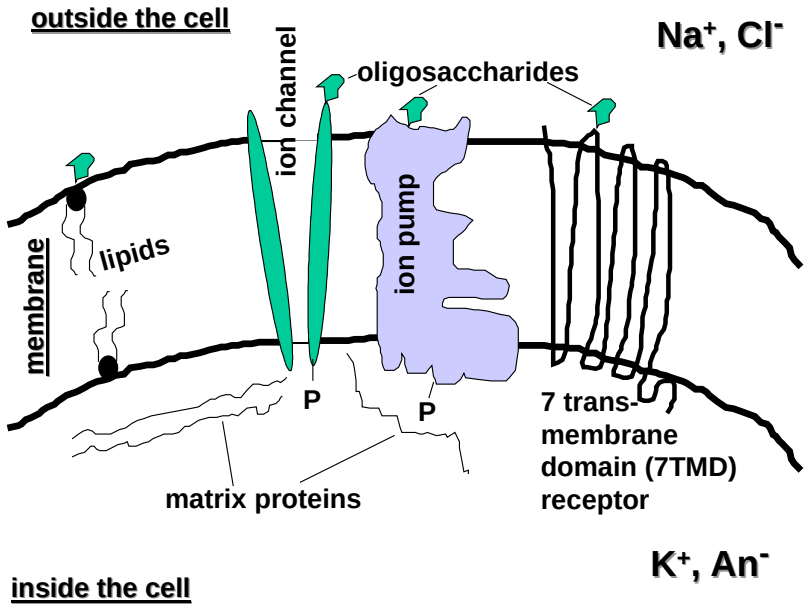
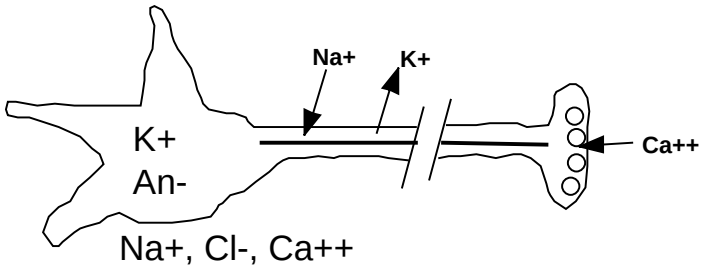
MEDS 370 -- INTRODUCTORY NEUROSCIENCE
Ionic Basis of the Resting and Action Potentials
August 31, 2001 Dick Mains mains@uchc.edu x8894

Reading one of the following
Kandel et al Principles 4th Ed, 2000. pages 19-32;105-169;1280-1285
Purves Neuroscience 2nd Ed. 2001. pages 43-98
Zigmond Fundamental 1999, pages 107-154
Alternative texts
Huguenard, McCormick 1997 Electrophysiology of the Neuron (Mac or Windows), Oxford
Siegel, Agranoff, Albers, Molinoff 1999 Basic Neurochemistry, 6th Ed., Raven Press
Aidley, Stanfield 1996 Ion channels: molecules in action. Cambridge Univ Press
Nicholls, Martin, Wallace 1992 From Neuron to Brain, 3rd Ed., Sinauer
Hall 1992 An Introduction to Molecular Neurobiology, Sinauer, pages 33-118

Basic membrane biology



Several of the functions of neurons that we will examine in detail in this course are performed by molecules like those diagrammed in this figure. Neurons, endocrine cells and muscle cells tend to have larger ion gradients across their membranes than other cells in the body, and the lipids in the membranes are probably a key part of that barrier to easy flux of ions. The ionic gradients are created by pump molecules which are transmembrane glycoproteins; these excitable cells capitalize on the ionic gradients by transiently opening ion channels, allowing a brief flow of ions and a brief change in membrane potential. The flow of ions and/or the change in membrane potential enables the excitable cells to perform some of their key functions, such as secretion or contraction.



The key thing about cells is that they ALL want/need to make the outside different from the inside of the cell. They need a permeability barrier, to keep ATP and DNA and RNA and proteins inside the cell, and to bring in goodies from the outside (like glucose, other nutrients), and then KEEP them inside.

- nervous system specializes in signaling using action potentials (APs)
  sensing environment, motion, making glands secrete
  making decisions
  these require cells to communicate; using APs from one end to the other
  chemicals at the end to excite or inhibit the target; INTEGRATION
- endocrine cells also fire APs, secrete, excite/inhibit targets
- muscle cells fire APs, cause motion
  difference --> nervous system does this in strings, cell-to-cell-to-cell-to-----
  e.g. for you to see this lecture, this simplified sequence of events occurs:

light falls on your cones; bipolar cells; ganglion cells; lateral geniculate nucleus; primary visual cortex, layer 4; other layers; to rest of visual cortex..... quite a cascade!

- later in course we will discuss the chemical signals
  and the anatomical organization
  and the development
  and the complex circuitry
- today, one of the simplest topics, membrane potentials and action potentials

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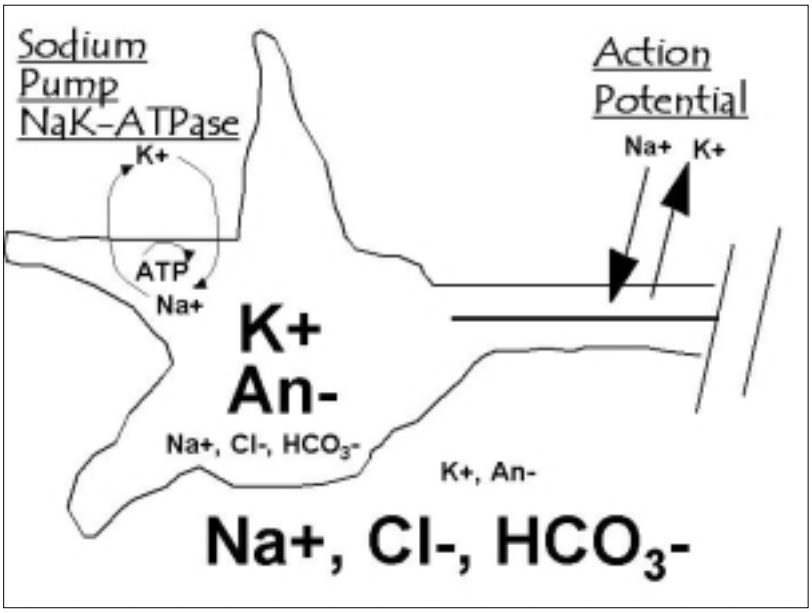
start with a creature (squid) from the ocean (extracellular):

500 mM NaCl
20 mM KCl
10 mM $CaCl_2$



- need a way to signal fast, cell-to-cell-to-cell-to-cell.....
- electrically is good – we use computers and telephones and radios and such every day
- so how to make an electrical signal? and a fast signal?

1. make a permeability barrier – cell already had to do that to hoard glucose and fat and special proteins and DNA and RNA – so why not a barrier to ions?

2. An obvious choice, make the interior high in K+ and low in Na+, and see what that does; and let's switch to mammalian cells vs. their blood:

	outside mM	inside mM
Na+	125	5
K+	5	125
Cl-	130	5
An-	~0	125
total	130+/- both places	

how to maintain that set of gradients?

easy way is Na/K ATPase exchange pump; high permeability to K+ and Cl+

and low/no permeability to Na+ (bacteria and plants use H+ gradient same way)

what does that mean?

Cl- gradient is 26:1 inward
K+ gradient is 25:1 outward

how can that be a steady state? let the ions start to flow a bit.....

1. K+ leaves, Cl- enters.....
2. interior of the cell gets a negative potential compared to outside
3. and electrical force on ions opposes the chemical force

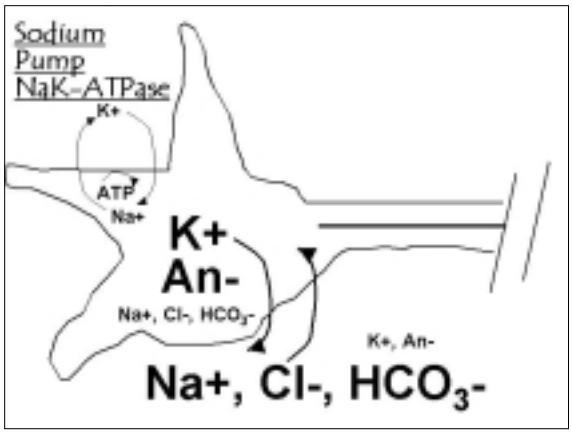
Nernst equation for that is

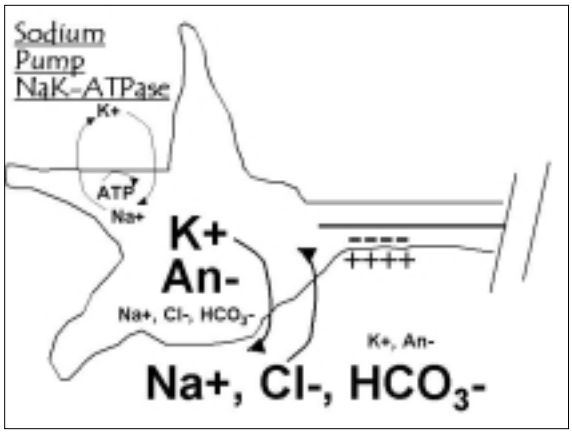
$E_K = -$ constant x $\log(K_{in}/K_{out})$

$\sim E_{Cl} = -$ constant x $\log (Cl_{in}/Cl_{out})^{-1}$

$= -$ constant x $\log (Cl_{out}/Cl_{in})$

- net is that RATIOS of ion concentrations matter; 10x ratio is 58 mV
- and that the "net driving force" on K+ and Cl- in the resting cell is close to zero
- leaks are fixed up by the Na/K ATPase exchange pump





- so..... how to use this to signal?

  change the permeabilities!

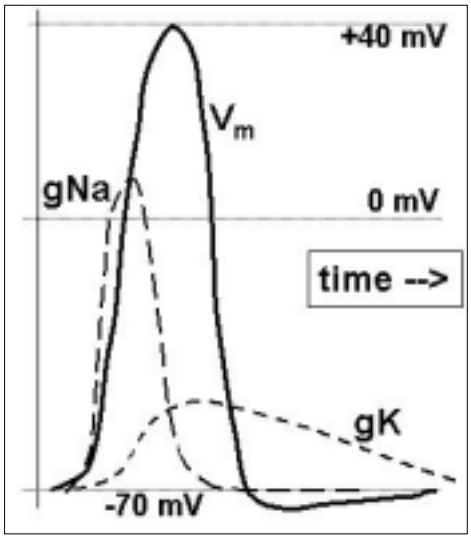
- this lecture is "all downhill from here"
- reminder that the electrical term we will use is “conductance” and the symbol is “g”
- [conductance and permeability are not quite the same thing, but close enough for now]
- so, what if conductance to K+ increased a lot?

  no big change

- or the conductance to Cl- increased a lot?

  no big effect

- could conceivably **decrease** the conductance, and is does indeed happen sometimes, but that is much harder to get a big effect, so we will ignore for now.



so increase g_{Na}? **NOW** we get something rolling!

- Na+ already has a huge inward gradient for concentration AND for electrical push – cell starts negative inside – so now you get a huge inward rush of Na+ ions..... fine, but that is like flipping to ***on***, need also to flip ***off***.....
- that is done by two mechanisms
  - g_{Na} inactivates
  - g_K activates, but ***later*** than g_{Na} turns on

we had said earlier that g_K bigger had no effect ***AT REST***.....

but in the activated cell, Vm = positive, so K+ has a big electrical force driving it ***out***

--> increasing g_K moves Vm down toward rest

➔ and turning off g_{Na} in a time-dependent manner helps a lot also

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Before we go on.....biochemical facts to keep in mind

- g_{Na} and g_K are separate proteins [net of 24 transmembrane domains per channel]
  - could have been one molecule changing its specificity
- there are a lot of similarities in the g_{Na} and g_K proteins
  - one can mutate g_{Na} into a g_K or g_{Ca} by changing a small number of residues
- the structure of the ion channel includes the voltage sensing (activation), the ion selectivity, and the inactivation; 3 functional, separate domains of the proteins
- we will now discuss some of the other electrical properties of neurons and axons/dendrites that are crucial to the rest of the course

- sign conventions

we have to understand the meaning of positive and negative, voltage and current

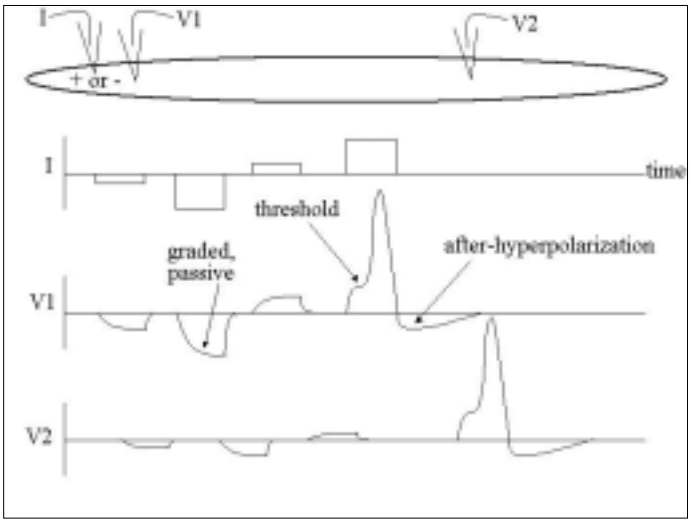
- outside cell = zero mV (0)
- inside minus outside is Vm = normally **negative at rest**
    - Hodgkin-Huxley in 1952 used the opposite convention
- then current is flow of positive ions
    - so outward current is positive
- comes from Ohm's Law

  V = I x R or as we will use it

  I = g x V since g = 1/R = conductance
- conductance increases with membrane area; resistance decreases

- capacitance
    - ability to store charge; across lipid membrane; at rest --- inside, +++ outside
    - increases with membrane area

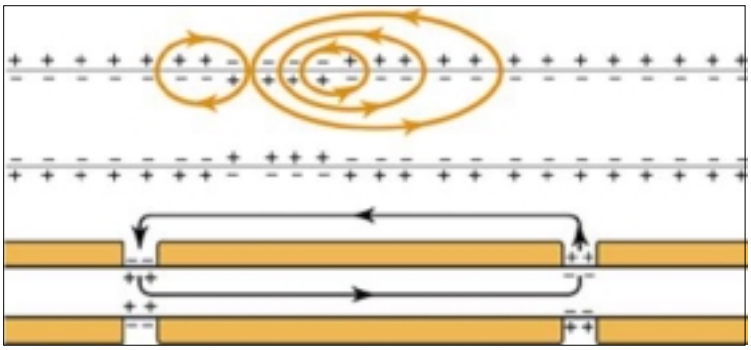
- time constant – time for Vm to reach (1 – 1/e) = 67% of final value during step change
    - product of membrane resistance x capacitance
    - τ = R x C

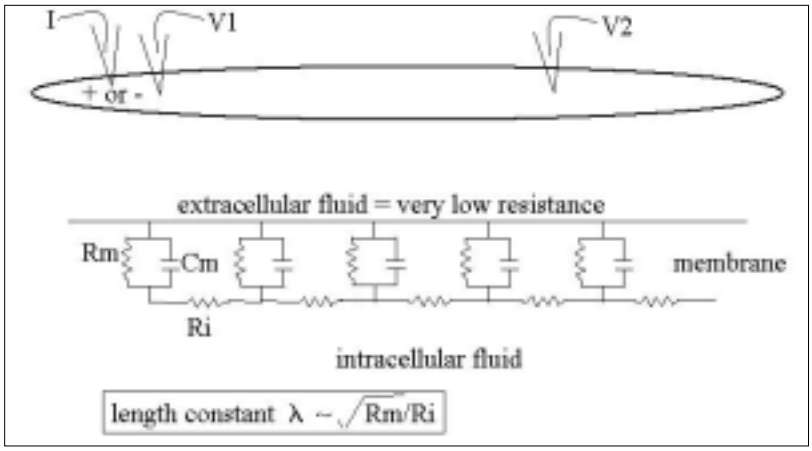


- cable properties; electrotonic spread of potential
    - basic idea is that voltage is debased and degraded farther from the source
- see passive and active properties
- threshold; all-or-nothing nature of the action potential
- afterhyperpolarization, lingering g_K, plus Na+ pumping, results in refractory period

- electrogenic Na+ pump
  - pump out 3 Na+, bring in 2 K+, use 1 (2?) ATP
  - net movement of +1 OUT of cell, drives Vm more negative (hyperpolarizes)
  - can actually affect AP signaling, especially in small fibers

- myelin; saltatory conduction; currents only flow at nodes of Ranvier
  - Figure from Siegel et al





- circuit models
  - resistors (oppose ion flow)
  - capacitors (store charge)

- length constant – distance over which Vm decays to 1/e = 37% of initial value

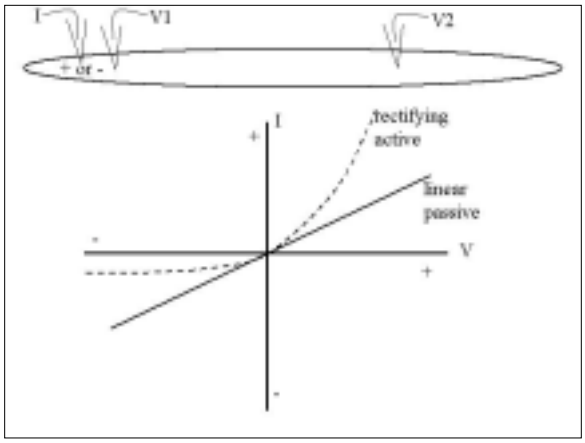
- Nernst; Goldman equations
  - Nernst was given above; balance of chemical and electrical driving forces
  - 10x ratio of concentrations (monovalent) = 58 mV

$$E_{ion} = \text{constant} \times \log (Ion_{in} / Ion_{out})$$

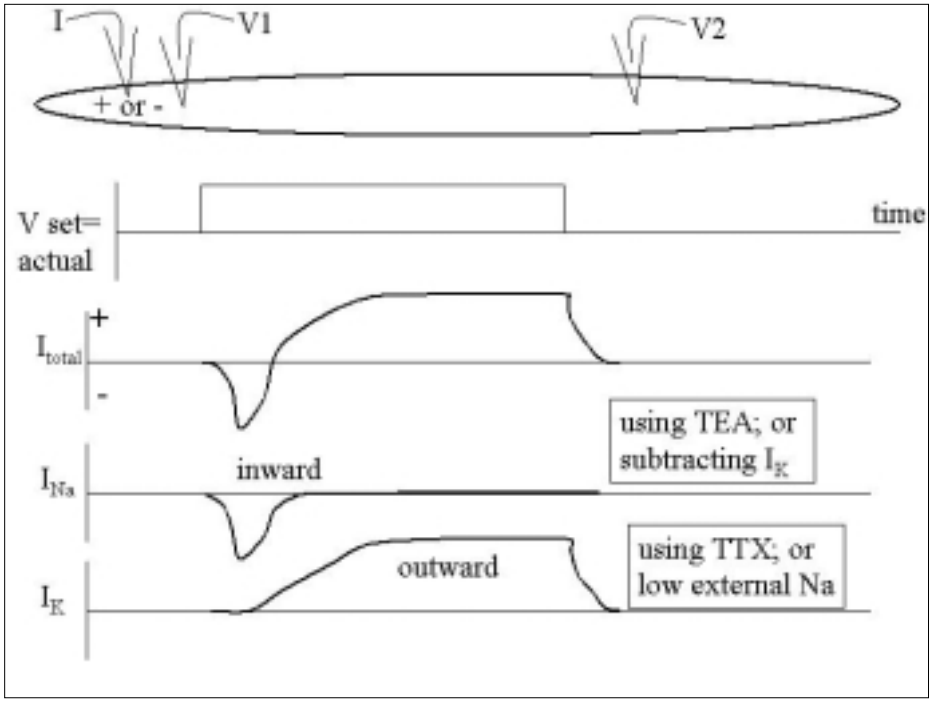
- so that defines the **reversal potential** for an ion; the membrane potential at which the NET driving force is zero (electrical opposes chemical)

- numbers to remember
  - E_K ~ -90 mV
  - E_{Na} +50 mV
  - E_{Cl} ~ -90 mV

I-V plot



- voltage clamp
- set "demand voltage"
- read actual membrane potential
- inject current to achieve demand voltage
- record current

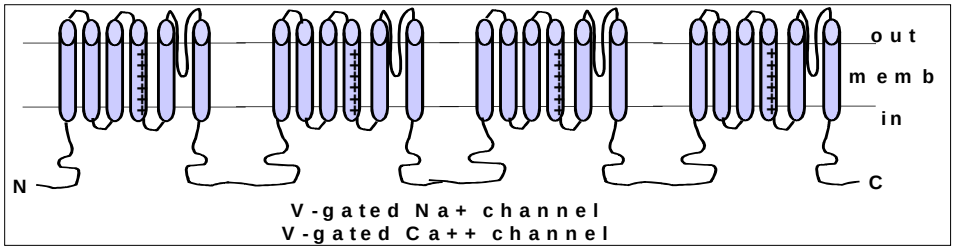


- tetrodotoxin and tetraethylammonium
- TTX blocks g_{Na}
- TEA blocks g_K

- patch clamp

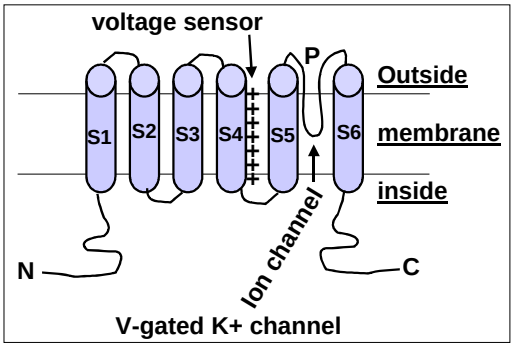
cell attached patch
whole cell recording
inside-out patch
outside-out patch

Other cool stuff not included in the lecture

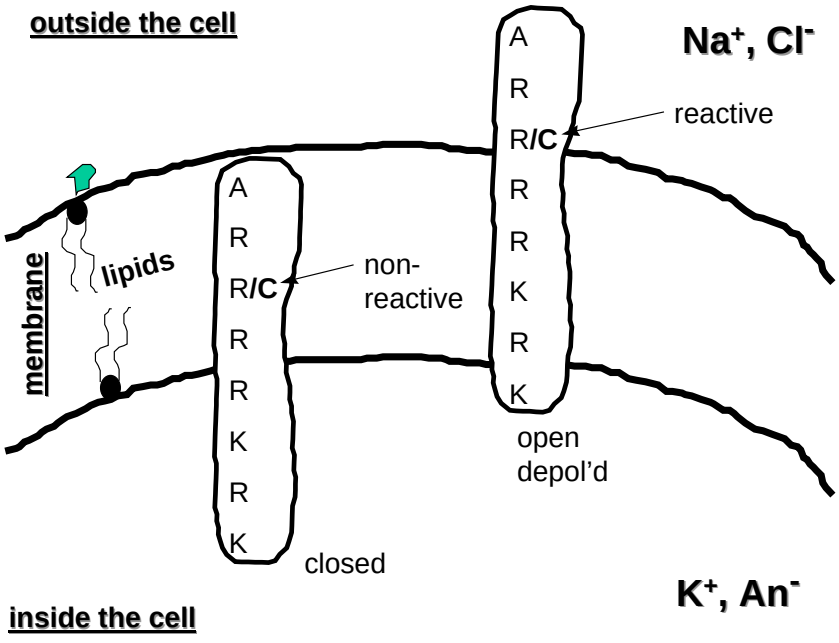


Ion channels are aqueous pores.
Ion channel proteins of special interest in this course come in many forms, one of which is diagramed here.

Channels fall into several families with surprisingly consistent properties within a family, ranging from bacterial to viral through mammalian channels. Note that in general it takes 20-24 TMD to make a channel: 2 CLC, 4 g_K, 5 neurotransmitter-gated, 6 gap junction, 1 g_{Na}, 1 g_{Ca}. Viral capsid channels are simpler, with only a single TMD for each subunit; they open at low pH when the virus arrives in an endosome, enabling the ribonucleoprotein to dissociate.



Ion Channels in general need 20-25 transmembrane domains, arranged in groups of 4-6, often referred to as "barrel staves". For voltage-gated channels, a really key point is that Na+ and K+ currents are **biochemically separable** (as well as electrically). The K+ inward rectifier has the same structure as the bacterial K+ channel, whose structure is well established by crystallography and for which the motion of M1 past M2 was demonstrated using site-directed mutagenesis coupled with spin labeling.



Pores are diffusion-limited, passing ions at 10^7 to 10^8 ions/sec. **P** forms the pore. **S4** is the Voltage-Sensor

Molecular basis of voltage sensitivity

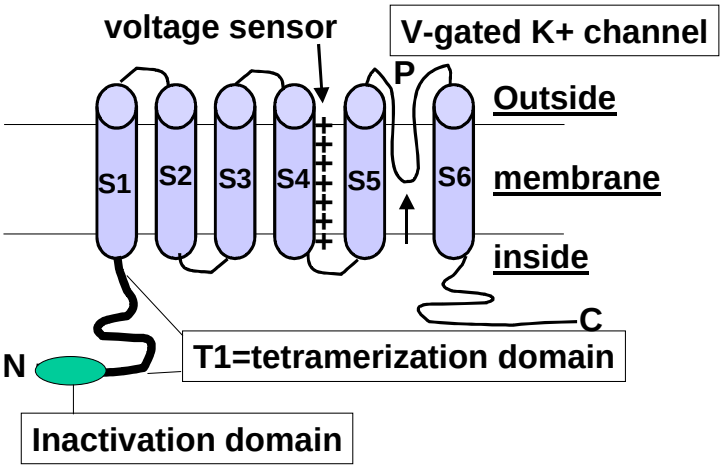
Probing gK or gNa residues important to voltage sensitivity by replacing positively charged residues in S4 with Cys; then react with methanethio-sulfonate and record changes in current. Result shows that 2-3 positively charged residues move far enough to become exposed transiently on the outside of the membrane during depolarization. In addition, by inserting a Cys at the outside face of S4 in each of the four domains of gNa, and then labeling the Cys with a fluorescent tag, the data showed that all four S4 TMDs normally move in response to voltage changes. Further, the S4 TMDs in domains III and IV moved less well when gNa was inactivated, while the S4

TMDs in domains I and II moved in response to voltage changes, regardless of the state of inactivation.

Drugs that block channels often are organic, roughly the size of hydrated Na+ or K+
Thus go only part way into mouth of channel
(which seems narrowest in the middle of the membrane)
Thus, many drugs work only from the outside OR the inside, not both

Inactivation of channel conductance

gK inactivation is at N-terminal; works as separate peptide "ball and chain". When a Cys residue is inserted in the correct site the C-terminal loop, and cells are exposed to intracellular Cd++, the C-terminal loop binds (via Cd++) to a His residue in the N-terminal inactivation domain of the adjacent subunit, preventing inactivation.



gNa; cytosolic loop between III and IV; peptide mimicks; "hinged lid" in both cases, "inactivation domain" works as a separate protein

Channels in myelinated axons

Squid giant axon ~15,000 more volume than myelinated axon, uses 1000 times energy
Node is 1-2 μm long, **internode** can be 1.5 mm = 1500 μm
myelin sheath up to 40 wrappings
Axon constricts down at node to smaller size, as low as 20% of internode value
Intramembranous particles at node are really thought to be gNa!
gNa density at node 1500/μm^2.....and <25/μm^2 elsewhere
==>>gNa ~ONLY present at nodes; held by spectrin and ankyrin
debate on role of oligos or Schwann in localizing gNa
also studies showing gNa clustering in absence of supporting cells
gK is ~only present under myelin!
[OK since glial cells are good K+ buffer and capacitance of myelin is low]
Regulation of channel expression
Anchoring to cytoskeleton IMPT; e.g. gNa at Nodes of Ranvier bound to ankyrinR/spectrin
Timing more important than pure speed
Hence myelinated and nonmyelinated, fatter and thinner
Timing in electrocytes of eel; visual and auditory systems; motor coordination too

Defective channels

- Often autosomal dominant; temperature (overheating) sensitive or $[K+]_o$ often (perhaps other defects would be fatal). Often inactivation defective; get persistently active channel.

- Several forms of deafness are caused by defective gK in the cells surrounding the scala media, a part of the ear with very high (100 mM) K+
- People with myotonia and periodic paralysis
  Mendelian inheritance, often DOMINANT since subunits must work together
- Myotonia (hyperexcitable; delayed relaxation after voluntary contraction) Can be due to defect in CLC-1 Cl- channel; muscle has LESS gCl than normal and thus excites more readily; no CNS effects since neuronal gCl much less important. Between attacks, muscle function seems quite normal
- Hyper (high) kalemic (K+) and hypo (low) kalemic periodic paralysis
  mutations in S4 voltage sensor of gNa
  and mutations in inactivation region of gNa
- Heart has many channels
  Nearly all have been shown to be defective in some variety of arrhythmia
  Some reports argue defective ion channels in heart = 50% of cardiac deaths!
  e.g. leave out 3 amino acid residues between III and IV in gNa
  → no inactivation!
  And defects in gK tetramerization domain can be dominant negatives
  although they might seem as though they should be recessive

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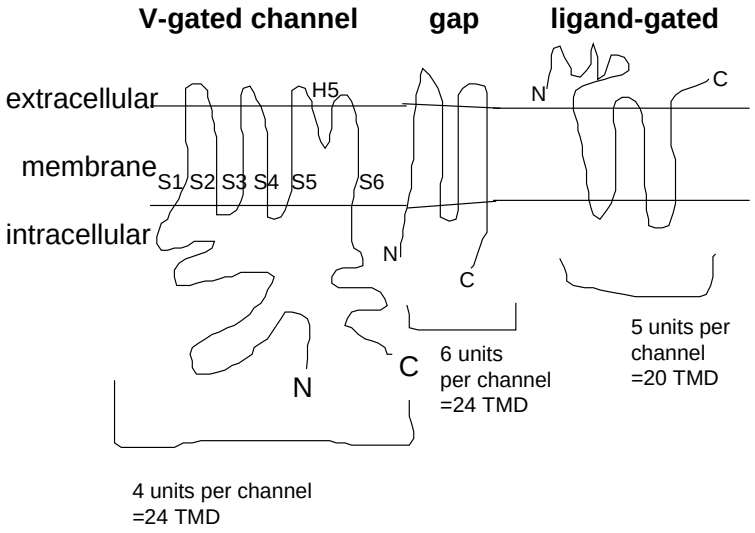

Pores are diffusion-limited, passing ions at 10^7 to 10^8 ions/sec.
Many channels in this **family**
Ion selectivity: Gap junction is formed by connexins; the resulting pore is ion-nonselective due to large pore (1.2 kDa cut-off).
Gap junctions are 6-mers of units with 4 TMD (N and C termini in cytoplasm); 20+ members of the gene family form heteromultimers. Structure quite solidly established with EM, X-ray; because the apposed channels are rotated 30^o with respect to each other, each subunit meets 2 subunits on the apposing membrane. These channels permit rapid cell-to-cell transfer of electrical signals (e.g. in heart) and nutrients among cells. Complexity comes from paired cells expressing DIFFERENT apposing subunits, and 2+ different subunits in each cell. Electrical connections between cells using these proteins (Cx; connexins) are thought to be crucial in coordination of "tissue behaviors". There are many Cx mutants causing deafness (dominant and recessive alleles; progressive deafness), demyelination, cardiac problems, infertility, cataracts, and more!