Monocular Sensory Processes of Vision:

Color Vision

*Mechanisms of Color Processing*
VI. Retinal fundamentals

A. Retinal fundamentals and cone photopigments
B. Properties of cone photopigments
C. Subjective methods of defining the cone fundamentals
D. Objective methods of defining the cone fundamentals
E. Spatial organization of cone types
Retinal mechanisms, because color as a stimulus attribute must be encoded initially by the cone receptors.

Normal color vision is trichromatic and must depend on three types of cone photopigments.

Three cone types
2. Medium wavelength or M-cones *(chlorolabe)* - peak sensitivity in the green wavelengths (535 - 540 nm).
3. Long wavelength or L-cones *(erythrolabe)* – peak sensitivity in the redish-yellow wavelengths (565 – 570 nm).

http://conesandcolor.net/
Cone Photopigments

- The photopigments have broadband absorption spectra - the absorption of each photopigment covers most of the visible spectrum.

- Each cone photopigment is composed of a chromophore and an opsin.

- All rod and cone photopigments in mammalian retinas have the same chromophore.

- Spectral absorption properties occur as a result of structural differences in the opsin molecule.

http://www.photobiology.info/
Normal Human L, M, and S Cone Photopigments Opsins

- The chromophore molecule is retinal.
- All photopigment differences arise in the opsin, which determines the ability of the photopigment, and the photoreceptor cell that contains it, to respond to light.

- Colored circles show the amino acid differences in molecular structure
- S-cone and M-cone pigments are ~50% similar
- L-cone and M-cone pigments are 96% similar

Variation in Human Cone Opsins

- The human M/L cone opsins have 364 amino acids.
- There are 18 known polymorphic variations in the M/L cone opsin.

- Amino acid variations at the 2 sites shown as red cause large shifts in spectral absorption.
- Altered amino acids at one of the 5 sites shown in yellow cause small spectral shifts (~2 nm).
- Changes at any of the 11 blue sites do not influence spectral tuning.
- The chromophore is attached at position 312.

Cone Photopigments

- All cone photopigments have broad spectral absorption properties, but differ in their relative position along the visible spectrum.

- A precise knowledge of the properties of the shape and location of the cone photopigments is essential to the understanding of normal and abnormal color vision.

- The shapes of the absorption spectra of all cone photopigments must be similar.

- Each photopigment must obey the “principle of univariance.”
Properties of Cone Photopigments

- All mammalian photopigment spectra have identical shapes, if they are compared on a wavenumber (1/\(\lambda\)) axis, where \(\lambda = c/\nu\) (speed of light/ frequency).
- In the examples, the absorption spectra of two photopigments, plotted on a wavelength axis (left), were shifted to make the wavelength at maximum absorption \((\lambda_{\text{max}})\) coincide on the wavenumber axis (right).
Properties of Cone Photopigments

• Principle of univariance - the neural output from any cone is a univariant, i.e., a color-blind signal.

• A signal from a single photopigment is related to the quantum catch (intensity).

• Absorption of light causes bleaching of the photopigment and a reduction of the probability of quantum catch at all wavelengths.

• A change in the state of adaptation causes an identical, proportional change in sensitivity at all wavelengths.

• The change in sensitivity of a photopigment as a function of adaptation, without a change in shape, is an application of the principle of univariance.
Principle of Univariance

- Spectral sensitivity functions S-cones (left) and M-cones (right) under two states of adaptation.
- S-cones (left figure) - the change in shape of the function with increased level of adaptation indicates a lack of univariance and the upper data do not represent a single photopigment across the full function.
- M-cones (right figure) - the shape of the function is identical under both states, demonstrating univariance.

Harwerth & Sperling, Vis Res 15:1193-204, 1975
**Methods of Studying Color Vision Fundamentals**

1) Subjective methods
   - Increment-threshold spectral sensitivity with selective adaptation.
   - Noninvasive, *in vivo* measurements
   - High precision, but they cannot differentiate between separate photopigments in individual cones or all three photopigments in each cone.

2) Objective methods
   - Microspectrophotometry
   - Suction electrode methods
   - *Ex vivo* measurements
   - Less precision, but they can measure the photopigments in separate cones over a large dynamic range.
Subjective Methods of Defining the Cone Fundamentals

- Stiles’ two-color, increment-threshold spectral sensitivity.
- Assumptions:
  1. In any area of the retina there are several different photopigments, but the one with highest sensitivity will determine the visual threshold.
  2. Photopigments that differ in their spectral locations will adapt at different rates for a given wavelength of the background.
  3. Cone mechanisms can be identified by “threshold-versus-intensity” (TVI) functions.

Stiles’ TVI Functions

- With the correct choice of test field and background wavelengths, the color vision mechanisms can be isolated and their spectral sensitivities investigated.

- The example shows two independent color vision mechanisms, one more sensitive to the 480 nm test field at absolute threshold and which adapts more rapidly to the 540 nm background field.
Stiles’ TVI Functions

- Measurements of TVI functions across wavelengths provides data to derive the spectra response function for each mechanism.
- Initially, Stiles called the functions cone photopigments, but later called them the “pi mechanisms.”
Stiles’ Pi Mechanisms

- Stiles presented data for 5 independent photopic mechanisms and 1 scotopic mechanism.

\( \Pi_0 \) - rod photopigment
\( \Pi_1 \) - SW mechanism
\( \Pi_2 \) - SW & MW mechanism
\( \Pi_3 \) - SW mechanism
\( \Pi_4 \) - MW mechanism
\( \Pi_5 \) - LW mechanism

Stiles, WS. *Proc Nat Acad Sci USA.*, 1959;45:100-114.
Selective Chromatic Adaptation

- The limiting condition of Stiles’ two-color threshold technique - use background wavelengths that selectively adapt two mechanisms, so that the third can be measured.

Adaptation field
wavelength = 620 nm  460 + 620 nm  460 nm

Harwerth & Sperling, Vis Res 15:1193-204, 1975
Recent Data on Human Cone Spectral Sensitivities

- Cone spectral sensitivities based on measurements in normal trichromats, dichromats, and monochromats.
Suction Electrode Methods

- Rod or cone outer segments are drawn into a suction electrode for recording membrane current produced by light absorption. The photograph shows a beam of light on a single cone outer segment.

- Measurements of the photocurrent with the suction electrode technique are the most sensitive method of studying photopigments.

Suction Electrode Methods for Measuring Cone Photopigments

- The spectral sensitivities of the three cone types in the primate retina.
- The sensitivities were determined over a 6 log unit range.
- The MW and LW cone sensitivities overlap across the entire visible spectrum, but the SW cone sensitivity is more separated (λmax’s at 435, 535, & 570 nm).

**Corrections for Pre-Retinal Light Absorption**

- A comparison of the results of subjective and objective measures of the retinal fundamentals requires correction for pre-retinal light absorption by the macula leuta (a) and crystalline lens (b).

- The absorption spectra of both the macular and lens pigments have their highest density at short wavelengths.
Human Cone Fundamentals

- With correction for pre-retinal light absorption (macula leuta and crystalline lens) the data from psychophysical and suction electrode methods are in substantive agreement.
Spatial Organization of Primate Cones

- In addition to the spectral absorption of cones, it is important to know the spatial organization and relative proportions of the cones.

- The figure is a model of the probable organization of human cones in the central fovea.

- Less than 10% of cones are SW cones and they are totally absent in the foveola.

- The ratio of LW to MW cones is modeled as about 2:1 throughout the entire retina.
Variations in Cone Mosaics of Subjects with Normal Color Vision

- Adaptive optics views of the L-cone (red), M-cone (green), and S-cone (blue) mosaics of four human subjects with normal color vision.
- The ratios of S-cone to L- or M-cones is constant across subjects.
- The ratios of L-cones to M-cones varies across subjects.
Variations in Cone Mosaics of Subjects with Normal Color Vision

- False color images showing the arrangement of L (red), M (green), and S (blue) cones in the retinas of different human subjects. All images are shown to the same scale.
- All of the subjects had normal color vision.
- The proportion of S-cones is about constant across subjects, but the ratio of M-cones to L-cones varies by about 40X.

DR.Williams, Vis Res 51:1379–1396, 2011
**Spatial Organization of SW Cones**

- S-cones can be identified by metabolic dyes (upper figure).
- The S-cones are a relative small proportion of the total cone population and are arranged in hexagonal arrays (lower figure).
- In contrast, the M- and L-cones appear to be randomly distributed.

Relative Proportions of Cone Types

- The relative proportions of cone types have been studied in the baboon retina.

- The peak density of S-cones is 1.5 deg from central fovea.

- The peak densities of the M- and L-cones is at central fovea with a 2:1 ratio for M- to L-cones at all eccentricities.

- The M-:L-cone ratio may vary across species or across individuals of a single species.

- Many models of human color vision incorporate a 1:2 ratio of M- to L-cones.

The Third Class of Photopigments in Mammalian Retinas

1. Rods – Rhodopsins
2. Cones – Photopsins
3. Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCs) – Melanopsin.

- $\lambda_{\text{max}} = 467$ nm
- The giant RGCs of the primate retina are examples of photosensitive ganglion cells.
- When a quantum of light is absorbed by melanopsin, a response is elicited but the photopigment also becomes desensitized. In contrast to rod and cone photopigments that require the enzymatic retinoid cycle to restore their light sensitivity, melanopsin uses the absorption of a second photon to regenerate the photopigment.


http://www2.cnrs.fr/
Melanopsin regulates a wide range of non-visual functions.

1. The synchronization of the circadian rhythms and the sleep-wake cycle with the light-dark cycle. Light detection by melanopsin acts directly on non-circadian mechanisms that interact with circadian mechanisms.

2. Adjustments in pupil size for homeostatic retinal adaptation. Rods and cones allow an initial transient constriction of the pupil, while melanopsin produces a stabilized state of sustained constriction in response to light because melanopsin remains photosensitive even during extended exposure to light.
Mechanisms of Color Processing

Recap

- Properties of cone photopigments
- Principle of Univariance
- Subjective/objective measurements of absorption spectra
- Pre-retinal light losses
- Variations of photopigment classes in normal color vision

http://albaalfonsogarcia.com/home/2012/10/