INTRODUCTION

Vision is an important topic in neurobiology, for both intellectual and practical reasons. Primates are peculiarly visual animals. In fact, there was a time when taxonomists used the characteristics of the visual system as one way to define a primate: primates have expanded visual centers. Partly because of that, we depend heavily on our vision. A blind rat doesn't do too badly; it uses its whiskers to navigate and never depends strongly on vision. A blind person is very badly handicapped. People are acutely aware of their vision and are sensitive about it. A whole medical specialty -- ophthalmology-- is concerned with curing and preventing diseases that affect vision.

Vision is also important in this course because, in effect, vision will stand for many other systems. Lectures on somatic sensation and hearing will concentrate on the periphery. You heard in anatomy that the cortex has layers, but we haven't said anything about what the cortex does. Rather than go into the details of cortical function for each of those systems, we are going to let vision illustrate the generalized function of the cortex. It turns out that the cortex is really quite uniform in its fundamental organization, so that the principles that one sees in vision are also correct principles for somatic sensation, for audition, and for that matter, for the motor system.

OVERALL STRUCTURE OF THE EYE

Vision begins with the retina, which lines the back of the eye. The retina curves around quite far anteriorly--as anyone sees who tries to look at it through the pupil with an ophthalmoscope. It is easy to see in the back of the eye but hard to see at the sides.

Images are formed on the retina by the optics of the eye. The refractive power of the eye is divided into two components. The cornea--the front surface of the eye--accounts for about 40 diopters of magnification, and the lens accounts for about 20. This is a very powerful lens system, with a short focal length. We vary the refractive power of the eye by varying the focal length of the system. That is done by muscles that pull on the edge of the lens and change its curvature. When the muscles are relaxed, the lens gets fatter. When the muscles are pulling, the lens gets thinner. That changes the focal length of the system. (The focal power of the cornea can't be voluntarily changed. It may change as people get older and can be artificially changed by laser keratotomy. That's part of the reason why one's near vision gets worse with aging; the other is that the lens gets stiffer, so it is less readily bent by pulling on it.)

Since the two lenses (the cornea and lens proper) are close together, they act as a single lens. The image, therefore, is inverted on the retina. It is also turned right for left. That is important for clinical diagnosis of brain injury, because one must remember the effects of the eye's optics in figuring out where lesions happen inside the brain.

The chambers of the eye are filled with fluid. The anterior chamber is filled with a saline fluid called aqueous and the posterior chamber is filled with a gel, with the approximate
consistency of Jello, called the vitreous. Because of its stiffness the vitreous may be important in keeping the retina closely apposed the back of the eye. The aqueous is a flowing solution that nurtures the inside of the eye (somewhat like eye lymph).

BASIC CIRCUIT OF THE RETINA

There are two types of photoreceptor cell, called rods and cones. There is an intervening cell called the bipolar cell, and there is an output cell called the retinal ganglion cell. The retina, as it turns out, is positioned in the eye “backwards”. The photoreceptor cells (the rods and cones) face back toward the outer layer of the retina, the sclera. The sclera is a tough, semi-rigid structure that contains much collagen and has the consistency of very fine shoe leather. It forms a rigid protective coat; and it also helps contain the intraocular pressure. The inside of the eye is under a slight pressure. If the outside of the eye were distensible, then it would be hard to keep the length of the eye constant in the face of any changes of pressure--and the image would often be out of focus. In between the sclera and the retina, there are some supportive tissues. I will return to them later, because the interplay between the retina and the surrounding structures is critical to important pathologies of the retina. Retinal ganglion cells are the cells whose axons form the optic nerve. They line the anterior surface of the retina. Their axons run across the retinal surface and then join together to form the optic nerve.

I will come back later to the reasons why the retina is arranged as it is. The arrangement means that light has to pass through the layers of retinal circuitry before it reaches the light sensitive elements. It can do that because the retina is very thin. In man, the retina is around 200 micrometers thick. The retina itself is basically translucent. When you look into the eye with an ophthalmoscope, you can see at least the crude appearance of the underlying tissue--and that tells you right away that light can shine through the retina without major interruption. After passing through the retina, light is finally caught by the rod and cone photoreceptive cells.

PHOTORECEPTOR STRUCTURE

Rods and cones are slightly different and I will later consider those differences. The basic principles of phototransduction that we see in rods are also true for cones. Figure 4 shows the overall structure of a rod photoreceptor cell. This region is called the outer segment. The “outer segment” of a rod contains disks of membrane, enclosed by an envelope of plasma membrane. These are the structures which contain the light sensitive pigment, which in rods is called rhodopsin. The synapse of the cell is at the other end. The inner segment is a supportive structure. It contains many mitochondria, and the endoplasmic reticulum. Protein is synthesized in the inner segment and membrane components -- protein inserted into phospholipid-containing vesicles-- are synthesized there. They are exported to the outer segment of the rods.

New membrane is added to the rod outer segment at the base. The disks are formed by in-pinching of the outer segment membrane. The newly synthesized membrane, containing rhodopsin and phospholipids, can't move directly into the disks. Instead, what happens is that the vesicle fuses with the plasma membrane. The new membrane then flows up the plasma envelope. Then the envelope is thrown into a fold, after which the membrane pinches off, resulting in a free floating disk inside the outer segment.

If that kept on happening, the outer segment would get longer and longer without limit.
Instead, the outer segment membrane is renewed by an unusual and virtually unique process in which the tip of the outer segment pinches off. The membrane again infolds itself to form two separate entities. One is the main structure of the rod and the other is the pinched off tip. In a primate, about 10% of the rod's length is shed every day. As a consequence, the disks migrate from proximal to distal. When old material is pinched off, it gets phagocytosed by the retinal pigment epithelium, which is a neighboring tissue.

This unusual and exotic structural machinery is important for phototransduction. To understand why, realize first that the disks drawn in textbooks — little oval things — are a gross distortion of the actual structure of a rod. The rod disks are extremely thin. You can barely see in an electron microscope any space between the disks. The outer segments really look more like a stack of CD’s, roughly 10 feet high.

Why might you want to do that? Remember that rhodopsin is contained mainly in the disks. The effect of the outer segment’s arrangement is to expose a huge surface of light sensitive membrane to the world. Not only is the rod specialized to contain lots of membrane, it is specialized to contain lots of membrane in a very narrow space. Remember that light enters the rods and cones from their ends. A long tube allows lots of membrane to be contained within tightly packed individual elements.

PHOTOTRANSDUCTION
How does a rod or cone send a signal about light? The subject is called phototransduction — energy from light is transduced into an electrical signal. The problem is how light can be caught by rhodopsin and end up making a signal come out of the other end of the cell at the synapse. I will start with some history, because it may make it easier to remember how these cells work.

INTRACELLULAR RECORDING FROM CONES
When Tomita penetrated individual cones and flashed light, what he saw was that the membrane potential of an individual cone rested at about –40mV. It was somewhat depolarized. When he flashed light he found that the cone cells hyperpolarized. This was a counterintuitive result, initially hard to believe, but turned out to be correct.

EXTRACELLULAR RECORDING FROM RODS
The correctness of Tomita’s experiment was confirmed by extracellular recording of currents that flow alongside the rod photoreceptor. Since the resistance of the extracellular medium is not zero, it is possible to measure currents flowing radially beside the cell. This is done by placing one electrode near the end of the outer segment and another near the inner segment. It was found that a substantial current flows along this path. The current is mediated by cations (primarily sodium) entering the outer segment, flowing along the length of the photoreceptor cell intracellularly, and being pumped out of the inner segment.

Because this current is largest in the dark, it was called the “dark current”. The dark current is interrupted by light; less cation enters the outer segment. Because an influx of cation is interrupted, the inside of the cell becomes more negative when the cell is stimulated by light — just as was reported from intracellular recording.
The magnitude of the dark current is remarkably large. It has been calculated that the dark current replaces all the intracellular sodium in a rat rod every six seconds. A rod photoreceptor cell is more active metabolically, on a per weight basis, than active muscle. For this reason, the dark current of the photoreceptor cells dominates the retina’s overall metabolism, accounting for approximately 80% of it. As will be discussed below, one reason for this large current to exist is that it allows great amplification of signals initiated by photons.

It may seem odd that rods and cones hyperpolarize in response to light, since “excitation” traditionally is associated with depolarization. However, the paradox that hyperpolarization is supposed to be inhibition, not excitation, is only semantic. Later you will learn how these cells signal to the rest of the retina. For the moment, don't worry about the fact that the response is hyperpolarization. Just remember that a cell can signal either by depolarizing or by hyperpolarizing. It doesn't matter as long as the cell changes its rate of neurotransmitter secretion.

THE PHOTOTRANSDUCTION CASCADE

The outer segment contains disks that contain rhodopsin and the disks are suspended within the outer segment. But, we have said that the effect of light is to interrupt the sodium current. How can that happen if the rhodopsin is contained inside the outer segment? Somehow a signal has to get from rhodopsin, which is contained in the membranes of the outer segment disks, to the plasma membrane, which is where the sodium current is entering.

When light hits rhodopsin, its 11-cis retinal undergoes a conformational change to all-trans retinal. This is the first step in phototransduction. When 11-cis retinal becomes all-trans retinal, rhodopsin becomes enzymatically active. It catalyzes the exchange of GDP for GTP on a G protein called transducin. Transducin then diffuses within the membrane. Transducin alpha GTP diffuses within the membrane and binds to a phosphodiesterase. The phosphodiesterase then becomes enzymatically active. It removes an inhibitory subunit from the phosphodiesterase and the phosphodiesterase can hydrolyze cyclic GMP to 5’ GMP. The flow of sodium into the outer segment is controlled by a cyclic GMP gated cation channel. The channel is held open by cGMP. When the concentration of cGMP falls, the channel closes, decreasing the dark current.

Something has to turn this sequence of events off again, or else the cascade would be permanently activated. The way that is done is still only partly understood, but some of the steps are known. Almost as soon as transduction starts to happen, the system starts turning itself off again. It does it in at least two different ways, which are shown here. The first is that the cytoplasm of the rod outer segment contains a kinase, Rhodopsin kinase, which phosphorylates activated rhodopsin. It won't phosphorylate native rhodopsin. But as soon as rhodopsin is activated, rhodopsin starts getting phosphorylated. Phosphorylated rhodopsin can bind another soluble protein, arrestin. At that point it can no longer bind to transducin. At the same time, transducin contains an intrinsic GTPase activity. So, it spontaneously reverts to transducin alpha GDP which cannot activate phosphodiesterase.

AMPLIFICATION

Rods can sense single photons, and send a message about them to the next cell. To do that, some kind of very major amplification is needed and that amplification occurs because the
reactions I have just been telling you about are reactions in which the signaling molecules act as enzymes. Rhodopsin can catalyze the exchange of many GTPs for GDP. In a second -- in the one second following the absorption of a single photon by a single molecule of rhodopsin -- a hundred molecules of transducin alpha GTP are created. Those then bind to phosphodiesterase, without further amplification. But, phosphodiesterase when activated catalyzes the hydrolysis of about 1000 molecules of cyclic GMP. So the total molecular amplification is around $10^5$ molecule per second.

Another more global way to think about this is just as energy gain. The cell uses a lot of energy to run the dark current. A smaller amount of energy (a photon) gates a much larger one.

CONE STRUCTURE

Cone structure are structurally different from rods and they have important differences in physiology. But their fundamental mechanisms, as far as we know, are the same as those in rods. The most striking difference between rods and cones is in the structure of the outer segments. The outer segment of a cone, instead of having enclosed disks has membrane infolding. What that means is that the membrane simply comes back and forth like this, so it forms a continuous membrane throughout. The cone, like the rod, is exposing a large surface to the world, but not nearly as large as the rod. It is not as important for the cone to expose a huge area to the world because the cone works under conditions in which there is lots of light.

DIFFERENCES BETWEEN RODS AND CONES

The physiological differences between rods and cones really are three. One is that the cone works more quickly than the rod. The response of a cone, also shown, is initiated more quickly and recovers more quickly. At one time, we used to think that this was because the cone outer segment is smaller and because the membrane that contains the sodium channels is the same membrane that contains the cone pigment. The diffusing distance is less and that might explain why it is quicker. However, it turns out that the difference in the diffusing distance isn't enough to matter very much. More likely, different speeds result because their cyclic GMP gated channels are different. Recently it has been discovered there is a different gene for the cation channel in cones and in rods; it may account for some of the differences in timing.

Another difference, and one of the most important ones, is that the sensitivity of rods and cones is different. The working range of vision is gigantic, around $10^8$. The way the retina spans this range is partly to divide the work between two different kinds of photoreceptors, rods and cones. Rods are dim light receptors and cones are bright light receptors.

The third difference between rods and cones is that cones don't contain rhodopsin. They contain different photopigments collectively called cone opsins. A very small amino acid substitution can create the differences in spectral absorption that make the difference between a rod, which contains a single pigment and a cone, which contains one of three different pigments. They get called short, middle, and long wave lengths by purists. You can call them blue, green, and red. The pigments have different absorption maxima and one cone contains only one pigment. An individual cone might contain the blue pigment, the green pigment, or the red pigment, but it won't contain more than one.
The way the brain computes color is by comparing the inputs from the three classes of cones. You compare the input of the red cones to the inputs of the green cones or the input of the blue cones with the longer pair (red and green, which equals yellow). People who are “color blind” are missing one (usually) or more (rarely) of the three cone opsins. Rods are dim light detectors and contain a single pigment. That is why you don't see colors in dim light -- because you can't unambiguously specify wavelength with only one pigment.

THE TOPOGRAPHY OF RODS AND CONES

The different sensitivities of rods and cones to different intensities of light, together with the different color sensitivities of cones, are important determining factors for vision. The third critical fact is the differential distribution of rods and cones across the retina. In the central retina, cones are very dense. In fact, the central retina contains a zone, the fovea, in which rods are entirely absent. Moving away from the fovea, rods begin to be intermixed and by the time one is 4-5 mm away from the fovea, rods predominate. Cones are present throughout the retina, but their density rapidly falls to a low level, roughly 1% of the level at the peak of the fovea.

Vision is very bad in the retinal periphery, because of the falling density of cones. Under ordinary lighting conditions (ordinary room light, for example) the rods are not useful. Rod function reaches saturation at a level of light below ordinary room light. At that intensity, the rods are responding maximally, so that changes in light intensity cannot modulate their membrane potential. Even though your retina has many rods in the periphery, they are useless because at ordinary indoor lighting levels they are always saturated. That means that your vision in ordinary indoor lighting conditions is served entirely by the cone system, and the cone density falls substantially toward the periphery.

If that is so, why can you not see sharply in the periphery under dark-adapted conditions? To understand the reason, we need to understand the subsequent processing of information by the retina. The total density of rods and cones is roughly constant across the retina—nature has packed as many photodetectors as it can into the retinal surface at all eccentricities. In the periphery the space between the now-sparse cones is filled with rods, each of which synapses on bipolar cells. However, the density of ganglion cells is low in the periphery: many bipolar cells synapse upon an individual ganglion cell. Visual acuity is determined by the packing density of the least dense element in the chain. In the peripheral retina, the ganglion cells become sparse, and acuity is low – because the activity of many rods and cones is pooled at the level of single ganglion cells. Since the density of ganglion cells becomes limiting in the peripheral retina, it does not matter in the periphery whether you are using your rods or your cones – vision will be equally poor.

PHOTOTRANSDUCTION DOMINATES THE RETINA'S STRUCTURE AND METABOLISM

As was noted earlier, the energy cost of phototransduction is very high. This probably explains some of the retina’s specialized features and is important in pathological conditions affecting the retina.

The apparent reason for the retina to be positioned in the eye “backwards” is to get the photoreceptor cells close to their supply of oxygen and substrates. They need a rich perfusion because of their high rate of metabolism. However, were they to get a conventional perfusion,
via penetrating blood vessels, there is the disadvantage that blood scatters light. The blood vessels would create lacunae in vision, where they interrupted the light path. Instead, a solution is to give the photoreceptors a separate circulation and to place it behind the photoreceptors, after light has passed through the photoreceptors. This circulation is called the choroidal circulation. It consists of a rich, anastomosing supply of vessels located immediately anterior to the sclera.

The retina has a second, independent circulation termed (confusingly) the “retinal circulation”. This consists of a major artery and vein that enter the eye along the course of the optic nerve (the choroidal circulation enters and leaves by vessels that penetrate the sclera). The retinal artery and vein branch across the retinal surface. These are the blood vessels that are seen clearly via an ophthalmoscope. They send branches that penetrate into the middle layers of the retina. Because they are relatively sparse, they do not interfere much with vision. They can be sparse because the inner retinal neurons need much less blood supply than the layer of photoreceptor cells.

Intervening between the photoreceptor cells and the choroidal circulation is the retinal pigmented epithelium. This is a classic epithelium with its basal surface connected by tight junctions. It serves as the blood-brain barrier at the level of the retina and has other roles in controlling the movement of substrates from the choroidal circulation to the retina. It also serves important functions in photoreceptor cells’ specialized metabolism. (1) It re-isomerizes all-trans retinal to 11-cis retinal. (2) It phagocytoses the shed tips of old outer segments. (3) The pigment in the pigment epithelium cells is melanin; this serves to prevent back-scattering of light once the light has passed through the retina. (4) It apparently serves as a biochemical barrier between the choroidal vasculature and the retina; when the retinal pigment epithelium is damaged, blood vessels from the choroid can invade the retina, eventually destroying it. This happens in macular degeneration and in diabetic retinopathy.

RETINAL DETACHMENT

The retina is very dependent on the apposition between the retinal photoreceptor cells and the supporting tissues. One of the places where that becomes critical is retinal detachment. That can happen spontaneously or it can happen in response to an injury -- for example a blow by a hockey puck or a squash ball. The retina is prone to detachment because there is no physical connection between the tips of the rods and cones and the retinal pigment epithelium. They are simply interdigitated. That tends to make them stay together, but if one gets a hit on the eye, they can come off.

What happens is that the patient becomes blind in the area that is detached. The reason the person loses vision is because the distance between the outer segments and the choroidal circulation has increased. Diffusion, you remember, is very effective for short distances and very ineffective across long distances -- long here meaning 100 or 200 micrometers. In retinal detachment, the rods and cones are ischemic.

The inner retina has a second blood supply. The “retinal circulation” runs across the retinal surface, penetrates to the inner retina, and then comes back out to veins that run again on the retinal surface. If your retina becomes detached, the inner retina is preserved because it has the retinal circulation to supply it. The lack of perfusion of the photoreceptors doesn't kill them
right away. Therapy is to surgically reattach the retina and this usually restores most of the lost function.

GLAUCOMA

In glaucoma, there is degeneration of the retinal ganglion cells. All of the causes of glaucoma are not known; one cause is high intraocular pressure and this can be treated with drugs that lower the pressure. Because of the spatial topography of rods and cones, described above, glaucoma is a particularly insidious disease. The disease usually progresses from the retinal periphery toward the center. However, because our peripheral vision is poor, and because the disease progresses slowly, major ganglion cell loss can occur without the awareness of the patient. There are clearly documented cases of patients who have lost 80% of all their ganglion cells without knowing it. (They commonly come to treatment when they notice that they are bumping into things.)

Because many forms of glaucoma are treatable, it is particularly important to test the visual fields of patients, particularly middle-aged ones or older, as part of a routine physical examination. This can be quickly checked by asking the patient to detect the examiner’s moving fingers in the peripheral visual field. Any patient with a hint of field loss should be referred to an ophthalmologist for measurement of the intraocular pressure. Both eyes should be tested individually. This is particularly true in the case of African-Americans, among whom glaucoma is especially common and who as a group are medically underserved.

RETINITIS PIGMENTOSA

Retinitis pigmentosa is a group of diseases, many of them inherited, in which the photoreceptor cells degenerate. Roughly half of all cases of retinitis pigmentosa are due to mutations in the rhodopsin molecule. The initial manifestation is a degeneration of the rod photoreceptors. This is often revealed only by specialized testing, because rods are relatively little used in our over-lighted modern world. However, rod degeneration is later followed by degeneration of cones. Apparently the cones require trophic support from the rods; in the absence of the rods, the cones degenerate. This tends to occur from the retinal periphery toward the center. The initial deficit is a weakness of peripheral vision, at first only rod vision and later both rod and cone. It then can proceed to tunnel vision and finally to complete blindness.

MACULAR DEGENERATION

Macular degeneration is a diverse collection of conditions, most often affecting individuals in their 60’s or 70’s. It is a degeneration of the cones in the central retina. Commonly, the peripheral retina is spared. However, the condition is debilitating because the patient loses the ability to read, watch television, or recognize faces. The cause of macular degeneration is not known. In advanced macular degeneration, there is breakdown of the retinal pigment epithelium followed by neovascular invasion of the retina. This is treated by laser photocoagulation of the invading blood vessels, which can slow but not halt the progression of the disease. Similar therapy is also used to slow the progression of diabetic retinopathy, in which the major damage is also done by neovascularization.