ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

Professor Paul Selvin
Physics 498

Announcements

April 21, 23, 28—Lecturers as usual.

Wednesday April 30th:

10:30-11:50 am--In class– Present your final talk

6-8ish pm– Pizza and Final talk.

Friday May 2nd: Take home Final Exam. Pick up in 364 Loomis between 4 and 6pm.

(You must turn in the final exam as well as your answers! You will also be asked to sign a statement saying you have not made a copy nor shared the test or answers with anyone else.)

Monday, May 5th, 5pm: Final Exam due. Turn in Exams and your answers to Rm 364 Loomis.

Friday May 9th 5pm: Turn in final paper to Rm 364 Loomis.

Homework (due Monday 4/ 28): Read P498Bio Library (on Web):

1. Bezanilla, F., *How membrane proteins sense voltage.* Nat Rev Mol Cell Biol, 2008.
2. Single-photon detection by rod cells of the retina … F. Rieke et al., Reviews of Modern Physics,1998.

Write a ½ page EACH stating: What was major point; What was one thing you did not know; What question do you have that is unanswered?

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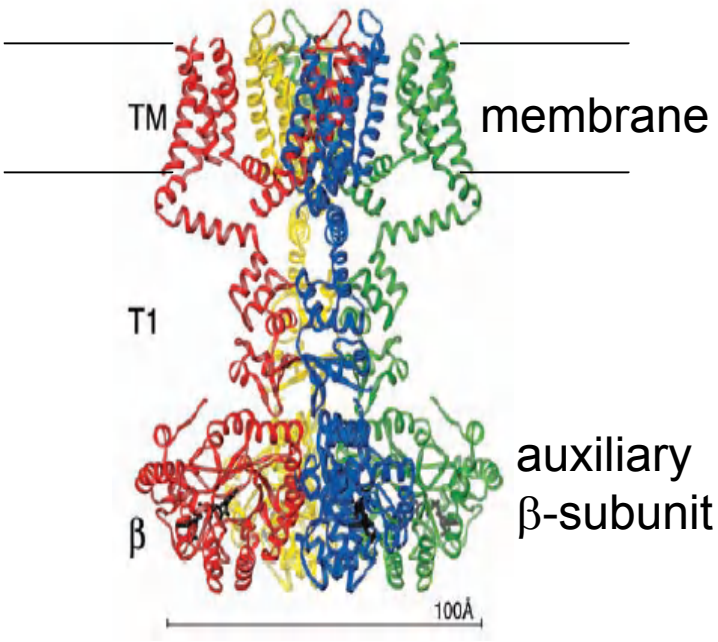
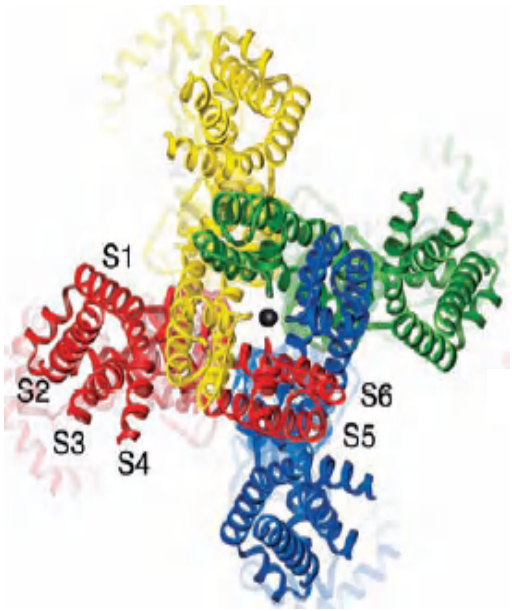
Summary of Ion Channels

How Shaker Potassium Ion Channel Reacts to Voltage

Structure of the Kv 1.2 Channel (a mammalian channel)



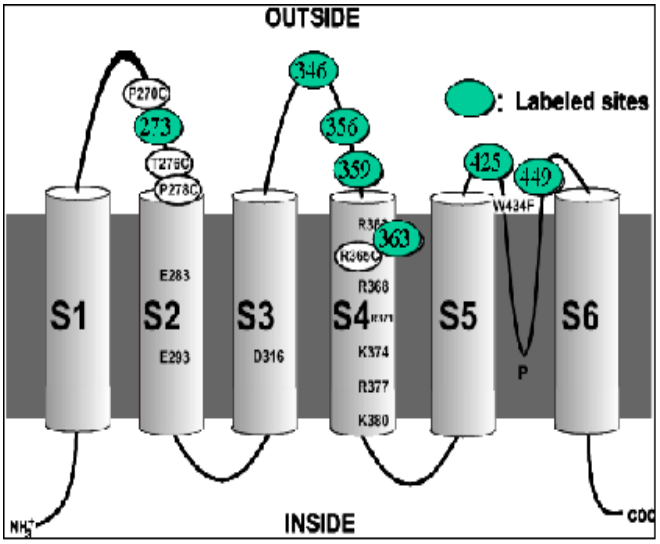


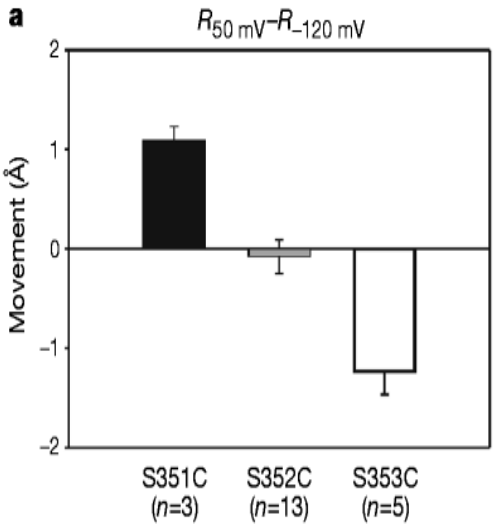
Channel is in open conformation – cannot settle Paddle movement debate

MacKinnon believes S4 movement large (Originally believed 15-28 Å)

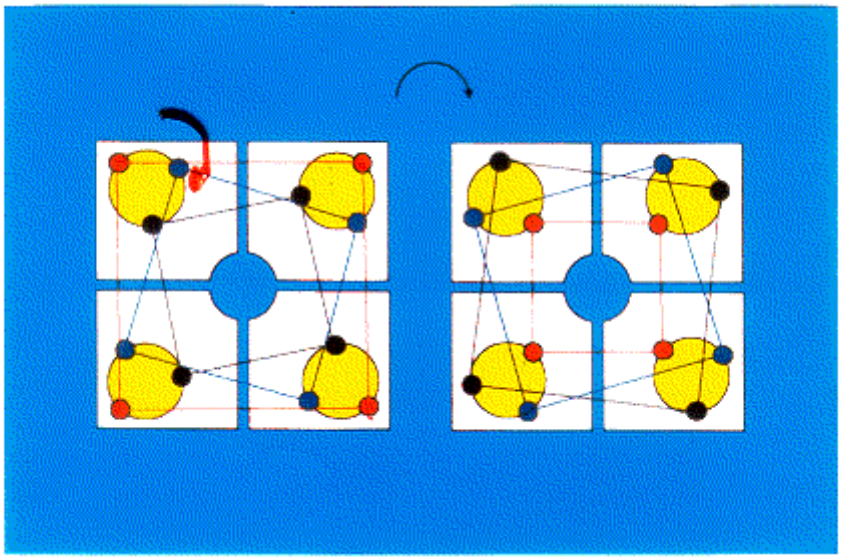
Figure adapted from Long and MacKinnon, Science, 309, 897-903, 2005.

Three neighboring residues,351, 352, 352 Move in different directions





- S351C gets closer
- S352 C unchanged
- N353C get farther



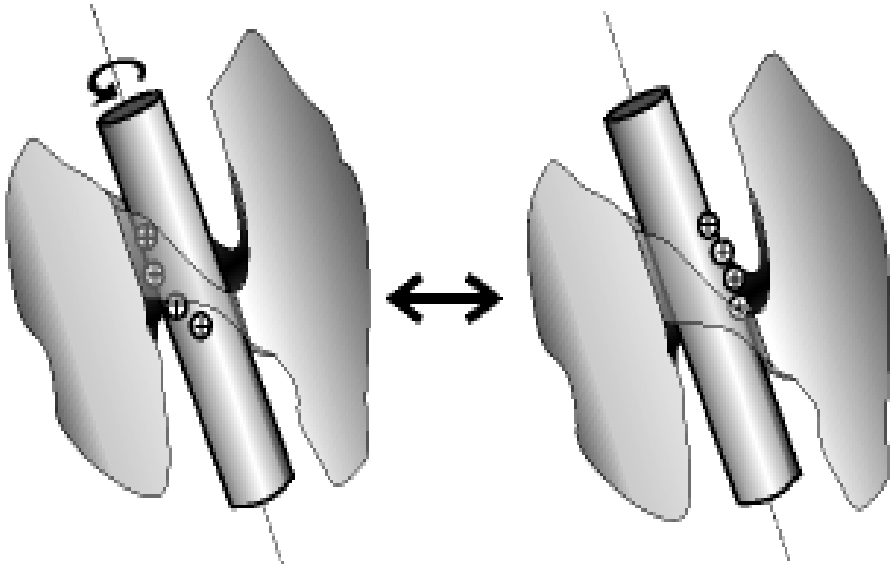
Red residue (S351C) initially far, then close.
Blue residue unchanged.
Black residue initially close, then far.

Shaker voltage sensor twists, does not translate too much.

How it all adds up:

Shaker voltage sensor twists, does not translate too much.

S4 Resting S4 Activated



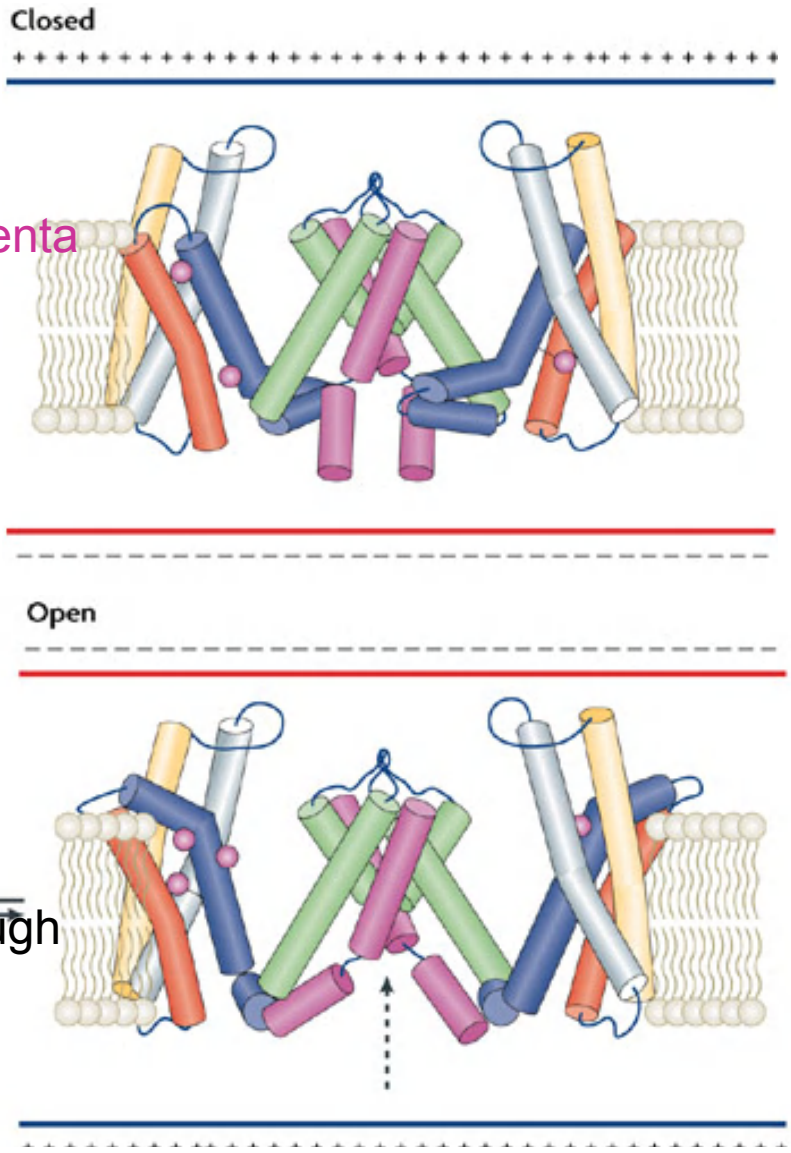
Cha, Nature, 1999

How the Pore Opens(Pancho Bezanilla Model)

Arginines in magenta
S4 in Blue
S6 in magenta

only two subunits shown

dashed arrow represents ion conduction through the open pore.



When the membrane potential changes from hyperpolarized (closed) to depolarized (open), segment S4 rotates 180°, changes its tilt by about 30° and moves towards the extracellular side by about 6.5 Å. Movement of the S4 segment is transmitted through the S4–S5 linker to the intracellular part of S6 (magenta). The ion conduction pore is formed by the S5 and S6 segments and the main gate of the channel is formed by the intersection of all four S6 segments. The gate opens when the S6 segment breaks in the Pro-Val-Pro region (PVP motif) of the S6 segment, splaying apart all four segments and thereby allowing ion conduction. When the membrane is depolarized the translation rotation and tilting of the S4 segment is transmitted through the S4–S5 linker, which is in contact with the intracellular part of the S6 segment. This causes the PVP motif to bend, which opens the gate and initiates ion conduction. In the closed position S6 is a straight -helix, whereas in the open position it is bent at the PVP motif, thereby opening the gate. S1, white; S2, yellow; S3, red;

Vision

1. Basic structure of Eye.

Lenses, Retina.

2. Making an image

Cornea vs. lense.

Why we can't see well underwater.

When we need glasses.

3. Sensitivity of vision

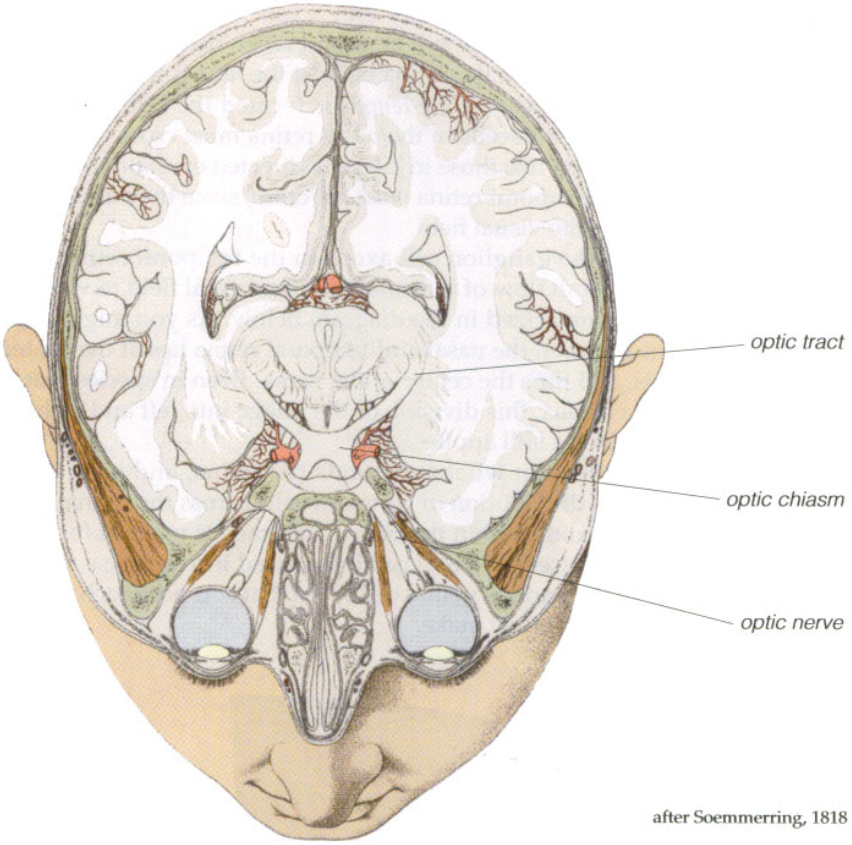
a. Can you see single photons?

b. Why we can't see stars during the day:
Signal/noise.

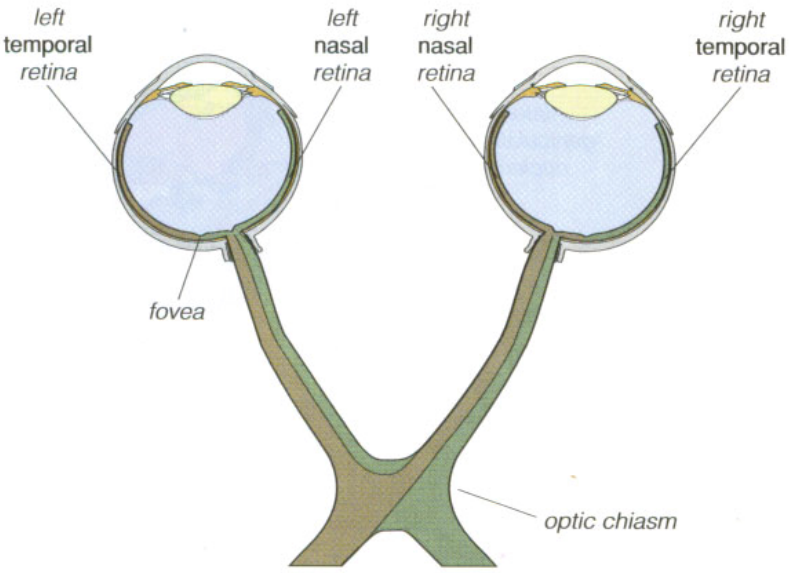
4. How light energy is converted into electrical (nerve) impulses. (This time or Next time ?)

Ion channels.

Visual System



Optic tract– right side of both sides goes into right side of optic tract



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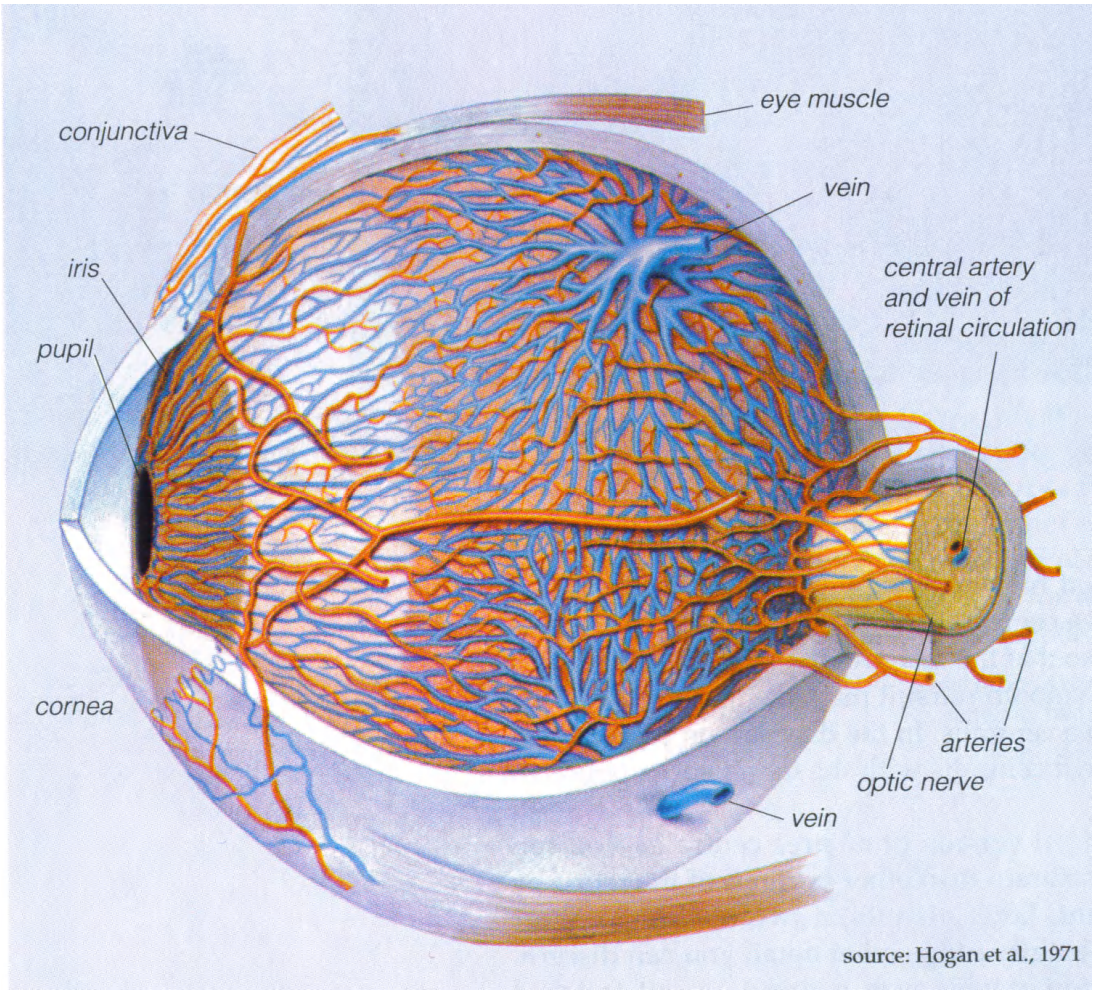
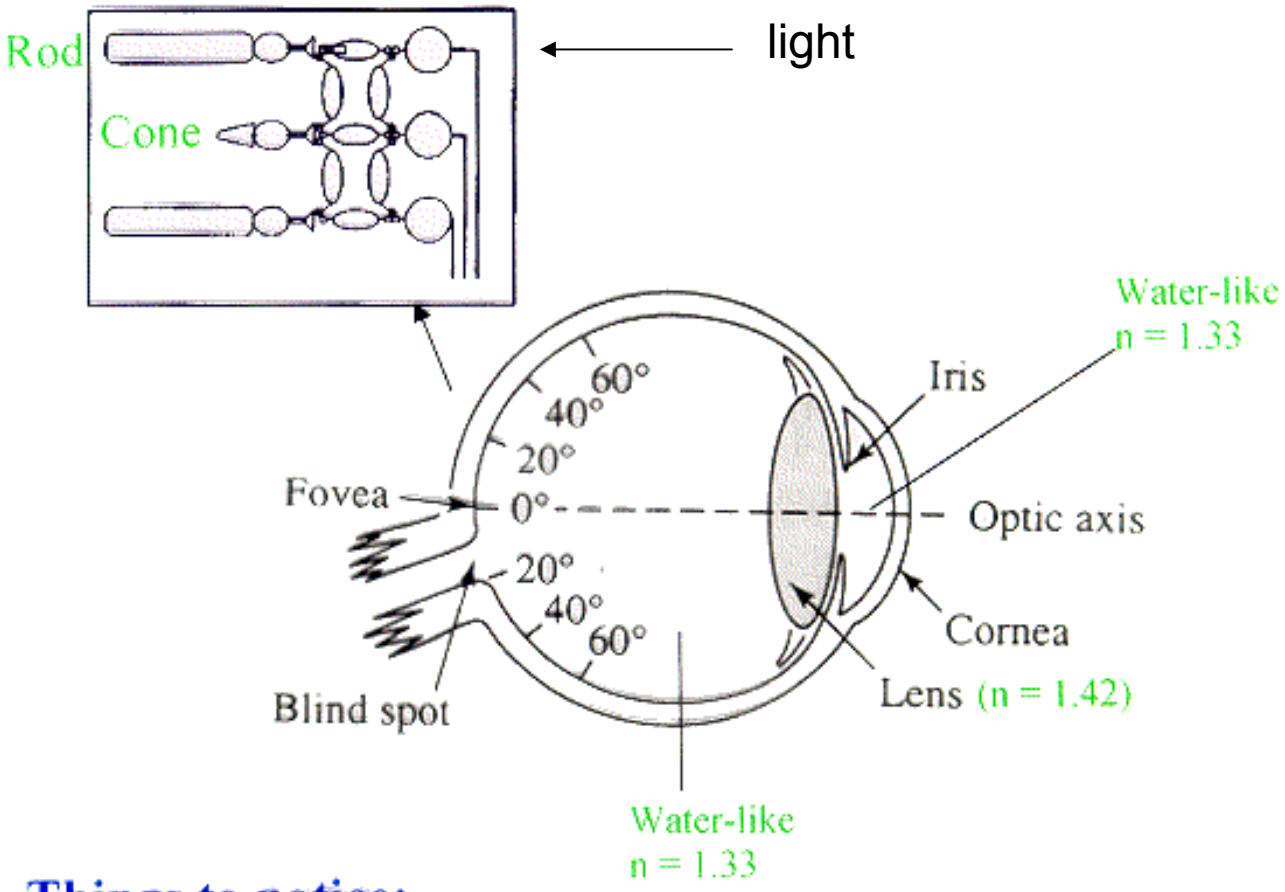
The Eye



Tons of blood vessels cause lots of Oxygen use.

Basic Structure of the Eye



Things to notice:

Image formation:

1. Two lenses, not one! **One fixed, one variable**
   Big index mismatch at cornea/air; some at lens.
2. **Image distance is fixed.**
3. **Normal eye: object at ∞, focussed on retina with lense relaxed.**

Light (and color) detection:

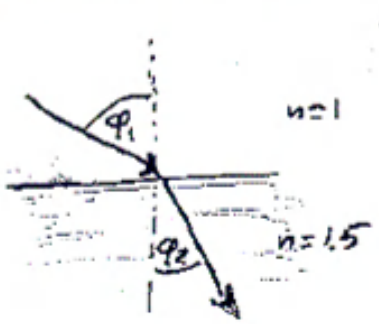
4. Sensitivity of retina = function position
5. **Rods and Cone Backwards!**

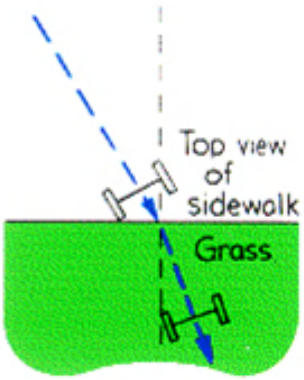
Forming an image…

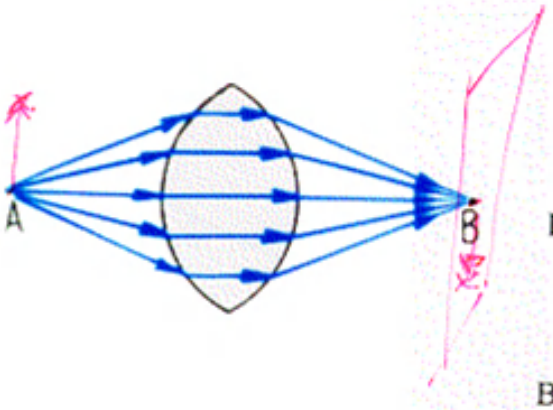
Light going from one media (index of refraction)to another.

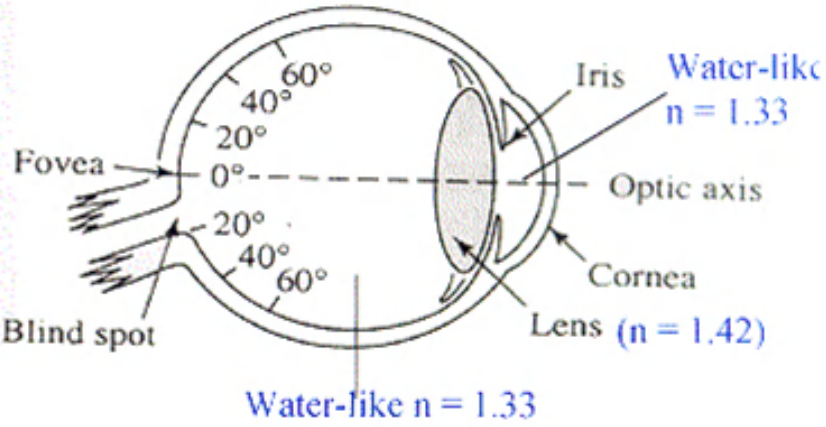
(Snell's Law)

$n_1 \sin\theta_1 = n_2 \sin\theta_2$









Amount of bending depends on index mismatch and angle of incidence.

Cornea is responsible for 2/3 → ¾ of light bending.

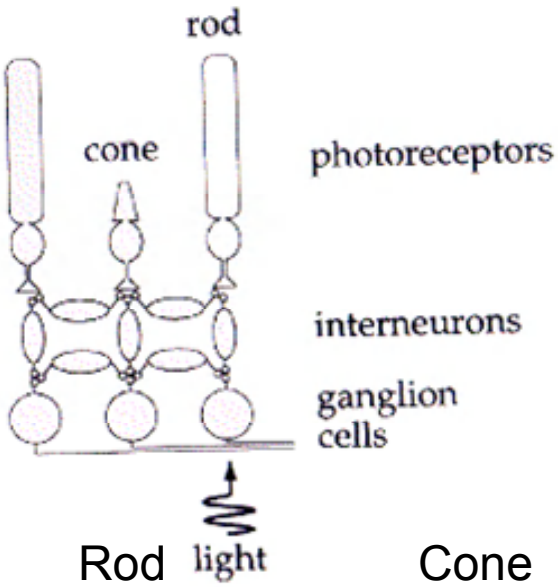
Why can't you focus well under water?

Why is image formed upside-down, but not left-to-right?

Retina

Two types of photoreceptors:

- **Rods:** dark-level sensitivity
- **Cones:** color, most of day-vision

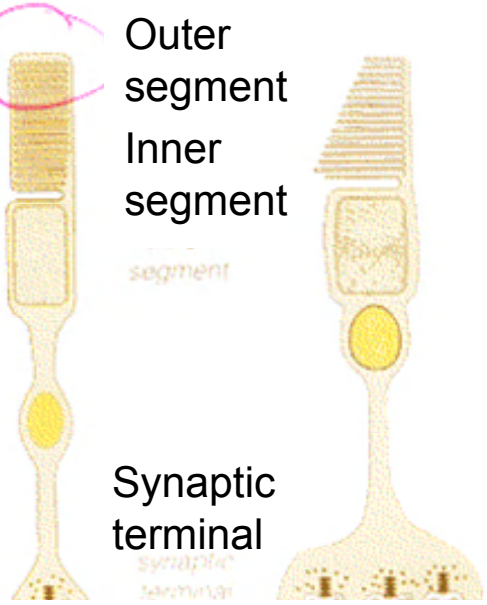


Mammalian rod contains ~ 10^8 rhodopsin molecules

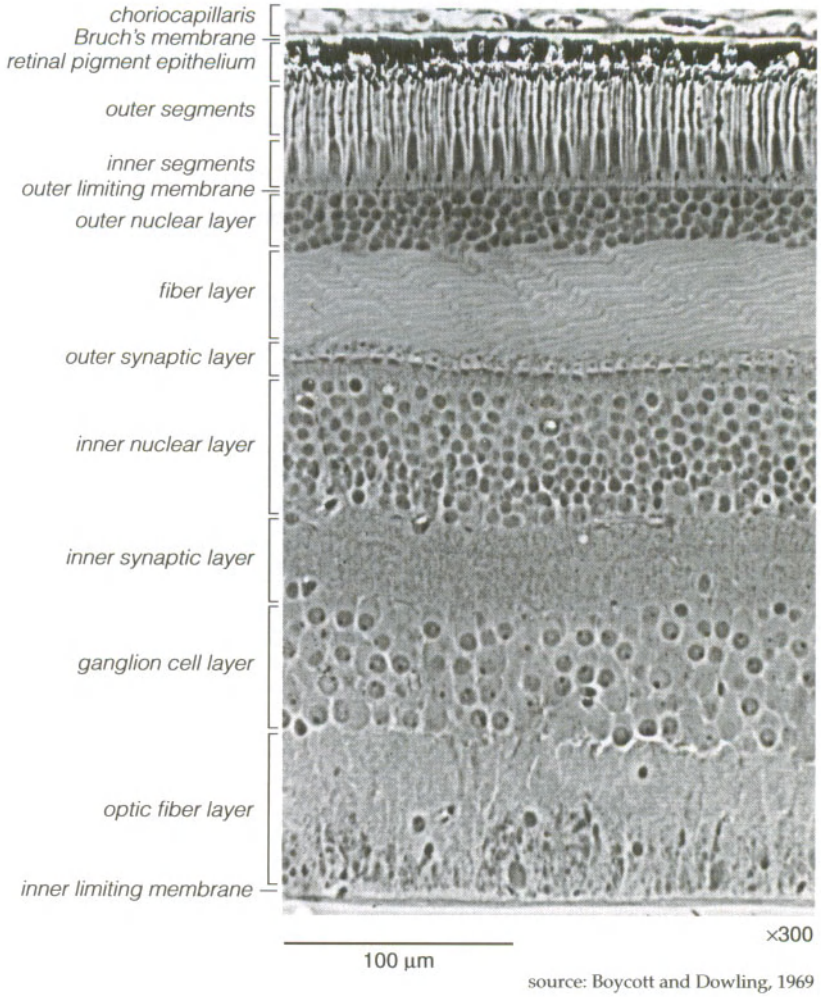
Rod ~3 μm

Cone

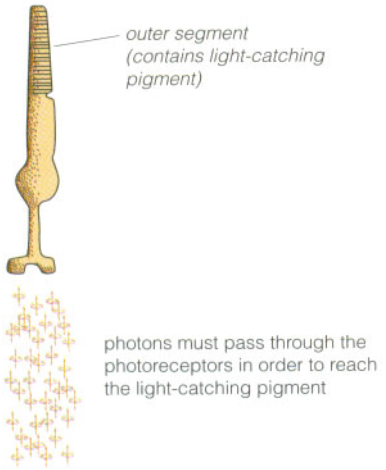
Rhodopsin– pigment that absorbs light→ changes shape: bent → straight that ultimately leads to signal, i.e. ion channel opening, nerve firing.



Your receptors face "backwards"



source: Boycott and Dowling, 1969



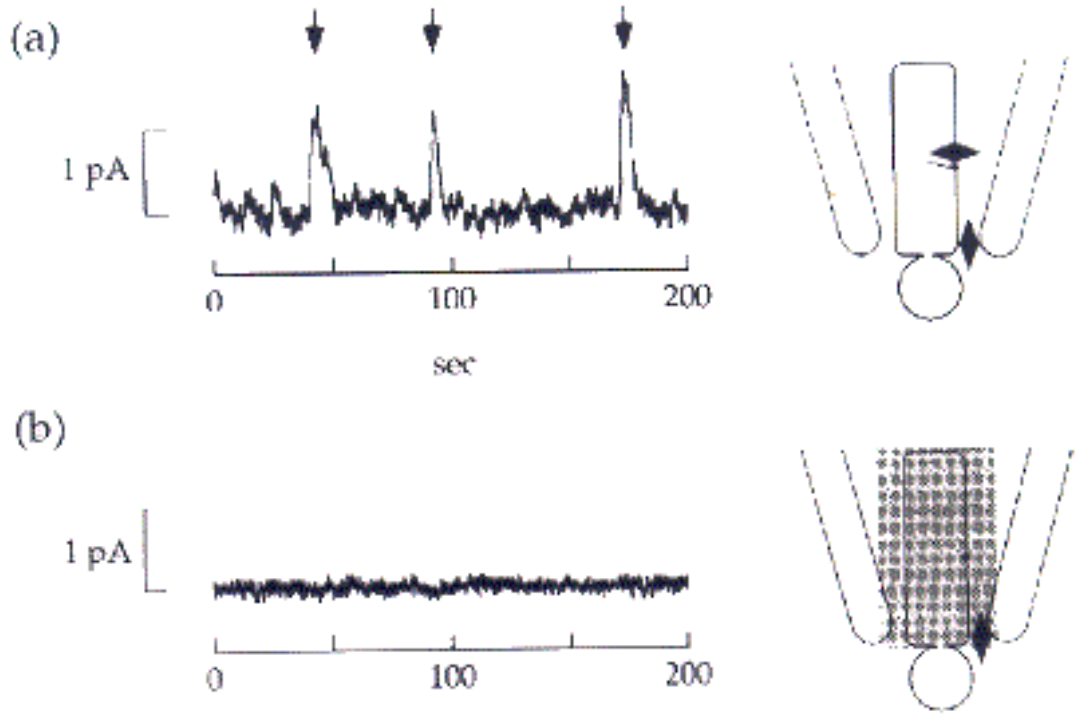
1.25 mm from Fovea, Roieck, pg38

Why?

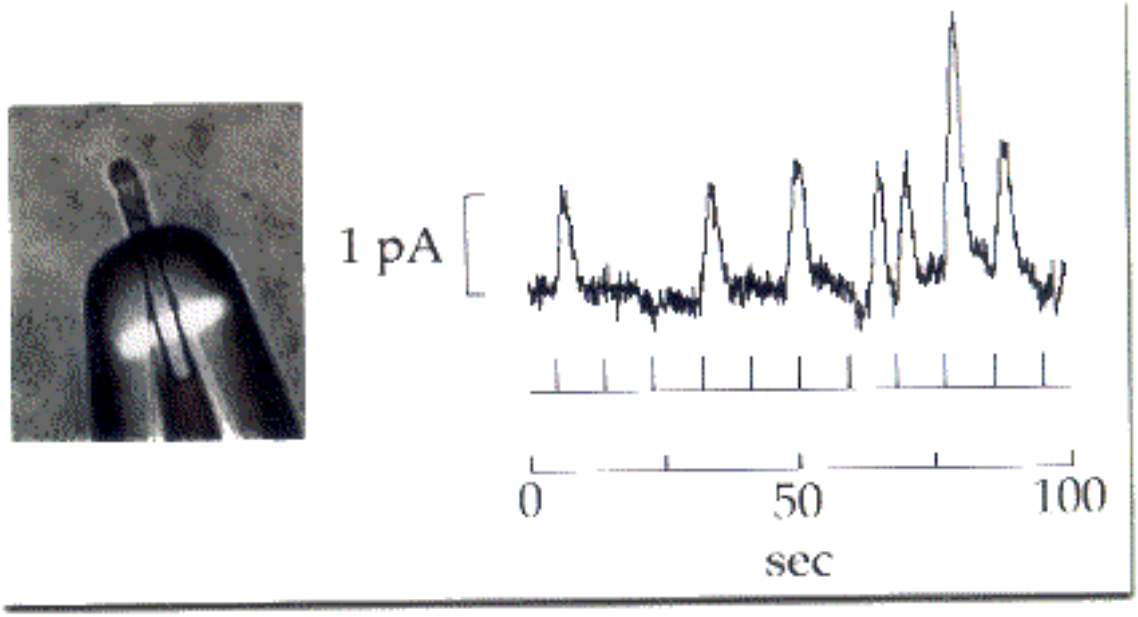
1. May be consequence of neural tissue development.
2. Outer portion of photoreceptors, which are very metabolically active, are near epithelium which supplies oxygen and nutrients. (Rodieck, pg 39.)

Rods

Isolated rod can "see" single photon!



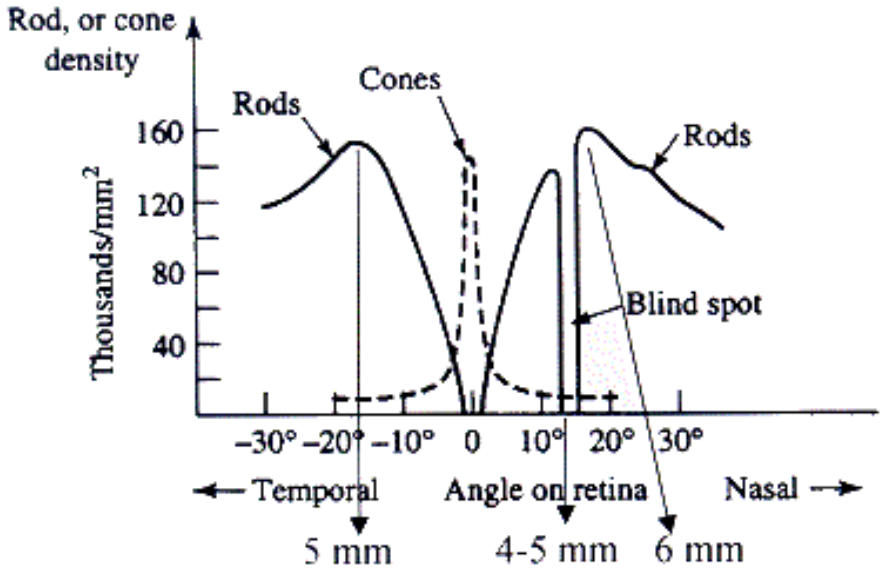
Rieke, Rev. Mod. Phys Fig. 6

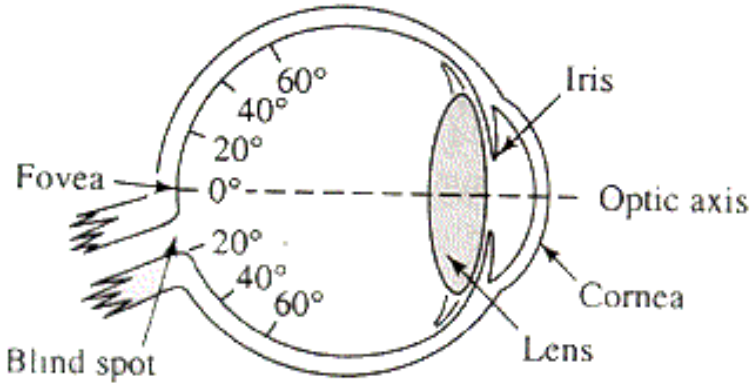


From Fred Rieke (UWash.) home page: http://depts.washington.edu/pbiopage/rieke/

Distribution of Rods and Cones are *not* uniform on retina.

Density of rods and cone in left eye [Benedek 3.10]



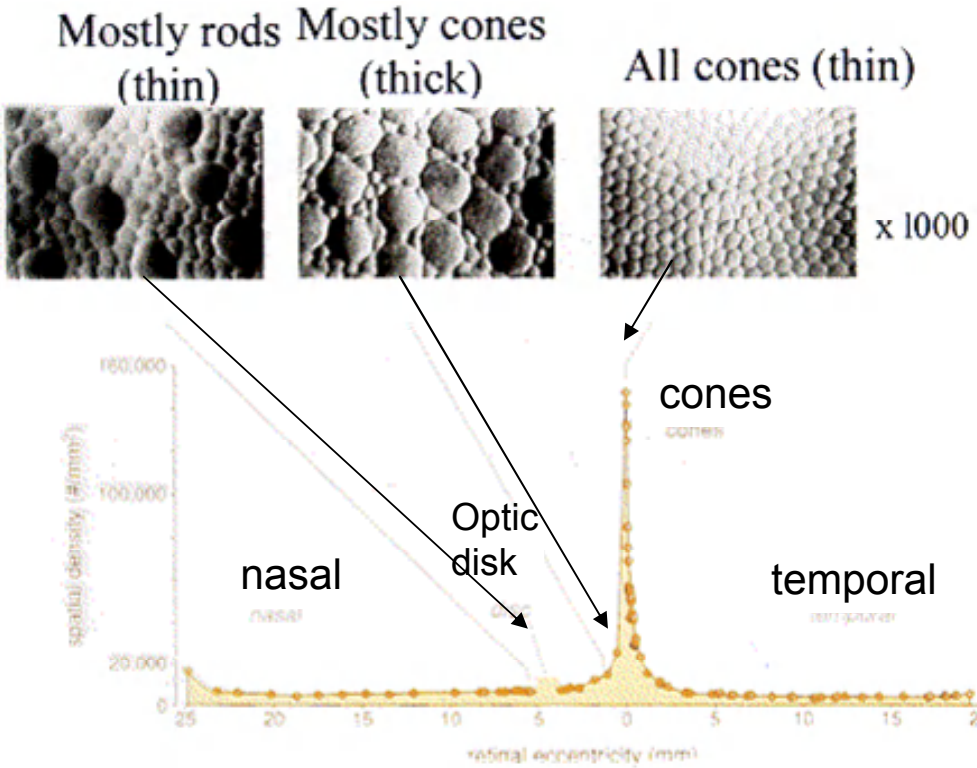


Each peak density @ > 100,000 receptors/mm^2

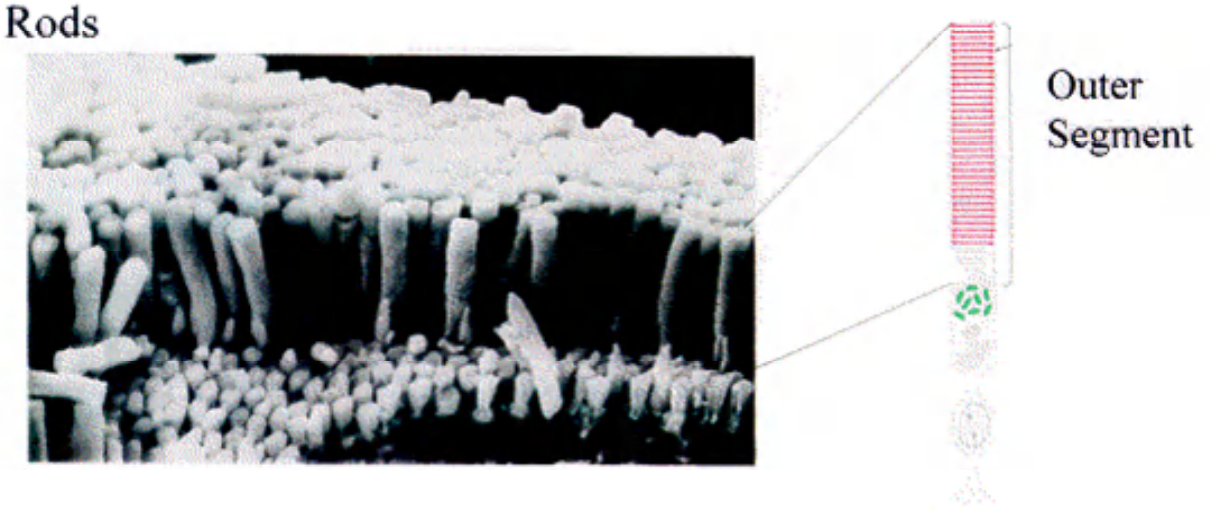
For max. sensitivity in low light, where should you look?

Distribution/pictures con't

Cones



Rods

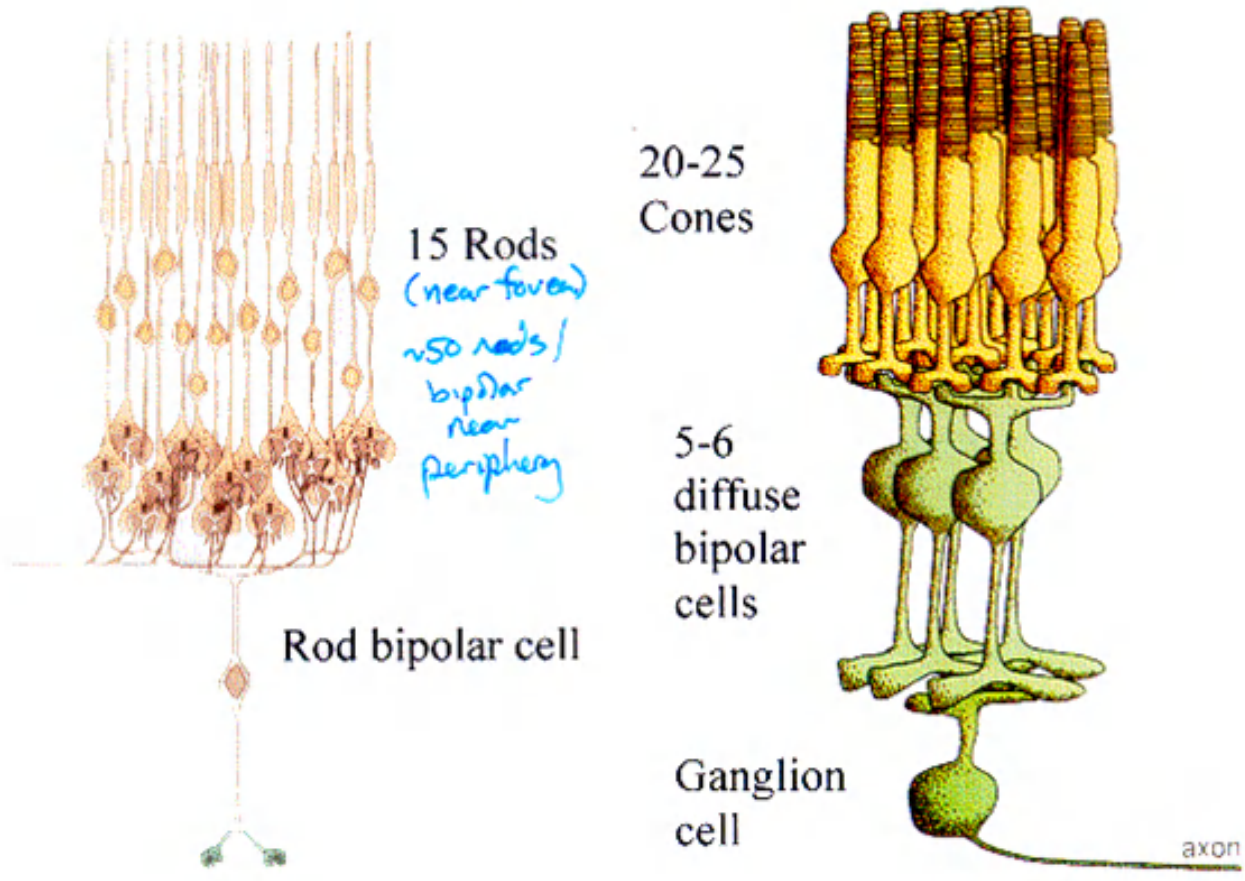


Rods & Cones are organized in groups

Order of magnitude…**Retina has**
$\approx 10^8$ photoreceptors,
$\approx 10^7$ intermediate cells (e.g. bipolar cells)
$\approx 10^6$ ganglion cells, which ultimately form optic nerve.

20 rods contact a second order cell. Between 25 and several hundred bipolars provide input to a ganglion cell (depending on where in the retina you are). [source: Prof. Fred Rieke, UWashington]

Rods (Cones) connected to a single ganglion cell act as a functional unit.

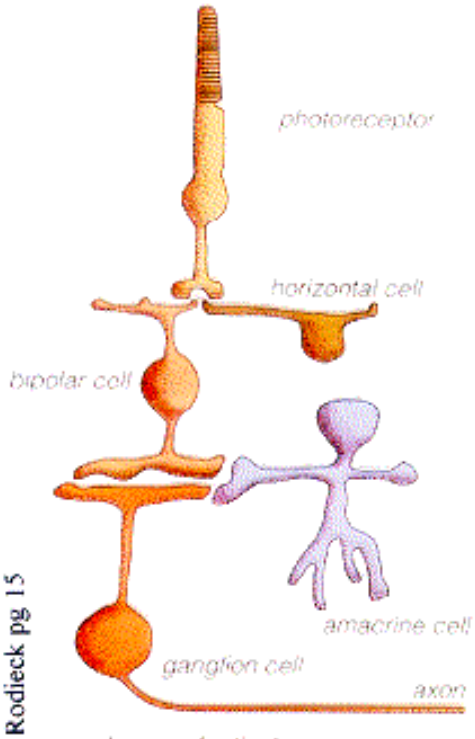


Signal processing begins in retina.

Output of rods can be modulated by horizontal cells

Important in dynamic range; Seeing differences in light.

[Rodieck. pg 144. 185]



Rodieck pg 15

classes of retinal neurons

1. **Dynamic range:** can see over range of intensities of $10^{\wedge 10}$.
(Uses both rods and cones.)
Rod vision: 10^6 dynamic range

Any one cell can't respond over this range.
Rod output can change by 100x.
Bipolar cell output can change by 100x.

- **If light level low,**
rod output → bipolar input.
- **If light level high,**
horizontal cell attenuates rod output.

Your eye responds logarithmically to light
Allows you to see over huge range of intensities.

2. Horizontal cells & other processing makes visual response more sensitive to differences in light intensity in image, than absolute light levels.

Absolutely uniform illumination into eye – see nothing!

Light (Intensity) levels

Definition of intensity? Power/area = (Energy/time)/area: Watts/m^2

Units? Photons/area/time x energy/photon

Energy/photon= $h\nu$/photon

You can see over enormous range: 10 orders of mag.!

Entering your eye:
$< 10^{-2}$ photons/μm^2/sec (starlight) to
$> 10^{8}$ photons/μm^2/sec in bright sunlight. (Rieke, rev. mod. Phys)

You can see very little light!
At retina: 4×10^{-4} photons/μm^2/sec.
Some sense of light and dark.
Can navigate a bit. [Rieke, UW]

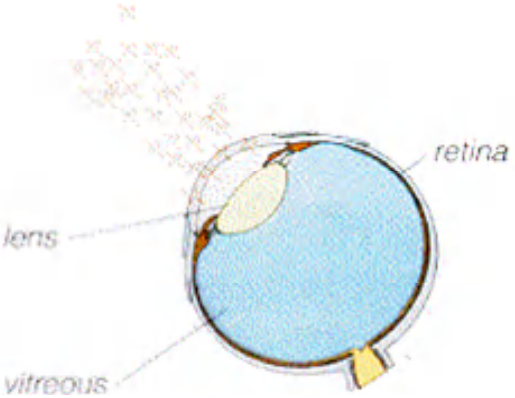
If photoreceptor (rod) is about 3 μm diameter, this corresponds to 0.004 photons/rod/sec or 1 photon per 250 rods / sec!

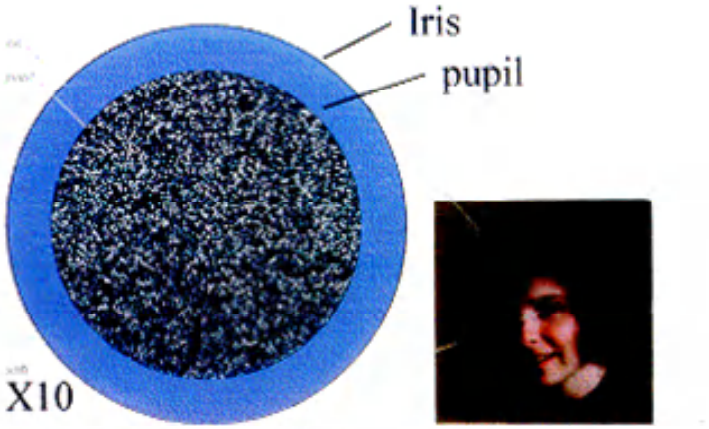
Relevant integration time: 0.1 sec.

Can see at 1 photon per 2500 rods per 0.1 sec!

Starlight: ≈100,000 photons/sec

Quick calculations…





10,000 photons/0.1 sec at pupil
[Low I – photon nature of light.]

Starlight: About 2,800 visible photons/s/mm^2 hit surface of earth/eye from North star (Polaris).

Pupil (in dark) ≈ 40mm^2, 6 mm diam. = 112,000 photons/sec

Eye has a memory/shutter time of about 0.1sec
= 11,200 photons/shutter time.

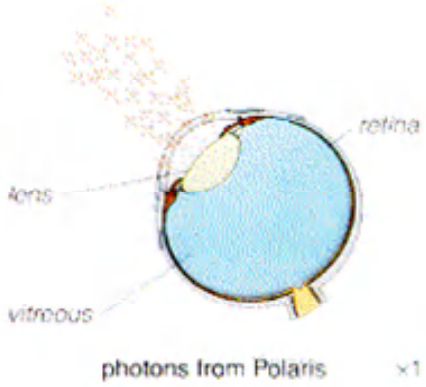
rod cell keeps emitting current providing

Memory time determined by length of time of ~~action potential~~ induced by photon in photoreceptor.

(lack of current)

Losses of light going through eye.

Only ≈ 10% of the photons that hit front of your cornea actually get absorbed by photoreceptor (rod/cone) and only 2 out of 3 of these photons lead to a chemical reaction (photoisometerization of rhodopsin from bent to straight form).



Losses… going through parts of eye …

Start with 11,200 photons/0.1 sec

Loss: Eye part (for cones, but total very similar for rods)

10%: Cornea (2.5% front reflection, 9% absorbed/scattered inside)
9.400 photons ledt.

42%: lense. vitreous humor. pigment in retina < photoreceptors
5.500 photons left.

46%: Hit inner segment (front) of photoreceptor but don't hit outer receptor (back) where photopigments (rhodopsin) are.
2.900 photons left.

64%: 1 out of 3 photons entering outer-segment get absorbed.
1.044 photons absorbed.

33%: 2 out of 3 absorbed photons leads to isomerization of rhodopsin (bent → straight)

700 photoisomerizations/0.1 sec

6% of photons entering your eye actually create signal.

Can you see a single photon?

Classic experiment: Hecht et al., 1942.
(Followed by Sakitt, 1972).

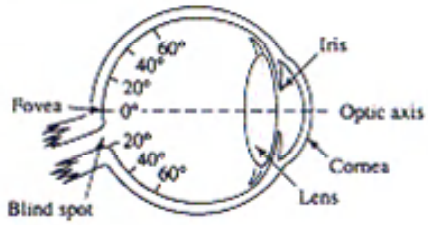
Experimental Arrangements:

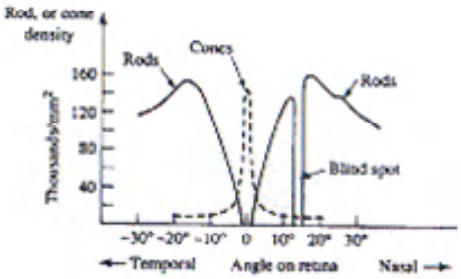
Short, dim light flash into eye of average N photons.
Ask patient: "yes" saw it, "no" didn't.
Keep reducing light level until can't see it.

Light flash:

Measure ave. # photons at cornea by thermopile.

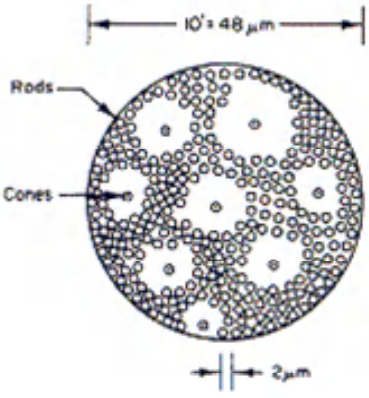
Thermopile: detector that heats up when photon absorbed; know heat capacity very well.





To maximize sensitivity:

1. Position: Shine 20° off-center.
2. Flash time < 0.1 sec: actual 1 msec.
3. Flash color: 510 nm (blue-green)
4. Spot size? If light < 10 minutes of arc, "receptive field": maximum sensitivity. [If shine light bigger than field, lower response/light intensity.]
5. Dark Adaptation. Sensitivity of eye > 30 min. in dark. Hecht waited 40 min. (2000x more sensitive > 30 min. in dark compared to bright room.)



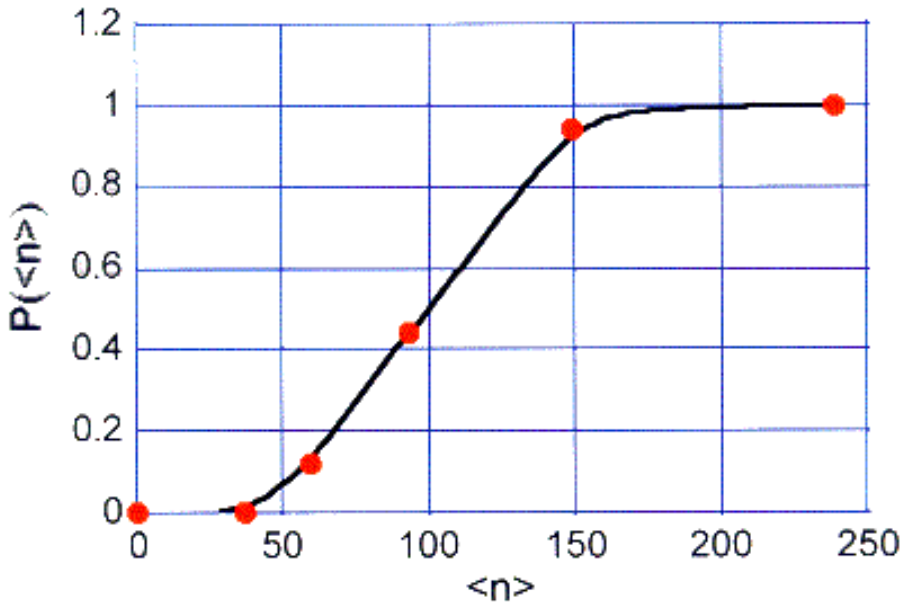
Data of Hecht et al. (1942)

Present series of flashes of <n> photons/flash entering eye (at cornea).

P(<n>) =Probability of seeing flash with <n> photons.
("yes/no" decision)

Table 3.1. Frequency of Seeing ($P(\bar{n})$) Versus $\bar{n}$, the Number of Photons at the Cornea (from Hecht et al. [6]).

$P(\bar{n})$	$\bar{n}$
0	37
.12	59
.44	93
.94	149
1.0	239



Why can't we see a single excitation?

Answer: photopigment spontaneously isomerize even in absence of external light.

5 spontaneous excitations per receptor field per 0.1 seconds.

This creates a "dark noise".

External Signal must be one this order or greater to see it.

Mammalian rod: 10^8 rhodopsins.

A rhodopsin spontaneously isomerizes 1/300 years!

[Remember: normally 2 eV photon to cause transition: = 80kT! Think ΔG!]

In each rod, 1 rhodopsin isomerizes every 90 sec.

One isomerization leads to rod firing. If 500 rods in visual field, 0.5 isomerization/0.1sec/ receptor field. Each isomerization leads to a nerve firing.

rod

Class evaluation

1. What was the most interesting thing you learned in class today?

2. What are you confused about?

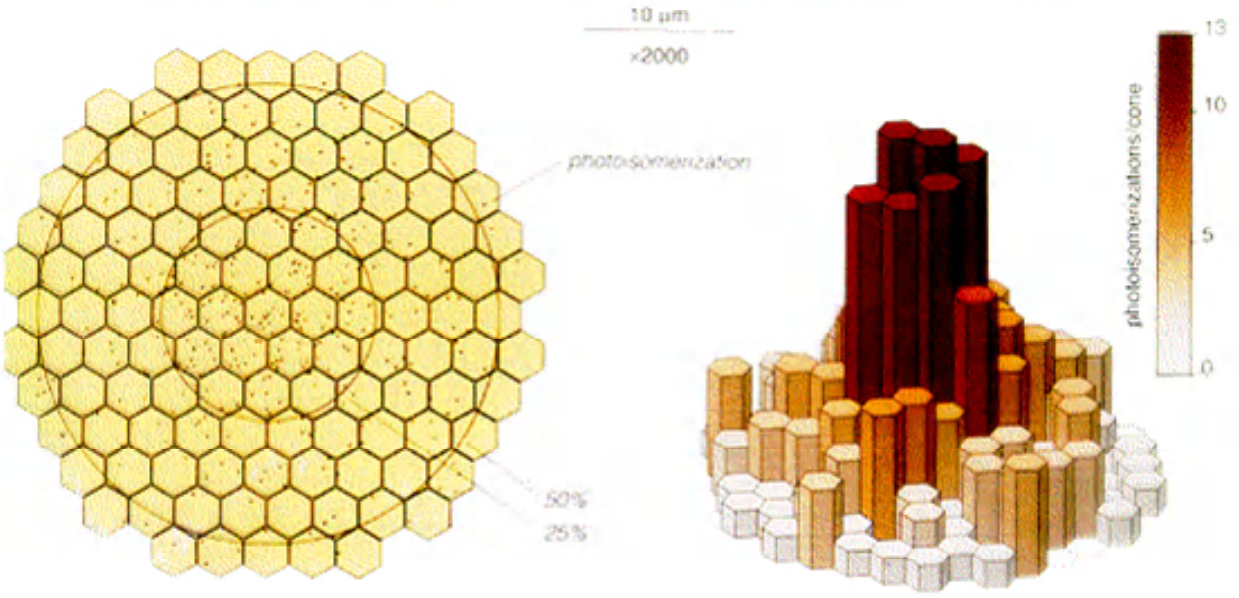
3. Related to today's subject, what would you like to know more about?

4. Any helpful comments.

Answer, and turn in at the end of class.

Light from star is focussed to a spot on retina.

700 photon isomerizations
– ½ within 25 um of center of spot on retina.



Cone about 3 µm diameter.

Central cones each have 10-15 photoisomerizations in 0.1sec.

≈ Same brightness as looking at grey rock in moderate daylight conditions (e.g. morning in diffuse sunlight).

Spot size

Diffraction limited for small pupil; larger for larger pupils due to spherical & chromatic abberations of eye.

Can a single isomerization be detected? I.e. if photon is absorbed, does it produce a measurable/detectable signal?

Ans: debatable, but probably not quite.