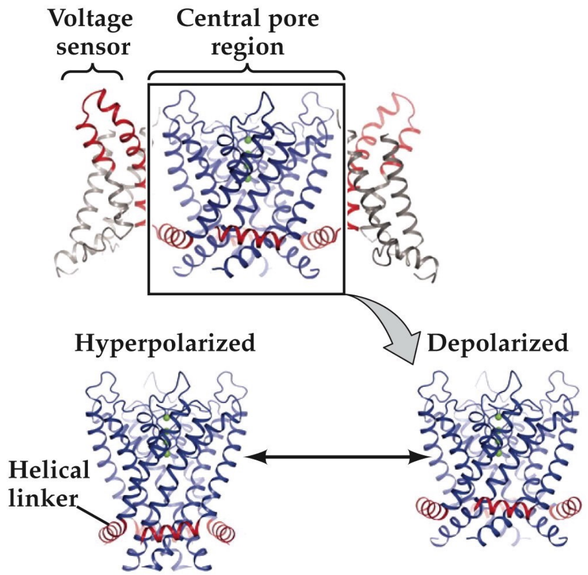
> *To pass through the potassium channel, an ion must remove most of its surrounding water molecules, leaving only two - one at the front and another at the back.*

The selectivity filter of the sodium channel is slightly larger than that of the potassium channel. It may accommodate a Na^+ ion attached with three water molecules, but not enough for a K^+ ion attached with three water molecules.

one more quote from http://web-books.com/MoBio/Memory/Channel.htm:

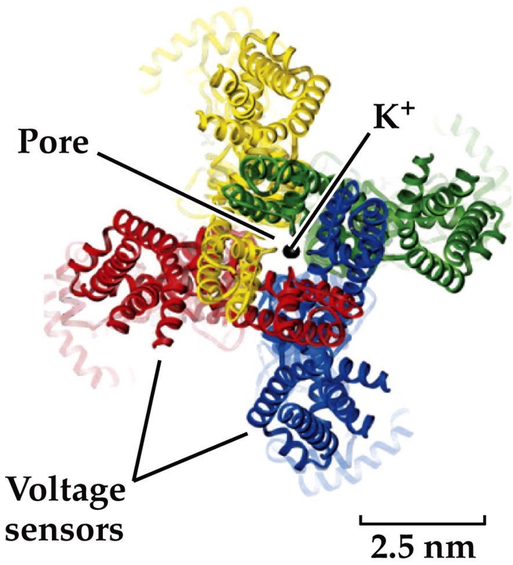
Structure of a mammalian voltage-gated K^+ channel

side view



Neuroscience 5e Fig. 4.8

top view



Neuroscience 5e Fig. 4.8

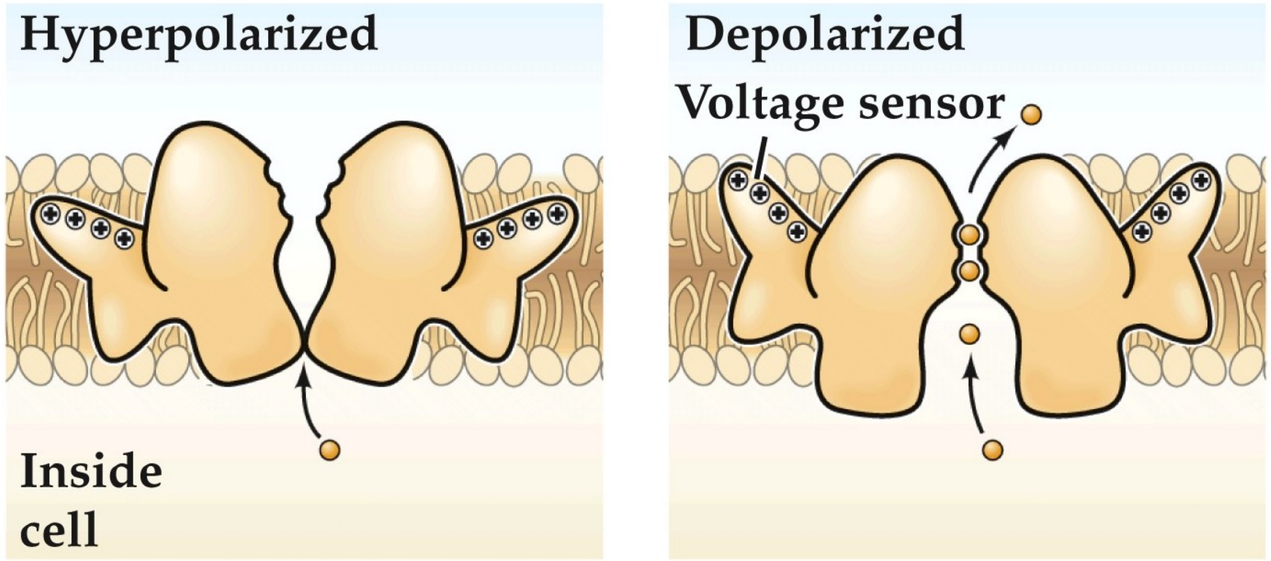
Speaker notes

Now we know from what we've learned over the past couple classes that unlike bacteria, neurons have K^+ channels that are **gated by voltage**

http://web-books.com/MoBio/Memory/Channel.htm :

> *There are many types of potassium channels. The one involved in the generation of action potentials is composed of four subunits, each is homologous to the Shaker protein (Fig. 3.2). The hydrophobicity profile indicates that it contains six hydrophobic segments, designated as S1 - S6. These segments are likely to be the transmembrane domains. Other experimental results suggests that the P-region is lining the channel pore.*

Structure of a mammalian voltage-gated K^+ channel

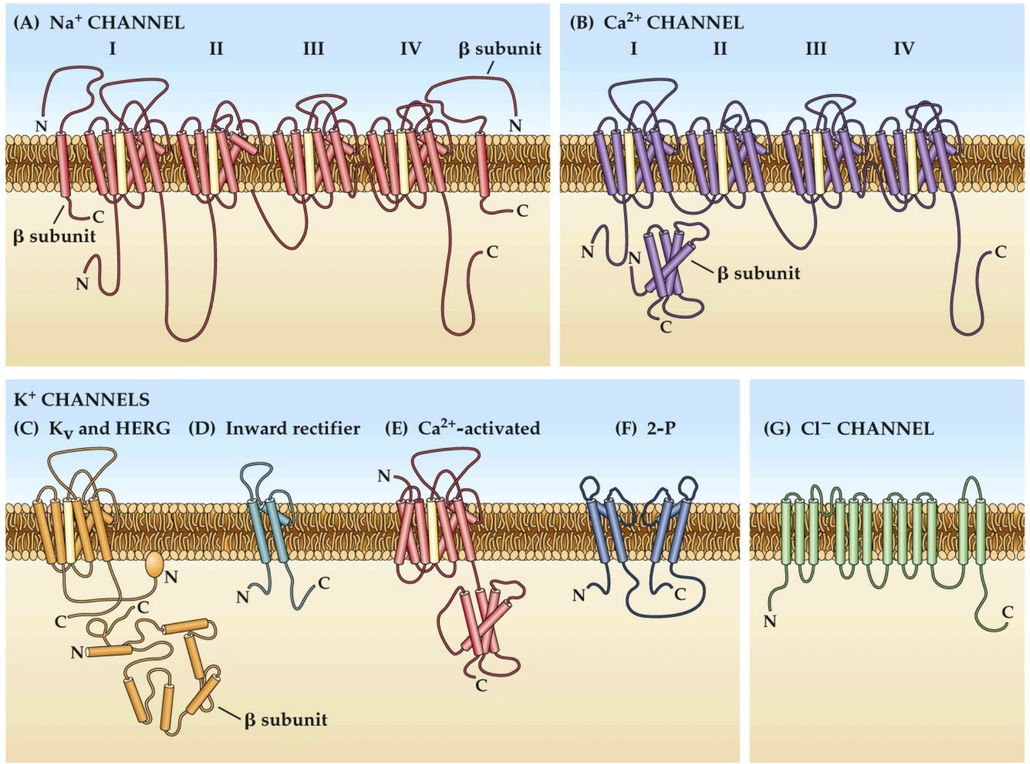


Neuroscience 5e Fig. 4.8

Speaker notes

No notes on this slide.

Molecular structures of ion channel proteins



Neuroscience 5e Fig. 4.6

Speaker notes

Yellow are voltage sensing tm domains

4–8 positively-charged amino acids in the S4 domain. Experiences force in a transmembrane electric field. Is the electric-field sensor for voltage-dependent gating.

K channels are diverse

How do Na^+ channels inactivate?

- Contains an activation gate that binds to the channel in the intracellular region and blocks the channel
- Activation gate changes conformation (closes/swings shut) to block channel only during the channel's open state
- Therefore, at resting V_m channel is closed and activation gate is open
- After depolarization, the channel opens and Na^+ ions go through. After a little bit of time (~ 1 ms) the activation gate swings shut to block channel

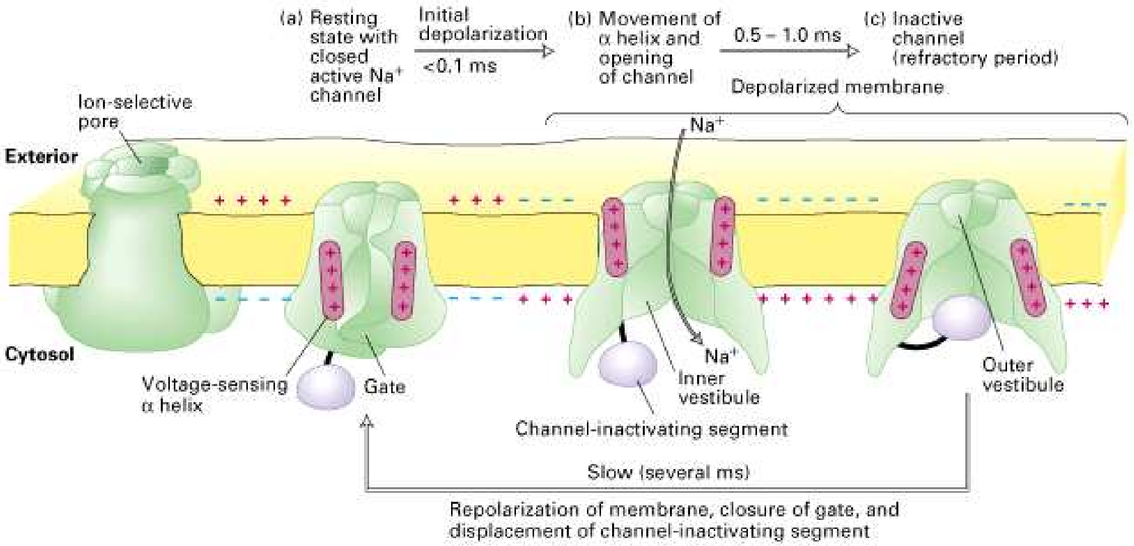
http://www.nature.com/nature/journal/v475/n7356/full/nature10238.html

Speaker notes

The theory is that the inactivation gate "swings" shut, turning off the channel

The physical structure of voltage gated Na channels has only recently begun to be solved, with the results so far fitting the models for Na channel opening and inactivation.

Sodium channel inactivation cycle



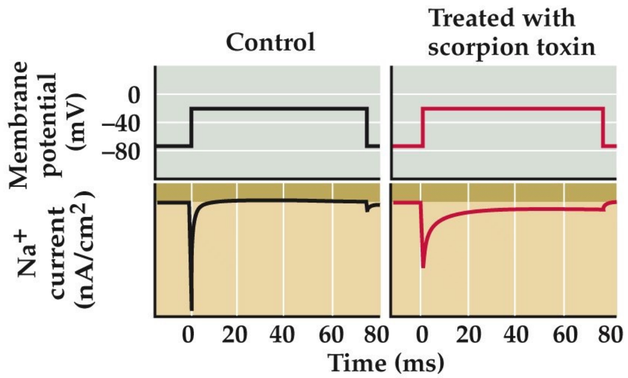
Lodish *Mol Cell Biol* 5e Fig. 21.13

Speaker notes

Figure 21-13 Lodish 4th edition OR Figure 7-33 Lodish 5th edition. Structure and function of the voltage-gated Na^+ channel.

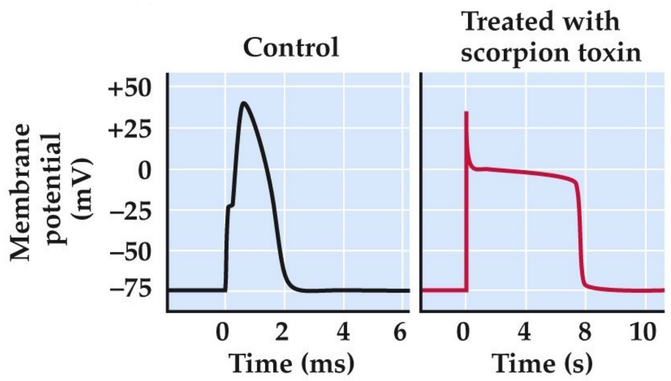
Toxins that poison ion channels

prolongs Na^+ currents by messing up channel inactivation



Neuroscience 5e Box 4B

AP profile reflects the shift in Na^+ conductance



Neuroscience 5e Box 4B

http://www.nature.com/news/rodent-immune-to-scorpion-venom-1.14014

Speaker notes

already learned about tetrodotoxin from puffer fish. blocks voltage gated Na channels underlying the AP

saxitoxin similar (homologue) to ttx, produced by dinoflagellates and possible effects from ‘red tide’ or eating shellfish that have injested these dinoflagellates.

scorpions paralyse prey by injecting alpha-toxins (left panels). Slow inactivation of Na channels, prolonging the AP and messing up information flow in CNS. Beta-toxins in scorpion venom shift the voltage dependence of Na channel activation (right panel), causing Na channels to open at potential much more negative than normal inducing uncontrolled AP firing.

Some alkaloid toxins (batrachotoxin, produced by S. American frogs) do both of these mechanisms.

Similar toxins from plants (aconitine from buttercups, veratridine from lilies) and insecticidal toxins (pyrethrins) produced by chrysanthemums and rhododendrons.

dendrotoxin from wasps affects K channels

apamin from bees K channels

charybdotoxin from scorpions K channels

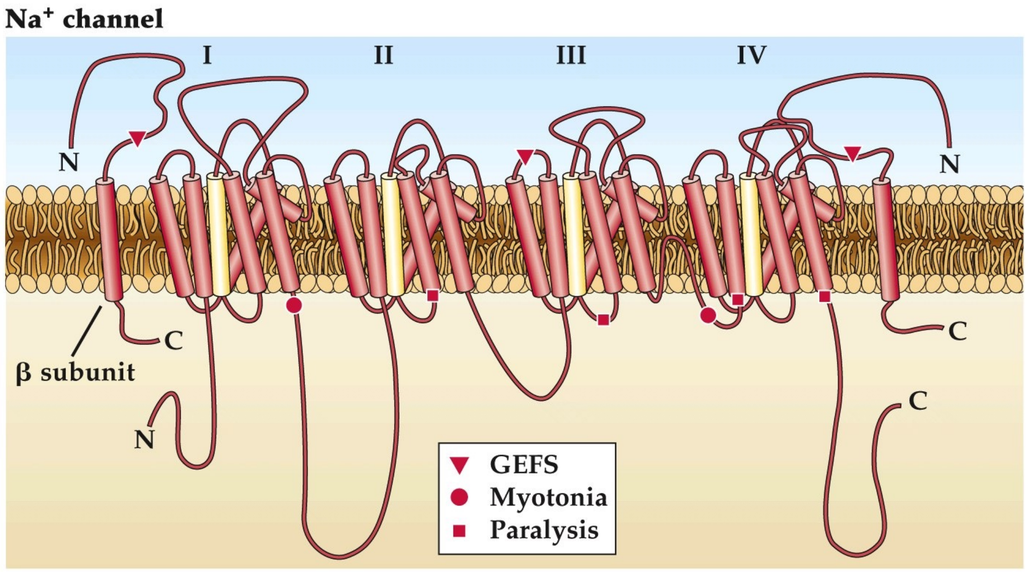
Diseases caused by ion channel mutations

- Channelopathies: genetic diseases resulting from mutations in ion channel genes
  - e.g. >50 neurological disorders, >40 cardiac disorders (Kim 2014)

GEFS: generalized epilepsy with febrile seizures, begins at infancy and continues through puberty. Mapped to two mutations, one on an alpha Na channel subunit and one on a beta subunit. Cause slowing of sodium channel inactivation

Myotonia: muscle contractions

Paralysis: muscle weakness



Neuroscience 5e Box 4D; see also Neuroscience 6e 'Clinical applications' p. 75-77

Speaker notes

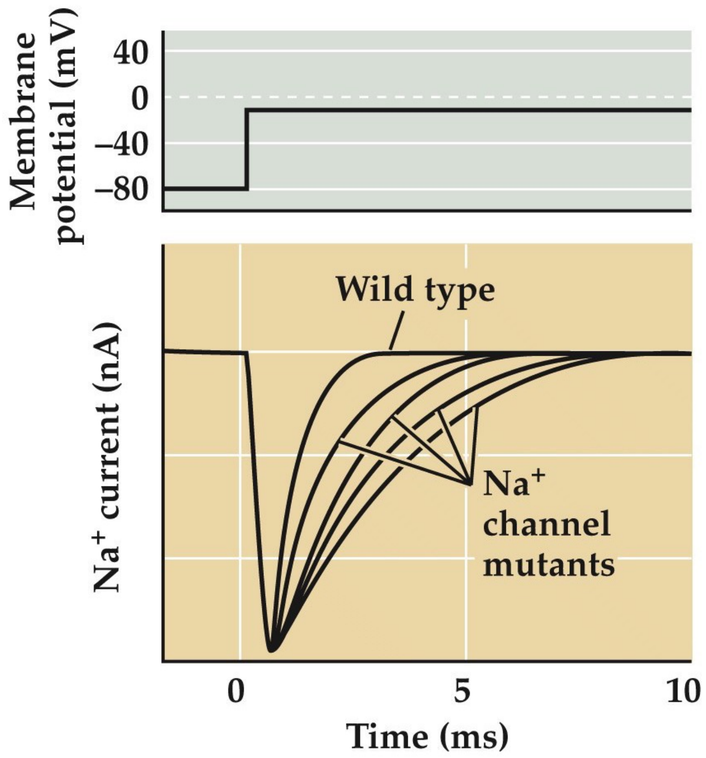
More than 20 different inhereited diseases from from mutations in Na channels alone. Cystic fibrosis results from chloride channel dysfunction (and altered fluid movements, chloride gradients often used for cell volume, fluid movements).

ataxia: greek for ‘without order’ or ‘incoordination’. Movement coordination problems.

paralysis: muscle weakness

myotonia: muscle contraction

Epilepsy can result from mutated Na^+ channels



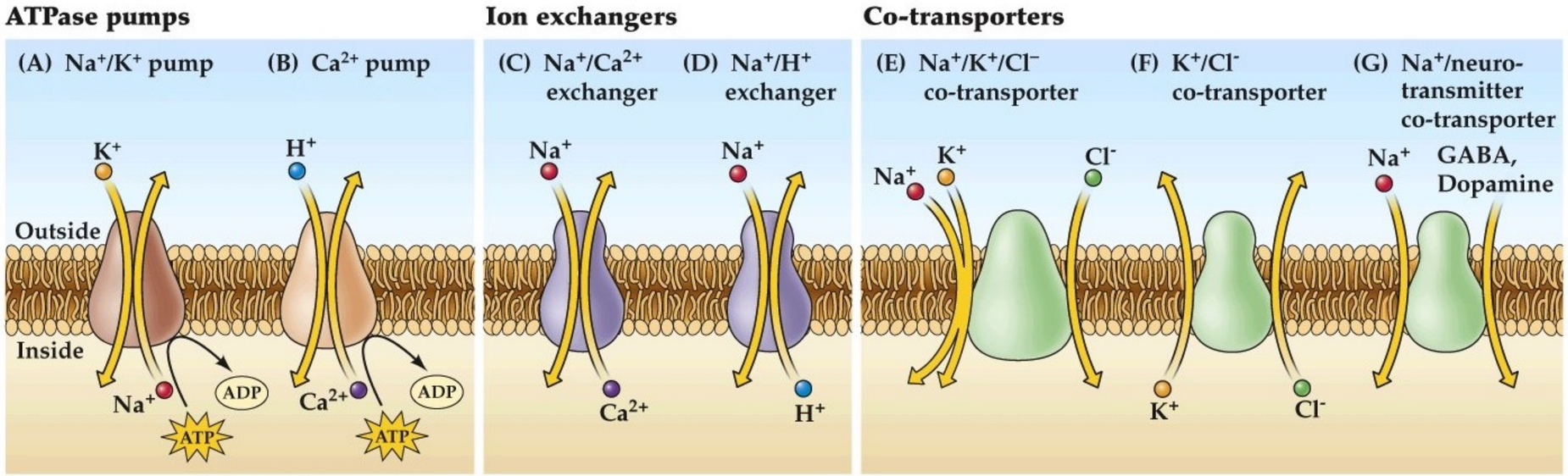
Neuroscience 5e Box 4D; see also Neuroscience 6e 'Clinical applications' p. 75-77

Speaker notes

You can see the the slower inactivation kinetics in this figure here in patch clamp recordings from normal and a number of different Na channel mutants. This slowing of Na inactivation is just enough to mess up spike patterns in single neurons and elicit hyperexcitability that results in seizures in networks of connected neurons.

GEFS: generalized epilepsy with febrile seizures

Ion transporters



Neuroscience 5e Fig. 4.9, 6e Fig. 4.13

Speaker notes

Lastly let's remind ourselves of the importance of ion transporters in maintaining the concentration gradients across the nerve cell membrane. We've previously discussed the active transporter the Na/K pump that is crucial for maintaining Na/K gradients but there are others that maintain gradients for other physiologically relevant ions like Cl, Ca.

Remember these transporters are all very slow compared to ion channels, **requiring several milliseconds to move a few ions** compared to **thousands of ions per second** conducted across the membrane for an ion channel.

Crystal structure for Na/K channel with either K bound in the central pore or Na was just solved in 2009 and 2013 respectively (Shinoda et al, Nature 2009) Nyblom et al. Science 2013)

Ouabain, plant 'arrow' poison traditionally from africa from the Acokanthera schimperi and Strophanthus gratus plants. Binds to the Na+/K+ pump. Cardiac dysfunction ensues.

Used together with

Radioactive Na efflux measurements and radioactive K influx measurements used with ATP synthesis inhibitors (e.g dinitrophenol) to help demonstrate that an active Na/K pump is responsible for producing ion conentraiton gradients in squid axon (Hodgkin and Keynes 1955).