Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN

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Photosensory System: Rods

Photopigments, found in disc membranes, drive light signal transduction:

- **Rods**: Rhodopsin
- **Protein**: opsins - membrane protein with multiple transmembrane helices
- **Chromophore**: 11-cis-retinal

Light photoisomerizes 11-cis-retinal to all-trans-retinal

- Retinal leaves opsins binding site, opsins undergoes conformational change, all-trans retinal detaches, opsins activates transducin... leads to photoreceptor decreased glutamate release → response!
Photosensory System: Cones

Photopsins vs Rhodopsins:

- Same chromophore - 11-cis-retinal
- Different Opsin Structure = wavelength sensitivity

Cones:

- Photopsin III → S cones: short wavelength receptor, detect blueish light
- Photopsin II → M cones: medium wavelength receptor, detect greenish light
- Photopsin I → L cones: long wavelength receptor, detect redish light
Photosensory System: Ganglion Cells

Light information passes through ganglion cells before going to brain: no-latency image pre-processing:

- Visual perception is not a result of cortical processing of raw photoreceptor light data
- Ganglion cells add vital and immediately processed information about image features
Photosensory System: Ganglion Cells

Ganglion cells as edge detectors:

RGC neural signals remain segregated up to the LGN, but merge in the primary visual cortex (complex cells)

Heeger Lab, NYU
Photosensory System: Ganglion Cells

“Hawk detector” - Mouse W3-RGC activity

The most numerous ganglion cell type of the mouse retina is a selective feature detector. Meister et al. 2012
Color Perception: Opponency Theory

- Basis: trichromatic theory of color vision - RGB combination = color
- Meaning, yellowish red = orange, reddish blue = purple...
- But what about yellowish blues or reddish greens?
- Color perception is not just based on trichromacy

Hering’s Opponency Theory

- Blue and Yellow are opponent colors
- Red and Green are opponent colors
- Two independent pipelines process color: blue-yellow | red-green
Color Perception: Opponency Process

- Midget Cells (RGC) → LGN parvocellular subtypes \([LxM] [Sx(L+M)]\)
- Parasol Cells (RGC) → LGN magnocellular cells, perception, motion, details
- The LGN contains color-cells for each of the two pipelines
- Opponent cells exhibit center-surrond receptive fields
Color Perception: Opponency Process

Opponent Theory
L*a*b colorspace

Trichromatic Theory
RGB colorspace
Photosensory Pathways

Visual Pathway Targets

- Lateral Geniculate Nucleus - in the Thalamus, receives most of the input, sends to visual cortex
- Pretectum - located at the thalamus-midbrain boundary, for pupillary light reflex
- Superior Colliculus - midbrain, head and eye movement coordination
- Suprachiasmatic Nucleus - hypothalamus, circadian functions

Image-Forming Pathways - Information with high spatial and temporal resolution: for “seeing”

Non-Image-Forming Pathways - Information about ambient luminance for circadian photoentrainment, pupil size, etc.
Study Overview

- A photosensory mechanism, that is not rod or cone, was discovered in nocturnal rodents: melanopsin-expressing ganglion cells
- Primates contain such a population, of anatomically distinct “giant” melanopsin-expressing ganglion cells that are photosensitive
- These cells exhibit an interaction with rods and cones, displaying an S-Off, (L+M)-On color opponent receptive field
- The combined information gives a signal of irradiance across the full dynamic range of human vision
- These cells project to the LGN... showing a convergence of non-image-forming and image-forming visual pathways
Melanopsin-associated photodetection

- Comprise a small subset of retinal ganglion cells, ~1% in nocturnal rodents
- Photopigment is melanopsin, Absorption ~480nm, mainly blue
- Phototransduction mechanism not well understood, light response by increasing firing rate
- Highly conserved, signalling pathway similar to invertebrates
- Previously thought to only be a part of the non-image forming pathway - via retinohypothalamic tract to the suprachiasmatic nucleus and olivary pretectal nucleus for pupillary control
Identifying Melanopsin-Expressing RGC’s

- Immunohistochemistry with Anti-Melanopsin (green)
- Adult human and macaque retinas, flat-mounted
- Confocal imaging over whole flatmount
- Assessed distribution, amount, and morphology of cells
Identifying Melanopsin-Expressing RGC’s
Melanopsin-Expressing RGC’s IHC Results

- Flat-mounts revealed distinct population of ~3000 melanopsin expressing RGCs
- With ~1.5 million RGCs, this population represents 0.2%
- Morphology of these cells shows the largest dendritic trees of any primate RGC
- Dendritic trees formed a dense plexus around fovea
- Dendrites (+cells) localized in extreme inner, and extreme outer layers of IPL; two cell subpopulations
- Cells have a minimum density (3–5)/mm² in periphery
- Cells have a maximum density (20–25)/mm² in parafoveal regions
Using *in vitro* intact macaque retina, rhodamine dextran was injected into LGN and pretectal olivary nucleus for retrograde tracing.

Still *in vitro*, light exposure to transported rhodamine liberates it, then it can spread through the cell, to the dendrites (!).

Light patterns were used to elicit activity on the flat-mounts - spectral opponency was targeted with light stimuli specific for S or (L+M) isolation.

In order to isolate rod or cone specific interaction, retinas were bathed in L-AP4 and CNQX (block ionotropic and metabotropic retinal glut receptors).
Next: Exploring pathway outputs

- Further rod and cone specific activity was parsed using spectral tuning (in conjunction with pharmacologic isolation) using a “varispec” liquid crystal tunable filter: enabling them to select 20nm bands between 430-610nm for light exposure
- Using rhodamine to target, activity was also assessed via intracellular recording
- Immunostaining after with anti-melanopsin for colocalization
Results 1

- **(a)** - Tracing of an RGC. The cell was recorded *in vitro*. Voltage traces for 550nm monochrom light, photopic conditions

- **(b)** - showing an (L+M)-On, S-Off opponent field. Left shows spatial frequency response to variable gratings. Dark gray circles indicate L+M cone isolation, other shows S cone isolation
Results 1

- **(a)** - RGC show photopic response, at 550nm evoke On response
- **(a)** - Spike latency typical of cone-mediated RGC response
- **(b)** - Observed S cone-mediated Off response was antagonistic to an (L+M) mediated On response, displaying a color opponent receptive field
Results 2

- (c) - Pure rod-mediated response via 550 nm monochrom at scotopic levels
- (d) - L-AP4 and CNQX application to isolate inherent photoresponse at 550nm photopic
Results 2

- (c) - After 10-20min total darkness, scotopic light stimuli elicited a strong rod-driven response
- (c) - Spike latency typical of rod-mediated RGC response
- (d) - Post-pharmacological silencing of photopic cone response still elicited RGC response
Results 3

- **(a)** - Total spikes for 10-s pulses across wavelengths against log quanta (illuminance)

- **(b)** - Giant cell response to monochrom light step 470nm as a function of retinal illuminance. Grey (dark adapted), Black (light adapted), White (isolated intrinsic)
Results 3

- **(a)** - The precise “photon-counting capability” in the cells was shown by counting the total number of spikes elicited by a long-duration light pulse, including those after light offset.

- **(b)** - Mean spike rate also increases regularly with increasing irradiance.

- The isolated, inherent photoresponse transmits a signal that best measures total irradiance.
Results 4

- (d) - Cone-mediated response at 13.5 log quanta illuminance, measured after 60s On
- (e) - Isolated response at 13.5 log quanta, blocked cone input
Results 4

- (d) - With no pharmacological blockade of cone activity, a summation of cone response and intrinsic response was seen at 470nm

- (e) - While inherent response seems to elevate the cone response, sustained firing was observed after the light stimulus
Results 5

- (f) - Cone mediated response at 610nm at 12 and 15.2 log quanta (low and high brightness) photopic

- (g) - Summed cone and intrinsic response at 470nm pulse at 11 and 14.6 log quanta (low and high brightness) photopic
Results 5

- **(f) left** - With a long wavelength source, the (L+M) cone response is transient at threshold.
- **(f) right** - The (L+M) cone response is transient at highest photopic levels.
- **(g) left** - A short wavelength source makes a threshold response sustained, masking possible inhibition from S cones.
- **(g) right** - Higher light intensity shows continuous discharge after lights offset.
Conclusions

- A Melanopsin expressing RGC exists in the primate retina with distinct morphological characteristics
- This population is intrinsically photosensitive
- Apart from their intrinsic activity, they also exhibit an interaction with rods and cones
- The rod-cone-RGC relationship shows an S-Off, (L+M)-On color opponent receptive field (an indirect conclusion?)
- Tracing shows output connection to LGN and the pupillomotor nucleus.
A new photosensitive cell has been discovered in primates; a cell that is not a rod or cone.

This cell supplies the brain with information about the light it captures... and also integrates the input of local rods and cones as well; local = a wide parafoveal plexus.

It sends this information to regions of the brain that control circadian rhythms as well as regions that process visual detail.

How this information is used by the brain to modulate image detail or color perception is unknown...
Conclusions

- With a combined rod, cone, and inherent sensitivity this cell has the ability to signal irradiance across the full dynamic range of human vision.
- The chromatic opponency may have evolved to signal large spectral changes to the circadian systems (dawn to dusk).
- The wide intrinsic sensitivity range and a projection to the LGN suggests that these cells may be providing an irradiance input to the visual cortex.
  - Irradiance encoding units have been recorded in the visual cortex, but their sensory origin has not been determined. These cells could a part of what encodes a total irradiance signal.