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The emerging roles of melanopsin in behavioral adaptation to light

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Abstract

Adaptation of behavior and physiology to changes in the ambient light level is of critical importance to life. These adaptations include light modulation of neuroendocrine function and temporal alignment of physiology and behavior to the day:night cycle by the circadian clock. These non-image forming (NIF) responses can function independent of rod and cone photoreceptors but depend on ocular light reception, suggesting the participation of novel photoreceptors in the eye. The discovery of melanopsin in intrinsically photosensitive retinal ganglion cells (ipRGCs) and genetic proof for its important role in major NIF responses have offered an exciting entry point to comprehend how mammals adapt to the light environment. Here, we review the recent advances in our understanding of the emerging roles of melanopsin and of ipRGCs. These findings now offer new avenues to understand the role of ambient light in sleep, alertness, dependent physiologies and potential pharmacological intervention as well as lifestyle modifications to improve the quality of life.

Keywords

melanopsin (OPN4); retinal ganglion cells (RGC); intrinsically photosensitive retinal ganglion cell (ipRGC); retina; non-image forming (NIF) photoresponse; the circadian clock; opsin

Three types of photoreceptors- rod, cone and ipRGC

Rods and cones in the outer retina are the predominant photoreceptor cells of the mammalian retina. Their high temporal and spatial sensitivity to light forms the basis of image forming (IF) vision. Severe disruption of rod/cone function or rod/cone cell death leads to the loss of IF vision. However, for decades it has been known that many patients and animal models with substantial rod/cone loss could support some non-image forming (NIF) functions¹⁻³ (Box 1), which are abolished in subjects who have lost both eyes⁴. The peak spectral sensitivity of many of these NIFs in individuals and animal models lies in ~460-500 nm range in both normal and blind subjects with substantial rod/cone loss⁵⁻¹¹, thus suggesting alternative photoreceptors play an important role in NIF responses. The discovery of melanopsin in a small subset of retinal ganglion cells (RGCs) in the inner retina^{12, 13}, the intrinsic photosensitivity of these cells^{14, 15} and the genetic proof that rod, cone and melanopsin account for all ocular photoresponses in mammals^{8, 16-19} have now made it possible to comprehensively understand how both inner and outer retina photoreceptors function together to adapt to the ambient light environment. Recent advances have formally linked melanopsin function to several physiological and behavioral responses to light in various mammals. These studies will help

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scientists understand how effective lighting strategies, pharmacological intervention and current medical practices affect the quality of life.

Box 1. Light adaptation or NIF visual photoresponse

In addition to the image forming function, the eye also mediates several light dependent reflexes, physiologies and behaviors. These NIF responses include:

- *Circadian photoentrainment*: In most animals, an intrinsic circadian oscillator helps organisms temporally orchestrate behavior and physiology to the appropriate time of the day. In many organisms including mice and human, the intrinsic periodicity of the circadian clock is close to, but not exactly 24 hours. To be an effective timekeeping mechanism, the circadian clock needs to be synchronized with the ambient light:dark environment on a daily basis. Light perceived through the eye acts as a strong stimulus to entrain the circadian oscillator with the ambient light:dark cycle. The threshold sensitivity for shifting the phase the circadian clock is of several seconds or minutes – orders of magnitude less sensitive than IF vision. Such requirement for integration of light information over longer timescale helps maintain a robust circadian clock in the face of occasional light noise in nature such as lightening. Attenuation of light input to the circadian clock has been documented in the *Opn4^{-/-}* mice ^{16, 17}.
- *Pupillary light reflex (PLR)*: Acute constriction of the pupil in response to an increase in light intensity received at the retina. The response is consensual or in other words, shining a beam of light in one eye constricts the pupil in the other eye. Under high light intensity melanopsin supports sustained pupil constriction ¹⁸.
- *Light suppression of activity*: The acute reduction in activity of nocturnal animals in response to light during their activity phase. Mice lacking melanopsin show acute activity suppression at the beginning of a light pulse, but progressively increase activity under prolonged illumination ¹⁰⁶.
- *Alertness*: Diurnal organisms including human show improved alertness and mood under bright light ¹⁰.
- *Acute suppression of pineal melatonin*: In mammals, the major source of circulating melatonin is the pineal gland. In both diurnal and nocturnal animals, melatonin synthesis and circulating levels peak during the night. Light for several minutes to hours can acutely suppress pineal melatonin synthesis and secretion. Peak spectral sensitivity of this response is distinct from those of rod/cone photoreceptors ¹⁰⁷ and is intact in retinal degeneration (*rd*) mice ¹⁰⁸. Mice homozygous for the *rd* allele are visually blind as a result of a primary degeneration of the rods and a secondary loss of cones, but they retain melanopsin-containing RGCs. Mice deficient in both rod/cone and melanopsin system show no light suppression of pineal melatonin synthesis pathway ¹⁹.
- *Light modulation of sleep*: Light suppresses sleep in diurnal animals, whereas it enhances sleep in nocturnal animals. A light pulse during the dark period failed to induce sleep in *Opn4^{-/-}* mice. The involvement of melanopsin in the direct effects of light is restricted to the dark period. Furthermore, *Opn4^{-/-}* mice sleep approximately one hour less than the wild-type mice under a 12 hr/12hr light-dark schedule ¹⁰⁹⁻¹¹¹.
- *Light exacerbation of migraine*: Light exacerbates migraine headache, and this effect is intact in blind individuals with light perception but not in patients who

have no light perception. Direct projections of ipRGCs to the thalamic region, which is implicated in migraine pain, offers a neural mechanism for the light exacerbation response⁴³.

- *Allodynia to light or photophobia*: Several individuals and some blind people show aversion to light. Intact photophobia in blind patients implies potential involvement of the melanopsin system. Additionally, young rodent pups (less than 10-days old) show photophobia before the rod/cone system is fully functional¹¹². Fully active melanopsin system at this age⁶⁷ most likely mediates such photophobia.

Melanopsin is an opsin class of G-protein coupled receptor (GPCR) first discovered in the photosensitive skin melanophores of *Xenopus laevis* (and hence the name)²⁰, where it mediates adaptation of skin pigmentation to ambient light level. Subsequently, melanopsin was discovered in the retina of several vertebrates (reviewed in²¹). In primates and rodents, melanopsin protein (also called OPN4) is exclusively expressed in a small subset of RGCs of the inner retina that are intrinsically photosensitive (ipRGCs) (Figure 1). Research primarily in the mouse has demonstrated that the light response properties of melanopsin and the ipRGCs are distinct from those of the photopigments and photoreceptors of the outer retina. For simplicity, we will describe the molecular functions of melanopsin and cellular roles of ipRGCs as “the melanopsin system”. Specifically, the four defining properties of the melanopsin system are: i) spectral sensitivity; ii) retinoid use; iii) signal transduction; and iv) the unusual cellular architectures of the ipRGCs. Here, we describe our current understanding of the molecular function of melanopsin, the cellular architecture of ipRGCs and the integration of rod/cone and melanopsin function in determining NIF responses. We conclude by discussing the potential implications of these responses for human health and disease.

Melanopsin photopigment

Melanopsin photopigment shows peak spectral sensitivity at ~480 nm, which lies in the blue-cyan range of the visible light and is distinct from those of classical rod/cone opsins (Figure 1d²²). The peak sensitivity correlates with the photosensitivity of several NIF responses of animals or humans under natural condition of prolonged light exposure when rod and cones have saturated or adapted, thus suggesting an important role of melanopsin in several NIF responses.

Melanopsin, like other members of the opsin class of photopigments, uses 11-*cis* retinaldehyde as a chromophore (light sensing ligand, Figure 2), which upon light activation, photoisomerizes to all-*trans* retinal and thereby causes a conformational change in the protein and activates downstream signaling proteins. The light activated or meta-state of classical vertebrate rhodopsin photopigment is unstable at physiological temperature. *Drosophila* rhodopsin, on the other hand, after activation by blue light, transits into a relatively stable meta-state that continues to activate downstream signaling proteins and upon excitation with long wave length orange light, the meta-state returns to the blue-sensitive basal state (reviewed in²³). Purified melanopsin from amphioxus is similarly bistable²⁴. Although direct experimental evidence with purified mammalian melanopsin is still lacking, some observations suggest a bistable nature of melanopsin^{25, 26}. In *Xenopus* oocytes expressing mouse melanopsin, the melanopsin photocurrent is sustained for >10 min after lights off. However, upon coexpression of arrestin the photocurrent is returned to baseline within approximately 2 min after lights off²⁷. Arrestin desensitizes activated GPCRs²⁸, and prolonged melanopsin photocurrent in the absence of arrestin is reminiscent of prolonged depolarization of *Drosophila* photoreceptor cells lacking arrestin²⁹. Therefore, the meta-state melanopsin might be stable. If the meta-state mammalian melanopsin behaves similar to the purified amphioxus melanopsin or *Drosophila* rhodopsin,

a long wave length light pulse might return the meta-state melanopsin to the basal-blue absorbing state. In support of this hypothesis, melanopsin driven photoresponses are potentiated by prior illumination with red-shifted light ^{26, 30, 31}. Despite these observations, direct proof of melanopsin bistability in the intact ipRGCs and its implication in natural light environment are yet to be addressed. Clear understanding of the meta-state melanopsin will impact how changes in spectral quality of ambient light modulate melanopsin signaling.

The retinal source for melanopsin

The photochemical and spectral properties of melanopsin have clear implications for human lifestyle and disease conditions. The initial source of 11-*cis* retinal and the steps leading to melanopsin pigment regeneration after photoactivation are not well known. Because defects in retinoid metabolism are implicated in many human diseases and conversely, components of retinoid metabolism pathway have been the focus of several therapeutic approaches ³², understanding retinoid use by melanopsin is important. In the retina, the retinal pigment epithelium (RPE) serves as the major local store of 11-*cis* retinal and also a site for regeneration of *cis*-retinoid from all-*trans* retinoid (Figure 2). 11-*cis* retinal from the RPE supports outer retina rod/cone photoreceptor functions ³². There is some evidence that the RPE also supports melanopsin function, but there is substantial debate on the mechanism by which RPE affects melanopsin function. Mice lacking critical enzymes of RPE retinoid metabolism (*Rpe65*^{-/-} or *Lrat*^{-/-}) produce limited quantities of *cis* retinoids, ^{33 34} which are largely used by the outer retina rod photoreceptors, leaving little retinoid for melanopsin function. Accordingly *Rpe65*^{-/-} and *Lrat*^{-/-} mice show reduced melanopsin photosensitivity; reduced PLR sensitivity and reduced sensitivity of the circadian clock to light. These NIF responses can be improved by the exogenous supplementation of *cis*-retinal or by the genetic ablation of the outer retina photoreceptors ³⁵⁻³⁷. These studies have clearly demonstrated that disruption of RPE function or survival might also affect melanopsin function. Therefore, it is likely that in visually blind patients carrying hypomorphic or null alleles of *Rpe65* ³⁸⁻⁴⁰, severe loss of PLR and poor sleep quality could arise from attenuated melanopsin function.

The residual photosensitivity in *Rpe65*^{-/-} and *Lrat*^{-/-} mice suggest melanopsin or the ipRGCs might utilize a self sustaining mechanism for recycling some all-*trans* retinal photoproduct to 11-*cis* retinal ³⁷. There is indirect evidence that melanopsin can photoisomerize all-*trans* retinal to 11-*cis* retinal and thereby can regenerate an active photopigment ²⁷. However, purified melanopsin from mouse retina was found to be in a complex with 11-*cis* retinal only ²². This suggests that either the all-*trans* retinal photoproduct is spontaneously isomerized to 11-*cis* retinal and stays bound to the opsin or the melanopsin-all-*trans* retinal complex is unstable and the all-*trans* retinal dissociates after initial light activation, leading to photobleaching of melanopsin. Support for both photoisomerization and photobleaching mechanisms in the mammalian ipRGCs exists. Several labs have shown that ipRGCs can be repeatedly photoactivated without exogenous retinal, which supports photoisomerization within the ipRGCs ^{14, 15}. However, some labs have also observed a reduction in photoresponses or photobleaching by up to 70% upon repeated photostimulation of the ipRGC ⁴¹. It is safe to conclude that some melanopsin is bleached upon illumination and regenerated in the intact retina from cell autonomous or extracellular sources, whereas some melanopsin can photoisomerize the all-*trans* retinal and regenerate functional photopigment. The mechanisms and molecules that determine the steady-state levels of melanopsin photopigment are yet to be discovered.

Partial bleaching of melanopsin and its dependence on the RPE for (at least some) retinal supply raises some interesting clinical issues. One of the emerging therapies for preventing or slowing the progression of some forms of blindness is based on attenuating the visual cycle in the RPE cells or reducing the availability of retinoid to the photoreceptors ⁴². One of such inhibitors of

visual cycle, all-*trans*-retinylamine, acutely reduces the available 11-*cis* retinal and consequently attenuates rod/cone function leaving the melanopsin system almost intact³⁷. However, the effect of chronic administration of these drugs on melanopsin system is currently unknown and needs careful assessment. If melanopsin is photobleached under prolonged illumination, the opportunity arises to develop melanopsin inhibitors, which can outcompete *cis*-retinal and lock melanopsin in an inactive state. Such inhibitors might induce a “pharmacological darkness” and could alleviate melanopsin-dependent exacerbation of migraine pain in normal and blind individuals (see⁴³).

Melanopsin protein shares sequence and functional similarities with invertebrate opsins

Molecular interaction with immediate downstream signaling proteins, subsequent signaling intermediates and effector channels are determined by the amino acid sequence of an opsin. Melanopsin shares more sequence similarity with invertebrate rhodopsins than with vertebrate rhodopsins¹². Several features of the ipRGC photosensitivity also exhibit characteristic features of invertebrate photoreceptors (Box 2). Specifically, ipRGCs depolarize upon light activation^{14, 15}; photoactivation causes a transient increase in cytosolic Ca^{2+} levels⁴⁴ and the photocurrent generated by ipRGCs exhibits a voltage-current relationship that resembles of the transient receptor potential (TRP) class of inward-rectifying cation channels⁴⁵. Furthermore, intact melanopsin phototransduction persists in mice carrying loss-of-function mutations in vertebrate rhodopsin signaling components, such as a downstream G-protein (*Gnat*^{-/-}), the signaling intermediate phosphodiesterase (*Pde6b*^{-/-} or *Rd1*) and an effector channel (*Cng3*^{-/-})^{8, 19}. Consequently, many blind patients carrying mutations in rhodopsin signaling components likely have intact ipRGC function.

Box 2. Rhodopsin phototransduction in vertebrates and invertebrates exhibit several key differences

In vertebrates (Figure 1a), a light-activated metarhodopsin triggers a pertussis toxin-sensitive class of G protein (Gt, transducin), which in turn, activates a PDE that hydrolyzes 3'-5' cyclic guanosine monophosphate (cGMP) to 5' cGMP. Light-activated hydrolysis of cGMP leads to closure of the cyclic nucleotide-gated (CNG) ion channels and hyperpolarization of the photoreceptor cells. In contrast, the invertebrate cascade (Figure 1b) is initiated by the activation of the pertussis toxin-insensitive *Gaq* class of G protein, which in turn activates phospholipase C- β (PLC β). Activated PLC β catalyzes the conversion of phosphatidylinositol-4,5-bisphosphate (PIP2) to inositol-1,3,5-triphosphate (IP3) and diacylglycerol (DAG). DAG is further catalyzed to produce poly-unsaturated fatty acid (PUFA). Events downstream of activation of PLC are complex and might involve IP3, DAG and PUFA as signaling intermediates to activate TRP cation channels, which results in the influx of Na^+ and Ca^{2+} as well as membrane depolarization (reviewed in¹¹³). IP3 can also trigger an increase in cytosolic Ca^{2+} level by release from Ca^{2+} stores²³.

Figure 1. (Box 2) Phototransduction mechanisms of classical vertebrate and invertebrate rhodopsins

The vertebrate rod/cone opsin phototransduction signaling cascade (a) is distinct from that of invertebrate rhodopsin (b).

The scarcity of ipRGCs makes it nearly impossible to employ the same set of biochemical approaches that proved successful in characterizing rhodopsin signaling processes from native photoreceptors. Instead, *Xenopus* melanopsin in native melanophores and heterologously expressed mammalian melanopsin have been important starting points to study melanopsin function. In both systems, light stimulated melanopsin triggers Gαq/Gα11 activation (Box 2) ^{27, 46, 47}, which in turn signal through PLCβ to trigger opening of a TRPC class of ion channel and increase in cytosolic Ca²⁺. A similar signaling cascade likely functions in the native ipRGCs. Melanopsin mediated photocurrent in the ipRGCs can be blocked by specific inhibition of Gq/G11 class of G proteins and of PLCβ ⁴⁸. Furthermore, light activation triggers increase in cytosolic Ca²⁺ in the ipRGCs ⁴⁹ and the melanopsin photocurrent shows

characteristic features of TRP class of ion channel⁴⁵. In summary, these observations indicate melanopsin employs a downstream signaling scheme similar to that of *Drosophila* rhodopsin, which is distinct from the vertebrate rod/cone signaling pathway.

According to the current model of the melanopsin signaling cascade, each critical component – the effector G protein, the signal amplifying cascade mediated by PLC, and the effector ion channels – is encoded by functionally redundant family members that are expressed in almost all mammalian cells. Furthermore, several GPCRs can promiscuously signal through nonpreferred G proteins and downstream signaling cascades. Accordingly, purified melanopsin also activates the transducin class of G proteins (Gt)⁵⁰ and open a CNG class of channel³⁰ – both of which are downstream effectors of the vertebrate rhodopsin cascade. This implies that the loss of any single signaling component might not completely abolish melanopsin initiated photoresponses. Conversely, ectopically expressed melanopsin in any mammalian cell will likely find a signaling cascade. The ectopic expression of melanopsin in RGCs renders them light sensitive with properties similar to those of the native ipRGCs⁵¹. This raises the potential for the application of melanopsin as a therapeutic optogenetic tool (Box 3) for treating several human diseases including blindness.

Box 3. Optogenetics

Optogenetics generally refers to combining optical stimulation of a light sensitive protein to probe or to alter cellular function. Prevalent applications involve expression of a bacterial or algal rhodopsin in a neuronal cell type of interest and precise millisecond scale optical stimulation. Because these rhodopsins are a natural chimera between light sensitive opsin and an effector channel, high-speed opening and closing of the channel by millisecond scale light pulses is achieved. The sluggish response of melanopsin makes it unsuitable for such popular optogenetic applications, but offers unique advantages for specific usage. As such, melanopsin can be used to mimic signaling by any Gq/G11 class of GPCR. Signaling events downstream of melanopsin increase intracellular Ca^{2+} level. High intracellular Ca^{2+} triggers phosphorylation of cyclic AMP/Ca response element binding protein (CREB) and thus leads to light mediated transcriptional activation of CREB targets¹¹⁴. Because melanopsin uses a multistep intracellular signaling cascade, significant signal amplification is naturally achieved at each step of the cascade. Recombinant adenoassociated virus-mediated expression of melanopsin in a large number of RGCs of mice with extensive degeneration of rod/cone cells restores some visual functions at normal indoor light levels⁵¹. Use of channel rhodopsin also restores some visual functions but only at high intensity light levels equivalent to midday sunlight¹¹⁵. These early successes along with the fact that melanopsin is naturally expressed in humans raises the hope that melanopsin might be the tool of choice in some optogenetics-based gene therapy approaches.

Photoresponses of the ipRGCs

In addition to the unique chromophore use and signaling properties of melanopsin, ipRGCs also have exclusive properties among photoreceptor cells. In the mammalian retina, up to 20 different types of RGCs can be distinguished based on their signaling properties and neuroanatomy⁵². The defining features of ipRGCs in different species are the expression of melanopsin protein and the resultant intrinsic photosensitivity. In each human eye, up to 3,000 RGCs out of ~1.5 million stain positively for melanopsin⁵³. In each mouse retina, up to 1,500 RGCs out of approximately 50,000 express melanopsin⁵⁴. Unlike the regionally concentrated rod/cone opsins in classical photoreceptor cells, melanopsin immunostaining does not show any regional preference within the cell; almost uniform melanopsin staining is observed along the soma, dendrites and to some extent in the axons of the ipRGCs¹³ (Figure 1c).

The intrinsic photosensitivity of ipRGCs distinguishes them from those of the rod/cone photoreceptor cells: ipRGCs have a high threshold for activation, a long latency to respond and take long time to return to base line¹⁵. Such response properties allow the integration of light information over long periods of illumination such that the ipRGCs function as irradiance detectors. The mechanism underlying the sluggish response of melanopsin is currently unknown. Single-photon responses of melanopsin have clearly shown that the photopigment is at least as sensitive as the classical rod/cone photopigments⁴¹. However, unlike classical vertebrate or invertebrate photoreceptors in which the photopigments and downstream signaling components are concentrated in subcellular compartments, the diffuse distribution of melanopsin in the ipRGC membrane and of other signaling components in the ipRGCs likely leads to the sluggish response. In support of this observation, ectopic expression of one of the fastest acting photopigments – *Drosophila* Rh1 rhodopsin – along with its downstream signaling components in mammalian neurons produces a sluggish response qualitatively similar to melanopsin⁵⁵.

The ipRGCs, like other RGCs, also transduce rod/cone-initiated light responses⁵⁶. Recordings from primate ipRGCs show distinct rod, cone and melanopsin-initiated responses in the ipRGCs⁵⁷. Under dim light conditions (scotopic conditions), rods primarily detect light, and the rod-initiated light response depolarizes ipRGCs and triggers action potentials that are sustained throughout the duration of the light pulse. With the gradual increase in light intensity within the working range of rods, there is a corresponding increase in ipRGC firing. As the light intensity increases to levels encountered during daytime, the rods are bleached. Under such lighting, both cones and melanopsin initiated light responses are detected in the ipRGCs. The L (long wavelength) and M (medium wavelength) cone-initiated light signals cause transient depolarization of the ipRGCs at onset and offset of light and, therefore, reliably encode lights on or off, but are unreliable for encoding light intensity over long duration. The intrinsic melanopsin-mediated photoresponses begin after a few milliseconds of cone-initiated response and are sustained for the duration of illumination. The activation of rods as well as L and M cones activate (“on”) ipRGCs, and the S (short wavelength) cones trigger an “off” response. However, under natural daylight, the intrinsic sustained melanopsin response likely overrides the S-dependent off response. Altogether, the primate ipRGC responses predicted that under dim light condition (approaching the limit of human vision), rod-initiated light signal likely supports NIF responses, whereas under natural daylight condition, melanopsin-initiated responses tonically encode light intensity information and support NIF responses⁵⁷. Accordingly, cone responses alone in mice are insufficient to sustain normal NIF responses under daytime light levels⁵⁸. A recent study in humans has carefully dissected the roles of cones and melanopsin in NIF responses⁵⁹. At the beginning of a long bright light pulse, cone signals are as effective as melanopsin signals in suppressing pineal melatonin release. However, with time the cone contribution decays exponentially and melanopsin functions as the predominant NIF photopigment under natural long-duration high intensity light. Under moderate light levels, both cones and melanopsin participate in setting the phase of the human circadian clock.

How do rod, cone and melanopsin photoresponses integrate? In rodents, specific ablation of the ipRGCs leads to almost complete loss of all NIF responses leaving the IF responses nearly intact (Figure 3.). This implies that rod- and cone-initiated light signal destined for NIF responses are predominantly transmitted through the ipRGCs and hence the ipRGCs constitute the principal cellular node integrating light responses from all three photopigment systems. However, it is still unclear whether melanopsin expression in ipRGCs affects its role in mediating rod/cone-initiated responses. For example, are melanopsin and outer retina responses simply additive or does melanopsin somehow augments rod/cone-initiated responses? In summary, the net light signal through the ipRGCs supports most NIF responses, and therefore, the ipRGCs constitute the central cellular framework for NIF responses.

Ontogeny, architecture and projections of the ipRGCs

The ipRGCs are born along with other RGCs in rodents, and melanopsin expression begins *in utero* long before the rod/cone photoreceptors are fully functional⁶⁰. In humans, the melanopsin system is also fully functional *in utero*, as premature babies born after 33 weeks show clear pupil constriction in response to light⁶¹. The genetic circuitry specifying ipRGC identity or melanopsin expression is still not understood. The master transcription factors Math5 and Brn3, which specify RGC differentiation, also specify ipRGC cell fate⁶²⁻⁶⁴, although the downstream regulators specifying ipRGC identity or regulating melanopsin expression are currently unknown.

The neuroanatomy of ipRGCs holds many clues of their functions. In the primate retina, ipRGCs are among the RGC cell types with the largest soma and the most extensive dendritic arborization⁵⁷. Unlike most other RGC cell types that are arranged in a nonoverlapping cobblestone pattern, the dendrites of the ipRGCs extensively overlap with each other^{13, 65}. By comparing the morphology of dendrites and light responsiveness, melanopsin expressing RGCs were classified into (at least) three subtypes, M1- M3 (Figure 1b. Reviewed in⁶⁶), although a recent paper doubts the definition of M3 subtype as a separate cell type⁶⁵. Physiological responses of the mouse ipRGCs also exhibit significant diversity with respect of threshold sensitivity, the magnitude of responses and deactivation rates⁶⁷. The M1 and M2 cell types in mouse retina exhibit distinct photosensitive properties⁶⁸. Despite these detail descriptions of morphological and physiological diversity, it is still unclear whether such diversity is conserved in primates and whether each cell type serves any specific purpose.

Dendrites of the predominant M1 and M2 subtype ipRGC stratify either in the on or off sublaminae of the inner plexiform layer (IPL) where they receive synaptic inputs from bipolar and amacrine cells^{69, 70}. Factors that specify dendritic stratification in the retina or factors that determine projections of RGCs to the target brain areas will also have significant effect on normal function of the melanopsin system. Accordingly, mice lacking a critical factor *Dscam* that specifies dendritic stratification and spreading show aberrant dendritic morphology of the ipRGCs⁷¹.

The axons of the ipRGCs exit the retina and project to distinct regions of the brain (Figure 4). Several studies in different rodents have mapped the central projections of the ipRGCs^{14, 72, 73}. The current and most comprehensive analysis of the central projections from the ipRGCs was done with *Opn4^{tau:LacZ}* mouse, a transgenic mouse with the reporter tau:LacZ knocked into the melanopsin locus¹⁴, in which the M1 subclass of ipRGCs are mostly labeled⁷⁴. Detailed projections of ipRGCs are described in Hattar et al⁷⁵. Unlike most other RGCs whose axons cross the optic chiasma and primarily project to the contralateral side of the brain, ipRGCs show interesting projections. Immediately after the optic chiasma, the ipRGCs from one eye almost equally innervate both left and right halves of the master circadian brain center – the suprachiasmatic nucleus (SCN). Beyond the SCN, the ipRGCs, like the other RGCs, project contralaterally to brain regions that directly or indirectly regulate other NIF processes. These regions include the intergeniculate leaflet (IGL), which indirectly entrains the circadian clock, and the olivary pretectal nucleus (OPN), the center controlling pupil constriction. Both the SCN and OPN predominantly receive ipRGC input, thus suggesting a unique axon guidance mechanism likely mediating such dominant connections. Several additional brain centers also receive sparse projections from the ipRGCs. They include: the lateral hypothalamus, the ventrolateral preoptic nucleus, the habenulla and the subparaventricular zones. These projections likely mediate effects of light on the hypothalamic regulation of sleep, behavior and physiology. Multisynaptic projections are responsible for the ipRGC regulation of pineal melatonin⁷⁶. Phenotypic assessments of mice lacking melanopsin or those with targeted ablation of ipRGCs also exhibit specific defects in light-dependent behaviors supported by

these brain centers (reviewed in ⁶⁶), thus validating functional significance of ipRGC projections. Recently described sparse projections of ipRGCs in the posterior thalamus found juxtaposed to dura-sensitive thalamocortical neurons are proposed to mediate light exacerbation of migraine pain ⁴³.

New mouse lines that comprehensively mark most ipRGCs have identified additional ipRGC brain targets. The superior colliculus (SC) and the ventral and dorsal lateral geniculate nucleus (vLGN and dLGN) receive substantial axonal projections from ipRGCs ⁷⁷ (Figure 4). Both SC and LGN receive extensive innervations from other RGCs and are primary relay centers for IF vision. ipRGC projections to the LGN is also found in the primate brain ⁵⁷. Accordingly, some human blind patients with substantial loss of rod/cone photoreceptors ¹¹ have rudimentary visual perception. In normal individuals, ipRGC projections to the LGN and SC might provide brightness information for IF vision. In summary, the ipRGCs extensively innervate several brain regions mediating NIF responses and also innervate the LGN and SC where they likely encode irradiance information for IF vision.

Genetics of the melanopsin system

Our knowledge about the role of melanopsin and ipRGCs in NIF responses has largely come from rodent genetics. Comparative analyses of light-dependent phenotypes of mice lacking melanopsin (*Opn4*^{-/-}), rod/cone function or ipRGCs have delineated the roles of the photopigments and of ipRGCs in NIF responses. In general, most of the NIFs that require light integration over a long period of time or acute NIF responses that activate at high-intensity light are attenuated in *Opn4*^{-/-} mice. These include light modulation of the circadian clock phase, general activity, sleep and pupil constriction (reviewed in ⁶⁶). These photoresponses are completely abolished in mice that lack both melanopsin and functional outer retina photoreceptors ^{8, 19}, which implies rod/cone photoreceptors can partially compensate for the loss of melanopsin protein. Specific acute or progressive loss of ipRGCs in mice with intact and fully functional rod/cone photoreceptors also leads to loss of NIF light responses (Figure 3), thus establishing the ipRGCs as the principal carriers for transmitting the light information originating from both melanopsin and outer retina photoreceptors ⁷⁸⁻⁸⁰.

Additional mouse models with specific inactivation of rod or cone photoreceptor function are helping the elucidation of the relative roles of rods and cones in NIF responses ^{58, 81}. As expected, both rods and cones can partially support some NIF responses including circadian photoentrainment ¹⁶. Surprisingly, even at photopic light level, cones appear to play a limited role in circadian photoentrainment. These mouse genetic studies support the overall response properties of primate ipRGCs in which a rod-initiated response supports irradiance encoding under dim light, whereas the transient responses of cones are insufficient to encode irradiance levels at high light levels. Hence, the relative roles of rod, cone and melanopsin in NIF responses are largely conserved between the nocturnal rodents and diurnal primates including humans.

Mouse genetics has also shed light on potential signaling mechanisms in the ipRGCs. Mice lacking the unconventional protein kinase C – PKCζ – phenocopy the reduced photosensitivity of the *Opn4*^{-/-} mice. Coexpression of PKCζ and *Opn4* in the ipRGCs raises the possibility that the kinase plays a critical role in melanopsin signaling cascade ⁸². Similar clues to the candidate downstream neurotransmitters in the ipRGCs have also come from mouse studies. Melanopsin expressing cells also express the neuropeptide PACAP (pituitary adenylyl cyclase-activating peptide) ^{53, 83, 84}, and PACAP receptors are expressed in the SCN ^{73, 85}. Exogenous application of PACAP at low concentration can phase shift the SCN clock, whereas mice lacking PACAP or the PACAP receptor show reduced response of the circadian clock to a phase resetting pulse of light ⁸⁶⁻⁸⁸. The residual circadian photosensitivity in the PACAP KO mice is likely mediated

via glutamate, which is also found in the ipRGCs⁸⁹. Analogous to PACAP, glutamate receptors are expressed in the SCN neurons⁹⁰ and references therein) and the exogenous application of glutamate to SCN slice culture can mimic light-induced phase shifts in the SCN clock^{91, 92}. Receptors for both PACAP and glutamate are excellent drug targets for several diseases^{93, 94}. Accordingly, specific pharmacological modulators of PACAP and glutamate signaling pathways could have significant impact on NIF responses.

Interaction between IF and NIF visual responses

Several animal models have painted a picture in which NIF responses and rod/cone-mediated IF vision are largely independent of each other; however, there are emerging data suggestive of interaction at various levels between these two systems. The melanopsin system can modulate the classical IF vision both in the retina as well as in the LGN. The ipRGCs signal to the dopaminergic amacrine cells might form a basis for the adaptation of the visual system to light intensity levels⁹⁵. Innervations of the LGN of primates⁵⁷ and rodents⁷⁷ by the ipRGCs suggest the melanopsin system might directly transmit ambient light intensity information to IF visual system.

There are several levels of potential regulation from the outer retina to the melanopsin system. As has been shown in *Rpe65*^{-/-} and *Irar*^{-/-} mice, general dysregulation of retinoid availability can affect the level of melanopsin protein and consequently the timing of activity rest in mice^{36, 37, 96}. As our knowledge on the relative contribution of rods/cones to NIF responses becomes clearer, it could have significant bearing on human health. Many diseases of the outer retina leading to blindness begins with the selective loss of RPE, rod or cone function and progress to significant cell death of the outer retina. Over a period of months and years, the surviving retina goes through profound remodeling in which the relative composition of cell types and their connectivity changes⁹⁷. Extensive remodeling of ipRGC dendritic structures in the retina has also been observed in old rats with outer retina degeneration⁹⁸. Therefore, the effects of several degenerative diseases of the outer retina on the melanopsin system might be complex and progressive.

Applications for improving health

The discovery of melanopsin now offers a mechanistic understanding of how light affects human physiology, behavior and sleep. Accordingly, effective utilization of light in improving the quality of life now offers new opportunities for interdisciplinary efforts among physicians and researchers of various branches of science that have so far remained largely nonoverlapping: circadian/endocrine biology, vision science, sleep and neuroscience, and architectural lighting. There are several areas in which the melanopsin photosystem can have direct implications in human health and diseases including (i) changing disease diagnosis procedures based on an evaluation of the melanopsin system; (ii) altering medical practices to address melanopsin-mediated photoresponses; (iii) developing pharmacological interventions for NIF responses; (iv) discovering gene alterations in patients; and (v) managing light exposure at work, home and at care-giving facilities.

Diagnostic procedures for the melanopsin system

Rodent data suggests perturbed melanopsin signaling might underlie several human disease conditions; these include sleep disorders, seasonal affective disorders, depression, aversion to light and light-exacerbated migraine pain⁴³. To begin to test whether the melanopsin system contributes to these ailments, it is necessary to have reliable diagnostic methods to quantify melanopsin function. However, nearly all current diagnostic procedures for the retina measure the structure and function of outer retina rod/cone photoreceptors. These procedures are unsuitable for evaluating the function of sparsely distributed ipRGCs in the ganglion cell layer

of the inner retina. A promising method would be the utilization of a PLR assay. It is now well established that from rodents to primates, melanopsin specifically contributes to the persistence of pupil constriction for several seconds following a brief pulse of light^{7, 18, 99}. A recent study succeeded in optimizing the spectral conditions to specifically measure melanopsin function in pupil constriction. In addition, the melanopsin response is attenuated in one form of blindness (Leber's Congenital Amaurosis) while normal or enhanced melanopsin response in another form of blindness¹⁰⁰. Such PLR response can also be used as a surrogate measure for the severity of another blinding disease - glaucoma, which involves progressive death of RGCs. In summary, evaluation of ipRGC function will be a starting point in further classifying blindness into patients with complete the loss of both NIF and IF vision and those with the loss of IF vision alone. Such classification will help determine whether normal ipRGC function in some blind patients might be beneficial in maintaining better quality of life than patients with no perception of light.

Medical practices

Several medical practices can now be evaluated in the context of melanopsin function, and these include the choice of cataract lens, pharmacological intervention aimed at the retinoid pathway for the treatment of other diseases, the decision for surgical bilateral enucleation as a prognosis for certain eye diseases including retinoblastoma, and the evaluation of gene therapy for improvement in the quality of life. For example, the human lens progressively loses transmittance in the blue range of visible light such that the lens of a 75-year old transmits 2-log units less light at 480 nm than the lens of a 5-year old^{101, 102}. Therefore, it is more important for elderly patients to have sufficient exposure to bright light. Furthermore, to improve visual function, to optimally activate the melanopsin system and consequently, to improve sleep quality in elderly patients, it might be ideal to implant an intraocular lens with sufficient transmittance in the blue range to restore function but minimal transmittance in the harmful near-UV range.

Pharmacological intervention

The distinct nature of melanopsin and rod/cone phototransduction pathways is encouraging ideas to manipulate signaling flux through the ipRGCs to manage human ailments dependent on lighting conditions. However, the point of intervention is still unclear. An ideal strategy would be to modulate melanopsin or a downstream signaling component in the ipRGCs without affecting the rod/cone signaling system. Compounds that either activate or inhibit the light flux through ipRGCs should mimic pharmacological light or darkness and have potential use. Activators would mimic light and offer a novel pharmacological intervention for mood uplifting effect, whereas inhibitors would mimic darkness and prevent light suppression of melatonin and hence improve sleep. Additionally, the recent observation that the ipRGCs might mediate light exacerbation of migraine pain⁴³ raises the possibility that pharmacological darkness alone or in combination with other drugs could offer novel strategy for pain management in normal and blind patients.

Gene and mechanism discovery from patients

Phenotypes of mice with altered signaling flux through the melanopsin system suggest a range of ailments in humans that might have an underlying defect in melanopsin function. Mouse models have also offered a range of mechanisms and genes that might affect the melanopsin system; these include melanopsin; putative downstream signaling components; and factors determining the differentiation of the ipRGCs, specifying their connectivity to the respective brain regions that mediate NIF responses and those in the outer retina affecting ipRGC function. As the cost of genome sequencing goes down and genetic association studies find loci associated with depression, seasonal affective disorders and sleep disorders, we might find

genes and mechanisms implicated in the melanopsin system. Indeed, an early success of this nature has already been demonstrated. A specific amino-acid changing mutation in melanopsin associates with a small subset of patients with seasonal affective disorder (SAD) ¹⁰³. SAD patients develop a form of depression that commonly begins with the short winter days and many patients find it helpful to have exposure to blue-enriched light (light therapy), thus further highlighting the relevance of light signaling for improved alertness in humans.

Light management

The general population in industrial nations is increasingly exposed to prolonged hours of artificial light that extends well into the night ¹⁰⁴. Furthermore, most hospitals and care-giving facilities often have 24-hour lighting. Although daylight-mimicking light during the day might be beneficial, such light in the night time can adversely affect circadian clock and dependent physiologies ¹⁰⁵. This is prompting lighting manufacturers and architects to adapt dynamic lighting for the workplace, care-giving facilities and the home.

Despite these potentials for leveraging the knowledge on melanopsin function to improving human health, significant barriers remain. Although melanopsin is expressed in the retina and is, therefore, the subject of vision science, the primary consequence of disrupted melanopsin signaling most likely lies in sleep disturbances, mood disorders, and consequent effects on metabolism, which are beyond the scope of vision science. Furthermore, these disorders are of complex etiologies and the patients are often in need of acute intervention. Nevertheless, the vision scientists have a clear role in evaluating the melanopsin system in the retina. Alternatively, sleep clinicians and psychiatrists could begin to interrogate the contribution of lighting conditions or the melanopsin signaling system to sleep and mood disorders in their patients.

Glossary Box

Circadian clock, suprachiasmatic nucleus (SCN) and photoentrainment a vast array of physiological and behavioral changes with daily periodicity are controlled by an endogenous oscillator, the circadian clock. The master circadian clock regulating the behavioral rhythms resides in the SCN of the hypothalamus. The intrinsic period length of the circadian clock is not exactly 24 hours, and the axial tilt of the planet produces natural changes in day lengths throughout the solar year. Thus, the phase of the clock is adjusted daily primarily by light. The adjustment (entrainment) of the phase of the intrinsic clock to the phase or timing of the ambient light is generally termed as circadian photoentrainment.

Photoreceptor the cells that sense light via the expression of photopigments that induce signal transduction pathways (phototransduction) to regulate light-dependent physiologies such as vision and regulation of circadian entrainment, seasonal reproduction and body-color changes. The vertebrate retina contains three types of photoreceptors, rods, cones and ipRGCs. Each vertebrate photoreceptor contains a photopigment consisting of a protein called an opsin and a Vitamin-A-based light absorbing molecule (chromophore), 11-*cis* retinal (see also Figure 2).

Retinal ganglion cells (RGCs) are found in the inner most layer (ganglion cell layer; GCL) of the retina (Figure 1a), working as output neurons to send the visual information to the central nervous system. At least 10-15 types of RGCs are classified by the morphological differences, although this number varies among species ¹¹⁷.

Melanopsin and intrinsically photosensitive retinal ganglion cells (ipRGCs) a small subset of mammalian RGCs is intrinsically photoreceptive and these cells express an opsin

protein called melanopsin (also called OPN4). Hence, they are generally referred to as ipRGCs or mRGCs.

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Figure 1. Melanopsin expressing RGCs and their spectral properties

(a) Schematic diagram of the mammalian retina showing different cell types and their connectivity. The rod and cone photoreceptors densely packed in the outer retina are the primary photoreceptors supporting IF vision. Light activated signals originating from the rod/cone cells are processed in the horizontal (H), bipolar (B) and amacrine cells (A) before reaching the RGC of the inner retina. A small percentage of RGCs express melanopsin and are intrinsically photosensitive (ipRGCs). The ipRGCs, like other RGCs also receive signals originating from the outer retina rod/cone photoreceptors. RPE: retinal pigment epithelium; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer.

(b) Melanopsin expressing RGCs in rodents exhibit diversity in their cellular architecture^{68, 116}. Dendrites of the M1 subtype primarily arborize in the outer half of the IPL, the sublamina a (OFF sublamina). Dendrites of the M2 subtype stratify in the inner sublamina of the IPL, the sublamina b (ON sublamina), whereas the M3 subtype stratify in both sublaminae a and b. In general, ON and OFF bipolar cells have its terminals in the sublamina b (ON sublamina) and sublamina a (OFF sublamina), respectively. However, the ON bipolar cells have unusual ectopic synaptic contacts with the M1 cell type in the OFF sublamina⁷⁰.

(c) Mouse retina flat mount stained with anti-melanopsin antibody (red). The distribution of melanopsin-staining cells is almost uniform across the mouse retina, whereas in the primate retina, the fovea is largely devoid of ipRGCs⁵⁷. The somata of melanopsin-positive RGCs have sparsely branching dendrites that are relatively long (up to several hundred microns). The dendritic fields of these RGCs in primate and mouse retina have an average diameter of 0.5 mm⁵⁷ and 0.3 mm⁶⁵, respectively. Thus, despite the limited expression of melanopsin in only

1–2% of RGCs, these RGCs form a diffuse photosensitive web that covers virtually the entire retina. Melanopsin immunoreactivity is found throughout the dendrites, soma and axons, which contrasts with rods and cones whose photopigment expression is restricted to the outer segments.

(d) Spectral sensitivity of rod, cones and ipRGCs and the spectral composition of indoor fluorescent lamps and sunlight. Maximum light sensitivities of human rods (R), S cones, M cones and L cones are to ~500 nm, ~420 nm, ~530 nm and ~560 nm wavelengths, respectively. The ipRGCs exhibit peak sensitivity at ~480 nm. The emission spectra for popular fluorescent lamps (black line) used for indoor lighting and sunlight (grey line; two hours after sunrise in San Diego, April 2010) were measured and analyzed by EPP2000 UV-VIS spectrometer and SpectraWiz software (StellarNet Inc.).

Figure 2. The visual cycle and phototransduction in the vertebrate retina

In the rod outer segment (ROS), light converts 11-*cis* retinal chromophore of rhodopsin to all-*trans* retinal. All-*trans* retinal is released from rhodopsin and undergoes an elaborate multistep enzymatic process (visual cycle) to regenerate 11-*cis* retinal. All-*trans* retinal is first reduced to all-*trans* retinol by retinol dehydrogenase 8 (RDH8) and RDH12. In the RPE, all-*trans* retinol is esterified by LRAT to all-*trans* retinyl esters. RPE65 mediates the conversion of all-*trans* retinyl esters to 11-*cis* retinol, which is oxidized to 11-*cis* retinal by RDH5. 11-*cis* retinal returns to the ROS where it binds to opsin to regenerate rhodopsin photopigment. In the ipRGCs, melanopsin is found in complex with 11-*cis* retinal²². The source of this chromophore and the mechanism for regeneration of 11-*cis*- from all-*trans*- retinal photoproduct is currently

not known. Evidence points to both photoisomerization of all-*trans* retinal to 11-*cis* by melanopsin itself and use of the RPE visual cycle.

Figure 3. The ipRGCs as the site of signal integration

The rod and cone photoreceptors of the outer retina signal via multisynaptic pathways to the RGCs of the inner retina. The RGCs, in turn, transfer the visual signals from the eye to the brain via their axonal projections. For NIF visual functions, the light information originating from the rods and cones are exclusively transmitted through the ipRGCs. The ipRGCs function as the node for integrating melanopsin and rod/cone initiated photoresponses. ipRGCs likely participate in the IF vision by two potential mechanisms. In the retina they also affect function of the dopamine-responsive amacrine cells,⁹⁵ which then affect adaptation of the rod/cone-initiated signals under prolonged illumination. The ipRGCs also send projections to the dorsal LGN (lateral geniculate nucleus) in the brain, which receives extensive innervations from other RGCs⁵⁷.

Figure 4. Central projections of the ipRGCs

A schematic diagram of summarizing the brain regions innervated with the ipRGC axons. SCN: suprachiasmatic nucleus; LH: lateral hypothalamus; AH: anterior hypothalamus, SPZ: subparaventricular zone; OPN: olivary pretectal nucleus; IGL: intergeniculate leaf; dLGN: dorsal lateral geniculate nucleus; vLGN: ventral lateral geniculate nucleus; SC: superior colliculus.