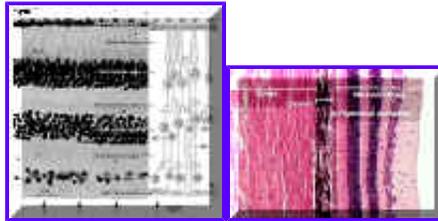


Anatomy and Physiology of the Retina

Retinal Microstructure

The figure to the left below is a light micrograph illustrating a cross section of the retina. To the right are drawn in complete outline a few of the cells whose cell bodies only are visible in the micrograph. The micrograph and drawing illustrate that the retina is organized both vertically (in columns) and horizontally (in layers). The principal "vertically oriented" elements are receptors (rods and cones), the bipolar cells and the ganglion cells. The "horizontally oriented" elements are the horizontal cells (labelled HC in the diagram) and the amacrine cells (labelled AC). Miscellaneous (and for our purposes less important) retinal cells include the interplexiform cell (not shown) and the Muller cell (MC).



The human retina is approximately 0.2 mm thick, and has an area of approximately 1100 mm² (about the size of a silver dollar). Each retina possesses about 200 million neurons. Note that light impinges on the retina from below in the diagram.

- Inner limiting membrane (ILM): is the boundary between the vitreous humor in the posterior chamber and the retina itself.
- Ganglion cell layer: comprises the cell bodies and axons of ganglion cells.
- Inner plexiform layer (IPL): contains the synapses made between bipolar, amacrine and ganglion cells. The thickness of this layer varies considerably across species, where "simpler" organisms (such as frogs, pigeons and squirrels, for example) possess thicker IPL's than "higher" organisms like primates. The thicker IPL indicates that these retinas perform more peripheral and specialized image processing.
- Inner nuclear layer (INL): contains bipolar cell, horizontal and amacrine cell bodies.
- Outer plexiform layer (OPL): contains bipolar cell, horizontal cell and receptor synapses.
- Outer nuclear layer (ONL): contains the nuclei of photoreceptors.
- Outer limiting membrane (OLM): a membrane which coincides with the base of inner segments of photoreceptors.
- Photoreceptor layer: contains the inner and outer segments of rod and cone photoreceptors.
- Pigment epithelium (PE): darkly pigmented cells which absorb light not captured by photoreceptors, thus reducing scattering; also plays a role in "trimming" photoreceptors -- cones are "trimmed" at dusk, and rods are "trimmed" at dawn-- how convenient. Diurnal species (active in bright light environments) typically possess dark PE's; nocturnal species (active in dim light environments) possess an adaptation called a tapetum. the tapetum is a mirrorlike layer behind the photoreceptors which reflects photons not captured by the photoreceptors back out the eye, thus giving the receptors a "second chance" to capture them. Sensitivity to light in these animals is thus increased by approximately twofold. The dominant wavelength of light reflected by the tapetum is usually close to the absorbance peak of rhodopsin (the photopigment contained by rods). Thus, the "eyeshine" seen in deer, opossums, dogs and the cat below appears greenish to us. (On the left below is our very

tolerant shiny-eyed cat, Alexandra (or "fur-creature" as she is nicknamed, a Russian Blue, in a Halloween costume -- some sort of furry wizard I think -- conceived and designed by my two daughters). On the right is Jen Nodes' (incredibly cute) nephew -- illustrating the phenomenon of "red eye" (see below for explanation)..

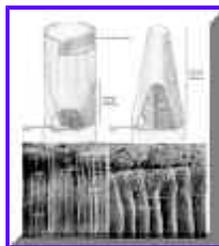


- Choroid: highly vascularized layer which supplies nutrients and oxygen to the retina. The eyeshine sometimes observed in flash photographs of humans is caused by light reflected from the choroid layer. The hue is red because oxygenated blood absorbs light of shorter wavelengths.

The vertebrate retina is said to be "inverted", because the photoreceptor layer is actually the furthest from the pupil. In other words, light must travel through all the layers of neural tissue just described before reaching the photoreceptors, which are the only cells in the retina which transduce light energy. Fortunately the neural retina is nearly invisible (being mostly water), and as we will see, nature has "cleared a path" for light reaching the foveal receptors, where our vision is best.

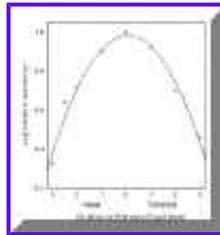
Rods and Cones

- Outer Segment: Rod and cone photoreceptors are easily distinguished by their outer segments. The outer segment contains photopigment in free-floating disks (rods) or folded layers (cones). Cone outer segments have a continuous outer membrane, whereas rods have discs, stacked like coins, in a sleeve. The rod and cone outer segment membranes are constantly being replenished (like fingernails, they just keep growing). This is why the pigment epithelium must trim off the excess, a process known as [phagocytosis \(movie from Kolb, Fernandez & Nelson\)](#).



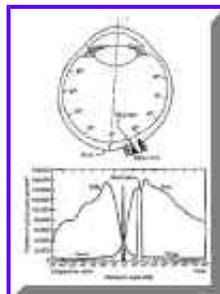
- Inner Segment: Photoreceptor inner segments contain the nucleus, support organelles (mitochondria, ribosomes, endoplasmic reticulum, synaptic vesicles, etc), and the axon terminal (where neurotransmitter is released). The capture of individual photons by the photopigment molecules in the disk membranes is what initiates neural signalling. Photoreceptors are actually specialized hair cells, and the inner and outer segments are connected by the cilium.

- Stiles-Crawford Effect: For light to reach the outer segment (and be absorbed by a photopigment molecule) it must first pass through the inner segment. Cone inner segments are actually exquisitely engineered waveguides (i.e., fiber optic structures) which capture light and funnel it into the outer segment. Because these inner segment waveguides capture light shining straight on them better than light from shallower angles, we can measure what is called the Stiles-Crawford effect, published in 1933. The Stiles-Crawford effect refers to the fact that that cones are more sensitive (by a factor of 10) to light which enters the eye from the center of the pupil (axial light) than we are to light entering from the margins of the pupil (off-axis light). This is good, since light which enters through the center of the pupil forms sharper images than light which enters from the sides of the pupil (because the eye's optics are much better there). This evolutionary strategy of "ignoring" (by being less sensitive) the blurred image produced by off-axis light in favor of the sharp high-contrast images produced by axial light only works, however, if you've got lots of light to begin with. This is generally true for cones, since they operate best under high luminance conditions (i.e., daytime). Rod photoreceptors, on the other hand, are designed to operate under dim luminance conditions (i.e., night) where photons are scarce. Rods must make every photon count, they can't afford to throw any of them away, and hence are not directionally selective like the cones.

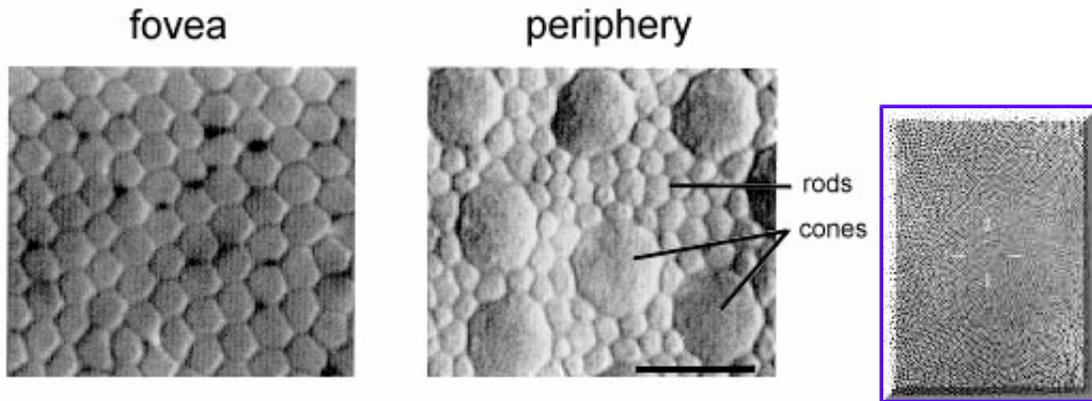


Distribution of Photoreceptors

The human retina contains approximately 120 million rod and 1 million cone photoreceptors. The figure below illustrates that the distribution of photoreceptors across the retina is not uniform. Cone density is highest at the fovea, where recent estimates place it considerably higher than the figure above suggests -- approximately 300,000/mm²; rod density is highest at about ± 18 degrees eccentricity. Rods are actually absent in fovea (which is why dim stimuli, such as stars, cannot be seen when gazed at directly, but become visible when images slightly eccentrically -- to the side -- where rods are plentiful). Primates and birds have true foveas (some birds, notably hawks and eagles, actually possess two foveas per retina!); other species such as squirrels, cats, dogs, deer, etc., have a less dramatic regional specialization, and possess what is called an area centralis or a visual streak.



Foveal cones are very tiny (2.3 micron in diameter) and are packed very close together (2.5 micron intercone spacing) in a hexagonal (honeycomb) matrix. The image labelled "fovea" below shows a cross section through the human fovea at the level of the inner segments. The dense hexagonal packing is obvious. With increasing distance from the fovea the cones become larger and are packed less densely (shown in rightmost figure). Rod photoreceptors fill up the spaces between cones, as illustrated in the image labelled "periphery". Despite the high density of foveal cones, the small area of the fovea means that only about 1% of all cones are contained in the fovea.



scalebar on central image=10 microns

Photoreceptors, Sampling and Aliasing

[Moire patterns](#)

Phototransduction

Photopigment contained in the disk membranes of the outer segment absorbs photons and undergoes a biochemical change. Photopigment is a complex of two molecules: opsin and the chromophore. Opsin is a protein; the chromophore is the part affected by light -- called retinal (a derivative of retinol, i.e., vitamin A, which is why your mom encouraged you to eat carrots).

When retinal is bound to opsin it is in the so-called 11-cis configuration (i.e., the molecule is "bent"). When energy is absorbed by the chromophore (in the form of a photon) it "unbends" the molecule, and converts it to an all-trans configuration. This process is called photo-isomerization. Ultimately, the all-trans isomer is converted back into the 11-cis form so that vision is possible again.

[Kent Wilson, photisomerization, the movie](#)

The isomerization of 11-cis retinal to all-trans begins the process of phototransduction. Interestingly, in contrast to other neurons, the result of transducing light energy is photoreceptor **hyperpolarization**. The exact chain of events is: isomerization of photopigment

breaks apart a molecule called transducin, which activates an enzyme called phosphodiesterase. Phosphodiesterase, in turn, breaks cGMP into its inactive form, which causes Na⁺ channels (which are open in the resting state) to close. Closing Na⁺ channels hyperpolarizes the neuron. Light stimulation thus causes **less** transmitter to be released at the synapse!

The hyperpolarization of the outer segment spreads to the inner segment by electrotonic conduction. Since receptors are so small, the receptor potential is still large at the axon terminal in the inner segment. Thus, most retinal neurons transmit information using only graded potentials. Some amacrine cells and all ganglion cells use action potentials.

[Kolb, Fernandez & Nelson, phototransduction, the movie](#)

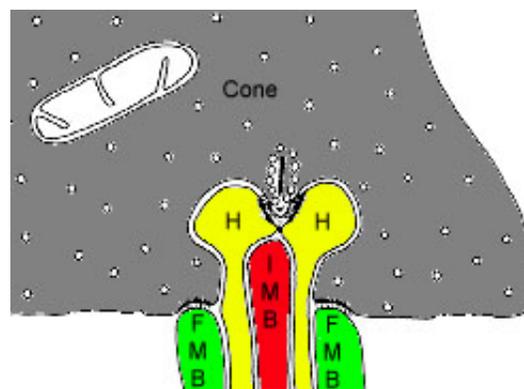
Retinal Neuron Response Properties

[Mapping rf's](#)

The outer plexiform layer is where photoreceptors make synaptic contact with both bipolar cells and horizontal cells. There are eight different types of cone bipolar in human retina. Five of these are called diffuse bipolars and make synaptic contact with many cones (up to 20). The other three types contact only single cones and are called midget bipolars (MBs).

On- versus Off-Responses

As illustrated in the figure below, cone pedicles possess invaginations in which triple synapses between the cones, bipolar cells and horizontal cells occur. **Horizontal cells (H)** are both pre- and post-synaptic to photoreceptors (meaning that they make synapses on to photoreceptors, and photoreceptors make synapses onto them). Bipolar cells are postsynaptic to both receptors and H-cells. Cones also make contact with bipolar cells at non-invaginating (flat) synapses.



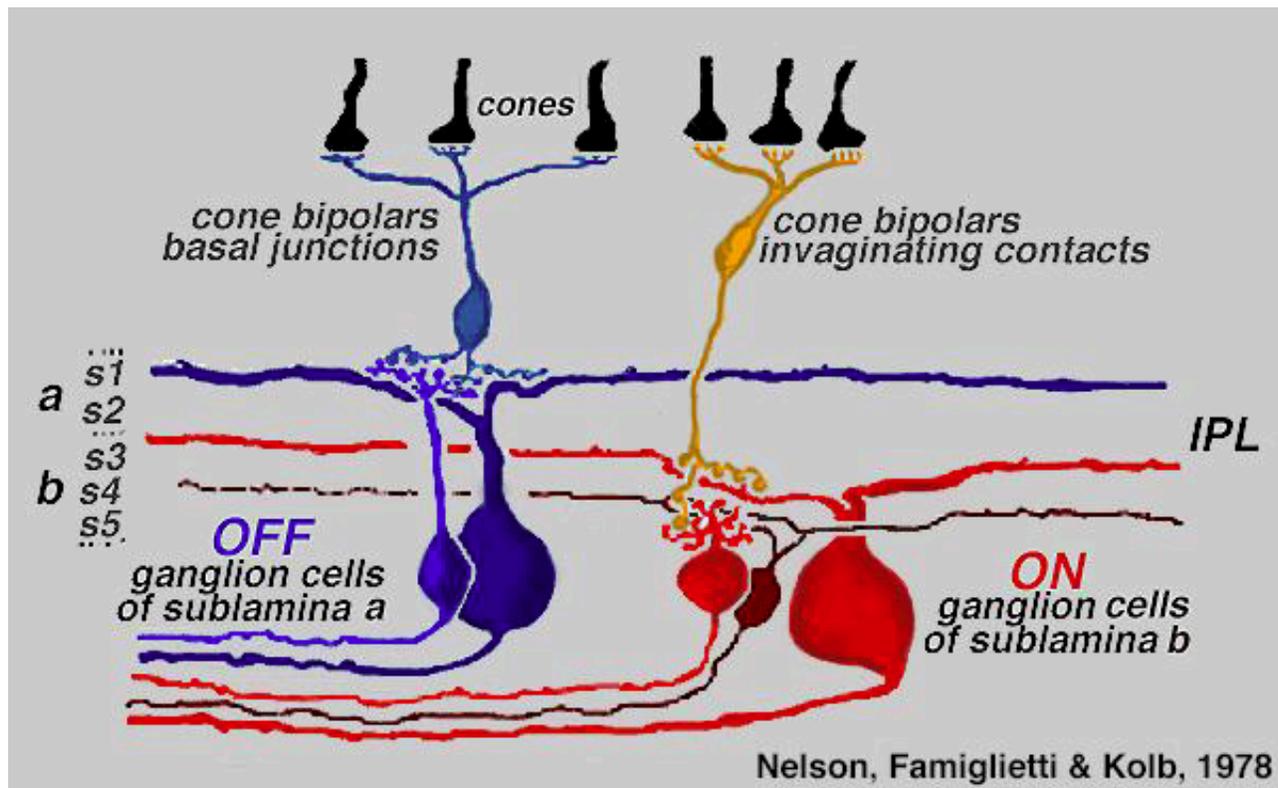
There are two types of midget bipolar which make different types of contact with the cone pedicle (pedicle is another name for the cone axon). The **invaginating midget bipolar (IMB)** contacts the cone pedicle as an invaginating dendrite sandwiched between **horizontal cells**. **Flat midget bipolars (FMBs)** contact the cone pedicle at its base, usually just to either side of the triad synapse.

The transmitter released by photoreceptors is glutamate (an excitatory amino acid)

neurotransmitter). Keep in mind that photoreceptors are depolarized in the dark (and, like typical neurons, they release transmitter when they are depolarized). When stimulated by light the photoreceptor hyperpolarizes, and the rate of transmitter release decreases. Bipolar cells, however, respond to light with either hyperpolarization or depolarization of their membranes. The **flat midget bipolar (FMB)** hyperpolarizes to light (like the receptor), and is said to possess an **off-center response**. The **invaginating midget bipolar (IMB)** has a sign-inverted response, and depolarizes to light. It is said to possess an **on-center response**. The different responses of these types of bipolar cells is due to different types of post-synaptic glutamate receptors. Whether visual neurons inhibit (off-pathways) or excite (on-pathways) to light is, as we shall see, a fundamental property of the responses of all visual neurons. This property has its origin in the very first visual synapse, that between photoreceptors and bipolar cells.

[Kolb, Fernandez & Nelson: movie illustrating cone \(& horizontal\), invaginating and flat bipolar cell responses](#)

The flat midget bipolar cells (off-responses) contact ganglion cells in sublamina a of the inner plexiform layer, whereas the invaginating midget bipolars (on-responses) contact ganglion cells in sublamina b. The on- and off-type bipolar cells confer their response properties to the ganglion cells -- thus, there are two types of ganglion cells: those possessing on-responses and those possessing off-responses.



[Kolb, Fernandez & Nelson: movie illustrating on- and off-center ganglion cell responses](#)

[Up one level](#)