ELEC ENG 3BB3:
Cellular Bioelectricity

Notes for Lecture 7

Tuesday, January 21, 2014

3. MEMBRANE CHANNELS

We will look at:

- Channel structure
- Biophysical methods for measuring channel properties
- Macroscopic channel kinetics
- Channel statistics
- Introduction to the Hodgkin-Huxley membrane model

Channel structure:

The coarse structure of *membrane channel proteins* can be determined by electron microscopy or X-ray diffraction.

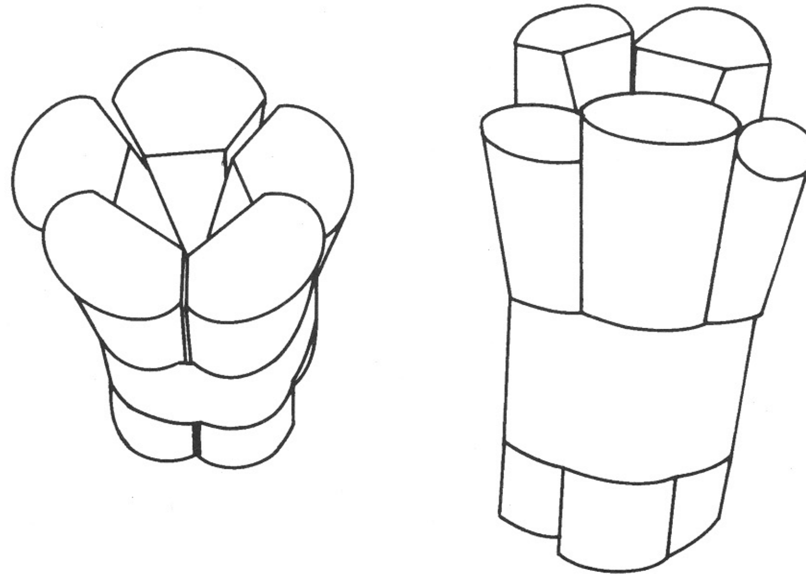
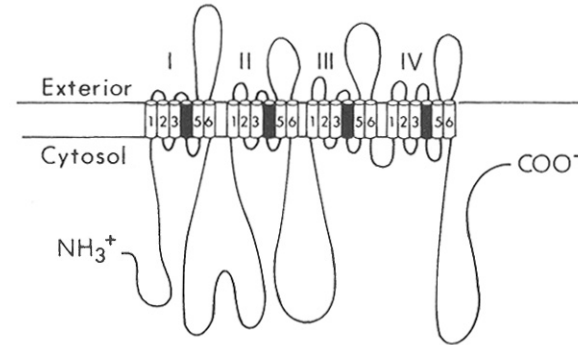


Figure 4.1. A model of the acetylcholine receptor which shows the five component subunits and the aqueous pore. The band locates the membrane bilayers through which the molecule passes; the lower part is cytoplasmic. [From R. M. Stroud and J. Finer-Moore, Acetylcholine receptor structure, function, and evolution. Reproduced with permission from *Annu. Rev. Cell Biol.* **1**:317–351 (1985). Copyright 1985, Annual Reviews, Inc.]

Channel structure (cont.):

- *Molecular genetics* can be used to express a channel protein in a cell that does not normally make that protein.
- The resulting channel properties can be evaluated to determine whether the protein synthesized is indeed the desired protein.



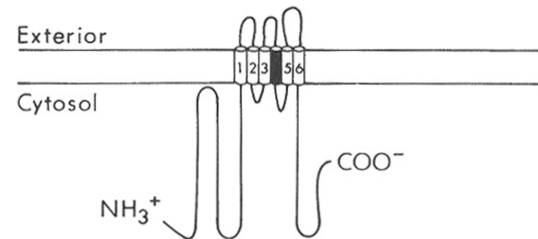


Figure 4.2. Proposed transmembrane structure of (a) voltage-gated Na^+ channel protein and (b) voltage-gated K^+ channel protein. The sodium channel arises from a single gene; it contains 1800–2000 amino acids, depending on the source. About 29 percent of the residues are identical to those in the voltage-gated Ca^{++} channel protein. There are four homologous domains indicated by the Roman numerals. Each of these is thought to contain six transmembrane α helices (Arabic numerals). The helix number 4 in each domain is thought to function as a voltage sensor. The shaker K^+ channel protein (b) isolated from *Drosophila* has only 616 amino acids; it is similar in sequence and transmembrane structure to each of the four domains in the Na^+ channel protein. [From J. Darnell, H. Lodish, and D. Baltimore, *Molecular Cell Biology*, 2nd edn., Scientific American Books, New York, 1990. Adapted from W. A. Catterall, Structure and function of voltage-sensitive ion channels, *Science* **242**:50–61 (1988). Copyright (1988) American Association for the Advancement of Science.]

Traditional functional description of channel:

Functional regions:-

- *Selectivity filter* – for determining which ions can pass through a channel
- *Gate* – for opening and closing channel
- *Sensor* – for detecting transmembrane potential to control gating

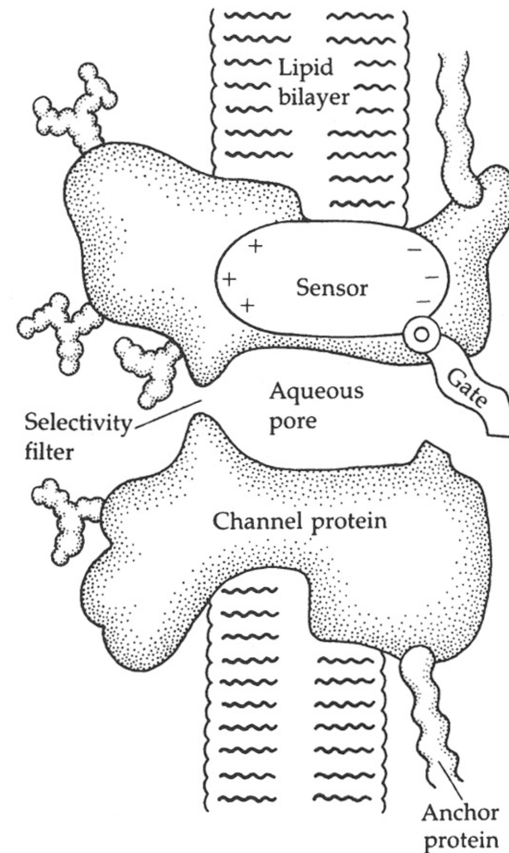


Figure 4.3. Functional description of membrane channel. “The channel is drawn as a transmembrane macromolecule with a hole through the center. The functional regions—selectivity filter, gate, and sensor—are deduced from voltage clamp experiments and are only beginning to be charted by structural studies. We have yet to learn how they actually look.” [From B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Associates, Sunderland, MA, 1992.]

Channel inactivation:

- In some channels, *inactivation* is thought to be achieved via a voltage-sensitive molecule that can block the channel opening.
- This is referred to as the "ball-and-chain" or "swinging gate" model.

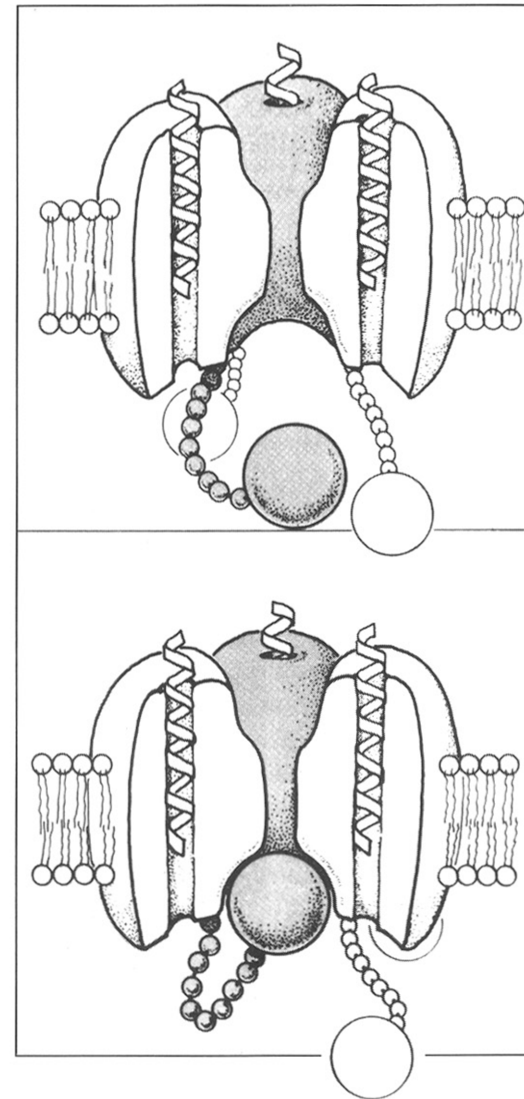
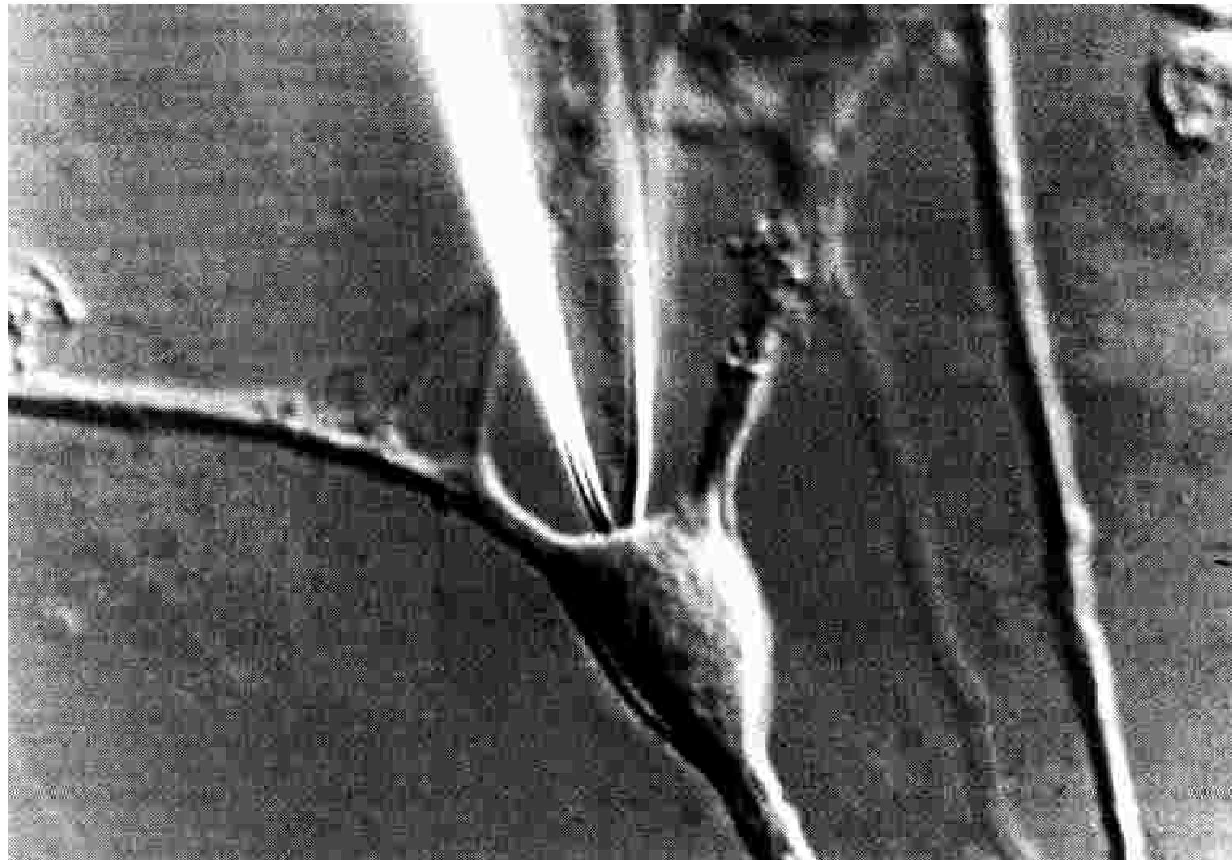


Figure 4.10. A protein ball pops into a pore formed by the bases of four membrane-spanning proteins (one not shown), thereby stopping the flow of potassium ions out of a nerve cell. Based on Hoshi T, Zagotta WW, Aldrich RW. 1990. Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. *Science* **250**:506–507, 533–538, 568–571.

Biophysical methods for measuring channel properties:

Micropipette electrodes are used to measure ionic currents or transmembrane potentials.



Biophysical methods for measuring channel properties (cont.):

- In *patch clamp* recordings, a micropipette forms a tight seal with a membrane patch.
- High leakage resistances are needed to obtain a good signal-to-noise ratio.

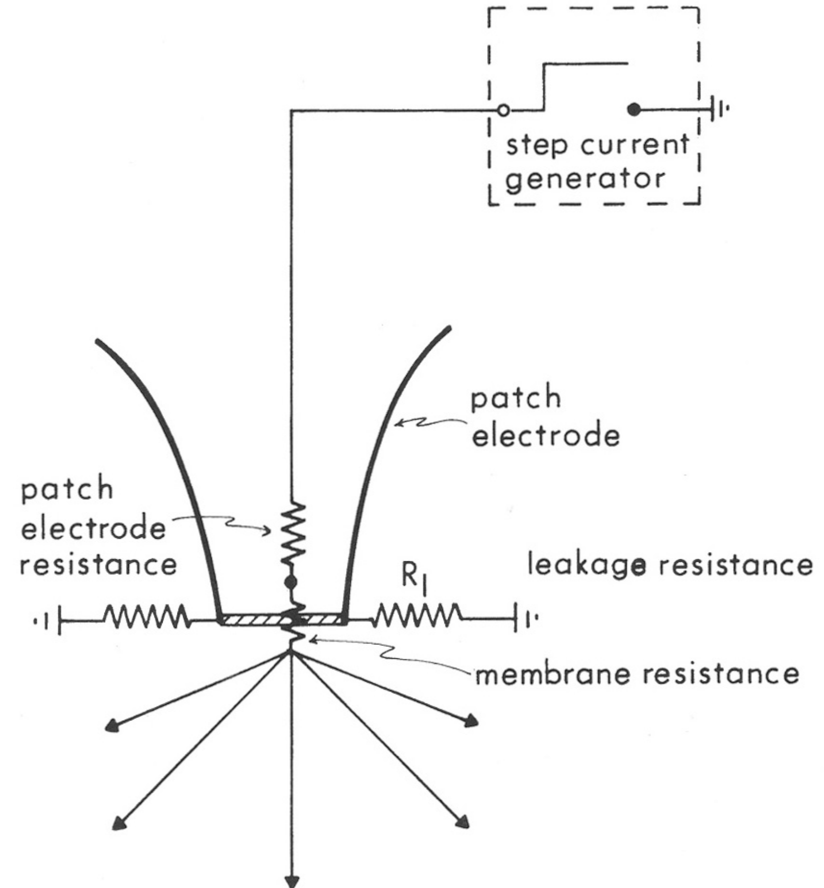


Figure 4.4. Inside-out patch clamp configuration. The desired current path through the cell is challenged by the alternate (leakage) pathway available in the region of electrode–membrane contact. A single open channel is assumed to give a membrane conductance equal to or greater than 20 pS (a resistance of ≤ 50 GΩ). To keep leakage current low (hence minimal loss of signal strength as well as reduced Johnson noise) this resistance should be in the tens of gigaohms; fortunately, patch electrodes with 100 GΩ leakage resistance are currently available.

Four common configurations for patch clamp recordings:

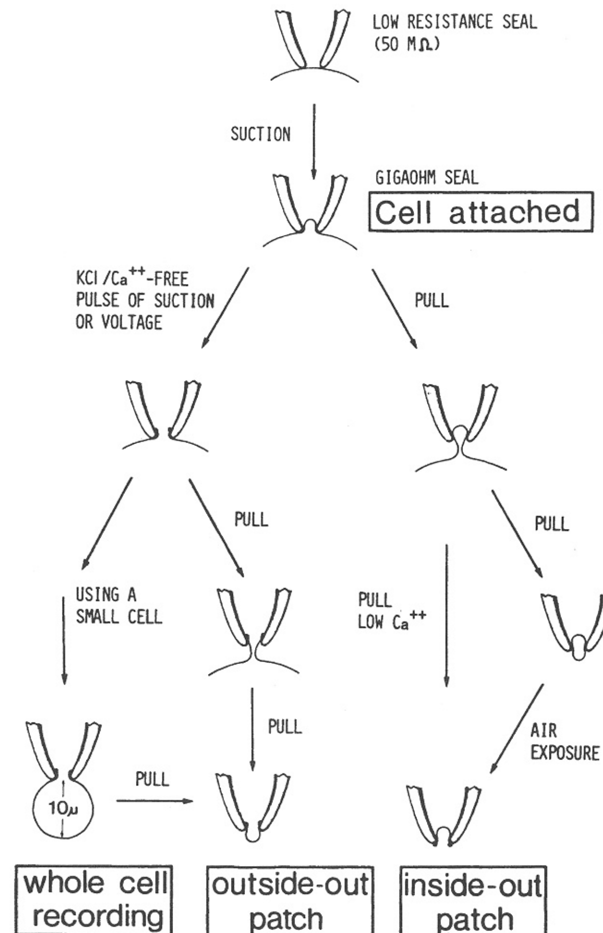


Figure 4.6. Four Configurations for Patch Clamping are described. The clean pipette is pressed against a cell to form a tight seal using light suction, and produces the *cell attached* or *on-cell* configuration. Pulling the pipette away from the cell establishes an *inside–out* patch. Application of a suction pulse disrupts the membrane patch, allowing electrical and diffusional access to the cell interior for *whole-cell* recording. Pulling away from the whole-cell arrangement causes the membrane to re-form into an *outside–out* configuration. From Hamill OP, et al. 1981. Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflugers Arch* **391**:85–100.

Single channel recordings:

- Single channels exhibit a *unitary current* when open and zero current when closed.
- Channel opening and closing is stochastic.
- Example single K-channel data:

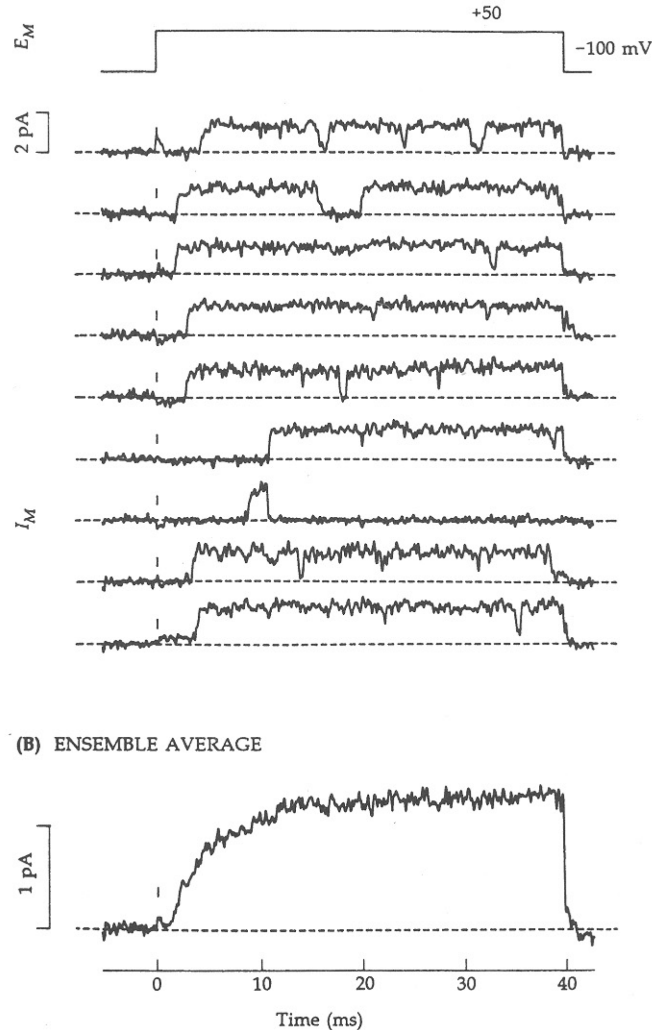
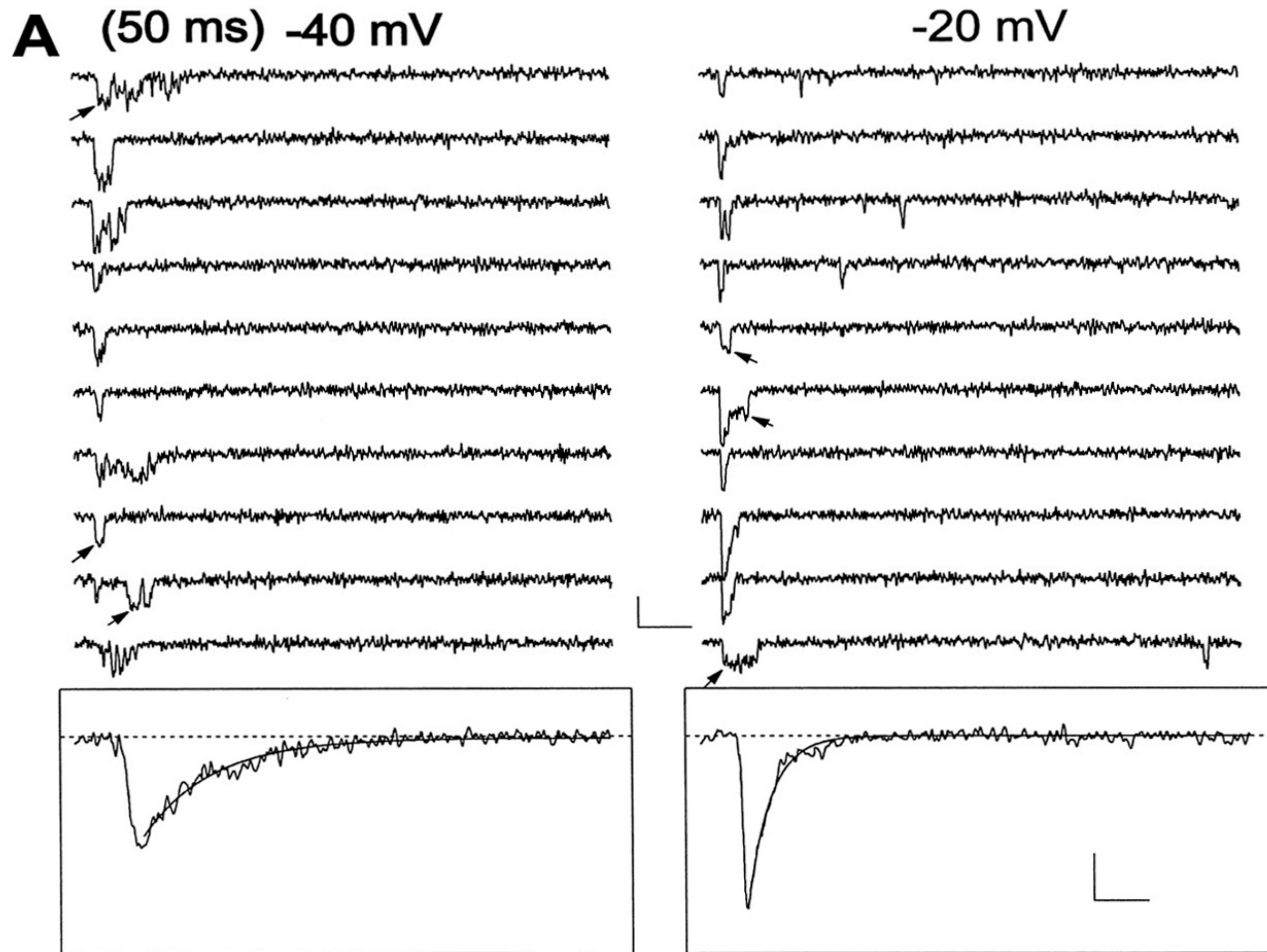


Figure 4.7. Patch-Clamp Recording of unitary K currents in a squid giant axon during a voltage step from −100 to 50 mV. To avoid the overlying Schwann cells, the axon was cut open and the patch electrode sealed against the *cytoplasmic* face of the membrane. (A) Nine consecutive trials showing channels of 20 pS conductance filtered at 2 kHz bandwidth. (B) Ensemble mean of 40 repeats; these reveal the expected macroscopic behavior. $T = 20\,°C$. From Bezanilla F, Augustine GR. 1992. In *Ionic channels of excitable membranes*, 2nd ed. Ed B Hille. Sunderland, MA: Sinauer Associates.

Single channel recordings (cont.):

- Example Na-channel recordings:



Electrical circuit representation of a single channel:

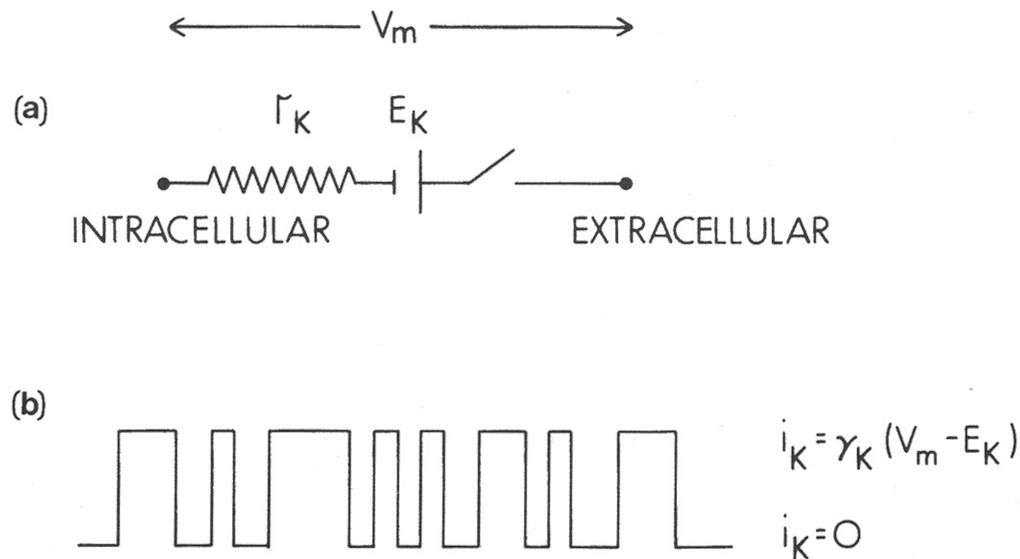


Figure 4.8. (a) Electrical circuit representation for a single (potassium) channel showing fixed resistance r_{K}, potassium Nernst potential E_{K}, and the transmembrane potential V_m. The closing and opening of the switch simulates the stochastic opening and closing of the channel gate. (b) Single-channel current corresponding to (a), where $\gamma_{\mathrm{K}} = 1/r_{\mathrm{K}}$. This is an idealization of the recording shown in Figure 4.7.

Single channel current-voltage relationships:

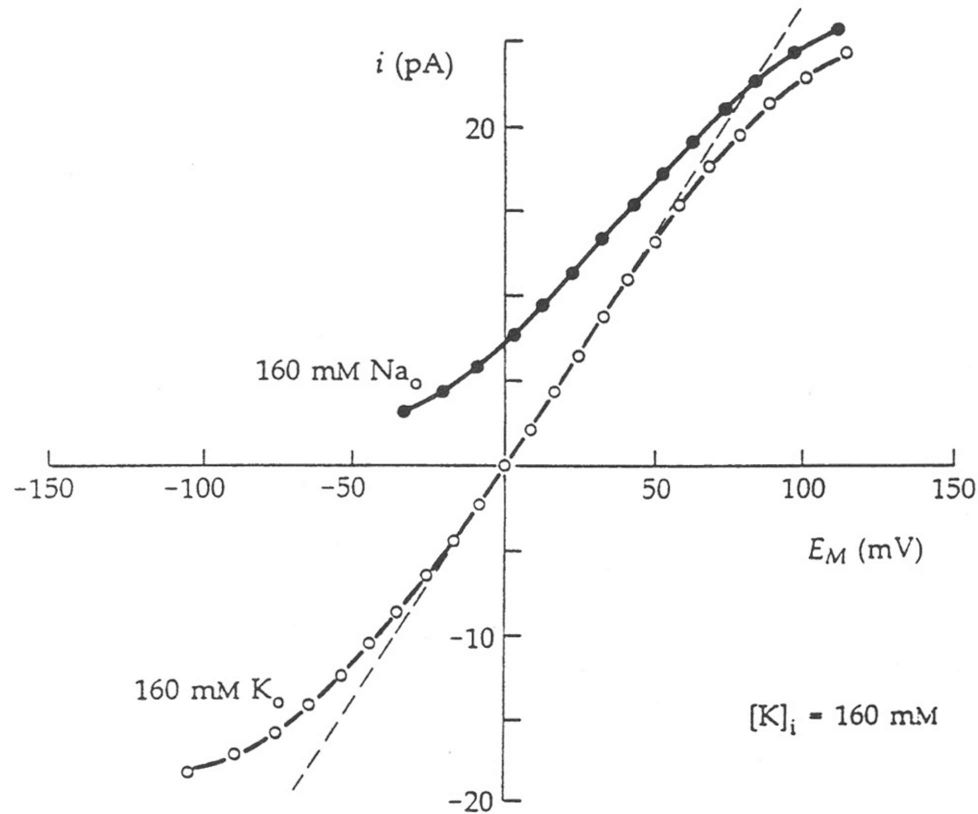


Figure 4.9. Current–Voltage Relations for a single BK K(Ca) channel of bovine chromaffin cell. The excised outside–out patch was bathed in 160 mM KCl or NaCl and the patch pipette contained 160 mM KCl. In symmetrical K solutions the slope of the dashed line is $\gamma = 265$ pS; $T = 23\,°C$. From Hille B. 1992. *Ionic channels of excitable membranes*, 2nd ed. Sunderland, MA: Sinauer Associates. Based on measurements of Yellen G. 1984. Ionic permeation and blockade in Ca^{2+}-activated K^+ channels of bovine chromaffin cells. *J Gen Physiol* **84**:157–186.

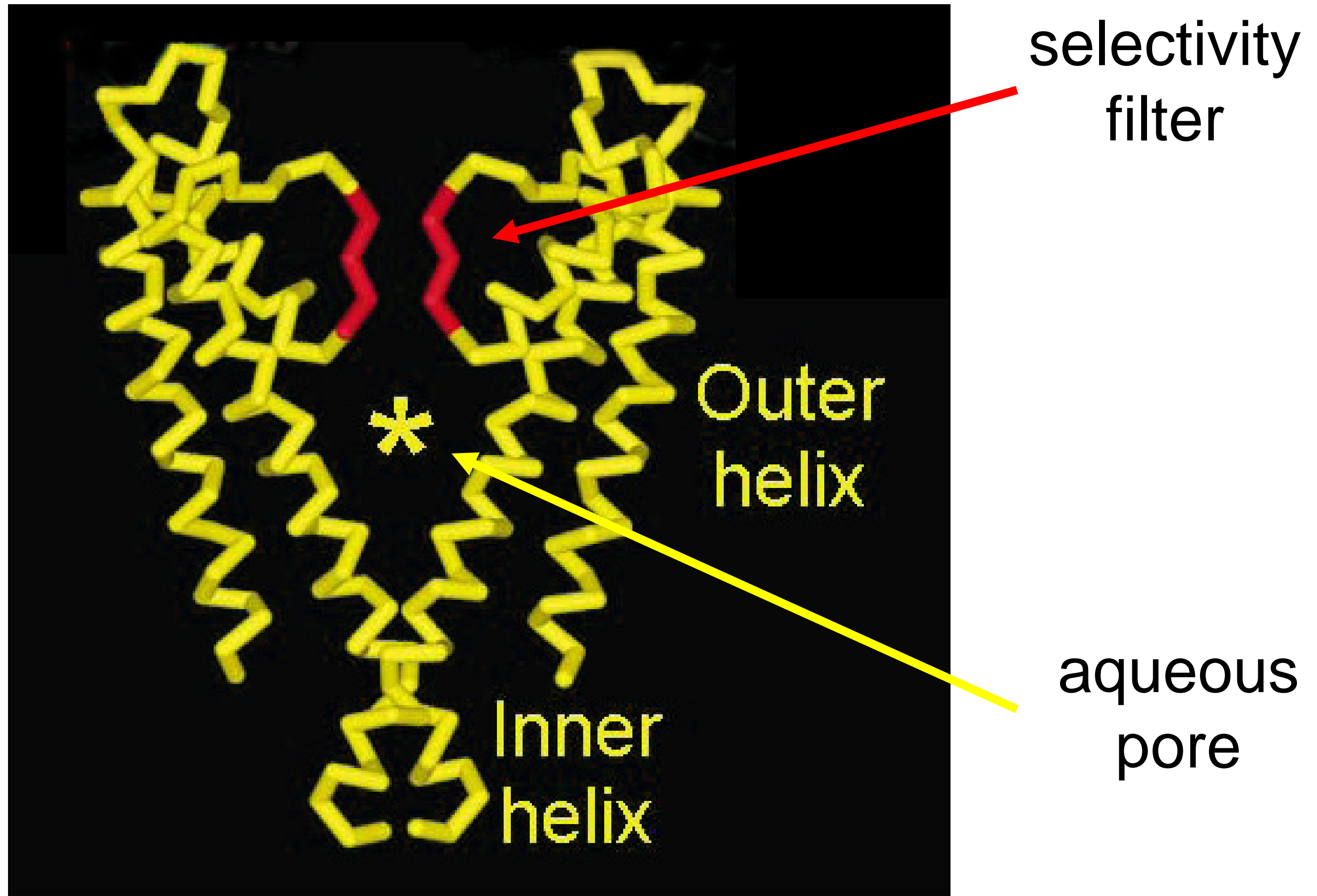
Single channel conductances and channel densities:

Table 4.1. Conductance and Density of Sodium and Potassium Channels[a]

Preparation	γ (pS)	Channels (number/μm^2)
	Sodium	
Squid giant axon	4	330
Frog node	6–8	400–2000
Rat node	14.5	700
Bovine chromaffin	17	1.5–10
	Potassium	
Squid giant axon	12	30
Frog node	2.7–4.6	570–960
Frog skeletal	15	30
Mammalian BK	130–240	—

[a]From B. Hille, *Ionic Channels of Excitable Membranes*, 2nd edn., Sinauer Associates, Sunderland, MA, 1992, based on data from a number of published measurements.

Channel selectivity:

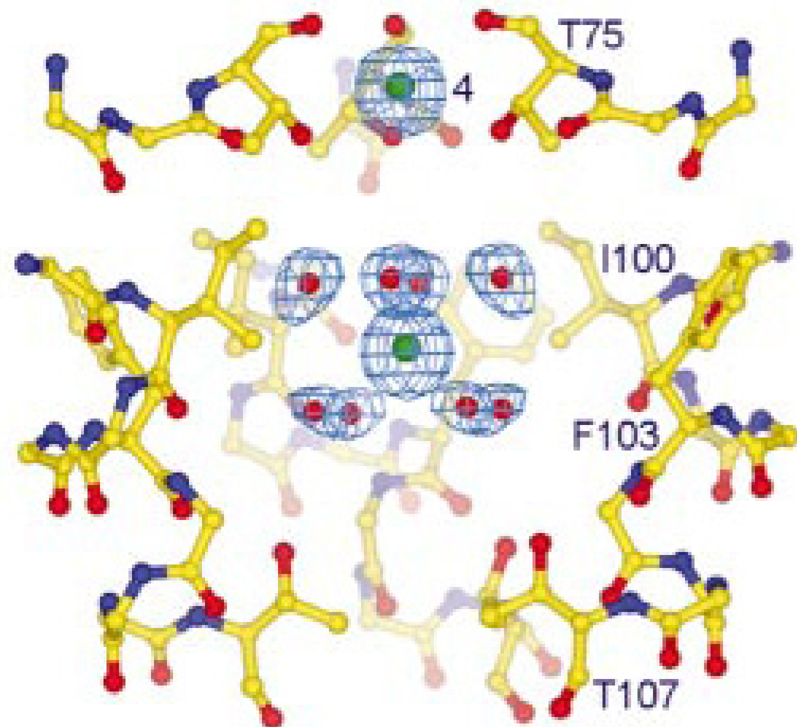


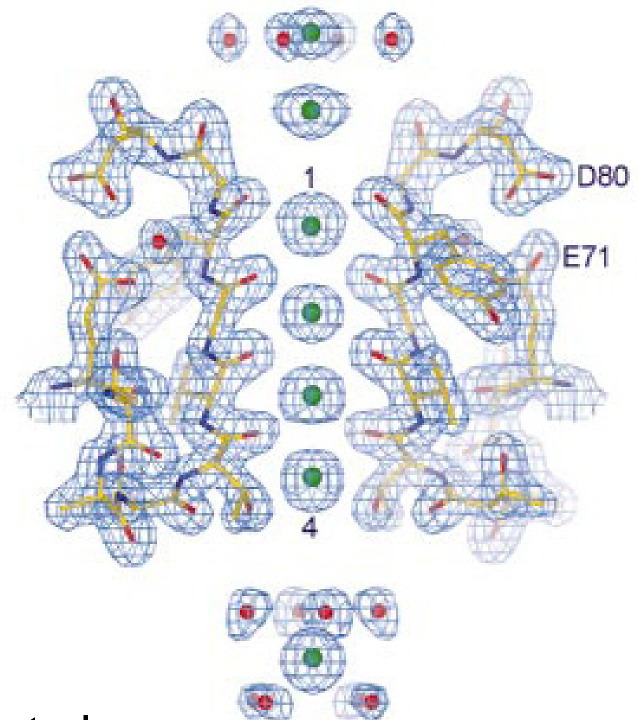
(adapted from Zhou et al., Nature 2001)

Channel selectivity (cont.):

K^+ ions in the aqueous pore

K^+ ions passing through the selectivity filter

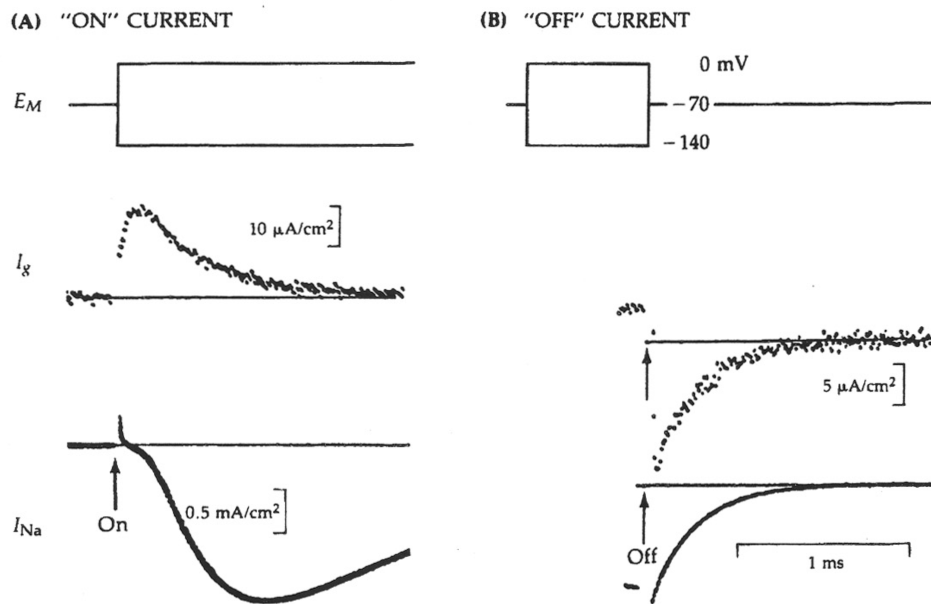




(Zhou et al., Nature 2001)

Channel gating:

Movement of a *charged gating particle* can be measured as a small *gating current*.

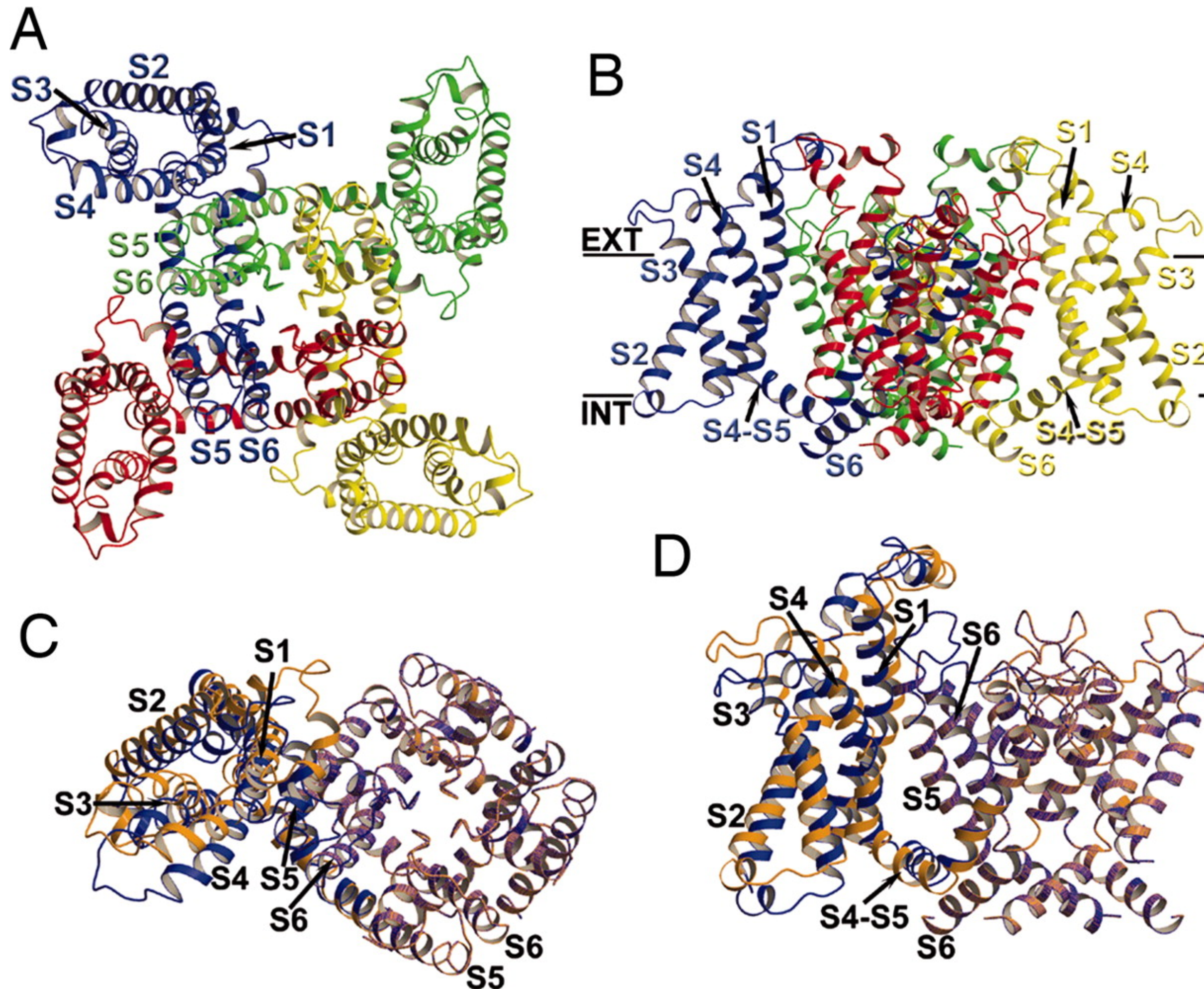


(from Johnston and Wu)

Figure 6.22 Gating current (I_g) and I_{Na} recorded by adding responses to symmetrical positive and negative pulses applied to the squid giant axon. I_g was measured in Na^+-free solutions with TTX to block Na^+ channels and internal Cs^+ to block K^+ channels. Since I_g is small, 50 traces had to be averaged in the recording computer to reduce the noise. I_{Na} is measured in normal artificial sea water without TTX. (A) Depolarization from rest elicits an outward "on" I_g that precedes opening of Na^+ channels. (B) Repolarization elicits an inward "off" I_g coinciding with closing of channels (a different axon). (From Hille 1992, adapted from Armstrong and Bezanilla 1974 by copyright permission of the Rockefeller University Press.)

Channel gating (cont.):

Structural model of *open* K-channel:



(Yarov-Yarovoy *et al.*, PNAS 2006)

Channel gating (cont.):

Structural model of *closed* K-channel:

(Yarov-Yarovoy *et al.*, PNAS 2006)