Visual Pigments

Today's lecture will be about "How do we see?"
The doctor wrote down some references (slide 2), but said we should rely mostly on the lecture.

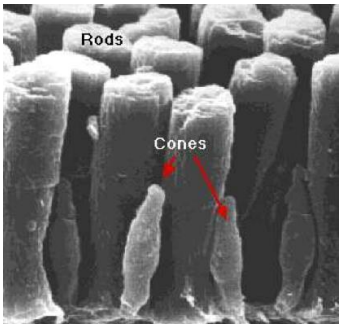
Lecture Outline:

- Mechanism of vision (transduction, cellular players and how they're activated, how the signal is amplified and terminated and why this happens.)
- Color Blindness
- Vitamin A

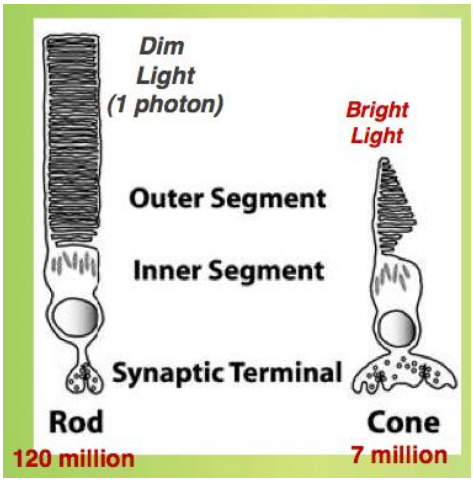
Let's start by noting that we only see a very short range within the wavelength spectrum. However, how do we see these colors? How do we see in the dark? How do we see in the light? These questions will all be answered within the lecture.

There are 2 cell types for vision:

1. **Rod cells** (thin and elongated)
2. **Cone cells**



The cells are given these names because of their shape.



<u>Structure of rod and cone cells:</u>
They have a cell body, which contains the nucleus, mitochondria, vesicles, ER, Golgi and all cellular components. This is also where protein synthesis takes place. The cell body is found within the inner segment. There is also an outer segment made up of many layers of discs, which can reach up to a 1000.

<u>Differences between rod and cone cells:</u>

- 120 million rod cells vs. 7 million cone cells.
- Rod cells are very sensitive to light; they can detect light as low as 1 photon.
- Rod cells are responsible for vision in the dark and cone cells are responsible for vision in the light.

*This is why when you go from a bright room to a dark room, it takes you around 2 seconds to be able to see again. This is because of the timing of transmission from

the activation of cone cells to the activation of signal in rod cells. Note that both of these types of cells are mixed together in the retina and connected to neurons. Rod Cells are composed of discs in their outer segment. In the disc's membranes, all the molecular components that are necessary for vision are found (receiving light, sending a signal and so on).

Signaling in rod cells:
In rod cells there are Na+ gated channels that are always open in the dark. However, when they are stimulated by light, they close causing them to become hyperpolarized. How do they work? In darkness, the channels allow a certain amount of Na+ to go in (Na+ influx). To a lesser extent also, some Ca++ ions can get into the cell via the same channel. This stream of Sodium and Calcium is the basis of vision. At the same time there's an efflux of K+ ions, which results in a balance. Because the channel allows entry of Ca++ and Na+ all the time, glutamate is always secreted in large amounts, which signals to the brain the dark current. When light hits the cells however, these channels close and secretion of glutamate reduces → this is the signal that there is light.

What are the components (molecules) found within the disc?

- Rhodopsin
- Transducin
- Phosphodiesterase
- Na+ gated channels

*All of them are regulated by a number of regulatory proteins
*Rhodopsin activates transducin, which activates phosphodiesterase and phosphodiesterase actives the Na+ gated channels.

Rhodopsin is a receptor (protein) with 7 trans-membrane domains. In the 7^{th} trans membrane domain, you have the attachment of chromophore (a small retinal molecule derived from vitamin A, responsible for the initial signal of light). The 7 transmembrane domains are hydrophobic.

The retinal molecule exists in 2 forms:
1) cis-retinal (11-cis… the double bond between Carbon #11 and #12 are in the cis formation)
2) trans-retinal (4-trans, it has 4 double bonds which are all in the trans formation)

When it is attached to inactive rhodopsin, the retinal molecule is in the 11 cis-retinal form. The cisretinal form has 5 double bonds (the cis part is responsible for the kink). When this molecule absorbs light (transfers the energy from light energy to electronic energy), its shape changes from the cis orientation to the trans orientation making it an all trans molecule. This occurs rapidly in about 200 femtoseconds (10^-13).

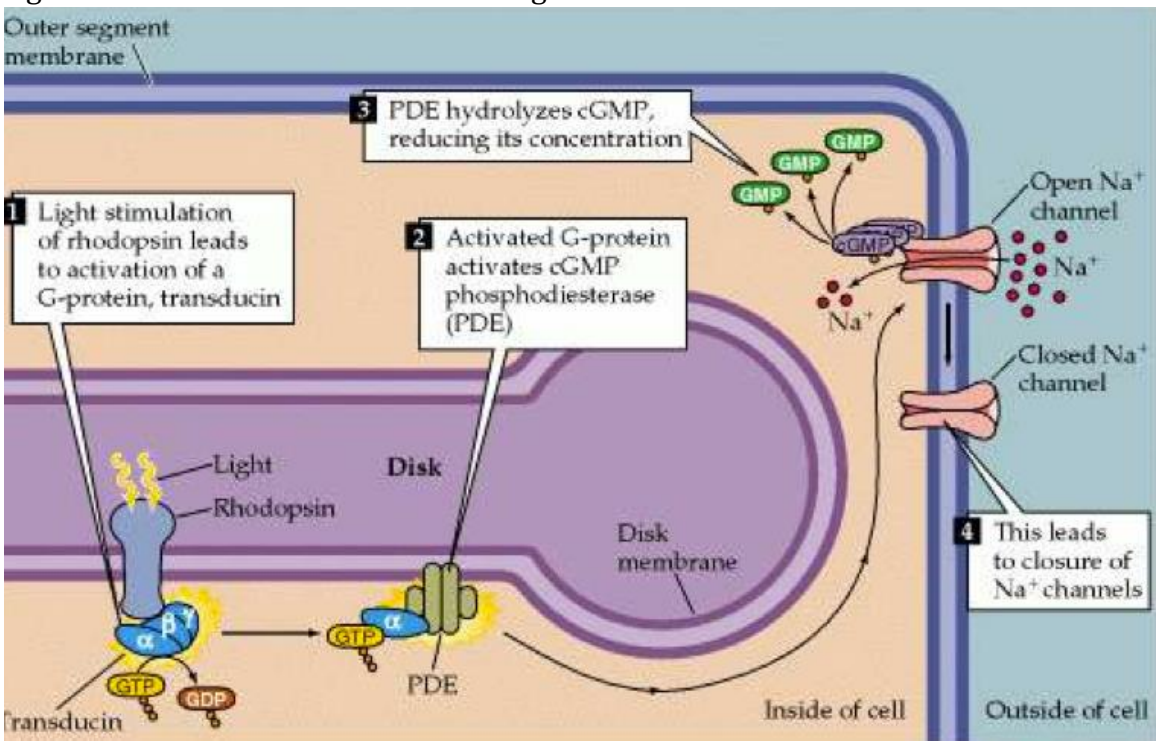
*Remember changing a molecule even in small forms makes a drastic change (deoxhemoglobin→oxyhemoglobin)

The change in the rhodopsin molecule does not follow an on-off mechanism. IT is rather a continuous change depending on the length of the waves of light. Rhodopsin can then be transformed to different conformations (in stages). In each one of these stages, the molecule absorbs light at a different wavelength. For ex, it starts at 498nm→570nm→543nm→477nm, etc. Each change allows the absorbance of light at a different wavelength.

Eventually the trans molecule will be released allowing another 11 cis retinal to bind to rhodopsin.

So in summary, when light is absorbed by rhodopsin, its structure changes from cis to trans. Afterwards, rhodopsin interacts with another protein called transducin (G protein) composed of 3 subunits (alpha, beta, and gamma).

When rhodopsin is activated, it interacts with transducin allowing GDP to be released and GTP bind to it. When GTP binds to the alpha subunit of transducin, the alpha subunit is released from the inhibitory beta and gamma. It then travels on the membrane surface and interacts with an enzyme called cGMP phosphodiesterase. What happens next is that when phosphodiesterase is activated by the alpha subunit, it converts cGMP to GMP. The importance of this reaction is that cGMP is needed for the Na channel to stay open. Therefore, when cGMP is depleted, the channel closes and prevents the entry of Na+ and Ca++ ions to enter the cell. This signal sends to the brain that there is light.



animation: http://www.ncbi.nlm.nih.gov/books/bookres.fcgi/webvision/photomv3-movie1.mov

The thing about rod cells is that they can absorb as little as 1 photon of light, making them easily activated. Now how can 1 photon activate many rod cells? By amplification. For example, 1 rhodopsin molecule can activate 500 transducin molecules. Each one of these transducins however, can only activate 1 phosphodiesterase. This is because the phosphodiesterase depends on immediate interactions with transducin. Each one of these phosphodiesterases can also convert 1000 cGMP to GMP.
There's more to the story however. We have to make the signal transmit easily. This is done by different mechanisms. Now remember that all of these molecules are found within the membrane, so it's different than being in 3D space. If they were found in 3D space, it would take a long time. Therefore it's easier for the molecules to move in 2D space rather than 3D. What also helps the signal transmit easily is that the membrane of rod cells is very fluidic just like oil. It's fluidic because it has a low concentration of cholesterol and a high amount of unsaturated fatty acids.

A single photon is able to close around 200 channels at a time. This indicates how sensitive the signal is.

Signal Termination

There are 7 different mechanisms by which the signal is terminated. Termination of the signal is important for us to be able to see movement. If signal was not terminated efficiently, what we will see is a still image of our surroundings.

Mechanism I: Inactivation of the rhodopsin molecule
Once rhodopsin (the receptor of light) is activated, two things happen.
1) It gets phosphorylated and arrestin binds to the phosphorylated site.
2) Arrestin then immediately inactivates rhodopsin.
*When arrestin bind to rhodopsin, it facilitates and makes it easier for all the retinal trans molecules to be released from rhodopsin (cooperativity).

Mechanism II: Arrestin and transducin distribution
If we look at the rod cell in the dark, what is usually found is transducin on the outside on the outer segment to absorb light and arrestin the inhibitor to be in the cell body (inner segment). But when the signal is activated (there's light), what happens is the reverse. Transducin goes in and arrestin goes out to inactivate the signal.

Mechanism III&IV: GTPase
Transducin has an intrinsic GTPase activity (GTP→GDP) to inactivate the signal. However its activity alone is slow. That's why there's another protein called GTPase

activating protein (GAP) to speed up the process. It's a regulatory molecule, which binds to transducin when it's activated and speeds up the GTPase activity. This facilitates the inhibition of transducin and termination of the light signal.

Mechanism V: Unstable all-trans rhodopsin complex
When 11 cis retinal binds to rhodopsin, the binding is strong. When it changes to the trans form, the interaction becomes weaker (unstable). When the trans forms are all released, only the 11 cis retinal forms stay which inactivate rhodopsin and terminate the light signal.

Mechanism VI UNCLEAR PLEASE REVIEW
There is a role for calcium ion. It is a sodium gated, but there is some calcium that can go in. as Activated cGMP amounts decreases; this will cause the closure of ion channels. Intracellular calcium decreases from 500 nm to 20 nm. This will activate guanylyl cyclase. What happens is that guanylate cyclase is activated which is responsible for synthesizing cGMP. Guanylate cyclase has a protein bound to it which is guanylate cyclase activating protein (GCAP). This protein is Ca++ bound when inactive. Therefore, when Ca++ is decreased, it's released from the activating protein allowing guanylate cyclase to be activated. This produces a lot of cGMP. This restores the amounts of cGMP. The restoration of cGMP will cause the channels to open again. This is what terminates the signal.

Mechanism VII: Ca-calmodulin
Now remember channels must be open in the dark, but that's not always the case. There's a protein called calmodulin (its activity is modulated by Ca++). When calmodulin is bound to Ca++, it binds to the channel and closes it. This terminates the signal. Now, when intracellular Ca++ decreases, it doesn't bind to calmodulin and calmodulin can't bind to the channels, therefore making the channel open up and allowing Ca++ to reenter the cells to reactivate the signal again.

**THERE WILL DEFINITELY BE A QUESTION ON THE MECHANISMS

Color vision

There are only 1 type of rod cells, and 3 types of cone cells. These cone cells are responsible for color vision. There are different types of cone cells because they have different protein compositions. These proteins like rhodopsin however, have the same chromophore (11 cis retinal). Because of their different amino compositions, they can absorb light at different wavelengths. The range of absorption of light in one cells is less than the range in rod cells.

Types of cone cells:
1) Blue (absorbs short wavelengths)

2) Green
3) Red (absorbs long wavelengths)

Each one has its own peak, and there's an overlap between the 3 different ranges. For example, if we absorb green light all 3 of them will be activated but to different extents. Meaning their overlap and combinations are what allow us to see different colors.
*Notice that cone cells are connected to neurons and that is how the signal is transmitted to the brain.

Absorbing light at different wavelengths depends on the differences in the amino acid sequences in rhodopsin. The cis and trans retinoic acid are the same for both cones and rods. For example, if we compare between the red and the blue rhodopsin, we find 40% of amino acid sequences are identical. Also, between the green and the blue rhodopsin, we find 40% of amino acid sequences are identical as well. However, the green and red are 95% identical in their amino acid sequences. This is why they absorb light very closely to each other because of their huge overlap.

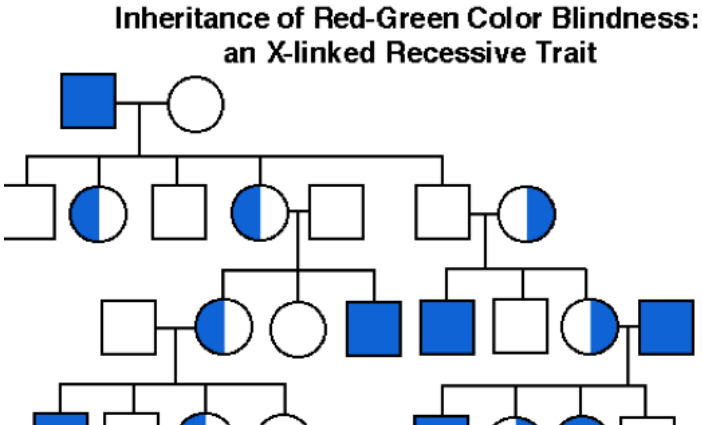
Differences between the red and green cones exist mainly in 3 amino acids present in the 7th transmembrane domain. In the green transmembrane domain, we have alanine180, phenylalanine, and alanine285. In red, its serine or alanine, tyrosine and threonine. The difference between these red and green amino acids is that three of them have hydroxyl groups (in the red type).
Note that if you take alanine from green and add a hydroxyl group to it, you will shift the absorption by 10 nm to a longer wavelength.

Remember, rod and cone cells differ in number, structure, light absorption, photoreceptors. However, they have the same chromophores.

There's a difference between sensitivity and sharpness when it comes to rods and cones. In the dark, the image you see is fuzzy and not sharp, while its sharp and has a high resolution in light. This is because in rod cells, there are about 10 or a 100 rod cells connected to only one neuron. However, in cone cells each cone cell is connected to a neuron. When the brain gets a signal in bright light, the signal comes from the red cone cell and your image is sharp. In rod cells however, when the many cells send a signal to the brain, the signal is strong but it doesn't know where exactly it came from and that's why its not sharp. Note also that rod cells are more sensitive than cone cells and that's because of their great amount and strong signal.

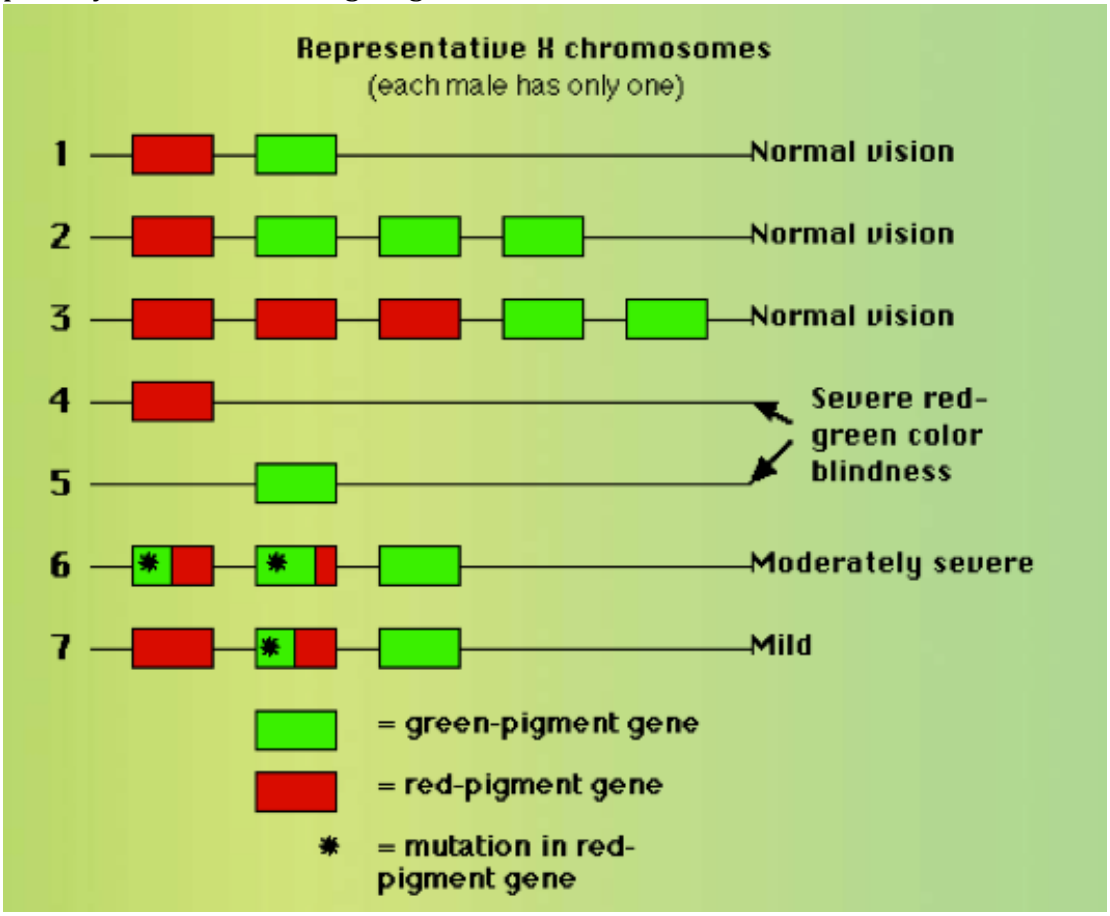
Color blindness

The blue opsin gene is found on chromosome 7. However, the



green and red opsin genes are found on the X chromosome. This makes males more prone to color blindness and that's why it affects males at a higher ratio.

Because red and green are 95% identical, they can combine with each other, Therefore, you can end up with an X chromosome that lacks the green gene or have 2 of the green genes, and you can also get combination of both. The effects are portrayed in the following diagram:



Note that there is severe red-green color blindness only when one of the opsins is deleted. Otherwise when you have 2 green opsins or more, the person will have normal vision. Also, when there is a combination of both, the color blindness will be moderate or mild, depending on the amount of combination.

There are individual differences in color perception because of single nucleotide polymorphisms. This is because nucleotide number 180 on the red gene differs from one individual to another. The resulting amino acid will be either serine or alanine. If you have serine (560nm), you will see the color red. And if you have alanine (530nm), you will see the color green.

Vitamin A

Vitamin A (retinol) is obtained from our diet (ex. B-carotene).
There are 3 forms of vitamin A:
1) 11-cis-retinal → contains an aldehyde group
2) Retinol → contains a hydroxyl group
3) Retinoic acid → contains carboxylic acid

Retinol is esterified by the attachment of bile acids. It is then carried by intestinal cells to liver cells by chylomicrons and stored there. When it is needed, it is carried by retinol binding protein from the liver cells to the different tissues. In tissues, it can be processed differently. In some target cells, retinol is converted to retinoic acid (it's a hormone, can change gene expression and be a carcinogen in some cases). In the retina, it's converted to retinal (11 cis retinal).

Deficiency of vitamin A may cause night blindness, hyperkeratinosis, susceptibility to infections, and anemia. Prolonged deficiency leads to eye tissue damage.

Please take a look at the slides, as the diagrams will help you understand better, specifically the mechanisms.
Thank you.