Melanopsin-Expressing ipRGCs, Location, Characteristics and Role in Physiology and Behavior

Laura Frishman

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Why do we need another photoreceptor?
Clinical implications of melanopsin-containing retinal ganglion cells

Image forming “pattern” vision and non image forming vision (NIF) when environment light is a regulator of physiology and behavior
Circadian entrainment in mammals
A little history

  - The eye is necessary.
- Mice with complete rod/cone loss still entrain to light/dark cycles (e.g, Ebihara & Tsuji, 1980; Foster et al., 1991)

Pupillary light reflex
- Mice with complete rod/cone loss also still have a strong pupillary light reflex (Keeler, 1920; Lukas et al., 2001)

Melanopsin (Opn4)

- An opsin-based photopigment discovered in the photosensitive skin melanophores of African clawed frog (Provencio et al., 1998)
- Present in cells in the inner retina of mammals (Provencio et al., 2000)
- Melanopsin is present in cell bodies, dendrites, and proximal axonal segments of a subset of rat RGCs (Hattar et al., 2002)
- Blind mice lacking rods and cones show circadian entrainment, but not if they lack melanopsin (Provencio et al, 2003; Panda et al., 2003)
Intrinsically photosensitive RGCs

A subpopulation of RGCs are intrinsically photosensitive (ipRGCs)
- Identified through retrograde labeling from SCN
- Responses are very slow and sustained

Berson, Dunn & Takao, 2002

Neurotransmission in the retina – block to eliminate postreceptoral pathways

- Metabotropic glutamate receptor: ON bipolar cell (L-AP4)
- Ionotropic glutamate receptor OFF bipolar (& HzCs), amacrine and ganglion cells (CNQX)
- Inhibitory amino acid receptors (GABA an Glycine) in inner retina
Establish intrinsically photosensitivity

Berson et al., 2002

SCN-projecting ipRGCs contain melanopsin

Tg mouse (tau-Lac-Z): beta-galactosidase

Rat: melanopsin antibody

Hattar et al., 2002
Light responses from cultured ipRGCs

Cultured ipRGCs, completely isolated from other retinal cell types, maintain their photosensitivity

Hartwick et al., 2007

ipRGCs are most sensitive to blue light
Peak sensitivity: ~480 nm

Rat ipRGCs: Berson et al., 2002  Primate ipRGCs: Dacey et al., 2005
ipRGCs predominantly project to ‘non-visual’ brain regions such as the SCN and the OPN (pupil light reflex).

Classes of melanopsin cells in mouse

Schmidt et al., 2011
Melanopsin cell types: projections

Schmidt et al, 2011
Types of ipRGCs in mice

M1  M2  M3

Schmidt et al., 2011

M1 ipRGC – feedback to dopaminergic amacrine cell (DAC)

Prigge et al., 2017
M4 cells: pre- and early postnatal influence

- Melanopsin-positive cell density decreases by 17% between post-natal days 8 and 15 and by 25% between days 8 and 30.
- Decrease due specifically to a decrease in melanopsin-positive cells co-labeled with a SMI-32, a marker for alpha-on ganglion cells (correspond to M4 ipRGCs).
- Type I responses:
  - Short Latency, high sensitivity, quick recovery from adaptation.
  - Type 1 responses disappear by P15.

Sexton et al, Neural Dev., 2015
Primate retina – geniculo-striate pathways in primate: form vision

Midget, On and OFF (70% RGCs)
Parasol, On and Off (10%)
SBS (BY) pathway (On-Off) (8%)
Off BY and melanopsin (0.2% ?)

(Dacey et al., 2005)

Giant retinal ganglion cells in primate retina

(Dacey et al., 2005)
Giant RGCs in primate retina: L+M-cone On and S cone Off responses

Dacey et al., 2005

Liao et al 2016 – primate retina
- Outer – M1
  input from DA ACs
- Inner – M2

macaque

human
Retinal ganglion cell density along horizontal meridian

ipRGCs: 15 cells/mm²

Liao et al, 2016 – ipRGCs have huge dendritic fields
ipRGCs: How many in one retina?

<table>
<thead>
<tr>
<th></th>
<th>Rat Retina</th>
<th>Human Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rods &amp; Cones</td>
<td>20,000,000</td>
<td>125,000,000</td>
</tr>
<tr>
<td>RGCs</td>
<td>120,000</td>
<td>1,500,000</td>
</tr>
<tr>
<td>Melanopsin RGCs</td>
<td>2,500</td>
<td>3,000</td>
</tr>
</tbody>
</table>

Hattar et al., 2002 (Dacey et al., 2005)

Pupillary response in primate

Gamlin et al., 2007
Post-illumination pupil response

• In humans, pupil takes longer to re-dilate with blue light stimulation versus red light stimulation – consistent with ipRGC prolonged responses

• Under pharmacological blockade, the persistent pupil constriction in primates shows a spectral sensitivity consistent with melanopsin (Gamlin et al., 2007)

Kankipati, Girkin & Gamlin, 2011
Human response

Lukas et al., 2014
Lukas et al., 2014 – which photoreceptors drive the pupil?

Pupil constriction and photoreceptor type: S-cone signals do not constrict the pupil

- Opponent melanopsin and S-cone signals in the human pupillary light response (Spitschan et al., 2014)

- Melanopsin- and L-cone–induced pupil constriction is inhibited by S- and M-cones in humans (Woelders et al, PNAS 2018)

- Silent substitution technique (Spitschan & Woelders, 2018)
Table 1. Photometric measures for each of the five potential photoreceptive inputs to circadian and neurophysiological light responses in humans

<table>
<thead>
<tr>
<th>Photoreceptor</th>
<th>Photopigment</th>
<th>Spectral sensitivity function</th>
<th>Unit of measure*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-wavelength (S) cones</td>
<td>S-cone photopigment</td>
<td>Cyanolite response function $N_\omega(\lambda)$</td>
<td>Cyanolic illuminance (cyanolic-lux)</td>
</tr>
<tr>
<td>Medium-wavelength (M) cones</td>
<td>M-cone photopigment</td>
<td>Chlorolite response function $N_\omega(\lambda)$</td>
<td>Chlorolic illuminance (chlorolic-lux)</td>
</tr>
<tr>
<td>Long-wavelength (L) cones</td>
<td>L-cone photopigment</td>
<td>Erythrolite response function $N_\omega(\lambda)$</td>
<td>Erytholic illuminance (erytholic-lux)</td>
</tr>
<tr>
<td>ipRGCs (intrinsic photopigment)</td>
<td>Melanopin</td>
<td>Melanopin response function $N_\omega(\lambda)$</td>
<td>Melanolic illuminance (melanolic-lux)</td>
</tr>
<tr>
<td>Rods</td>
<td>Rod opsin</td>
<td>Rod opsin response function $N_\omega(\lambda)$</td>
<td>Rhodolic illuminance (rhodolic-lux)</td>
</tr>
</tbody>
</table>

*Each unit of measure $(E_\omega)$, where $\omega$ specifies the retinal photopigment, is derived by convoluting the spectral power distribution of incident light $(E_\lambda)$ with the relevant spectral sensitivity function, which in turn is derived by the photopigment spectral sensitivity adjusted for pre-receptoral filtering in a standard observer $(N_\omega(\lambda))$ (see the supplementary material online for full functions and a detailed description of their derivation) according to the equation $E_\omega = \int E_\lambda N_\omega(\lambda) d\lambda$. Species-specific variants of the spectral sensitivity functions may be required for non-human applications to account for differences in pre-receptoral filtering and photopigment spectral sensitivities.

The ipRGC as a site of signal integration

![Diagram showing the role of ipRGCs in signal integration](image)

TRENDS in Molecular Medicine

Hatori & Panda 2010
Light considered a drug: potential for beneficial and deleterious effects*

• Circadian entrainment to light/dark cycle
• Pupillomotor responses
• Sleep/wakefulness systems
• Light avoidance
• Modulation of cognitive systems
• Metabolism, learning and memory

*Lukas et al., 2014

Light avoidance in neonatal mice
(Johnson…& Copenhagen, 2010)

• Neonatal mice avoid light by turning away from it before rods and cones are active, or eyes are open

• The mice do not actively avoid the light after melanopsin is knocked out

Effect of early melanopsin activity on development
Light affects retinal development in utero via melanopsin RGCs (Rao et al)

Dark-rearing, or opn4 KO: hyaloid vessel persists
Vascular overgrowth occurs.

Peripheral Sensory Neurons Expressing Melanopsin Respond to Light
Matynia et al, 2016
Matynia et al, 2016

- Melanopsin is expressed in mouse and human trigeminal sensory ganglion (TG) neurons
- Melanopsin-expressing TG neurons are intrinsically photosensitive (in vitro – mice) – and activated via nociceptors (C fibers)
- Calcitonin gene-related peptide (CGRP), a neuropeptide implicated in migraine (Eftekhari et al., 2015; Russo, 2015), and PACAP, which colocalizes with melanopsin
- Light aversion is present in nitroglycerin treated WT mice with optic nerve transection, but not in Opn4(-/-) mice