Photoreceptors and Phototransduction

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NEUR 3045 Visual Neuroscience
Background
Light

400 - 700 nm is important for vision
The retina is carpeted with light-sensitive rods and cones.

An inverted image is formed on the retina.
Retinal Cross-Section

Light

Retina 200 ×
Rods and cones

*Fig1b. Scanning electron micrograph of the rods and cones of the primate retina. Image adapted from one by Ralph C. Eagle/Photo Researchers, Inc.*
Human photoreceptors

- **Rods**
  - Achromatic night vision
  - 1 type

- **Cones**
  - Daytime, achromatic and chromatic vision
  - 3 types
  - Long-wavelength-sensitive (L) or “red” cone
  - Middle-wavelength-sensitive (M) or “green” cone
  - Short-wavelength-sensitive (S) or “blue” cone
Human photoreceptor mosaics

0.3 mm of eccentricity is about 1 deg of visual angle

The central foveola (c. 1.25 deg diam.) is rod free
The light-sensitive photopigment lies inside the rod and cone outer segments.
Arrangement of visual pigment molecules

The molecule consists of protein, opsin, forming 7 transmembrane $\alpha$-helices, surrounding the chromophore, retinal, the aldehyde of Vitamin A.

Human foveal cones each contain about $10^{10}$ visual pigment molecules.
Chromophore

**11-cis retinal**

(chromo- colour, + -phore, producer)

Light-catching portion of any molecule

The molecule is twisted at the 11th carbon.
Chromophore
(chromo- colour, + -phore, producer)
Light-catching portion of any molecule

11-cis retinal

A photon is absorbed
Chromophore

(light-catching portion of any molecule)

A photon is absorbed

11-cis retinal

The energy of which initiates a conformational change to...
Chromophore

*chromo- colour, + -phore, producer*

Light-catching portion of any molecule

A photon is absorbed

Think of the molecule as a photo-sensitive switch!

the energy of which initiates a conformational change to...

11-cis retinal

all-trans retinal

ON

OFF

ON

OFF
Phototransduction

Energy of absorbed photon is converted (transduced) to an electrical neural signal, the receptor potential.
Inspired by:

**Phototransduction**
- Activation
- Range extension
- Deactivation
Rhodopsin (R)
G-protein coupled receptor

Chromophore
11-cis-retinal

Transducin (G)
G-protein, trimer with \( \alpha, \beta \) and \( \gamma \) subunits

PDE6
Phosphodiesterase, effector molecule, with \( \alpha, \beta \) and two \( \gamma \) subunits

Main molecular players in the cascade...
In the Dark…
In the Dark

- Intracellular cGMP concentration high
- CNG channels open

CNG = Cyclic Nucleotide Gated channel
Activation steps
Activation steps

A photon is absorbed resulting in activated R*
Activated transducin, G*α, binds to and activates R* catalyses the exchange of GDP for GTP on the G-protein, producing the activated subunit G*α, which dissociates.

Activation steps
The drop in cGMP leads to closure of the CNG channels, which blocks the entry of Na\(^+\) and Ca\(^{2+}\) ions into the outer segment, causing the outer segment to hyperpolarize.
How many photons are needed for us to detect light (when fully dark-adapted)?
When fully dark-adapted, we can detect as few as 7-10 photons.

How is this possible?
Amplification

The absorption of a single photon is sufficient to change the membrane conductance. How?

A single R* catalyses the activation of c. 500 transducin molecules. Each G*α can stimulate one PDE6*, which in turn can break down $10^3$ molecules of cGMP per second. Thus, a single R* can cause the hydrolysis of $>10^5$ molecules of cGMP per second!
Amplification is beneficial at low light levels, but what negative effects might amplification have at high light levels?
An important function of the photoreceptor and the transduction cascade is:
Range extension and light adaptation
Why is light adaptation or sensitivity regulation important?
Because the visual system must maintain itself within a useful operating range over the roughly $10^{12}$ change in illumination: from absolute rod threshold to levels at which photoreceptor damage can occur.
It must do so despite the fact that that a typical postreceptoral neuron can operate over a range of only c. $10^3$. 
Rods that are optimized for low light levels

Cones that are optimized for higher light levels

Typical ambient light levels

Visual function

Sensitive
ROD SYSTEM
Lower range

Less sensitive
CONE SYSTEM
Upper range

Absolute rod threshold
Cone threshold
Rod saturation begins
Damaging levels
Typical ambient light levels

Photopic retinal illuminance  
(log phot td)

-4.3  -2.4  -0.5  1.1  2.7  4.5  6.5  8.5

Scotopic retinal illuminance  
(log scot td)

-3.9  -2.0  -0.1  1.5  3.1  4.9  6.9  8.9

Visual function

Scotopic levels  
(below cone threshold) 
where rod vision functions alone.  
A range of c. 10³

Mesopic levels 
where rod and cone vision function together.  
A range of c. 10³

Photopic levels  
(above rod saturation) 
where cone vision functions alone.  
A range of > 10⁶

Absolute rod threshold  
Cone threshold  
Rod saturation begins  
Damage possible
Adaptation and sensitivity…

System must ADAPT to changes in light level

Ideally, the system should be very sensitive at low light levels, so that it can detect a few photons, but then much, much less sensitive at high light levels.

How can this achieved within the transduction cascade?
At low light levels the sensitivity is very high: A single R* can cause the hydrolysis of $>10^5$ molecules of cGMP per second!

But as the light level increases, the system will saturate (as you run out of “stuff”).
Range extension (1)

Reduction in $[\text{Ca}^{2+}]$ causes Calmodulin (CaM) to dissociate from the CNG channels raising the affinity of the channels for cGMP.

$\text{Ca}^{2+}$ feedback
Reduction in [Ca^{2+}] causes Calmodulin (CaM) to dissociate from the CNG channels raising the affinity of the channels for cGMP.

Reduction in [Ca^{2+}] causes dissociation of Ca^{2+} from GCAP, allowing it to bind to GC increasing the rate of resynthesis of cGMP.

Ca^{2+} feedback
Increase in concentration of $G^\alpha$-PDE6* in light speeds up rate of reaction 2 and speeds up the visual response.
How does speeding up the visual response help light adaptation?
It reduces the integration time of the system...

Long integration time

Short integration time

Light adaptation
What are the benefits of this type of adaptation?

- **Long integration time**
  - Input
  - Output

- **Short integration time**
  - Input
  - Output
Deactivation

Speeding up deactivation also decreases temporal integration.
Rec-2Ca$^{2+}$ forms a complex with RK, blocking its activity. When [Ca$^{2+}$] drops, Ca$^{2+}$ dissociates and Rec goes into solution.
Deactivation steps

Ca\(^{2+}\) feedback

Free RK multiply phosphorylates R*
Deactivation steps

Ca\(^{2+}\) feedback

Arrestin (Arr) quenches the phosphorylated R*
Deactivation steps

RGS9 deactivates the activated $\text{G}^*\alpha$-PDE6* complex.
Deactivation steps

Deactivation (1)

Deactivation (2)

RK → Rec → P → Arr → PDE6
Second run through…
Phototransduction cascade
activation stages
Activation steps of the phototransduction cascade

A photon is absorbed resulting in activated R*
Activation steps of the phototransduction cascade

R* catalyses the exchange of GDP for GTP on the G-protein, producing the activated transducin, subunit G*α (Gα-GTP).
Activated transducin, G*α, in turn, binds to and activates phosphodiesterase (PDE6) by displacing γ inhibitory subunits to produce PDE6*. 

Credit: Pugh, Nikonov & Lamb
Activation steps of the phototransduction cascade

PDE6* (G*α-E*) activity produces a local drop in cytoplasmic cG (cGMP)
A drop in cGMP leads to closure of cGMP gated channels, blocking the entry of Na\(^+\) and Ca\(^{2+}\) into the outer segment. The ion exchanger continues to function lowering [Ca\(^{2+}\)] in the outer segment.

Credit: Pugh, Nikonov & Lamb
Phototransduction cascade inactivation steps
Inactivation steps of the phototransduction cascade

Removal of Ca$^{2+}$ activates guanylate cyclase activating protein, GCAP. Activated GCAP binds to guanylate cyclase, stimulating production of cG.
In the dark, when \([\text{Ca}^{2+}]\) is high, most of recoverin (Rec) is in the calcium bound form at the membrane; Rec-2\(\text{Ca}^{2+}\) forms a complex bond with rhodopsin kinase (RK) blocking its activity.
When $[\text{Ca}^{2+}]$ drops, $\text{Ca}^{2+}$ dissociates from Rec, which moves into solution. Free RK rapidly increases, increasing its interaction with $R^*$, and leading to its rapid phosphorylation.

Ca$^{2+}$ feedback

Credit: Pugh, Nikonov & Lamb
Arrestin (Arr) then binds quenching the activity of R*.

Ca^{2+} feedback

Credit: Pugh, Nikonov & Lamb
G*α-E* is inactivated when the terminal phosphate of its bound GTP is hydrolyzed, which occurs when the RGS9-Gβ5 protein binds to the complex.
Summary of molecular adaptation mechanisms

We’ll come back to these in the Sensitivity Regulation lecture
Mechanisms that shorten the visual integration time

\[ \text{[Ca}^{2+}\text{]} \text{ dependent activity of Rec} \]

\[ \text{[G}^*\alpha\text{-PDE6}^*\text{]} \text{ dependent Increased rate of hydrolysis of cGMP to GMP} \]
Changing the integration time of the system...

**INPUT**

<table>
<thead>
<tr>
<th>Input</th>
<th>Time</th>
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**OUTPUT**

<table>
<thead>
<tr>
<th>Output</th>
<th>Time</th>
</tr>
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- **Long integration time**
  - Light adaptation

- **Short integration time**
  - Light adaptation
Shortening the integration time of the system increases sensitivity to higher flicker rates...

INPUT

OUTPUT

Long integration time

Short integration time

Light adaptation
Human temporal response

- An excellent way of characterizing the effects of light adaptation psychophysically is to measure changes in the temporal response.

- Focus on changes in temporal sensitivity
Changes in temporal sensitivity

- Substantial relative improvements in sensitivity at moderate and high temporal frequencies

**LEVELS (log td)**
- 0.42
- 1.05
- 1.60
- 2.20
- 2.79

**ML**
- 5.69
- 5.28
- 4.75
- 4.16
- 3.39

**MM**
- 2.79
- 2.20
- 1.60
- 1.05
- 0.42
Mechanisms that simply decrease sensitivity

Photopigment bleaching (less photopigment available at high light levels)

Reduction in the number of open CNG-gated channels
Changing the gain (attenuation) of the system...

High gain (low attenuation)

Low gain (high attenuation)
Phototransduction – cones versus rods
Cones versus rods

Cones have different isoforms of:

Visual pigment, transducin, arrestin
PDE6, cGMP channel, and recoverin.

Quantitative differences. In cones:

(i) R* forms 4 times faster than for rods - faster onset of light response.
(ii) R* decays 10-50 times faster (lower amplification factor).
(iii) GTPase activating protein (RGS-Gβ5) expressed at much higher levels - shorter G*α (activated transducin) lifetime - faster recovery.
(iv) Clearance of Ca^{2+} from cone outer segments is several times faster than for rods.
(v) cGMP channels in cones are twice as permeable to Ca^{2+} than in rods.
Cones versus rods

- Cones are 25 - 100 times less sensitive to single photons.
- They catch fewer photons (less visual pigment).
- They respond with faster kinetics (isoforms of transduction cascade).
- They have a much greater ability to adapt to background light.
- They do not saturate at normal environmental light levels.
TRANSDUCTION AND UNIVARIANCE
Chromophore

(chromo- colour, + -phore, producer)
Light-capturing portion of any molecule

A photon is absorbed

11-cis retinal

the energy of which initiates a conformational change to...

all-trans retinal

Think of the molecule as a photo-sensitive switch!
Crucially, the event is binary or “all or nothing”.

If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

**Chromophore**

11-*cis* retinal

![Chemical structure of 11-cis retinal]

all-*trans* retinal

![Chemical structure of all-trans retinal]
Crucially, the event is binary or “all or nothing”.

If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.
Crucially, the event is binary or “all or nothing".

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Crucially, the event is binary or “all or nothing”. If a photon is absorbed it has the same effect as any other absorbed photon, whatever its wavelength.

11-cis retinal

all-trans retinal
Can this process encode wavelength (colour)?

11-cis retinal

all-trans retinal
No, it cannot encode wavelength (colour)!

It is “UNIVARIANT”

Chromophore

11-cis retinal

all-trans retinal
Vision at the photoreceptor stage is relatively simple because the output of each photoreceptor is:

“UNIVARIANT”

What does univariance mean in practice?

Use Middle-wavelength-sensitive (M) cones as an example...
Vision at the photoreceptor stage is relatively simple because the output of each photoreceptor is:

“UNIVARIANT”

What does univariance mean in practice?

Use Middle-wavelength-sensitive (M) cones as an example...
So, if you monitor the cone output, you can’t tell which wavelength (energy) of photon has been absorbed.

UNIVARIANCE

Crucially, the effect of any absorbed photon is independent of its wavelength.
Crucially, the effect of any absorbed photon is *independent* of its wavelength.

All the photoreceptor effectively does is to count photons.
What does vary with wavelength is the probability that a photon will be absorbed.

This is reflected in what is called a “spectral sensitivity function”.

UNIVARIANCE
Imagine the sensitivity to these photons...

In order of M-cone sensitivity:
If you have four lights of the same intensity (indicated here by their heights)

The green will look brightest, then yellow, then blue and lastly the red will be the dimmest
We can adjust the intensities to compensate for the sensitivity differences.

When this has been done, the four lights will look completely identical.
Changes in light intensity are confounded with changes in colour (wavelength)
Vision at the photoreceptor stage is relatively simple because the output of each photoreceptor is: UNIVARIANT
What is univariance?

- *Probability* of photon absorption depends upon wavelength

- But the response is *independent* of photon wavelength --
  - Response varies in ‘one way’—more or less photons are absorbed.
  - A change in output could be caused by a change in intensity or a change in colour.
  - Each photoreceptor is therefore ‘colour blind’, being unable to distinguish between colour and intensity changes.
Univariance in suction electrode recordings

4.16 THE CONE PHOTOCURRENT in response to a brief test flash is biphasic. The amplitude of the photocurrent response increases with the stimulus intensity. The response functions are the same for different wavelengths of light: (A) stimulus wavelength = 500 nm; (B) stimulus intensity = 659 nm. The stimulus time course is shown below the photocurrent plots. Source: Baylor et al., 1987.

4.17 THE PRINCIPLE OF UNIVARIANCE states that absorption of a photon leads to the same neural response, no matter what the wavelength of the photon. The principle predicts that two stimuli at different wavelengths can be adjusted to equate the photocurrent response throughout its time course. This is shown here as the match between photocurrents in response to 550 nm (shaded line) and 659 nm (solid line) test lights set to a 9:1 intensity ratio. Source: Baylor et al., 1987.
Univariance

If a cone is $n$ times less sensitive to light A than to light B, then if A is set to be $n$ times brighter than B, the two lights will appear identical whatever their wavelengths.
If we had only one photoreceptor type in our eyes, what colours would we see?
If we had only one photoreceptor, we would be colour-blind…

Examples: night vision, blue cone monochromats
What does vary with wavelength is the *probability* that a photon will be absorbed, and this relationship is different for the four different photoreceptors.

This is reflected in what are known as spectral sensitivity functions...
Four human photoreceptors have different spectral sensitivities

\[ \lambda_{\text{max}} \text{ (nm, corneal, quantal)} \]

- L (long-wavelength): 500 nm
- M (medium-wavelength): 541 nm
- S (short-wavelength): 441 nm
- Rods: λ_max varies across different species and lighting conditions

Log\(_{10}\) quantal sensitivity

-5  -4  -3  -2  -1  0

Wavelength (nm)

400  450  500  550  600  650  700
So, if each photoreceptor is colour-blind, how do we see colour?

Or to put it another way: How is colour encoded at the input to the visual system?
A change in colour from green to red causes a relative increase in the L-cone output but causes a decrease in the M-cone output.

A change in colour from red to green causes a relative increase in the M-cone output but causes a decrease in the L-cone output.

Thus, colour can be encoded by comparing the outputs of different cone types...
Colour is encoded by the relative cone outputs

[Graph showing Log₁₀ quantal sensitivity against Wavelength (nm) for blue light]
Colour is encoded by the relative cone outputs

Wavelength (nm)

Log$_{10}$ quantal sensitivity

S M L

Colour is encoded by the relative cone outputs

Blue light

Green light
Colour is encoded by the relative cone outputs.
Colour is encoded by the relative cone outputs

Blue light

Red light

Green light

Purple light

Yellow light

White light
If we want to be able to predict the relative responses of the three cones signals to lights of any spectral composition...
We need to know the three cone spectral sensitivities:

More in the lecture on “Achromatic and Chromatic Vision”.
Photopigments and spectral tuning
The spectral sensitivity of the photopigment depends on the energy required to initiate the rotation of the chromophore from its 11-cis form to its all-trans form.
The double bond is made up of a $\sigma$ and a $\pi$ bond.

Together, they prevent rotation around the double-bond axis.

Therefore there are different “stereoisomers”.
The absorption of a photon promotes a $\pi$ electron to a higher-energy orbital.
This "breaks" the π component of the double bond, allowing free rotation about the s bond and thus the conformational change from 11-cis-retinal to all-trans-retinal, (which has a lower energy).

The absorption of a photon promotes a π electron to a higher-energy orbital.
The absorption of a photon promotes a $\pi$ electron to a higher-energy orbital.

What is the optimal energy for each photopigment?

\[ E = \frac{hc}{\lambda} \]

\( h = 6.62606957 \times 10^{-34} \text{ J.s} \)

\( c = 2.99792458 \times 10^8 \text{ m.s}^{-1} \)

\begin{align*}
S & : 421 \text{ nm} & : 4.72 \times 10^{-19} \text{ J} \\
M & : 530 \text{ nm} & : 3.75 \times 10^{-19} \text{ J} \\
L & : 559 \text{ nm} & : 3.55 \times 10^{-19} \text{ J}
\end{align*}
But the same chromophore is used in all four human photopigments.

So how is the initiation energy modified?

Photopigment molecule (cone)

From Sharpe, Stockman, Jägle & Nathans, 1999
But the same chromophore is used in all four human photopigments.

The initiation energy is altered by changing the amino acids in the surrounding opsin molecule.

Photopigment molecule (cone)

From Sharpe, Stockman, Jägle & Nathans, 1999
Only 15 amino acid differences between L and M: 96% identical

From Sharpe, Stockman, Jägle & Nathans, 1999
Rods and S cones have 348 amino acids whereas L and M cones have 364 amino acids – 16 amino acid difference at the N terminal
Tuning Site

180 alanine

277 phenylalanine

285 alanine

MWS

LWS

serine $^{\text{OH}^-}$ ~ 5 nm

tyrosine $^{\text{OH}^-}$ ~ 25 nm

threonine $^{\text{OH}^-}$
Mean L- and M-cone spectral sensitivity functions

Note logarithmic scale

Wavelength (nm)

Log$_{10}$ quantal sensitivity

541 566

L

M

500 550 600 650 700

400 450 500 550 600 650 700