

Biochemistry of Vision

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ABSTRACT

All living things respond to the stimulus of light. Almost all multicellular organisms have specialized light receptor cells in which light energy can cause changes in a light-sensitive pigment. In most invertebrates, the light receptors do not function as eyes and as a result, they are unable to form images. However, they are able to perceive the presence of light and can detect any changes in light intensity. As a result, some of these receptors can give no indication of the direction of the light source and hence the animal responds mainly by random movements. However, there are some cases in which light receptors are arranged in such a manner as to indicate direction. One of the earliest forms of vision is known as phototaxis which is a light-controlled motion. This phenomenon has been observed in some photosynthetic bacteria such as *Chromatium*, which move selectively towards illuminated areas rather than dark places. The exact mechanism of this movement is unknown; however, it is likely that the energy needed to move is provided by the light which produces adenosine triphosphate (ATP) in the photosynthetic process. Hence, the bacterium cannot move in dark places since there is no production of ATP. However, the organism will start moving again as soon as it finds an illuminated area.

Keywords: phototaxi, Vision, receptor cells, light and rhodopsin

VISION

It is also referred to as "Visual phototransduction" - A process by which light is converted into electrical signals in the rod cells, cone cells and photosensitive ganglion cells of the retina of the eye. It is the sensory transduction of the visual system [1]. It is a process by which light is converted into electrical signals in the rod cells, cone cells and photosensitive ganglion cells of the retina of the eye. This cycle was elucidated by [2] for which he received the Nobel Prize in 1967. It is called "Wald's Visual Cycle" after him. The visual cycle is the biological conversion of a photon into an electrical signal in the retina. This process occurs via G-protein coupled receptors called opsins which contain the chromophore 11-cis retinal. 11-cis retinal is covalently linked to the opsin receptor via Schiff base forming retinylidene protein. When struck by a photon, 11-cis retinal

undergoes photoisomerization to all-trans retinal which changes the conformation of the opsin GPCR leading to signal transduction cascades which causes closure of cyclic GMP-gated cation channel, and hyperpolarization of the photoreceptor cell. Following isomerization and release from the opsin protein, all-trans retinal is reduced to all-trans retinol and travels back to the retinal pigment epithelium to be "recharged [3]". It is first esterified by lecithin retinol acyltransferase (LRAT) and then converted to 11-cis retinol by the isomerohydrolase RPE65. The isomerase activity of RPE65 has been shown; it is still uncertain whether it also acts as hydrolase. Finally, it is oxidized to 11-cis retinal before traveling back to the rod outer segment where it is again conjugated to an opsin to form new, functional visual pigment (rhodopsin).

The Sense of Vision

In general, all living things respond to the stimulus of light. Almost all multicellular organisms have specialized light receptor cells in which light energy can cause changes in a light-sensitive pigment. In most invertebrates, the light receptors do not

function as eyes and as a result, they are unable to form images. However, they are able to perceive the presence of light and can detect any changes in light intensity. As a result, some of these receptors can give no indication of the direction of the light source and hence

the animal responds mainly by random movements. However, there are some cases in which light receptors are arranged in such a manner as to indicate direction [4]. One of the earliest forms of „vision“ is known as „phototaxis“ which is a light-controlled motion. This phenomenon has been observed in some photosynthetic bacteria such as Chromatium, which move selectively towards illuminated areas rather than dark places. The exact mechanism of this movement is unknown; however, it is likely that the energy needed to move is provided by the light which produces adenosine triphosphate (ATP) in the photosynthetic process. Hence, the bacterium cannot move in dark places since there is no production of ATP. However, the organism will start moving again as soon as it finds an illuminated area [5]. In higher life forms, they have more complex eyes that generally have a lens which is capable of concentrating light onto a photosensitive area. This increases the sensitivity of the eye to dim light. It also increases the ability of the eye to perceive direction and movement. The light from each source is focused onto some of the receptor cells at any moment. There are basically two different types of image-forming eyes in animals; compound eyes and camera-type eyes. Many insects and crustaceans have compound eyes which utilise many closely packed lenses. Each

Photoreceptors

The photoreceptor cells involved in vision are the rods and cones. These cells contain a chromophore (11-cis retinal, the aldehyde of Vitamin A1 and light-absorbing portion) bound to cell membrane protein, opsin. Rods deal with low light level and do not mediate color vision. Cones, on the other hand, can code the color of an image through comparison of the outputs of the three different types of cones. Each cone type responds best to certain wavelengths, or colors, of light because each type has a

The Human Eye

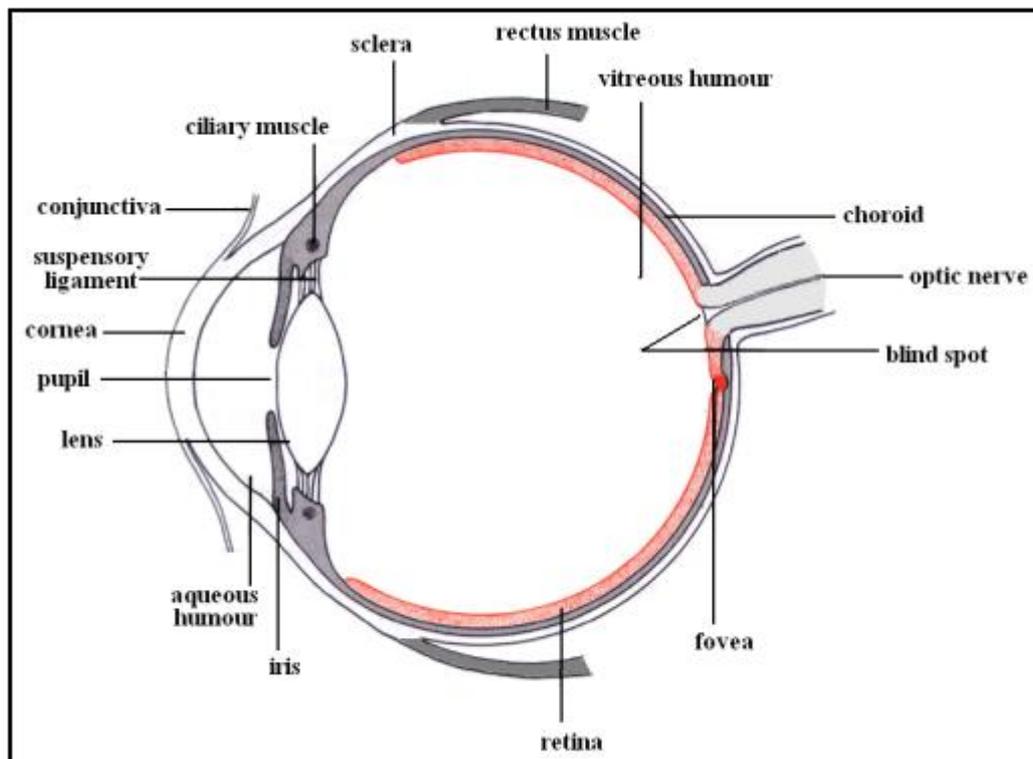
The shape of an adult human eye (Figure 1) is like a globe with a diameter of approximately 2.5 cm, that fits into the bony sockets in the skull. The globe of

lens is connected with a few sensory cells to form a functional unit known as the ommatidium. The formation of images depends on the pattern of light that falls onto the compound eyes surface [6]. The ommatidia point in various directions and as such will be stimulated by light from different points. Therefore, the brain integrates all the messages received from the various ommatidia and it apparently creates an image that corresponds to the total of many smaller images [7]. Various animals such as molluscs and vertebrates possess a camera-type eye which uses a single lens system to focus light onto a photosensitive surface, known as the retina, which functions similarly to a piece of photographic film [8]. The recognition of the shapes of objects involves the formation of an image on this photosensitive area [9]. For humans, the term „vision“ is a complex process of information regarding the environment of a living organism [10]. The human eye is capable of detecting a variety of colours, forming images of objects miles away, and responding to as little as one photon of light. However, it is actually the brain that sees. In order to understand vision, it is necessary to know how the eye generates sensations, and then follow these signals to the visual centres of the brain, where images are perceived.

slightly different opsin. The three types of cones are L-cones, M-cones and S-cones that respond optimally to long wavelengths (reddish color), medium wavelengths (greenish color), and short wavelengths (bluish color) respectively. Humans have a trichromatic visual system consisting of three unique systems, rods, mid and long-wavelength sensitive (red and green) cones and short wavelength sensitive (blue) cones [11].

the eye or eyeball is a three-layered structure which consists of sclera, choroid and retina [6].

Figure 1: Longitudinal section through the human eye.



The sclera is a tough but elastic, white outer layer of connective tissue. At the front of the eye, the sclera becomes the transparent cornea, which allows light to enter the interior of the eye and functions as the first constituent of the light-focusing system of the eye [4]. A delicate layer of epithelial cells forms a mucous membrane, known as the conjunctiva, which covers the outer surface of the sclera and helps to keep the eye moist [7]. The choroid is a layer of darkly pigmented tissue through which many blood vessels pass and is located just inside the sclera. The choroid is important since it provides blood to other parts of the eye and it functions as a light absorbing layer which prevents internally reflected light from blurring the image. At the immediate back of the junction between the main part of the sclera and the cornea, the choroid becomes thicker with smooth muscles embedded; this part of the choroid is known as the ciliary body. The front choroid forms the donut-shaped iris, which gives the eye its colour. The iris consists of smooth muscle fibres arranged in

circular and radial directions. By changing size, the iris regulates the amount of light entering the pupil, the hole in the centre of the iris. The pupil is reduced when the circular muscle fibres contract and it is dilated when the radial muscle contract [7]. Just inside the choroid, the retina forms the inner most layer of the eyeball and contains the photoreceptors (Clegg and Mackean 2000). The photoreceptors are of two types, referred to as rods and cones. The rod cells are abundant toward the periphery of the retina while the cone cells are abundant in the central portion of the retina. The bipolar cells which are short sensory neurons synapse with the photoreceptors in the retina. The bipolar cells synapse in the retina with longer neurons, i.e. ganglion cells, whose axons form the optic nerve that runs to the visual centres of the brain. There are several sets of synapses present in the retina, which allows the eye to modify the information transmitted from the receptor cells to the brain [8]. There are no rods and cones present in the optic disc, and as such this region on the lower outside of

the retina is a blind spot, i.e. light focused on that part is not detected. The theoretical line through the centre of the lens is referred to as the optical axis [10]. The fovea or "yellow spot" lies on the optical axis and is the place of most acute vision.

This portion contains many cones but few rods [10]. The second constituent of the light-focusing system of the eye is the lens which is suspended just behind the pupil by a suspensory ligament attached to the ciliary body [7]. The lens and the ciliary body divide the eye into two cavities. The ciliary body constantly produces the clear, watery aqueous humour that fills the front cavity of the eye. The back cavity, filled with the jelly-like vitreous humour constitutes most of the volume of the eye. The aqueous and vitreous humour function as liquid lenses that help focus light onto the retina. The lens itself is a transparent protein disc that focuses an

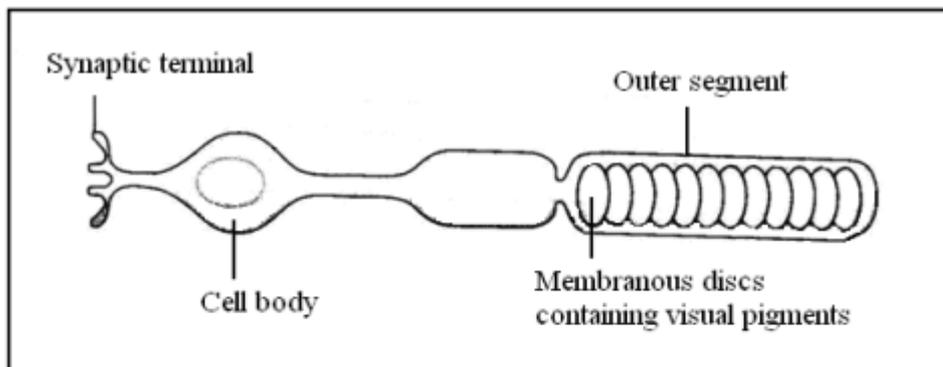
Photoreceptors: Rods and Cones

The retina contains millions of photoreceptor cells. These are referred to as rods and cones and the names of these cells come from their individual shapes. The human retina contains approximately 125 million rods and 6 million cones. The rod cells are abundant toward the periphery of the retina while the cone cells are abundant in the central portion of the retina [4]. Each rod cell or cone cell has an outer segment with a stack of folded membranes or discs, in which visual pigments are embedded [7]. The visual pigment in the rods is built into the membranes of the flattened vesicles in the outer segment and is referred to as rhodopsin [8]. In the cones, the visual pigment is known as iodopsin [3]. They

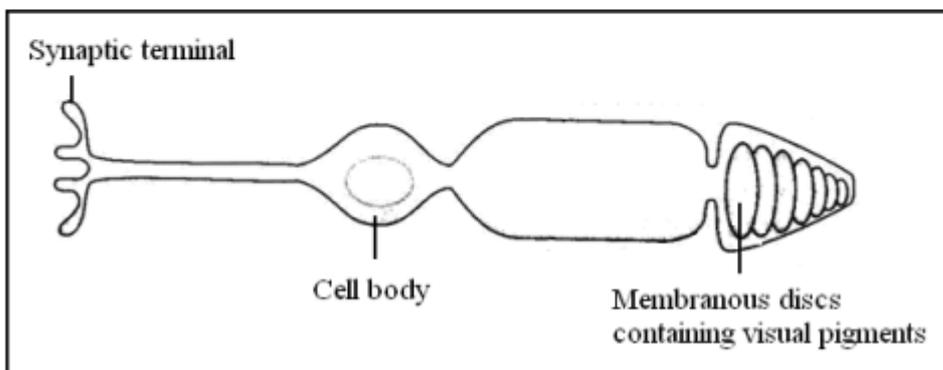
image on the retina [8]. The eye is similar to a camera. The cornea and lens, which are two constituents of the light-focusing system, form an inverted image on the retina. The iris regulates the opening of the lens while the eyelids prevent light from entering and also prevent any possible damage to the surface of the cornea. The ciliary muscle controls the lens so that objects from different distances may be brought sharply into focus. The focusing of light onto the retina can be accomplished by this mechanism and also by the curvature of the cornea [8]. The cornea has a refractive index of 1.38; the lens is 1.42 where as the refractive index of both humours is 1.33. The largest difference in refractive index occurs between the air and cornea and therefore it is essential for image formation. The delicate and accurate control is achieved by the lens which acts as a fine adjustment [8].

are thought to be three types of cone cells which contain different forms of iodopsin and as such they respond to light of different wavelengths [4]. One responds best to red light, one to green and the other to blue. In general, colours are detected as a result of the relative degree of stimulation of the three types of cones. The sensation of white light is observed when all three types of cones are stimulated equally [5]. The cone cells are concentrated in a central part of the retina, called the fovea, and as a consequence a person can only perceive the colour of an object if its image falls close to the fovea or in the direct line of vision [5].

Figure 2: Structure of the (a) rod cell and (b) cone cell.



2 (a)



2(b)

The brain receives information through an intermediary optical nerve and has no direct contact with the photoreceptor cells. Therefore, the ability to observe images is dependent on the brain, which determines the location of the photoreceptor cell that passes the impulse to any nerve fibre. Rod cells are more sensitive to small amounts of light. Cone cells provide sharper images and this is due to the ability of the brain to map images based on the position of the photoreceptor cells, which conveys the nerve impulse to the brain [6]. It is possible for a number of rod cells to share the same nerve fibres, in some cases as many as 150. On the other hand, each cone cell is connected to the brain by an exclusive nerve fibre. In some cases, few cone cells may share the same one. As a consequence, the fovea provides sharper and clearer

visual impression in the brain compared to the other parts of the retina. This image is precisely analysed by the tightly packed cones which individually or in small groups send separate impulses to the brain. An image that falls on the rods is not analysed in detail compared to the cones because rods are not as tightly packed and also as a large number of rods send only one set of impulses to the brain [7]. Therefore, when the brain receives an impulse from the fibres connected to the rods, it has no way of determining precisely from which one of the rod cells the impulse originated [8]. Consequently, the images are not as sharp as those of the cones. The signals from the rods are combined where as those from the cones are distributed between many nerve fibres. Hence, the rods have greater sensitivity in dim light

[4]. The rods located at the outermost edge of the retina are not able to form images. However, they function as a trigger reflex that turns the eyes in the

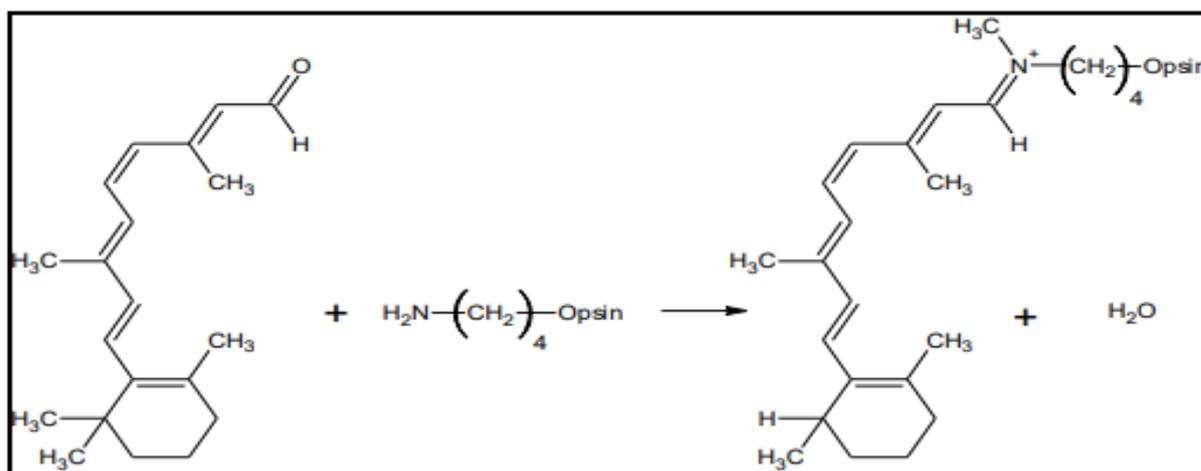
direction of the object which may lie just beyond the normal limits of sight [5].

Photochemistry of Vision and Signal Transduction

The process of vision is triggered by the photochemical isomerization of the light absorbing pigment molecule retinal, which is the aldehyde derived from Vitamin A, bonded to a membrane protein called an opsin [1]. Rods contain their own type of opsin, which combines with the conjugated polyenal, 11-cis-retinal, in the retina to form the red-purple, 11-cis-imine which is also known as rhodopsin or „visual purple“ (Figure 3)[2]. Rhodopsin consists of 348 amino acid residues that are grouped

mainly into seven hydrophobic, alpha helix segments which pass between the two sides of the photoreceptor membrane (Wayne and Wayne 1996; Casiday and Frey 2000). The 11-cis-retinal functions as a chromophore, and is the main receptor for photons which enter the eye. The 11-cis-retinal absorbs light in ultraviolet region. However, the maximum absorption is shifted to 498 nm when it is attached to an opsin molecule [3].

Figure 3: The reaction which links 11-cis-retinal to opsin



The initial stage in monochromatic vision after the photons hits the rod cell, is the photoisomerization of 11-cis-retinal to all trans-retinal (Figure 4) [2]. This occurs when a photon promotes the π electron in a $\pi \rightarrow \pi^*$ excitation. This excitation weakens the π components of the double bond thus allowing free rotation about the bond between carbon 11 and 12. This photoisomerization occurs in about 200 femtoseconds and causes the conjugated carbon chain to become straightened [4,6,7]. The irradiation of rhodopsin causes a number of subsequent changes in the conformation which are seen by the appearance and disappearance of a series of intermediates of varying colours [8]. These light induced changes in 11-cis-retinal and opsin are referred to as the „bleaching“ of rhodopsin. The initial isomerization does not cause any

change in the shape of the opsin protein, which is the twisted conformation of all trans-retinal that is referred to as bathorhodopsin. The bathorhodopsin is not stable enough to stay in this arrangement for long. Due to the rigidity and elongated shape of the all trans-retinal, the isomer does not fit into the site on the protein. As a result, the protein begins to change its shape in a very short period of time (10-9 s) [7,8]. A series of intermediates are produced (Table 1), each of which absorbs at different wavelengths. Eventually the protein expels the all trans-retinal, to give free opsin and all trans-retinal [3,5]. The energy of the excited state, which resulted from the interaction of opsin with retinal, is lowered and this causes a red shift. The shift will be larger for stronger interaction. The absorption maximum moves towards

the blue and the shifts are smaller as the more highly strained structures of

lumirhodopsin and metarhodopsins are formed [6].

Figure 4: The photoisomerization of 11-cis-retinal all trans-retinal.

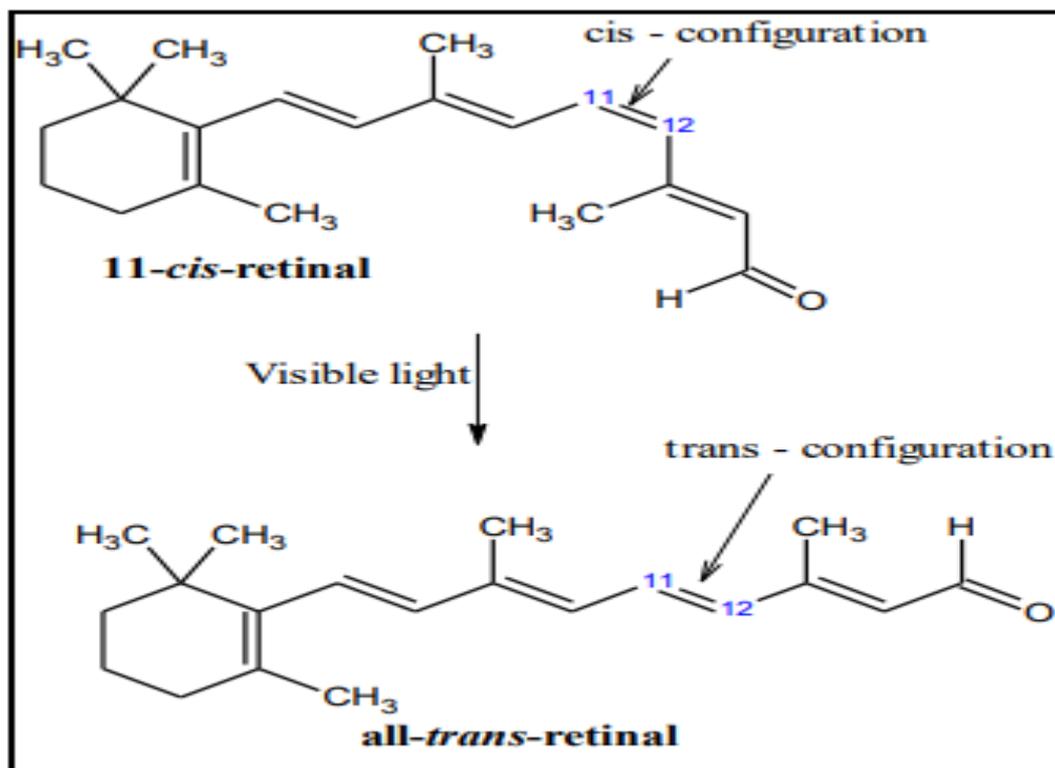


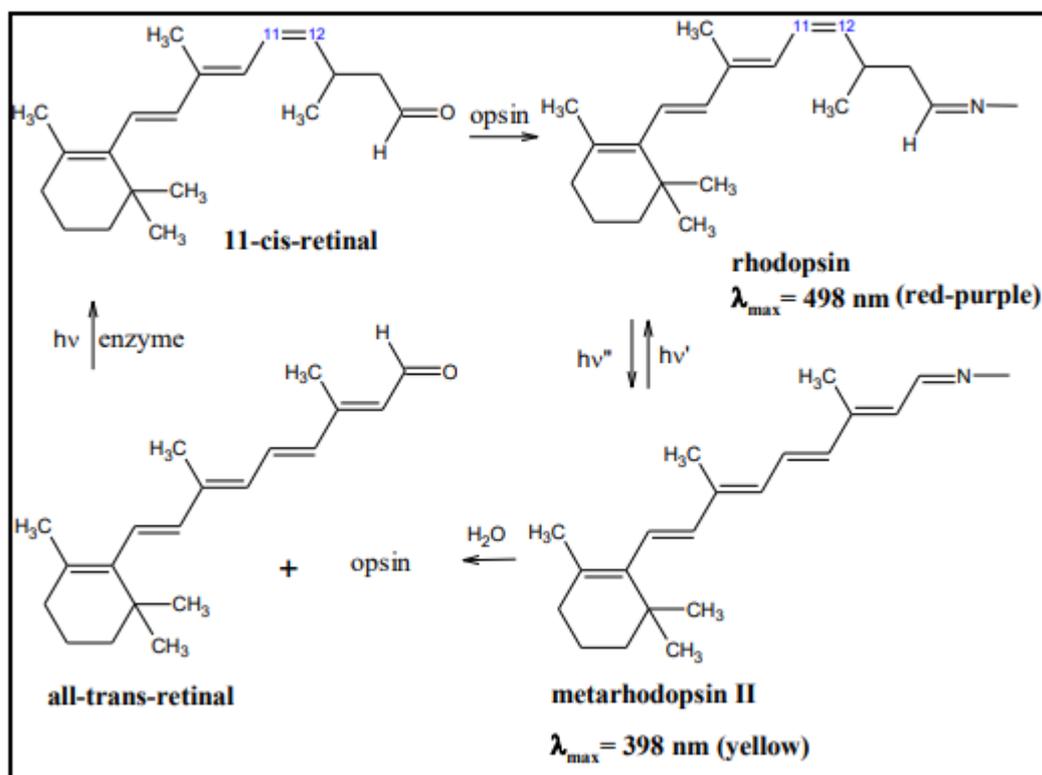
Table 1: Intermediates formed as rhodopsin changes its conformation following the cis-trans isomerization of retinal and their characteristic absorbance [3].

Name of Pigment	λ_{\max} (nm)
Rhodopsin	498
Bathorhodopsin	543
Lumirhodopsin	497
Metarhodopsin I	487
Metarhodopsin II	380
all <i>trans</i> -retinal (free)	370

The most important intermediate produced is the yellow all trans-metarhodopsin II (Figure 5), which is formed when the light absorbed causes the isomerization of the cis double bond in rhodopsin and it also triggers a nerve impulse [8,9]. In comparison to the 11-cis-imine, the trans-metarhodopsin II does not fit into the site on the surface of the protein. As a result the carbon-nitrogen bond becomes exposed and can

be readily hydrolyzed to produce all trans-retinal and opsin [7]. The trans-metarhodopsin II can be transformed to rhodopsin in the presence of bright light through trans-cis isomerization [8].

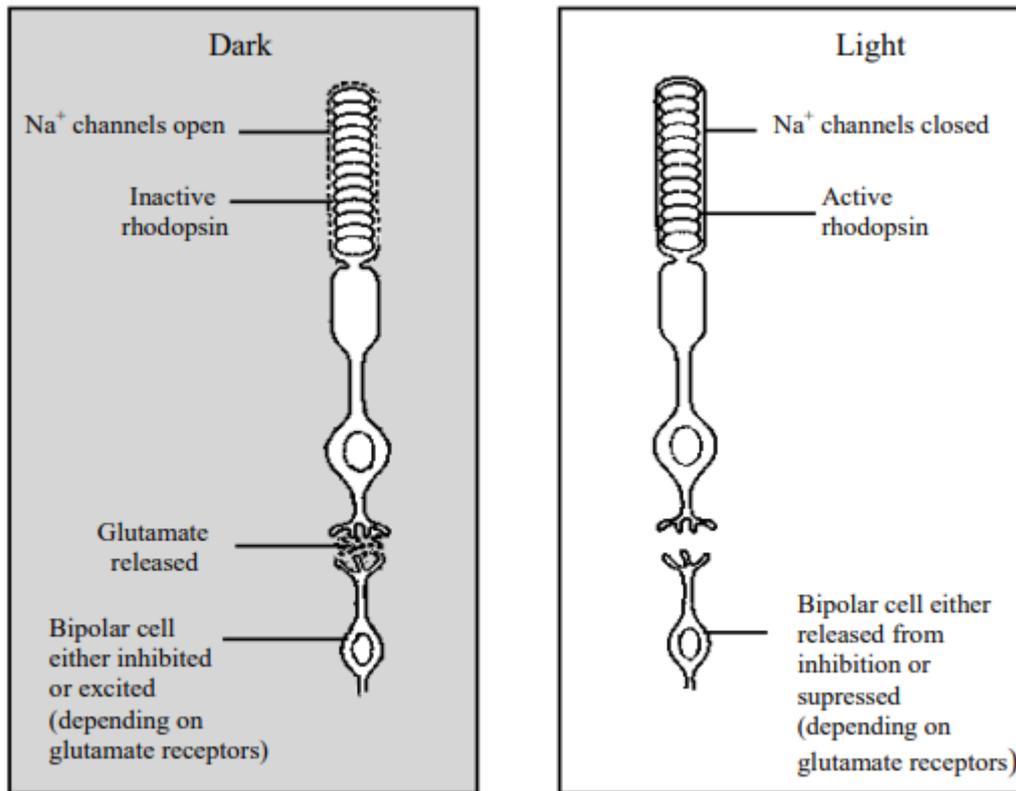
Figure 5: The reaction scheme showing the formation of trans-metarhodopsin II which hydrolyzes to all trans-retinal, which is reconverted to 11-cis-retinal, and opsin.



The rods and the cones have many Na^+ (sodium ion) channels in the plasma membrane in the outer segment and most of these channels are open in the dark. Therefore, Na^+ ions are continuously diffused into the outer segment and across the narrow stalk of the inner segment. The flow of Na^+ ions that occurs due to the absence of light is referred to as the “dark current” and this causes the membrane of the rods to become depolarized [11]. In this state, the rods cells releases glutamate and

regulates the “firing” of two different classes of bipolar cells that have opposite responses to glutamate [4]. In the presence of light, the Na^+ channels in the outer segment begin to close rapidly causing a reduction in the dark current and as a result, the rods become hyperpolarized. The synaptic terminal of the rods slows down the release of glutamate which consequently enhances the activity of one class of bipolar cells and suppresses the activity of the other type [5].

Figure 6: Structure of the rods when depolarized and hyperpolarized.



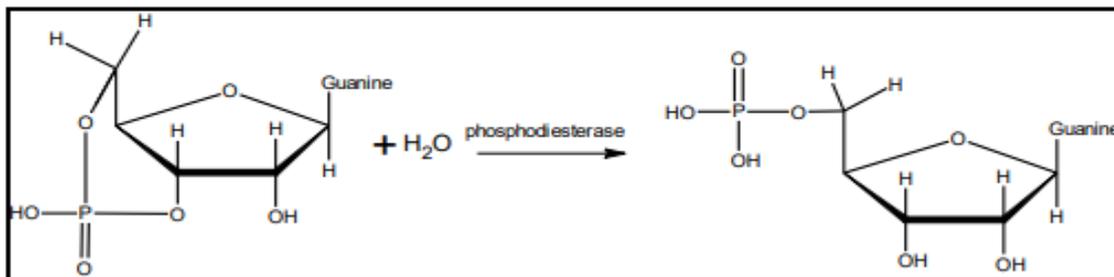
(a) Rod cell depolarized

(b) Rod cell hyperpolarized

Each of the opsin is related to over a hundred regulatory proteins known as G proteins [3]. The trans-metarhodopsin II activates the G protein called transducin, which is also contained in the disc membrane of the rods. This

transducin activates the enzyme phosphodiesterase, which catalyses the hydrolysis of the intracellular messenger, cyclic guanosine monophosphate (cGMP) (Figure 7) [5,6,7].

Figure 7: Hydrolysis of cyclic guanosine monophosphate by phosphodiesterase.



Rhodopsin is inactive in the dark and the cGMP which is bound to Na⁺ ion channels in the plasma membrane of the rod keeps those channels open. In the depolarized state, the rod cells release the neurotransmitter, glutamate, which prevents the transmission of an action potential in the ganglion cell [4,7]. The hydrolysis of cGMP by

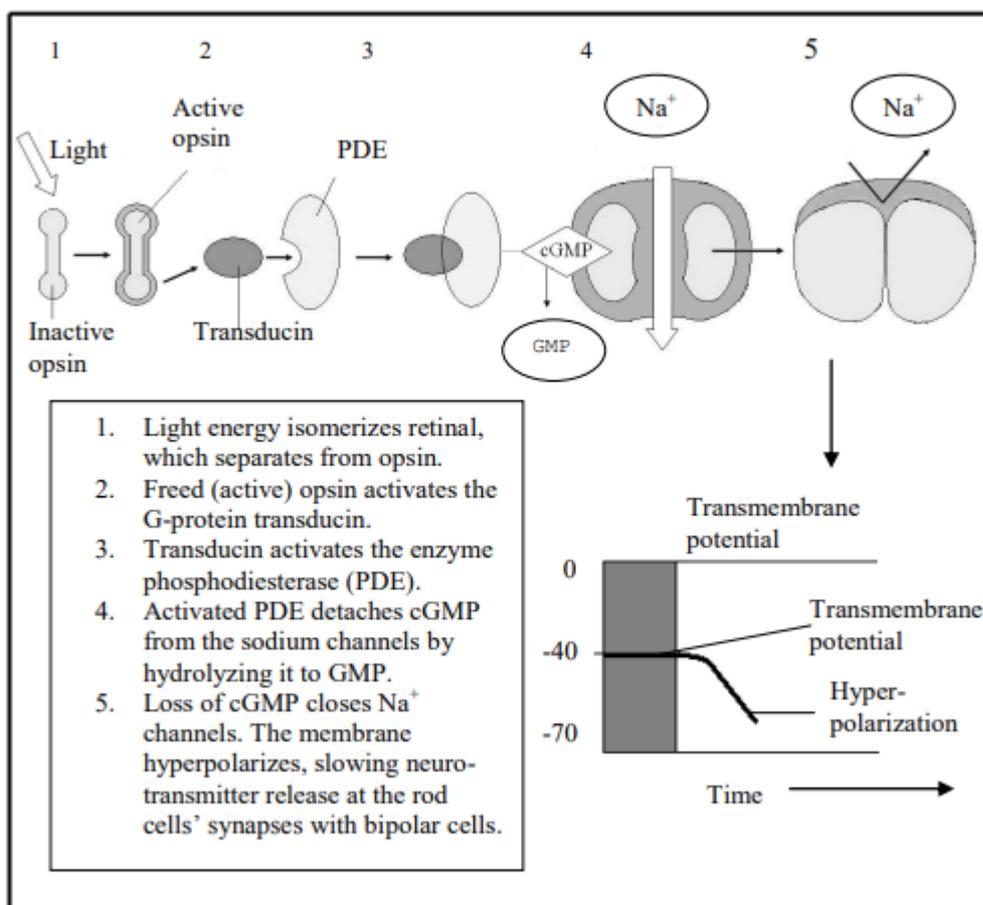
phosphodiesterase to guanosine monophosphate (GMP) causes the Na⁺ channels in the plasma membrane of the rod to close [8,9]. The potential of the cell becomes relatively lower than that of the external environment since the permeability of the plasma membrane to the sodium ions is reduced [2,4,6]. When a large charge difference across the

membrane builds up, the cell becomes hyperpolarized and this prevents the release of the glutamate. As a result of the large difference in potential, an action potential is produced which passes along the rod cell to the synaptic terminal as an electrical impulse [3]. This electrical impulse is then passed onto the adjoining nerve cell which transmits the impulse to the brain by means of the optic nerve. The brain determines the origin of the nerve impulse and the image is interpreted to produce the perception of sight [5]. The all trans-retinal is isomerized back by a number of slow thermal reactions to the 11- cis-retinal which can combine with opsin to reform rhodopsin [7].

The free trans-retinal is reduced to Vitamin A and re-isomerized in a dark, enzyme-catalysed reaction to the 11-cis form which is then re-oxidised to 11-cis-retinal in the rod. The 11-cisretinal reattaches to free opsin to reform rhodopsin which then waits for the next photon to begin the process again [8]. There are still a few not-so-well

understood processes related to vision. For example, the blue light filtering by the carotene-like pigment xanthophyll, present in the tinted region called the "macular pigment", which prevents damage to photoreceptor molecules, is not well explained in the literature. The $\pi \rightarrow \pi^*$ transition which takes place in the conjugated double bonds of xanthophyll is also responsible for reducing the "chromatic aberration", which is also not fully comprehended. The deactivation mechanism for the excited xanthophyll molecule is another area which is unclear. Another gray area of vision is how blue, red, and green absorbing cone cells with different forms of iodopsin combine to produce colour vision. Hence, in a way, this article attempts to emphasize the very fact that vision, particularly human vision, is not fully understood and further elaborative high level research work is an absolute necessity for a clearer picture to emerge.

Figure 8: Signal transduction pathway of a rod cell from light reception to receptor potential.



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