Biochemistry

Sheet

Lec No: 1

Subject: Visual transduction

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Neuroscience; Visual Transduction

Basics of human vision:

The retina contains receptor cells; these are **rods** and **cones.** They are connected to nerve cells to transmit signal

	Rods	Cones
Sensitivity	High	Low
	Night vision (in the dark)	Day vision (in bright light)
Acuity (image sharpness or resolution)	Lower visual acuity	Higher visual acuity
Color vision	No	Yes
Number of receptors	Over hundred million	Seven million only
Wavelengths	All wavelengths (wide range)	Narrow range
	Has an inner and an outer segment, the inner segment contains all the cellular components like nucleus, etc. The outer segment has flattened disks where you have visual transduction proteins	

- *Few cones are connected to a single neuron.* This arrangement is the basis for the **high sharpness** (acuity) and **low sensitivity** of the cones.
- *Many rods are connected to a single neuron.* As a result, the image would be **less sharp** in the rods than in the cones. There is also **greater sensitivity** in the rods because *more rods = amplification of the signal* and so more intense signal to the brain.

It takes you a few seconds until you are able to see when you get from a room that has light to a dark room, because rod cells need to start working

The dark current:

Normally, neurons maintain a resting membrane potential of about -60 to -70 mV. When excited **(activated)**, they open cation channels causing depolarization and opening of voltage-gated Ca^{2+} channels at the synapse. Ca^{2+} flows in and promotes fusion of synaptic vesicles, which release neurotransmitters.

Receptor cells' membranes work "backwards", they are **depolarized** in the **dark** (when they are **NOT** activated).

1. When there is no light, Na^+ and Ca^{2+} enter through cyclic nucleotide-gated channels in the outer segment membrane.
2. This leads to the release of K^+ through voltage gated channels in the inner segment.
3. Rod cells depolarize.
4. Glutamate (a neurotransmitter) is continuously released.

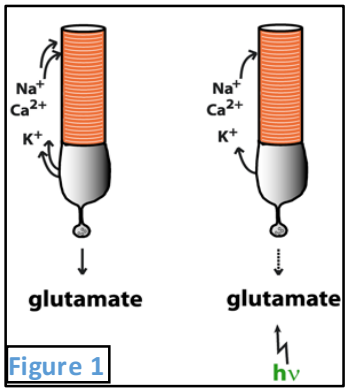


Figure 1

When light hits and excites receptor cells, channels in the outer segment membrane close which causes hyperpolarization of the cell membrane and that stops the release of glutamate (**glutamate decreases**).

Generaton of vision signals:

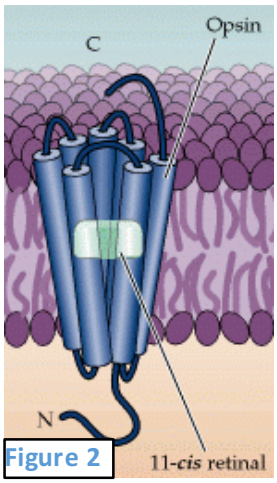


Figure 2

Photoreception in the rods:

- **Rhodopsin**: a receptor with 7 transmembrane domains that is coupled to the trimeric G protein **transducin**.
- Rhodopsin = Opsin (a protein) + chromophore (11-*cis* retinal, a derivative of Vitamin A, that's why vit.A is important for vision) Chromophore is bound to the last transmembrane domain and is responsible for initiating the signaling.
  - Figure 2 shows **Rhodopsin** while figure 3 shows **Opsin**.

Figure 3

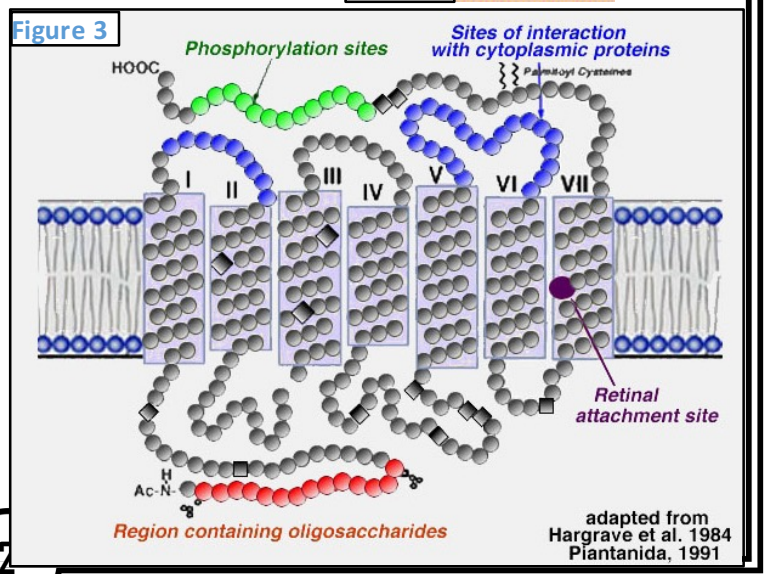
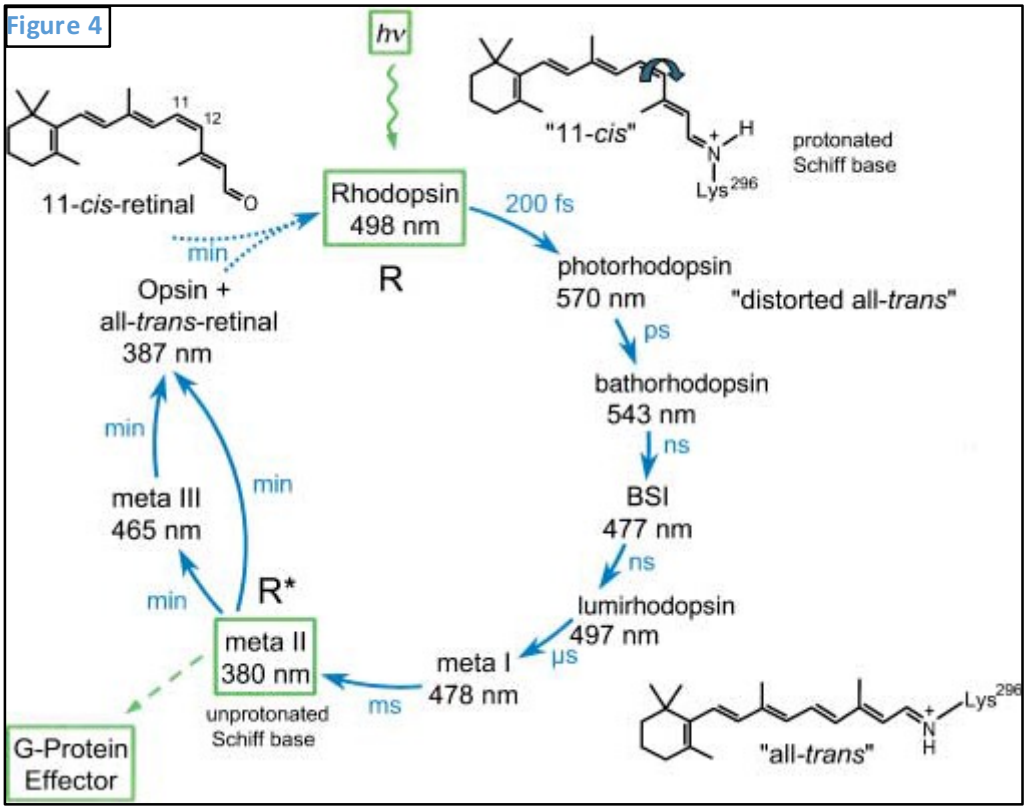


Figure 4



- Light converts **11-*cis* retinal** (inactive form) to **all-*trans* retinal**. This structural change causes the molecule to become rigid. A series of intermediates (figure 4) is then formed, one of which is **metarhodopsin 2 (R*).**

  This conversion is considered amazingly fast in the chemistry world.

  The conversion of 11-cis retinal to all-trans retinal changes the structure of the whole rhodopsin molecule (conformational change) allowing it to absorb light with all wavelengths from red to violate.

  This is important for us to be able to see in the dark, because in the dark, light is faint and there are different wavelengths.

  Put in another way: Each one of the rhodopsin intermediates can absorb light at a different wavelength.

In the slides it's mentioned that Opsin perturbs the distribution of 11-cis retinal's electrons which enables them to get excited with less energy (i.e. longer wavelength light)

Quick Reminder; G Protein

A trimetric protein. The 3 subunits are α, β and γ. In its inactive form, the α subunit is bound to GDP and is in complex with the β and γ subunits. When activated the GDP is replaced with GTP enabling the α subunit to dissociate from the β and γ subunits and do its assumed function (activating phosphodiesterase here)

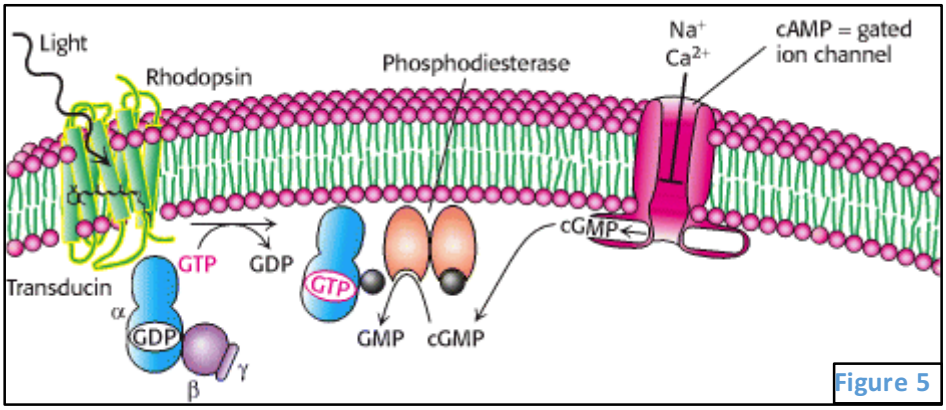


Figure 5

- Activation of phosphodiesterase:
    1. Metarhodopsin2 (R*) binds transducing.
    2. Dissociation of GDP.
    3. Association of GTP.
    4. Release of the α subunit.
    - *PDE consists of 2 catalytic subunits (1α & 1β) and each is inhibited by a γ subunit.*
    5. The α-GTP molecule binds to one of PDE's γ subunits which releaves the inhibition on a catalytic subunit.
- Phosphodiesterase catalyzes the breakdown of cGMP to GMP and **cGMP levels inside the rod decrease.**
- cGMP normally binds to channels to keep them open and so the decreased levels of cGMP causes the **closure of Na^+** channels, decreasing the inward flow of Na^+ and Ca^{2+} ions and as a result **hyperpolarization** of the photoreceptor cell membrane.
    - The large potential difference travels as an electrical impulse down the rod cell to the synaptic terminal, and is then transferred to an adjoining nerve cell.
    - The nerve cell carries the impulse all the way to the brain.
    - The brain determines where the nerve impulse originated and interprets the image.

Signal Amplification:

- 1 photon can close about 200 channels, How?
  A single Rhodopsin activates 500 transducin molecules; each activates a phosphodiesterase that would be able to break down 1000 cGMP molecules to GMP.

Facilitation of transduction:

1. All reactions take place in the plasma membrane which is a 2-dimensional surface rather than a 3D structure (smaller area increases the odds of the molecules meeting and interacting with each other).

2. Olive oil-like nature of the cell membrane (fluidic) allows molecules to collide more often because they are swimming.

3. Cooperativity of binding:

   The binding of one cGMP enhances additional binding while one cGMP release makes it easier for the next to be released. (same idea as O_2 cooperativity when binding to hemoglobin).

Signal termination:

Very quick signal initiation and termination enables us to see continuous movement.

- Mechanism I: *Arrestin binding*:

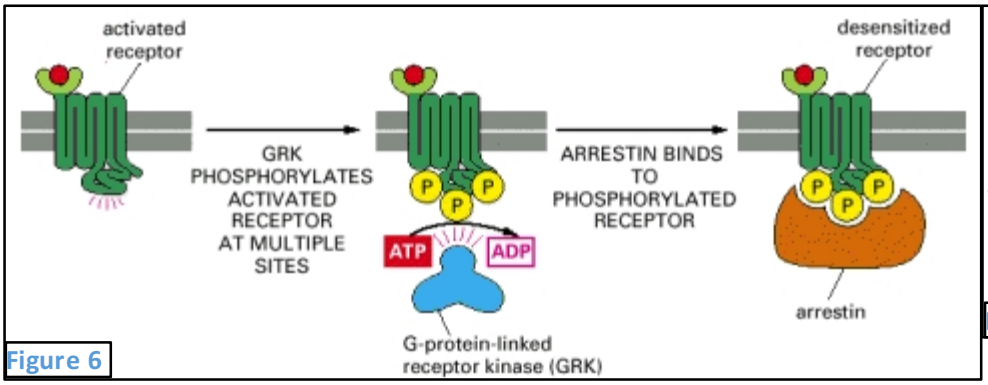


Figure 6

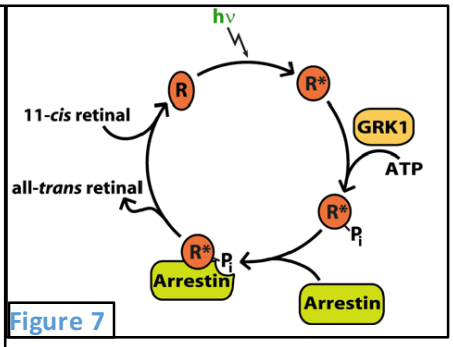
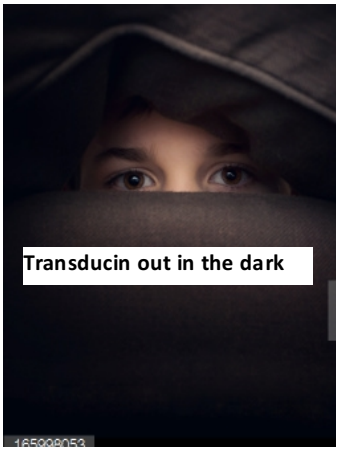


Figure 7

1. Rhodopsin kinase (G-protein linked receptor kinase (GRK1)) phosphorylates the c-terminus of R*.
2. This decreases transducin activation and facilitates R*'s binding to arrestin.
3. The binding arrests R*'s activity and facilitates the release of the all-trans retinal, this regenerates Rhodopsin
   (Arrestin arrests signaling..)

- Mechanism II: *Arrestin/transducin distribution*:
  When there is no light arrestin would be in the inner segment while transducin will be in the outer segment (low inhibition); it's so eager to be activated. In light they switch to terminate the signal (high inhibition).



- Mechanism III: *Intrinsic GTPase activity of G-protein*:

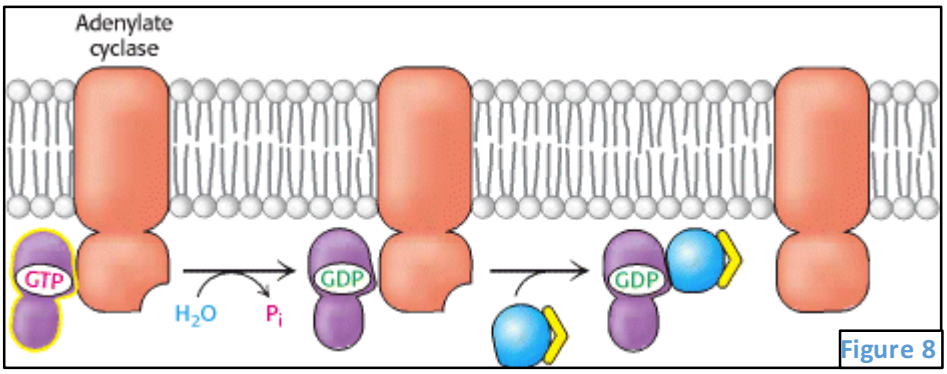


Figure 8

1. The α subunit of the G protein (transducin) has an intrinsic GTPase activity causing hydrolysis of GTP to GDP.
2. The α subunit releases the PDE γ subunit which re-inhibits the catalytic subunit.
3. α-GDP binds the β and γ subunits.

- Mechanism IV: *Facilitation of GTPase activity of G-protein*:

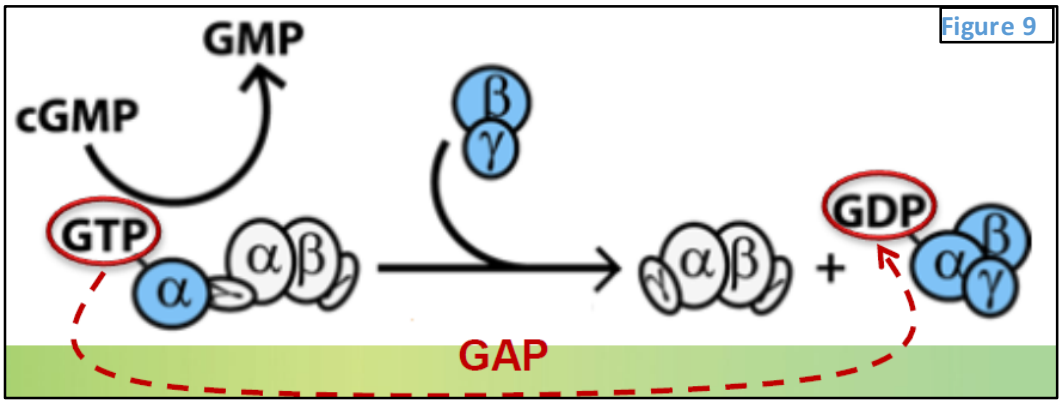
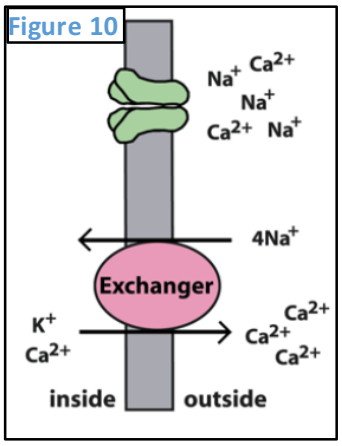


Figure 9

GTPase (transducing) activity is regulated by **GTPase activating protein (GAP)**, this protein can't bind the α subunit (they have a low affinity for each other) unless α subunit is bound to phosphodiesterase (phosphodiesterase is activated).

This ensures that transducin doesn't shut off before activating PDE.

- Mechanism V: *Unstable all-trans rhodopsin complex*:



All-*trans* retinal interaction with rhodopsin is unstable, thus all-*trans* retinal gets kicked out and rhodopsin is left as opsin that is inactive; can't interact with transducin.

Feedback regulation by calcium ions:
When channels close, Ca^{2+} ceases to enter, but the exchanger would still be active pushing calcium out. **Calcium** decreases so much inside the cell.

- Mechanism VI: *Guanylate cyclase*:

Normally Ca^{2+} binds to an enzyme called **guanylate cyclase activating protein** inhibiting it. Reduction of Ca^{2+} disinhibits guanylate **cyclase activating protein** allowing it to activate guanylate cyclase which causes the synthesis of **cGMP**; cGMP increases allowing for the **opening of the Na^+ channels.**

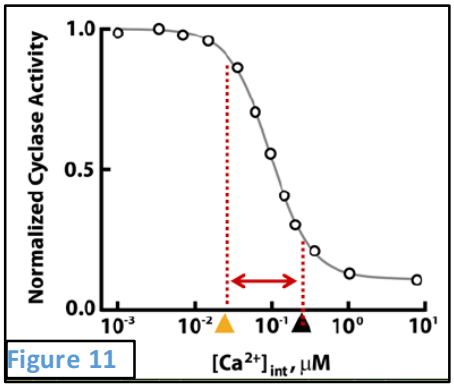
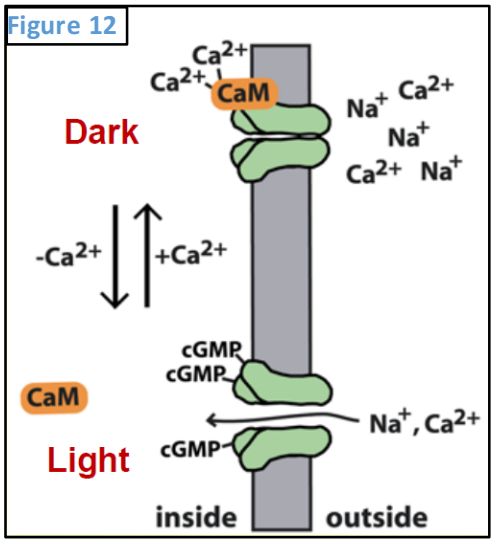


Fig 11: Notice that a small reduction in the amount of calcium causes a tremenduous change in the activity of guanylate cyclase.

- Mechanism VII: *Ca-calmodulin:*

Ca-Calmodulin (CaM) binds the cannels in the dark and keeps *some of them* closed, so not all channels are open in the dark. During visual transduction, when the level of calcium is down, CaM is released and channels open up to allow sodium ions to get into the cell again.

Figure 12



Color vision- Cone cells:

We got 3 different types of cone cells each is responsible for a certain range of wavelengths (we have 3 ranges: Red, green, blue). The wavelengths for the red and green are very close to each other.

- Chromophore is the same of that in rods but the opsin (protein) is different.

If you compare the primary structure (aminoacid sequence) of the opsin in rods vs cones: similarity ~40%

If you compare red and green vs blue ~40% homology

Red vs green >95%homology

i.e. there is a big similiraty between the two and this has important implications.

- The red and green opsins are present on the X chromosome.
- Chromosomes line up together in the metaphase of cell division and that is when genetic recombination happens.
- Because their structure is very similar, the red and green opsins genes could line up over each other resulting in faulty genetic recombination.

What are the important amino acids in the red versus green opsins?

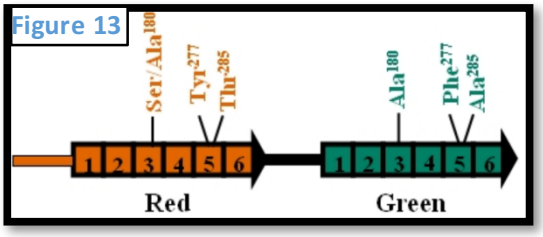


Figure 13

Three amino acids:

- In the red they are serine, tyrosine and threonine: these are polar amino acids with hydroxyl side group.
- In the green: Alanine and phenylalanine; Non polar amino acids.

So if you change alanine to serine you cause a shift of about 10 nanometers in wavelength.

Phenylalanine to tyrosine: another 10 nanometers.

Alanine to threonine: another 10 nanometer. And so green become more like red.

Color blindness:

The blue opsin gene is on chromosome 7.

Red and green opsins genes are on the X chromosome → males are more affected in red green color blindness than women.

Genetic recombination can occur between or within genes (inter vs intra genetic recombination).

- **Inter-genic recombination:**

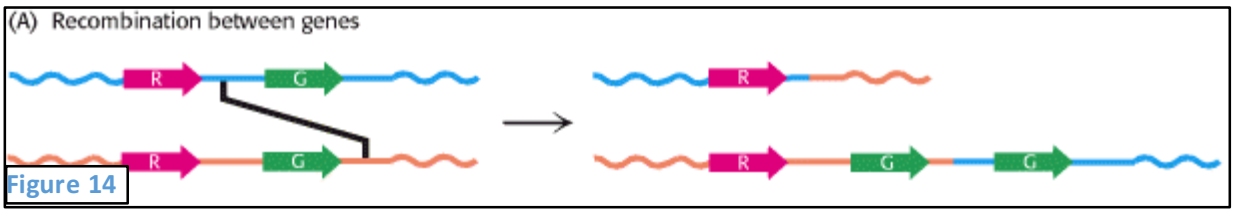


Figure 14

You can have loss of a whole gene, or you can have multiple genes (which would make you no extra good).

- **Intra-genic recombination**:

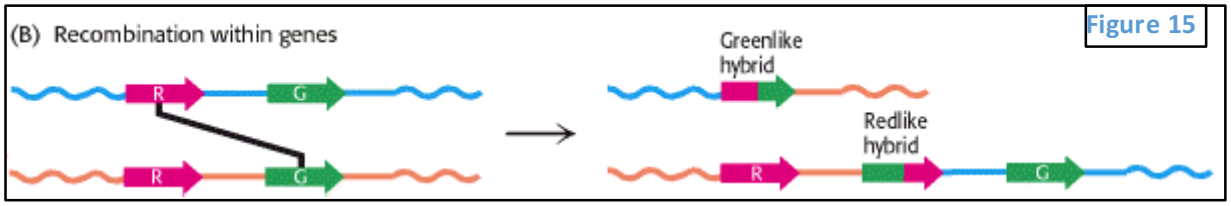


Figure 15

Gives you a hybrid gene; severity depends on how much of the gene you lost.

Final note: Genetic polymorphism plays a role in how we see colors, so we do not all see colors the same exact way.

End of lecture.