Review

The emerging roles of melanopsin in behavioral adaptation to light

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The adaptation of behavior and physiology to changes in the ambient light level is of crucial importance to life. These adaptations include the 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 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.

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. The 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 NIF functions [1–3] (Box 1), which are abolished in subjects who have lost both eyes [4]. The peak spectral sensitivity of many of these NIF responses in individuals and animal models lies in the ~460–500 nm range in both normal and blind subjects with substantial rod/cone loss [5–11], thereby 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 scientists understand how effective lighting strategies, pharmacological intervention and current medical practices affect the quality of life.

Melanopsin is an opsin class of G-protein-coupled receptor (GPCR) first discovered in the photosensitive skin melanophores of *Xenopus laevis* (hence the name) [20], where it mediates the adaptation of skin pigmentation to ambient light level. Subsequently, melanopsin was discovered in the retinas of several vertebrates (reviewed in [21]). 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 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

Glossary

Circadian clock, suprachiasmatic nucleus (SCN) and photoentrainment: A vast array of physiological and behavioral changes with daily periodicity is controlled by an endogenous oscillator called 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 circadian photoentrainment.

Photoreceptor: These are 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 the 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 (Figure 2).

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

Melanopsin and intrinsically photosensitive retinal ganglion cells (mRGCs / 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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Box 1. Light adaptation or NIF visual photoresponse

In addition to the IF 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 humans, 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 phases of the circadian clock is several seconds or minutes, orders of magnitude less sensitive than IF vision. Such a requirement for the integration of light information over longer timescales helps maintain a robust circadian clock in the face of occasional light noise in nature such as lightening. The attenuation of light input to the circadian clock has been documented in mice lacking melanopsin (*Opn4*$^{-/-}$ mice) [16,17].
- *Pupillary light reflex (PLR)*: The 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 humans show an 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. The 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 the rod/cone and melanopsin systems show no light suppression of the 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 fails 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:12 hr light:dark schedule [109–111].
- *Light exacerbation of migraine*: Light exacerbates migraine headaches, and this effect is intact in blind individuals with light perception but not in patients with no light perception. The direct projections of ipRGCs to the thalamic region, which is implicated in migraine pain, offer a neural mechanism for the light exacerbation response [43].
- *Allodynia to light or photophobia*: Several individuals and some blind people show aversion to light. Intact photophobia in blind patients implies the potential involvement of the melanopsin system. Additionally, young rodent pups (less than 10 days old) show photophobia before the rod/cone system is fully functional [112]. A fully active melanopsin system at this age [67] most likely mediates such photophobia.

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 [22] (Figure 1d). The peak sensitivity correlates with the photosensitivity of several NIF responses of animals or humans under natural conditions of prolonged light exposure when rods and cones have saturated or adapted, thereby suggesting an important role for 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 metastate of classical vertebrate rhodopsin photopigment is unstable at physiological temperatures. *Drosophila* rhodopsin, by contrast, after activation by blue light, transits into a relatively stable metastate that continues to activate downstream signaling proteins. Moreover, upon excitation with long wavelength orange light, the metastate returns to the blue-sensitive basal state (reviewed in [23]). Purified melanopsin from amphioxus is similarly bistable [24]. 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 the coexpression of arrestin the photocurrent is returned to baseline approximately 2 min after lights off [27]. Arrestin desensitizes activated GPCRs [28], and prolonged melanopsin photocurrent in the absence of arrestin is reminiscent of the prolonged depolarization of *Drosophila* photoreceptor cells lacking arrestin [29]. Therefore, the metastate melanopsin might be stable. If the metastate mammalian melanopsin behaves similarly to purified amphioxus melanopsin or *Drosophila* rhodopsin, a long wavelength light pulse might return the metastate 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 a natural light environment are yet to be addressed. The clear understanding of the metastate melanopsin will impact how changes in the 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

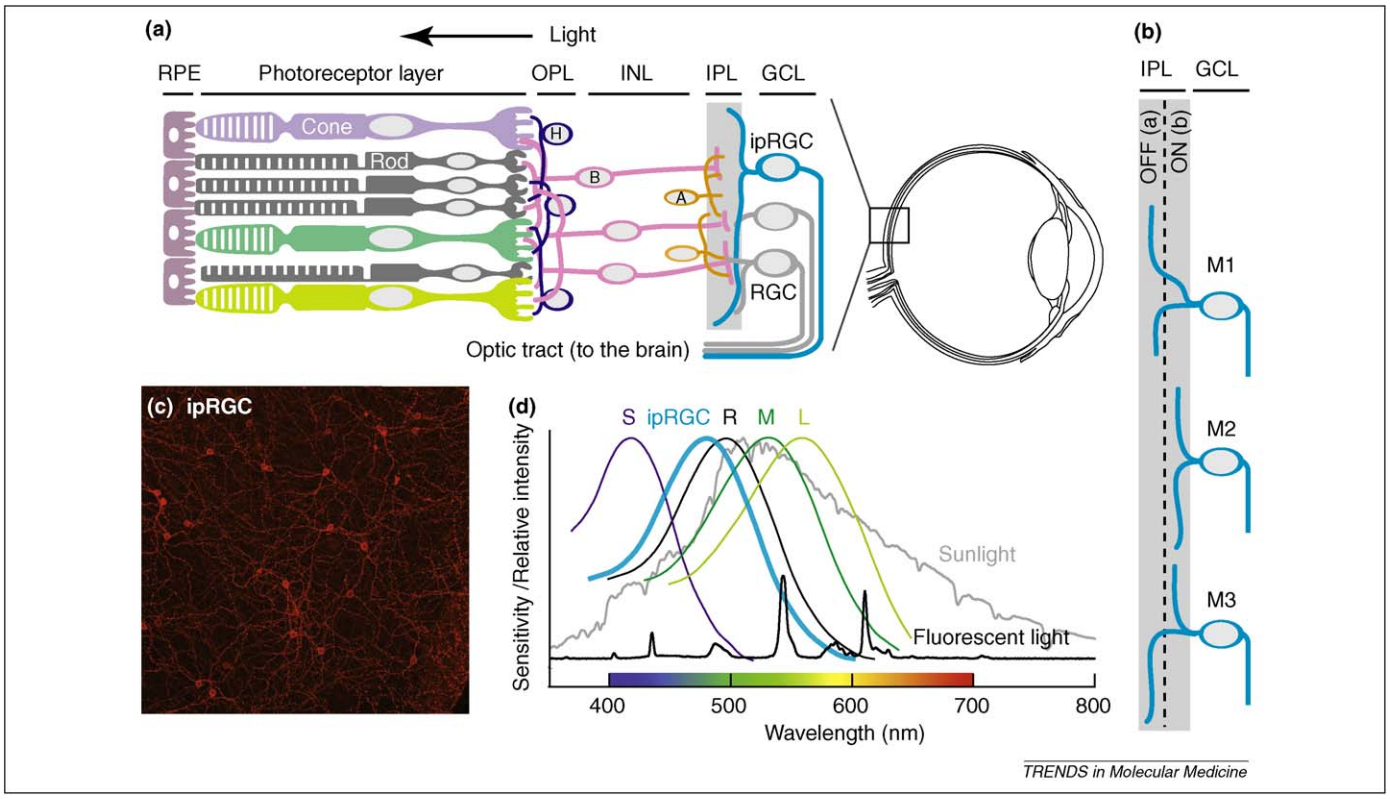

Figure 1. Melanopsin expressing RGCs and their spectral properties.
(a) Schematic diagram of the mammalian retina showing different cell types and their connectivities. The rod and cone photoreceptors densely packed in the outer retina are the primary photoreceptors supporting IF vision. Light-activated signals originating from the rod/cone cells are processed in the horizontal (H), bipolar (B) and amacrine cells (A) before reaching the RGCs 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, the ON and OFF bipolar cells have their 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 sublamina [70].
(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 ipRGCs [57]. 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 retinas have an average diameter of 0.5 mm [57] and 0.3 mm [65], 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 ~500 nm, ~420 nm, ~530 nm and ~560 nm wavelengths, respectively. The ipRGCs exhibit their peak sensitivity at ~480 nm. The emission spectra for popular fluorescent lamps (black line) used for indoor lighting and sunlight (gray line; two hours after sunrise in San Diego, April 2010) were measured and analyzed using a EPP2000 UV–VIS spectrometer and SpectraWiz software (StellarNet Inc.).

metabolism are implicated in many human diseases and, conversely, components of the 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 the 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 it 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 pupillary light reflex (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 the 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], the severe loss of PLR and poor sleep quality could arise from attenuated melanopsin function.

The residual photosensitivity in *Rpe65*$^{-/-}$ and *Lrat*$^{-/-}$ mice suggest that melanopsin or the ipRGCs might use 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 regenerate an active photopigment [27]. However, purified melanopsin from mouse retinas has been 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

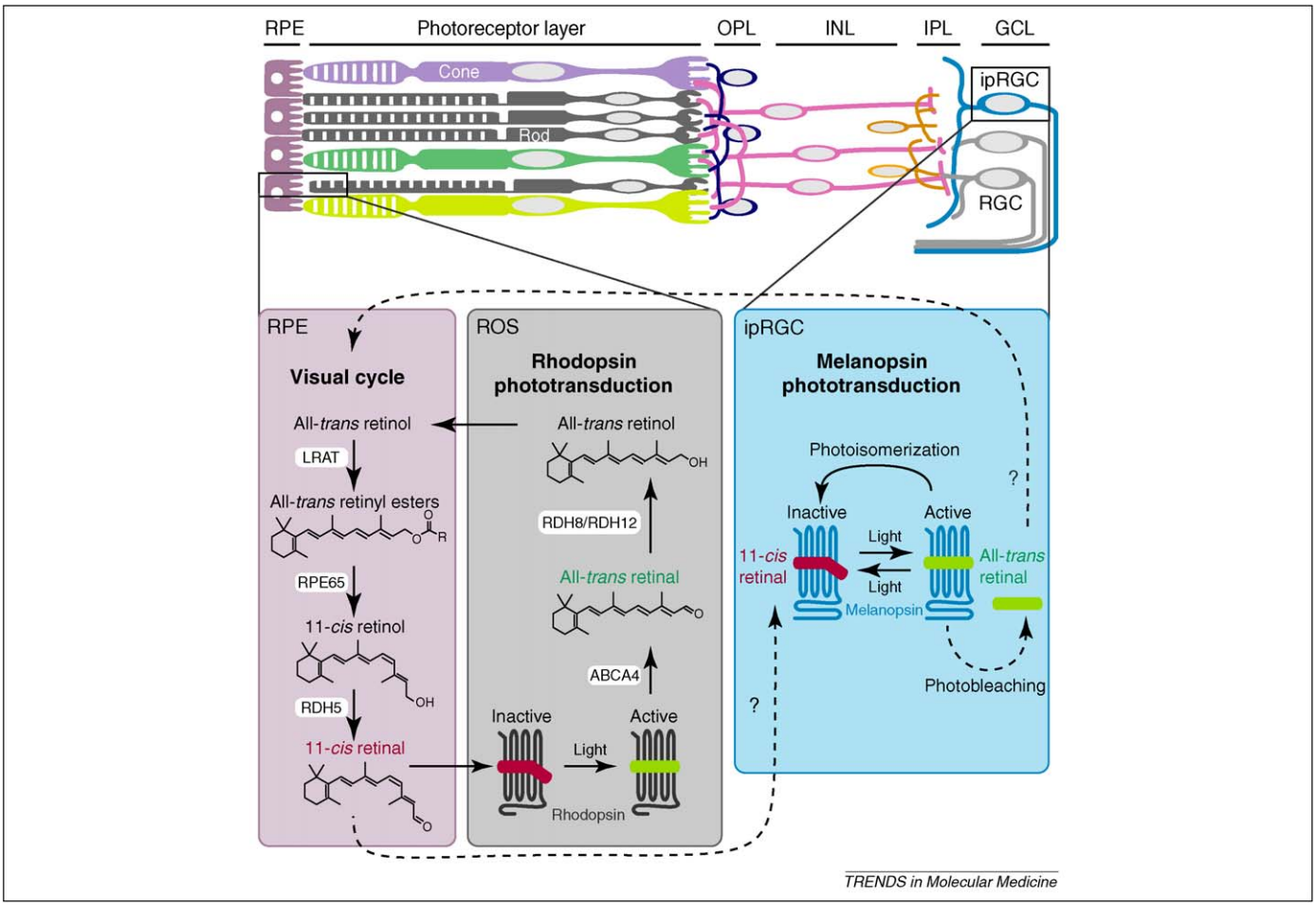

Figure 2. The visual cycle and phototranduction in the vertebrate retina.
In the rod outer segment (ROS), light converts the 11-*cis* retinal chromophore of rhodopsin to all-*trans* retinal. All-*trans* retinal is released from rhodopsin and undergoes an elaborate multistep enzymatic process (visual 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 (lecithin retinol acyltransferase) to all-*trans* retinyl esters. RPE65 (Retinal pigment epithelium-specific protein 65 kDa) 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 the rhodopsin photopigment. In ipRGCs, melanopsin is found in complex with 11-*cis* retinal [22]. The source of this chromophore and the mechanism for the regeneration of 11-*cis* from all-*trans* retinal photoproduct is currently not known. Evidence points to both the photoisomerization of all-*trans* retinal to 11-*cis* by melanopsin itself and the use of the RPE visual cycle.

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 the photobleaching of melanopsin. Support for both photoisomerization and photobleaching mechanisms in mammalian ipRGCs exists. Several labs have shown that ipRGCs can be repeatedly photoactivated without exogenous retinal, which supports photoisomerization within ipRGCs [14,15]. However, others have also observed a reduction in photoresponses or photobleaching by up to 70% upon the repeated photostimulation of ipRGCs [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 can photoisomerize the all-*trans* retinal and regenerate functional photopigments. The mechanisms and molecules that determine the steady-state levels of melanopsin photopigments are yet to be discovered.

The 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 RPE cells or reducing the availability of retinoid to the photoreceptors [42]. One such inhibitor of the visual cycle all-*trans*-retinylamine acutely reduces the available 11-*cis* retinal and, consequently, attenuates rod/cone function, leaving the melanopsin system almost intact [37]. However, the effect of the chronic administration of these drugs on the 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 the melanopsin-dependent exacerbation of migraine pain in normal and blind individuals (see [43]).

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

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

In vertebrates (Figure Ia), light-activated metarhodopsin triggers a pertussis toxin-sensitive class of G protein (Gt, transducin), which in turn activates a phosphodiesterase (PDE) that hydrolyzes 3′–5′ cyclic guanosine monophosphate (cGMP) to 5′ cGMP. The light-activated hydrolysis of cGMP leads to the closure of the cyclic nucleotide-gated (CNG) ion channels and hyperpolarization of the photoreceptor cells. By contrast, the invertebrate cascade (Figure Ib) is initiated by the activation of the pertussis toxin-insensitive Gαq class of G protein, which in turn activates phospholipase C-β (PLCβ). Activated PLCβ 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 polyunsaturated fatty acid (PUFA). Events downstream of the activation of PLC are complex and might involve IP3, DAG and PUFA as signaling intermediates to activate transient receptor potential (TRP) cation channels, which results in the influx of Na^+ and Ca^{2+} as well as membrane depolarization (reviewed in [113]). IP3 can also trigger an increase in cytosolic Ca^{2+} level by release from Ca^{2+} stores [23].

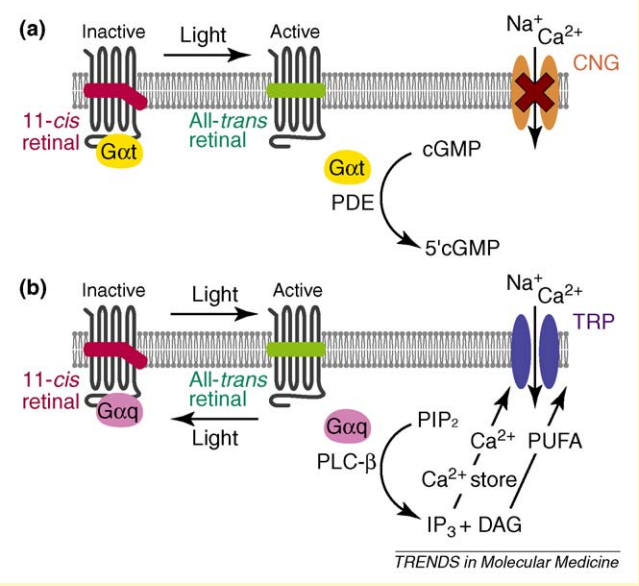

Figure I. Phototransduction mechanisms of classical vertebrate and invertebrate rhodopsins.
The vertebrate rod/cone opsin phototransduction signaling cascade **(a)** is distinct from that of the invertebrate rhodopsin **(b)**.

of an opsin. Melanopsin shares more sequence similarity with invertebrate rhodopsins than with vertebrate rhodopsins [12]. Several features of ipRGC photosensitivity are also characteristic of invertebrate photoreceptors (Box 2). Specifically, ipRGCs depolarize upon light activation [14,15]; photoactivation causes a transient increase in cytosolic Ca^{2+} levels [44] and the photocurrent generated by ipRGCs exhibits a voltage–current relationship that resembles that of the transient receptor potential (TRP) class of inward-rectifying cation channels [45]. 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 effecter channel (*Cng3*$^{-/-}$) [8,19]. Consequently, many blind patients carrying mutations in rhodopsin signaling components probably have intact ipRGC function.

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 [27,46,47] (Box 2), which in turn signals through PLCβ to trigger the opening of a TRP class of ion channel and increase in cytosolic Ca^{2+}. A similar signaling cascade is likely to function in native ipRGCs. The melanopsin-mediated photocurrent in ipRGCs can be blocked by the specific inhibition of the Gq/G11 class of G proteins and PLCβ [48]. Furthermore, light activation triggers an increase in cytosolic Ca^{2+} in ipRGCs [49], and the melanopsin photocurrent shows characteristic features of the TRP class of ion channel [45]. 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 effecter G protein, the signal-amplifying cascade mediated by PLC and the effecter 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 opens a cyclic nucleotide-gated (CNG) class of channel [30] – 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 is likely to find a signaling cascade. The ectopic expression of melanopsin in RGCs renders them light sensitive with properties similar to those of native ipRGCs [51]. This raises the potential for the application of melanopsin as a therapeutic optogenetic tool (Box 3) for treating several human diseases including blindness.

Photoresponses of 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 neuroanatomy [52]. 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 3000 RGCs out of ~1.5 million stain positively for melanopsin [53]. In each mouse retina, up to 1500 RGCs out of approximately 50 000 express melanopsin [54]. 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 ipRGCs [13] (Figure 1c).

Box 3. Optogenetics

Optogenetics generally refers to combining the optical stimulation of a light-sensitive protein to probe or to alter cellular function. Prevalent applications involve the 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 a light sensitive opsin and an effecter channel, the high-speed opening and closing of the channel 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 the phosphorylation of cyclic AMP/Ca response element-binding protein (CREB) and leads to the light-mediated transcriptional activation of CREB targets [114]. Because melanopsin uses a multistep intracellular signaling cascade, significant signal amplification is naturally achieved at each step of the cascade. Recombinant adeno-associated virus-mediated expression of melanopsin in a large number of RGCs of mice with the extensive degeneration of rod/cone cells restores some visual functions at normal indoor light levels [51]. The use of channel rhodopsin also restores some visual functions but only at high intensity light levels equivalent to midday sunlight [115]. 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.

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 a long time to return to baseline [15]. 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 photopigments [41]. 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 ipRGCs probably leads to the sluggish response. In support of this observation, the 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 melanopsin [55].

The ipRGCs, like other RGCs, also transduce rod/cone-initiated light responses [56]. Recordings from primate ipRGCs show distinct rod, cone and melanopsin-initiated responses in ipRGCs [57]. 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 ipRGCs. The L (long wavelength) and M (medium wavelength) cone-initiated light signals cause the transient depolarization of the ipRGCs at the onset and offset of light and, therefore, reliably encode lights on or off, but are unreliable for encoding light intensity over long durations. The intrinsic melanopsin-mediated photoresponses begin after a few milliseconds of cone-initiated response and are sustained for the duration of illumination. 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 is likely to override the S-dependent off response. Altogether, the primate ipRGC responses predict that under dim light conditions (approaching the limit of human vision), rod-initiated light signals are likely to support NIF responses, whereas under natural daylight conditions melanopsin-initiated responses tonically encode light intensity information and support NIF responses [57]. Accordingly, cone responses alone in mice are insufficient to sustain normal NIF responses under daytime light levels [58]. A recent study in humans has carefully dissected the roles of cones and melanopsin in NIF responses [59]. At the beginning of a long bright light pulse, cone signals are as effective as melanopsin signals in suppressing pineal melatonin release. However, over 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, the specific ablation of ipRGCs leads to the almost complete loss of all NIF responses leaving the IF responses nearly intact [78–80] (Figure 3). This implies that rod- and cone-initiated light signals destined for NIF responses are predominantly transmitted through ipRGCs, which thereby 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 mela-

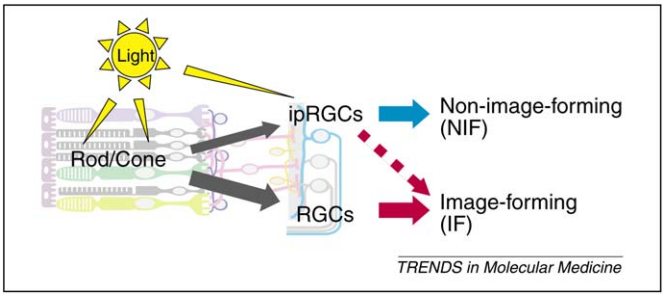

Figure 3. The ipRGCs as the site of signal integration.
The rod and cone photoreceptors of the outer retina signal via multisynaptic pathways to the RGCs of the inner retina. The RGCs, in turn, transfer the visual signals from the eye to the brain via their axonal projections. For NIF visual functions, the light information originating from the rods and cones is exclusively transmitted through ipRGCs. The ipRGCs function as the nodes for integrating melanopsin and rod/cone-initiated photoresponses. ipRGCs are likely to participate in the IF vision by two potential mechanisms. In the retina, they also affect the function of the dopamine-responsive amacrine cells [95], which then affect the adaptation of the rod/cone-initiated signals under prolonged illumination. The ipRGCs also send projections to the dorsal lateral geniculate nucleus (LGN) in the brain, which receives extensive innervations from other RGCs [57].

nopsin somehow augment rod/cone-initiated responses? In summary, the net light signal through ipRGCs supports most NIF responses and, therefore, ipRGCs constitute the central cellular framework for NIF responses.

Ontogeny, architecture and projections of ipRGCs

The ipRGCs are born along with other RGCs in rodents, whereas melanopsin expression begins *in utero* long before the rod/cone photoreceptors are fully functional [60]. In humans, the melanopsin system is also fully functional *in utero*, because premature babies born after 33 weeks show clear pupil constriction in response to light [61]. 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 fate [62–64], 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 somata and the most extensive dendritic arborization [57]. Unlike most other RGC cell types that are arranged in a nonoverlapping cobblestone pattern, the dendrites of ipRGCs extensively overlap with each other [13,65]. By comparing the morphology of dendrites and light responsiveness, melanopsin-expressing RGCs have been classified into (at least) three subtypes termed M1–M3 (Figure 1b; reviewed in [66]), although a recent paper doubts the definition of the M3 subtype as a separate cell type [65]. The physiological responses of mouse ipRGCs also exhibit significant diversity with respect to threshold sensitivity, the magnitude of responses and deactivation rates [67]. The M1 and M2 cell types in mouse retinas exhibit distinct photosensitive properties [68]. Despite these detailed 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 ipRGCs stratify in either the off or on 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 a significant effect on the normal function of the melanopsin system. Accordingly, mice lacking the critical factor Dscam that specifies dendritic stratification and spreading show the aberrant dendritic morphology of ipRGCs [71].

The axons of 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 ipRGCs [14,72,73]. The current and most comprehensive analysis of the central projections from ipRGCs was performed with a $Opn4^{tau:LacZ}$ mouse, a transgenic mouse with the reporter tau:LacZ knocked into the melanopsin locus [14], in which the M1 subclass of ipRGCs is mostly labeled [74]. Detailed projections of ipRGCs are described in Hattar *et al.* [75]. 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, 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, ipRGCs, like the other RGCs, project contralaterally to brain regions that directly

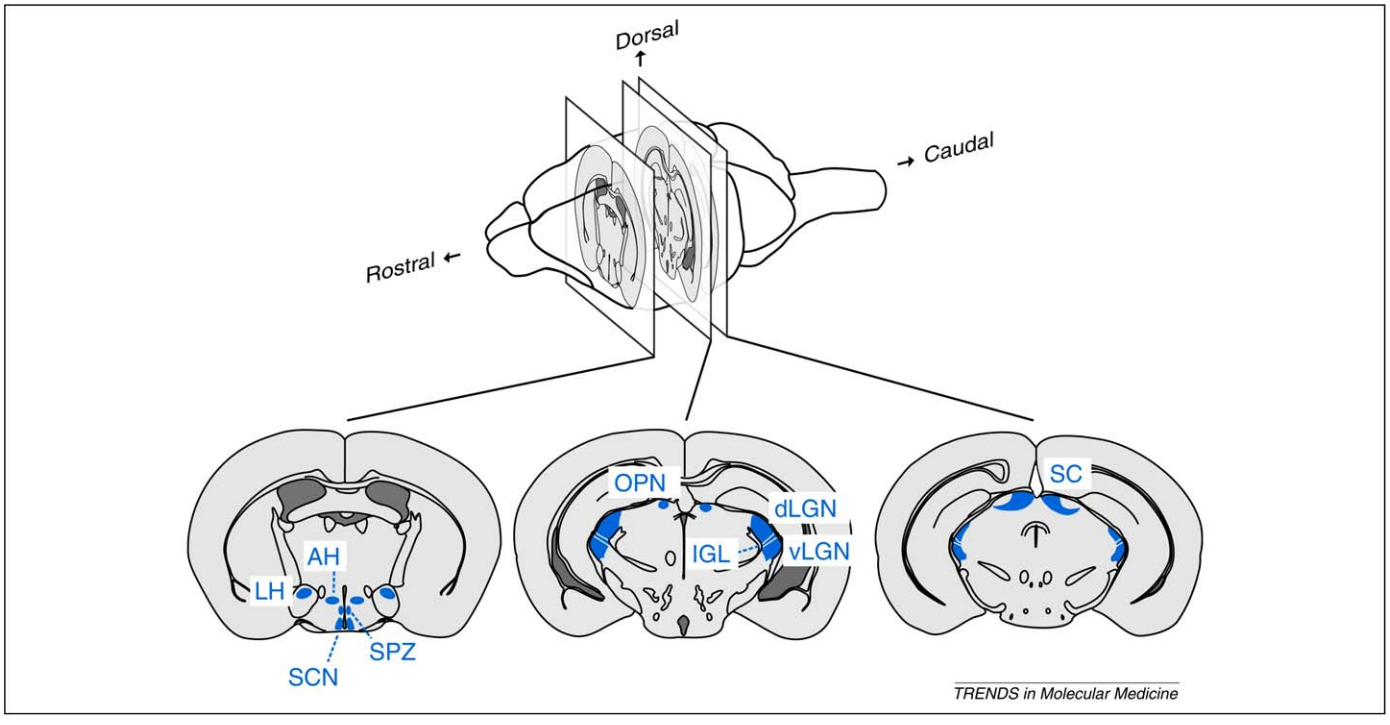

Figure 4. Central projections of ipRGCs.
A schematic diagram summarizing the brain regions innervated with ipRGC axons. SCN: suprachiasmatic nucleus; LH: lateral hypothalamus; AH: anterior hypothalamus; SPZ: subparaventricular zone; OPN: olivary pretectal nucleus; IGL: intergeniculate leaf; dLGN: dorsal lateral geniculate nucleus; vLGN: ventral lateral geniculate nucleus; SC: superior colliculus.

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, thereby suggesting a unique axon guidance mechanism mediates such dominant connections. Several additional brain centers also receive sparse projections from ipRGCs. They include the lateral hypothalamus, ventrolateral preoptic nucleus, habenulla and subparaventricular zones. These projections are likely to mediate the effects of light on the hypothalamic regulation of sleep, behavior and physiology. Multisynaptic projections are responsible for the ipRGC regulation of pineal melatonin [76]. Phenotypic assessments of mice lacking melanopsin or those with the targeted ablation of ipRGCs also exhibit specific defects in light-dependent behaviors supported by these brain centers (reviewed in [66]), thereby validating the functional significance of ipRGC projections. The recently described sparse projections of ipRGCs in the posterior thalamus found juxtaposed to dura-sensitive thalamocortical neurons are proposed to mediate the 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 the SC and LGN receive extensive innervations from other RGCs and are primary relay centers for IF vision. ipRGC projections to the LGN are also found in the primate brain [57]. Accordingly, some blind patients with the 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, ipRGCs extensively innervate several brain regions mediating NIF responses and also innervate the LGN and SC where they are likely to 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 photopigments and ipRGCs in NIF responses. In general, most NIF responses 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 the 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 the loss of NIF light responses (Figure 3), thereby establishing ipRGCs as the principal carriers for transmitting the light information originating from both melanopsin and outer retina photoreceptors [78–80].

Additional mouse models with the 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 a photopic light level, cones seem 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 nocturnal rodents and diurnal primates including humans.

Mouse genetics has also shed light on potential signaling mechanisms in ipRGCs. Mice lacking the unconventional protein kinase C (PKCζ) phenocopy the reduced photosensitivity of the $Opn4^{-/-}$ mice. The expression of PKCζ in ipRGCs raises the possibility that this PKC plays a crucial role in the melanopsin-signaling cascade [82]. Similar clues to the candidate downstream neurotransmitters in ipRGCs have also come from mouse studies. Melanopsin-expressing cells also express the neuropeptide PACAP (pituitary adenylate cyclase-activating peptide) [53,83,84], and PACAP receptors are expressed in the SCN [73,85]. The exogenous application of PACAP at low concentrations can phase shift the SCN clock, whereas mice lacking PACAP or the PACAP receptor show a reduced response of the circadian clock to a phase resetting pulse of light [86–88]. The residual circadian photosensitivity in PACAP knockout mice is likely to be mediated via glutamate, which is also found in ipRGCs [89]. Analogous to PACAP, glutamate receptors are expressed in the SCN neurons ([90] 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 a 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 that suggest an interaction at various levels between these two systems. The melanopsin system can modulate the classical IF vision both in the retina and LGN. The ipRGCs' signal to the dopaminergic amacrine cells might form a basis for the adaptation of the visual system to light intensity levels [95]. Innervations of the LGN of primates [57] and rodents [77] by ipRGCs suggest the melanopsin system might directly transmit ambient light intensity information to the 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 $Lrat^{-/-}$ mice, the 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 contributions 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 begin 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 changes [97]. The extensive remodeling of ipRGC dendritic structures in the retina has also been observed in old rats with outer retina degeneration [98]. 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, the effective use of light in improving 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 care-giving facilities.

Diagnostic procedures for the melanopsin system

Rodent data suggest that perturbed melanopsin signaling might underlie several human disease conditions including sleep disorders, seasonal affective disorders (SADs), depression, aversion to light and light-exacerbated migraine pain [43]. 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 (GCL) of the inner retina. A promising method would be using 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 has 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), but a normal or enhanced melanopsin response in another form of blindness [100]. Such a PLR response can also be used as a surrogate measure for the severity of another blinding disease glaucoma, which involves the progressive death of RGCs. In summary, the evaluation of ipRGC function is a starting point for further classifying blindness into patients with the complete loss of both NIF and IF visions and those with the loss of IF vision alone. Such a classification will help determine whether normal ipRGC function in some blind patients might be beneficial in maintaining a 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 lenses, 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 five-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 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 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 a mood uplifting effect, whereas inhibitors would mimic darkness and prevent the light suppression of melatonin and thereby improve sleep. Additionally, the recent observation that ipRGCs might mediate the light exacerbation of migraine pain [43] raises the possibility that pharmacological darkness alone or in combination with other drugs could offer a novel strategy for pain management in normal and blind patients.

Gene and mechanism discovery from patients

The 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 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, SADs 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 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), thereby 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 nighttime can adversely affect the 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 their consequent effects on metabolism, which are beyond the scope of vision science. Furthermore, these disorders have complex etiologies and patients are often in need of acute intervention. Nevertheless, vision scientists have a clear role in evaluating the melanopsin system in the retina. Alternatively, sleep clinicians and psychiatrists could begin to interrogate the contributions of lighting conditions or the melanopsin-signaling system to sleep and mood disorders in their patients.

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