

COURSE: OPTO 328 – Physiology of Vision I

UNITS: 2 + 0 = 2.

TUTOR: Dr. Ali A Abusharha

RECOMMENDED TEXTS:

- 1) Visual Perception by Cornsweet.**
- 2) Physiology of the Eye by Hugh *Davson*.**
- 3) OPTOMETRY by Keith Edwards and Richard Llewelyn**
- 4) Visual Development (2nd Ed.) by Nigel W. Daw**

Topics

- 1) Glossary of terms**
- 2) Retinal Structure and Organization**
- 3) Photochemical and Electrophysiological Aspects of Vision**
- 4) Sensory aspects of Vision**
- 5) Concepts of Threshold: Frequency of seeing curve**
- 6) Temporal phenomena**
 - I. Flicker**
 - II. Talbot-Plateau law**
 - III. Granit-Harper law**
 - 5.1.1 IV. Ferry-Porter law**
- 7) Spatial Phenomena**
- 8) The Electroretinogram**
- 9) The Visual Evoked Potential**

LECTURE 1

The primary stimulus for vision is **LIGHT**.

In this course, we will discuss extensively, the chemical processes that take place in the retina in response to light stimulation, and how this chemical changes are transformed into electrical impulses for further interpretation in the occipital cortex. All this results in *Visual perception*.

Before we begin our discussions, let us revisit some of the terms you may already be familiar with from the first course in this series.

FLUX The rate of flow of fluid or energy.

LUMINOUS FLUX The rate of flow of light (Lumens).

LUMEN (lm) Unit of luminous flux.

LUMINOUS INTENSITY

In the study of the effects of light on the eye, we are most interested in that quality of light called '*Brightness*'. This brightness (or luminosity) is based on an arbitrary standard (the *Candela*).

The luminous intensity of a light source can be vulgarly (commonly) defined as the degree of illumination (sensation of brightness), which it produces.

INTENSITY OF ILLUMINATION

The intensity of illumination refers the level of illumination on a particular surface. It depends on

- I. The luminous intensity of the source of light.
- II. The distance between the light source and the surface in question.
- III. The angle the surface makes with the direction of incident light.

$E = I/r^2$ where **I** is the luminous intensity of the light source, **E** is the intensity of illumination; and **r** is the distance between the light source and the surface.

The equation above is referred to as the Inverse-Square law.

The Inverse square law states: The intensity of illumination at a point, varies inversely as the square of the distance from that point to the light source.

LUMINANCE

Whereas intensity of illumination describes the amount of light that falls on a surface, luminance measures the amount of light reflected off a surface from a light source.

A 'perfect diffuser' can be imagined as a white surface that reflects all the light that falls on it.

Thus a perfect diffuser 1cm away (and perpendicular) from a standard light source of candela has a luminance of 1 Lambert.

This unit of measurement is too large for our purposes. The log micro-Lambert (mL) is therefore used for studies of light thresholds.

SURFACE INTENSITY

In this modality, the luminance of a surface is defined per unit area of that surface. (e.g. candelas per square foot or candelas per square centimetre.

EFFECTIVELY POINT SOURCE

An illuminated surface of given area forms an image of a definite area on the retina and the sensation of brightness, or rather the stimulus, depends on the amount of light falling on this area of the retina.

If the surface is moved closer to the eye, the amount of light entering the eye is increased in accordance with the inverse square law, but the surface does not appear brighter because the size of the image has increased. Therefore the actual *illumination* of the retina has not altered.

Thus the subjective brightness of an extended source is independent of its distance from the observer.

It may be argued that the brightness of a lamp decreases as it is moved away from the eye and eventually becomes so faint that it disappears if it is moved far enough away. This, however, is a consequence of the fact that at a certain distance from the eye the lamp

becomes an *Effectively point source* i.e. its image on the retina is of the same order of size as that of a receptor element.

Consequently, when it is moved further away still, although its image on the retina becomes smaller, the amount of light, per receptor element, now begins to fall in accordance with the inverse square law.

RETINAL ILLUMINATION

This modality just has to do with the simple truth that the level of illumination that falls on a particular retinal area has not only to do with the candlepower per unit area of the surface being viewed, but also on the pupil size.

The unit of retinal illumination is the *Troland*. A Troland is that intensity of stimulation that accompanies the use of a pupillary area of one square millimetre and an external stimulus surface luminance of one candela per square metre.

LIGHT SENSE

This refers to the appreciation or awareness of light and as such, the modification of its intensity.

COLOUR SENSE

This enables us to distinguish between the qualities of two or more lights in terms of their wavelengths.

FORM SENSE

This permits the discrimination of the different parts of a visual image.

LIGHT SENSE

Absolute Threshold

Threshold to white light i.e. the minimum light stimulus necessary to evoke the sensation of light, is the simplest way to study light sense.

To measure thresholds, the subject is placed in a completely dark room and the illumination of a test screen patch is increased until he is aware of its presence.

The patch may be illuminated all the time and its illumination steadily increased or it may be presented in flashes of varying luminance.

In the fully dark-adapted eye, the minimum stimulus necessary to evoke the sensation of light is the **absolute threshold**.

This threshold is not a fixed quantity, but varies from moment to moment, so that if a subject is presented with a luminous screen in the dark, we may find a range of luminance over which there will be some probability but not certainty, of his seeing it.

Experimentally, the screen is presented frequently at different levels of luminance. A plot is then made of the 'frequency of seeing' against luminance to give the frequency of seeing curve. Threshold is then computed from an arbitrarily chosen frequency.

LECTURE 2

Retinal Structure and Organization

Figures 2.1 a and b represent the path through which light information travels to the brain and back.

Please note the difference between the dotted lines and the solid ones.

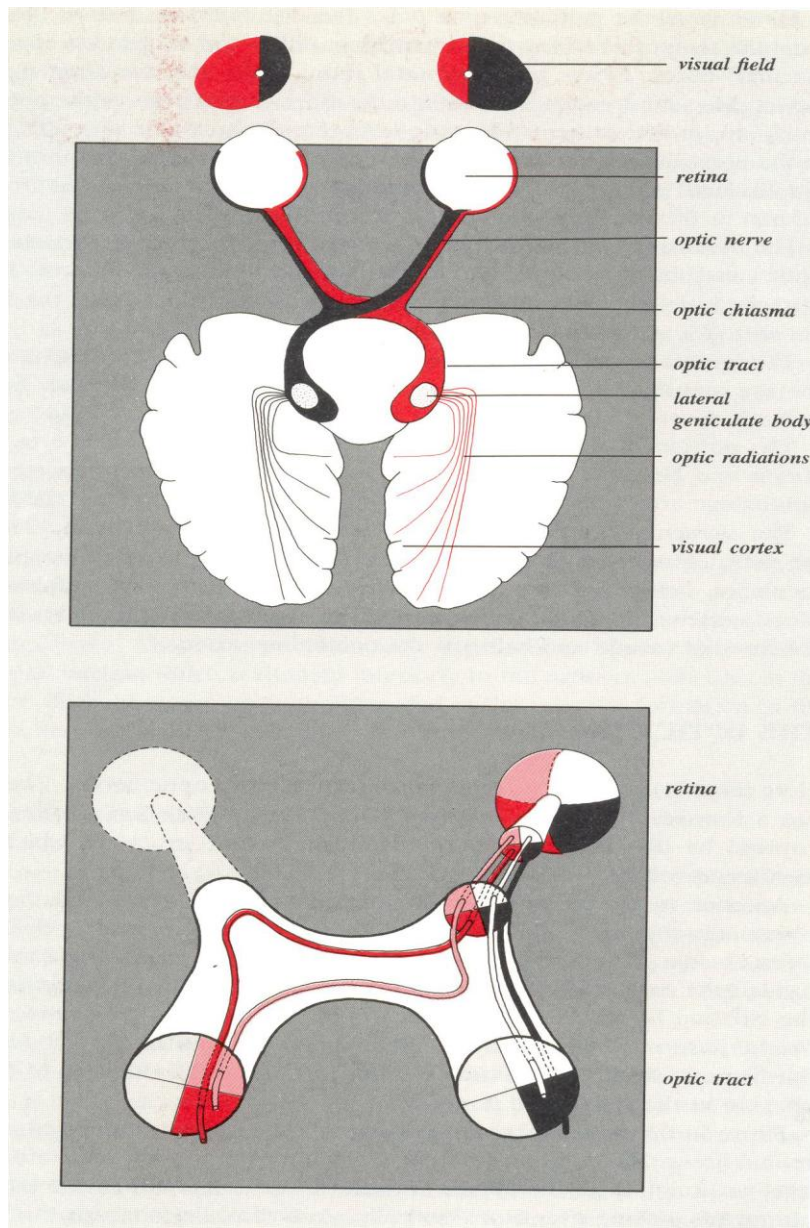
Question

What effect on vision would you expect if there were a lesion of the optic tract?

Figure 2.2 is a schematic representation of the retinal layers.

1. Retinal pigment epithelium (RPE)
2. Layer of the rods and cones
3. Outer limiting membrane
4. Outer nuclear layer
5. Outer plexiform layer
6. Inner nuclear layer
7. Inner plexiform layer
8. Ganglion cell layer
9. Nerve fibre layer
10. Inner limiting membrane

- **The RPE** is a single layer of pigmented cells, which is attached to the choroid. Its main functions are:
 - It nourishes the photoreceptors from the choroidal blood circulation.
 - Its melanosomes absorb light photons that pass through the rod and cone layer but which fail to excite the rods and cones. Normally, these light photons would be reflected back into the layer of rods and cones and thus, cause interference with vision. The RPE melanosomes therefore reduce back-scatter of light into the rod and cone layer which would otherwise have interfered with the processing of a clear image of the object of regard.
 - It digests the outer segment discs shed from the rods and cones.



Figures 2.1a and b.

- a** **Depicts the general path traveled by nasal and temporal fibres.**
- b** **Shows exactly how fibres from each quadrant of the eye cross the optic chiasma.**

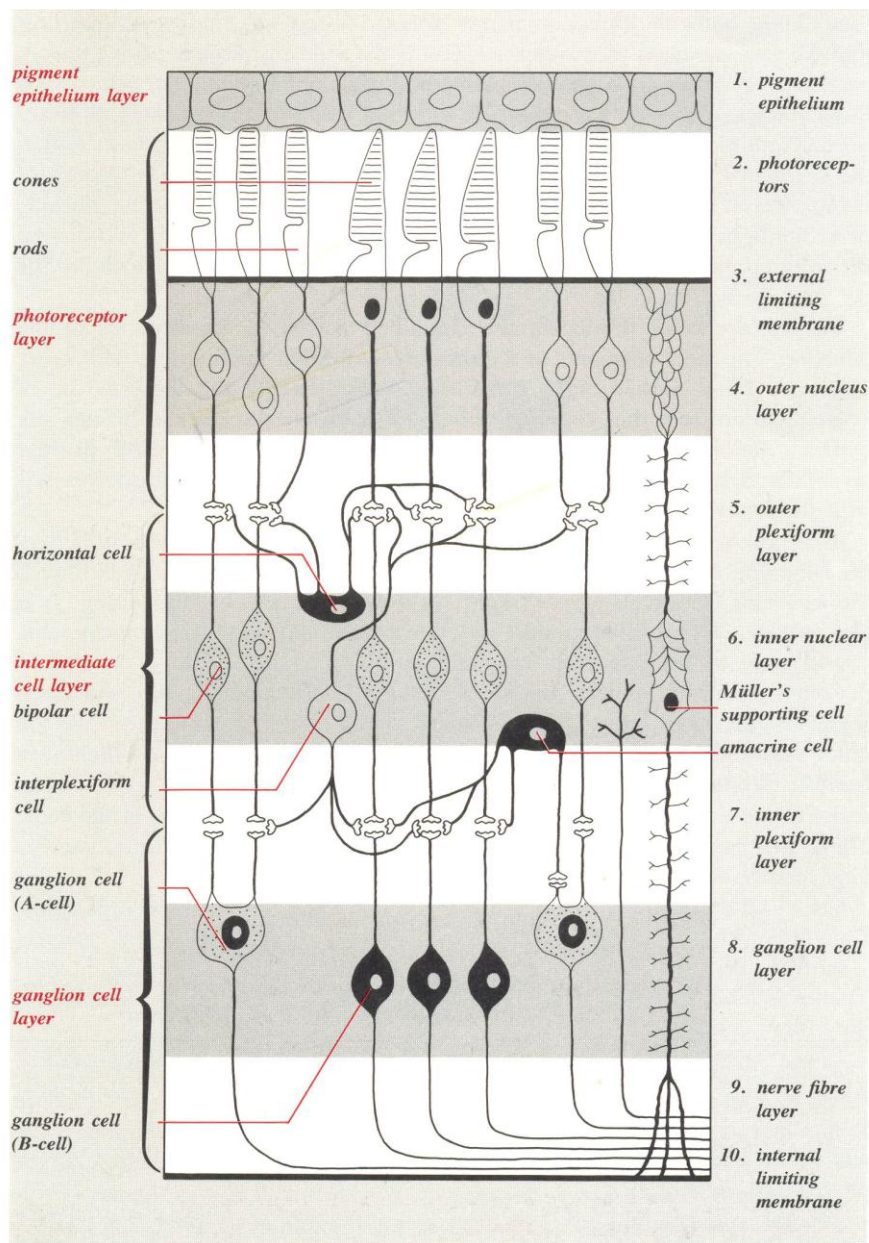


Figure 2.2

The Structure of the Retina

- **The rod and cone layer** contains the outer segment layers of the rods and cones. The cell bodies/nuclei of the rods and cones actually lie within the outer nuclear layer.
 - At the fovea, because there are numerous cones, the cones are very thin and look like rods.
 - There are about 110 to 125 million rods, and 6.5 million cones in the eye. At the central fovea, the cone concentration is maximum and is about 147,000 cones/mm². About 5mm from the fovea, the concentration of rods reaches a maximum of about 160,000 per mm².
 - Light from an external object passes into the eye and when it reaches the retina, it first passes through layer 10, then 9, then 8 etc., until it reaches the photoreceptor layer (rod and cone layer).
 - The light then bleaches the photoreceptor pigments of the rods and cones. One such pigment is Rhodopsin – which is the rod pigment.
 - There are three types of cone pigment in the human retina (red, green, and blue) and each single cone contains only one type of pigment.
 - Based on studies of other mammals, the cone distribution is as follows:

○ Long Wavelength-Sensitive Cones	-	33%
○ Medium Wavelength-Sensitive Cones	-	13%
○ Short Wavelength-Sensitive Cones	-	54%

The proportion of blue cones reduces to 3% in the central retina. This finding would at least partially explain the well known ‘blue-blindness’ (tritanopia) in the foveal region.

- **Receptor inner segments** are depicted in figure 2.3. The receptor inner segment is easily distinguished into two distinct zones:
 - The outer zone, called the ellipsoid, which is packed with mitochondria.
 - The inner zone, called the myoid, which contains free ribosomes, granular and agranular endoplasmic reticulum, vesicles, Golgi membranes, and neurotubules.

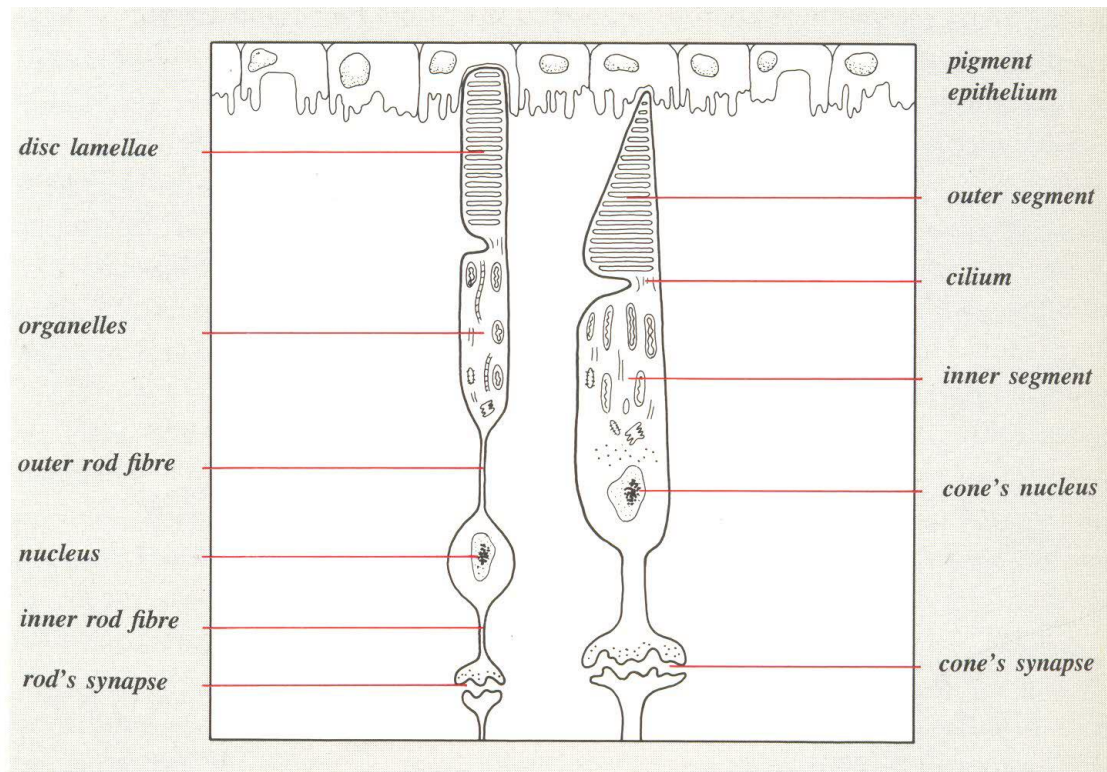


Figure 2.3 Photoreceptor Structure

- The myoid is responsible for the manufacture of most of the molecules involved in the production and repair of the outer segment discs of rods and cones.
- Opsin (the visual pigment) is also produced in the myoid, and then it is transported to the outer segment via the connecting stalk.

Note *A thin outer fibre connects the rod's cell body with its inner segment. The cone's cell body on the other hand, is connected directly to its inner segment.*

- **Outer plexiform layer** is the layer that contains the connections between the rods and cones on the one hand, and the horizontal and bipolar cells on the other.
 - Cones synapse (connect) with:
 - Midget bipolar cells
 - Flat midget bipolar cells
 - Flat bipolar cells
 - Rods synapse with:
 - Rod bipolars
- The important points to note about the synapses in the outer plexiform layer are as follows:
 - In the fovea only, one cone connects with one midget bipolar cell. Outside the fovea, this relationship does not exist.
 - Flat bipolar cells synapse with about six cones each.
 - One rod bipolar cells synapses with about 45 rods.
- It then becomes clear that the rods bipolar cells receive much more information than the midget bipolars, or the flat bipolars, both of which synapse with cones.
- The one-to-one relationship between the cone and the midget bipolar cell is necessary for the preservation of fine detail.
- Horizontal cells also synapse with rods and cones in the outer plexiform layer. One horizontal cell connects with 6 – 12 cones in the central retina, and 30 – 50 cones in the periphery. One horizontal cell connects with 80 – 150 rods.
- The likely function of the horizontal cells is to provide communication:

- ✓ Between the cones
 - ✓ Between the rods
- It is unlikely that the horizontal cells provide a pathway for rod-cone communication.
- The obvious advantage that the feedback mechanism of the horizontal cells provide is that the response of each cone, and each rod can be modified before being transmitted to the ganglion cells.
- ***The inner nuclear layer*** contains the cell bodies of the bipolar, horizontal and amacrine cells. The amacrine cells also provide a feedback mechanism for the transmission of signals from the rods and cones to the ganglion cells. This time however, they modulate the synapses between the bipolar cells, and the ganglion cells (in the inner plexiform layer).
- ***The inner plexiform layer*** contains complex interactions between the bipolar and ganglion cells and amacrine cells. There are many types of amacrine cells that synapse with multiple bipolar and ganglion cells, as well as with each other.
 - Interplexiform cells are a distinct type of amacrine cell, which also go back to the outer plexiform layer and synapse with bipolar and horizontal cells. They therefore provide a more complex type of feedback between the plexiform layers rather than within one plexiform layer.
- ***The ganglion cell layer*** contains the cell bodies of the ganglion cells. There is one-to-one relationship between a midsize bipolar cell and a midsize ganglion cell (in the inner plexiform layer). So, in the central fovea, the information from one cone travels along a pure path to the visual cortex
 - Another type of retinal ganglion cell (the diffuse ganglion cell) synapses with numerous bipolar cells.
 - Midsize ganglion cells are also referred to as beta cells or x-cells.
 - Diffuse ganglion cells are also referred to as alpha cells or Y-cells.
- ***In the nerve fibre layer*** the axons of the ganglion cells pass through on their way to the brain. Whilst passing through this layer, the ganglion cells are sheathed (covered) by Müller cell processes and astrocytes. These covering of the axons perform the following functions:
 - They nourish the nerve fibers.
 - They provide protection for the axons.
 - They provide insulation for the fibers so that they conduct electric impulses faster and more efficiently.

LECTURE 3

PHOTOCHEMICAL ASPECTS OF VISION

The visual pigment of rods is known as **Visual purple or Rhodopsin**. For the study of the photochemical aspects of vision, we will discuss the process by which Rhodopsin mediates the sensation of light.

- ✓ When light photons fall on a rhodopsin pigment molecule, this molecule enters into an 'excited' state. This 'excited state is very unstable and therefore leads to the formation of new molecules.
- ✓ These new molecules undergo a series of reactions (called ***thermal reactions***), which ultimately lead to the sensation of light.
- ✓ It stands to reason that these reactions should be reversible (at least in part) if not, the eye would have to produce large amounts of rhodopsin each time the pigment was excited by light.
- ✓ As far as the visual process is concerned, a simplified schema of events can thus be represented as follows:
 - Rhodopsin \leftrightarrow Activated state \leftrightarrow Bleached products
- ✓ These bleached products are the result of a thoroughly complicated series of events which involve rhodopsin changing its color from ***purple to orange to yellow, and finally to white when it is eventually converted to Vitamin A + Opsin***.
- ✓ Generally then, when the eye is fully dark-adapted we should find only rhodopsin within the rods. However, when the rods are excited by light, we would find a mixture of rhodopsin and its bleached products. This mixture will depend on the intensity of the light and the duration of its exposure.

Production And Turnover Of Outer Segment Disc Lamellae

- Taking a quick peep at the schematic diagram of rods and cones, we see that there are numerous ***Disc Lamellae*** in the rod and cone outer segments. These lamellae are oriented at 90^0 to incident light, an arrangement that maximizes the absorption of incident light.
- These lamellae are manufactured in the ***Myoid portion of the Rod Inner Segment***. The disc lamellae are then moved to the Golgi bodies, which then transport the lamellae into the ***Outer Segment***. As more and more lamellae are produced, the older lamellae climb higher up in the ***Outer Segment*** until they are secreted into the RPE.
- Estimates have been given of the replacement of the disc lamellae at 25 to 36 per day.
- It would appear however that the turnover in cones does not undergo a similar process to that in rods.

Note *Purkinje shift refers to the shift of maximum wavelength sensitivity of the eye from 500 μ m during scotopic (rod) vision, to 550 μ m during photopic (cone) vision.*

Bleaching of Rhodopsin

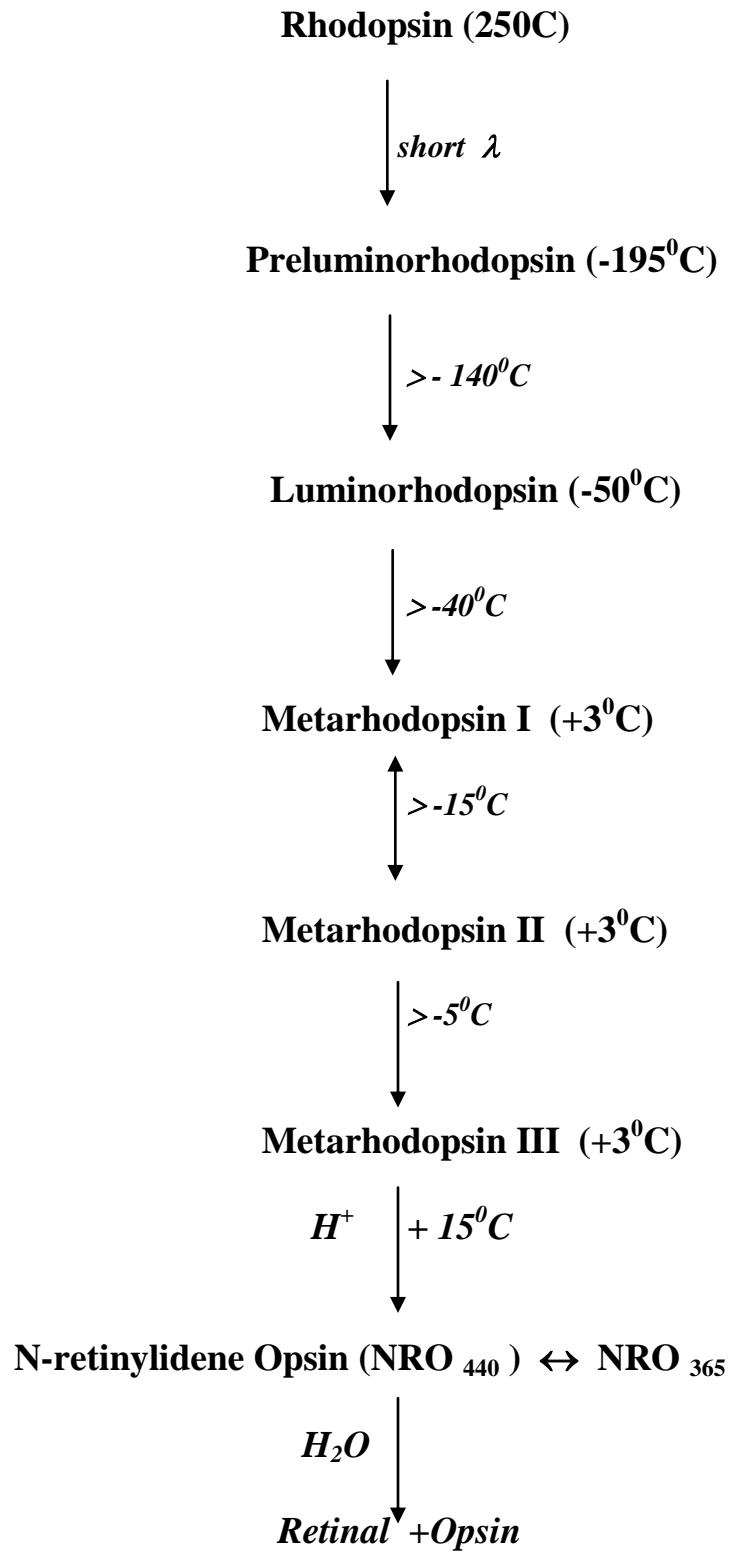
Early studies recognized that rhodopsin turned yellow on exposure to light.

The final products of the bleaching of rhodopsin are ***Retinene, and Vitamin A*** – into which retinene is converted if it is exposed to light photons long enough to prevent its re-conversion into rhodopsin.

Retinene is actually vitamin A aldehyde and is now (more correctly) referred to as ***Retinal***.

Retinal + Opsin = Rhodopsin (Visual purple).

The scheme proposed by Abrahamson and Ostroy (1967) of the chemical intermediaries in the thermolysis of rhodopsin is still valid today and is as follows:



Notes *The first step in the reaction above is the only step that requires the absorption of light.*

Luminorhodopsin remains stable at temperatures below -40°C , above which it breaks down into a series of metarhodopsin molecules, which vary only in their wavelength of maximum absorption (λ_{max}).

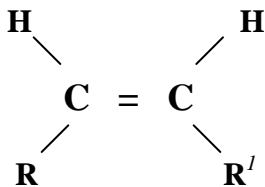
The final conversion (from NRO to Opsin) involves the splitting of retinene from Opsin. This reaction involves a process known as breaking a Schiff-base link.

Regeneration of Rhodopsin

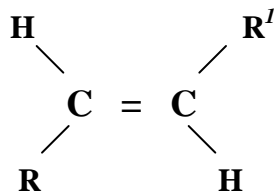
In the retina, the products of bleaching of rhodopsin (retinene and vitamin A) are re-combined to form rhodopsin in the dark.

Inasmuch as this combination must take place for the retinene to be recycled into rhodopsin, the transformation is not straightforward.

Retinene in its combined form with Opsin is in its 11-cis form. But when retinene is separated from Opsin, it exists in its all-trans form. Therefore, retinene in (retinene + Opsin) must first be converted (isomerized) into its 11-cis form (or to another form, which is equally capable of producing rhodopsin – 9-cis retinene). This isomerization will take place (in the dark) in the presence of an enzyme called ***Retinene Isomerase***, or by mere exposure to light.



Cis Retinene



Trans Retinene

The Role of the RPE

The RPE's role in the regeneration of rhodopsin cannot be overemphasized. It has long since been discovered that rhodopsin would not be regenerated in the absence of the RPE. The reason for this is now taken for granted to be due to the high concentration of vitamin A that has been found in the RPE after maximum light adaptation. Conversely, after full dark adaptation, no vitamin A is found in the RPE indicating that vitamin A, a vital precursor for rhodopsin, is stored almost entirely in this layer.

Note It is worthy of note that various researchers have reported that the scotopic pigments:

- *Vary between species.*
- *Could exist in multiple forms in the same animal*
- *Sometimes vary with the period of the year (season)*
- *Sometimes vary according to the habitat of the animal.*

OVERVIEW OF ELECTROPHYSIOLOGY OF VISION

When any receptor is stimulated, electrical changes take place that result in the transmission of electrical current along that receptor's nerve fibre.

This electrical current takes the form of *Action Potentials*.

The duration of any one action potential is very short. However, the greater the stimulus that causes the electrical current, the more action potentials per second there are. However, the magnitude (size) of the action potential itself never changes.

Therefore, once the stimulus is large enough to generate an action potential, a constant current is transmitted across the nerve fibre. What changes with the intensity of the stimulus is the frequency of action potentials. This phenomenon is called the *all-or-nothing-response*.

It is important to digress a bit to describe the processes that usually take place when an action potential is generated in a receptor.

The Action Potential

Below is a schematic representation of an Action Potential in progress within a nerve fibre or neuron.

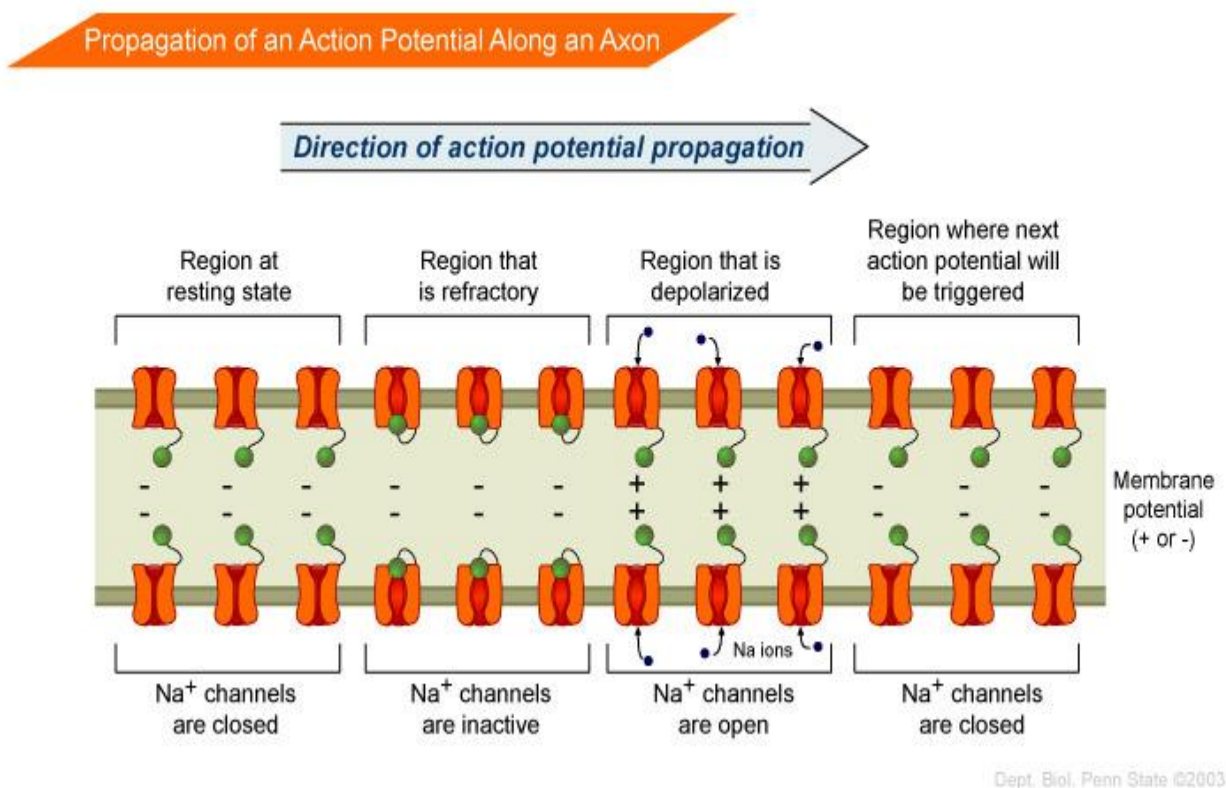


Figure 3.1 **An Action Potential in Progress**

The resting membrane potential is -70 mV. When a (local) stimulus causes a reduction of this RMP to -59 mV, an action potential is generated. A stimulus causing a reduction of the RMP to less than -59 mV has no effect. A *generator potential* then moves this action potential along the entire length of the membrane.

Once the RMP depolarizes (increases) to -59 mV, Na^+ channels are opened and Na^+ ions which normally are more outside the membrane rush into it and cause a massive increase in the membrane potential to about $+30$ mV.

K^+ are normally much slower to respond and do so after the membrane potential has reached $+30$ mV. The K^+ ions then rush out of the membrane and eventually restore the membrane potential back to (and a little bit greater than) its original resting state of -70 mV. This is called ***Re-polarization*** of the membrane.

In the eye however, a sufficiently large stimulus causes a ***hyper-polarization*** (rather than a depolarization) of the photoreceptors – so that the membrane potential becomes more negative instead of more positive. This is how the electrical current carrying vital information to the brain is generated.

Notes *It is pertinent to note at this juncture that the crucial electrical event that leads to a discharge of electrical current along the nerve fibre is called a Generator Potential. This potential is a much slower mechanism in which the hyper-polarization of the cell lasts longer. If the generator potential is maintained long enough, it causes the discharge of electrical current. The generator potential (as well as the action potential frequency) increases in direct proportion with the intensity of the light stimulus.*

In the photoreceptors, the outer segment is usually more negative than the inner segment in the dark. This causes a flow of positive current from the inner to the outer segment.

When a sufficient light stimulus excites the photoreceptor, the outer segment increases its resistance and thus prevents the flow of positive ions into it and thus increases its negativity. This ***hyper-polarization*** reverses the direction of the current and is the first stage of the action potential that sends visual impulses to the cortex from the photoreceptors.

Nature of Photoreceptor-generated Action Potentials

To understand the nature of these action potentials, we need to discuss a number of terms and effects.

The optic nerve fibres can be categorized into three groups according to their reaction to light stimuli.

On Fibres	These fibres (about 20% of the total) display a strong response immediately the light impulse is received and stop this response immediately the light stimulus is removed.
On-Off Fibres	These comprise about 50% of the total fibres. They respond to a light stimulus with an initial burst of activity when the impulse is received, and with another burst of activity when the impulse ceases.
Off Fibres	These comprise the remaining 30% of the fibres and respond only when the light stimulus stops.

The Electroretinogram is a record of the slow changes of potential that occur in a radial direction across the retinal surface when light falls on it.

This potential, according to the classic analysis of Granit displays three components:

- a) P I This is a slowly developing positive component of the potential which tends to disappear with strong stimuli or after light adaptation.
- b) P II This is a positive component that is turned on rapidly. This component is strongly associated with the passage of impulses along the optic nerve.
- c) P III This is a negative component that comes on rapidly and is responsible for the return of the membrane potential back to the base line (in short, it is responsible for the 'off' effect). This component is mainly associated with cone impulses and is believed to function in connection with the inhibition of impulses.

Once the generator potential has set off electrical impulse transmission in the photoreceptor cell, the following are summaries on how the major fibres involved in this transmission respond:

Horizontal Cell	<i>It also hyperpolarizes but its response to the stimulus is much slower.</i>
Bipolar Cell	<i>Response is also slow here. It responds to the stimulus in an opponent (explain) fashion i.e. centre hyperpolarizes, surround depolarizes.</i>
Amacrine Cells	<i>Response here is depolarization. Some receptive fields respond in an opponent fashion, others in a uniform fashion.</i>
Ganglion Cells	<i>Respond with an initial depolarization. Like bipolar cells, centre-surround antagonism (opponent receptive fields) is very much a characteristic of their response.</i>