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Non-image-forming ocular photoreception in vertebrates

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Abstract

It has been accepted for a hundred years or more that rods and cones are the only photoreceptive cells in the retina. The light signals generated in rods and cones, after processing by downstream retinal neurons (bipolar, horizontal, amacrine and ganglion cells), are transmitted to the brain via the axons of the ganglion cells for further analysis. In the past few years, however, convincing evidence has rapidly emerged indicating that a small subset of retinal ganglion cells in mammals is also intrinsically photosensitive. Melanopsin is the signaling photopigment in these cells. The main function of the innerretina photoreceptors is to generate and transmit non-image-forming visual information, although some role in conventional vision (image detection) is also possible.

Introduction

Vertebrate eyes mediate both image-forming and non-image-forming visual functions. Image-forming vision, with its high spatial and temporal resolution, enables the animal to detect and track objects in the visual world. Non-image-forming vision, however, provides a measure of the ambient luminance for the purposes of synchronizing the animal's biological clock with the surrounding light–dark cycle (circadian photoentrainment), controlling the pupil size, and other functions such as acute suppression of locomotor behavior (negative masking) in rodents (reviewed by Foster and Hankins [1]). Circadian photoentrainment, in turn, affects a whole host of functions such as melatonin release, body-temperature regulation and feeding behavior.

Retinal rods and cones, the classical ocular photoreceptors, are responsible for image-forming vision. In mammals, it now appears that non-image-forming vision is mediated not only by rods and cones but also by a small subset of retinal ganglion cells (RGCs) that is intrinsically photosensitive (ipRGCs). In lower vertebrates such as fish, a subset of inner retinal neurons (not necessarily RGCs) might also be photoreceptive [2]. Here, we summarize the current knowledge of the workings of these non-rod and non-cone ocular photoreceptors and their photopigments.

The quest for novel ocular photoreceptors

The quest for novel photoreceptors in the eye began in the field of circadian biology. Almost all organisms exhibit circadian rhythms in physiology and behavior, with roughly 24-hour cycles. Although many tissues are now known to have intrinsic rhythms [3,4], the overall bodily circadian rhythm in mammals is synchronized by a master pacemaker in the hypothalamus called the suprachiasmatic nucleus (SCN). To be in phase with the solar day, the biological time in the SCN in turn has to be synchronized with—or 'entrained' to—the environment.

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Ambient light adjusts the phase of the circadian rhythm in the SCN via the retinohypothalamic tract, a monosynaptic pathway connecting a small population of RGCs to the SCN [5–7]. Circadian photoentrainment disappears after removal of the eyes, suggesting that ocular photoreceptors are exclusively responsible for photoentrainment [8–10]. Until recently, rods and cones were assumed to be the exclusive circadian photoreceptors, even though photoentrainment was known to exhibit certain properties different from those of rods and cones, such as long-term temporal integration [11]. Beginning in the 1980s, researchers started to challenge the 'rod–cone dogma' through a series of behavioral studies on genetic mouse lines with retinal degeneration [12,13]. The most convincing experiments were performed on transgenic mice with an almost complete elimination of rods and cones [14,15]. Surprisingly, these mice showed an apparently normal circadian photoresponse to light, strongly suggesting the existence of circadian photoreceptors other than rods and cones. These experiments set the stage for 'hunting' for the novel non-rod and non-cone photoreceptors.

Intrinsic photosensitivity of a subset of retinal ganglion cells

To look for the elusive circadian photoreceptors, Berson *et al.* [16] labeled the RGCs that project to the SCN by retrograde transport of fluorescent microspheres injected into the rat SCN. Remarkably, they found that this subset of RGCs remained light-sensitive even after synaptic inputs from rod and cone pathways had been pharmacologically blocked. Several key properties distinguish these intrinsically photosensitive RGCs from rods and cones: first, light depolarizes ipRGCs but hyperpolarizes rods and cones; second, the ipRGCs are much less sensitive to light than rods and cones; and third, the kinetics of the light responses of ip RGCs are ~100-fold slower than rod and cone responses, with an overall time course (triggered by a flash stimulus) lasting as long as 1 min. A subsequent study has identified giant primate ipRGCs with similar properties [17••]. These ipRGCs steadily fire action potentials throughout a light stimulus (Figure 1), thus faithfully encoding the irradiance over time and providing a neural representation of the steady light intensity that is useful for photoentraining the circadian system [16,17••].

Identity of the circadian photopigment

Rod and cone visual pigments consist of an opsin, the protein moiety, covalently linked to a vitamin A-based chromophore, typically 11-*cis*-retinaldehyde. Upon photon absorption, 11-*cis*-retinaldehyde isomerizes to all-*trans*-retinaldehyde, after which the opsin undergoes conformational changes to become active as a signaling molecule, eventually splitting into opsin and all-*trans*-retinaldehyde. In the past decade, several novel, opsin-like proteins have been identified by molecular cloning from the eye and other tissues. One of these, melanopsin, was originally found in dermal melanophores of *Xenopus laevis* [18], but also localized by *in situ* hybridization to the iris and inner retina of *Xenopus* [18]. Subsequently, melanopsin was found in the inner retina of monkey and mouse [19]. These unusual findings no doubt prompted the pioneering experiments by Berson *et al.* [16] mentioned earlier. More recently, melanopsin has been found in the retina and brain of fish and chicken [2,20–22]. Melanopsin has also been reported to be present in the mouse retinal pigment epithelium [23], but this result has not been verified by others [24••].

In rodents, the expression of melanopsin in SCN-projecting RGCs was established by combined retrograde labeling from the SCN and *in situ* hybridization [25] or immunocytochemistry [26]. Evidence also came from co-immunolabeling of melanopsin and pituitary adeny-late cyclase-activating peptide (PACAP, which is present in SCN-projecting RGCs) [27], and from targeting of the axon-labeling marker tau-LacZ to the melanopsin gene locus [26]. As mentioned before, RGCs that project to the SCN show intrinsic photosensitivity [16,26]. In this review, melanopsin-expressing RGCs and ipRGCs will be used

interchangeably. Genetic ablation of melanopsin eliminated the intrinsic photosensitivity of mouse ipRGCs without altering their genesis or axonal projections [28]. In addition, mice lacking melanopsin showed an incomplete pupil light response [28] and attenuated circadian photoentrainment [29,30]. Finally, removing melanopsin in mice with non-functional rods and cones [31] or with degenerated rods and cones [32] completely abolished the pupillary light reflex and circadian photoentrainment, indicating that ipRGCs, rods and cones together account for all major non-image-forming functions.

Although melanopsin is clearly essential for the intrinsic light response of ipRGCs, it was unclear for a while whether it is indeed the photopigment. One concern was that the first reported absorption spectrum of heterologously expressed melanopsin had a λ_{max} at 424 nm, and an action spectrum with λ_{max} in the same region [33]. These measures are both considerably shorter than the 480 nm value derived from the action spectra for the photosensitivity of ipRGCs [16,17••], circadian phase-shifting and pupillary light reflex in the absence of rods and cones [31,34]. This discrepancy, together with the fact that melanopsin shares only ~27% amino-acid identity with known vertebrate photopigments, has led to the suggestion that melanopsin is not the signaling pigment in question but, instead, a photoisomerase that binds all-*trans*-retinaldehyde and uses light energy to convert it back to 11-*cis*-retinaldehyde for regenerating the still-unidentified pigment in ipRGCs [35].

Contributing to the confusion about melanopsin is cryptochrome, a blue-light-sensitive flavoprotein that functions as a circadian photopigment in *Arabidopsis* and *Drosophila* (reviewed by Van Gelder [36]). Cryptochrome was first found in plants, with high sequence homology to DNA photolyases [37]. In contrast to opsin-based photopigments, which use retinaldehyde as chromophore, cryptochromes use flavin adenonucleotide and/or a pterin [38]. In mammals, there are two cryptochromes, both of which are widely expressed in the body, including in most cells in the inner retina [39]. This retinal location has prompted the proposal that cryptochromes function as circadian photopigments in mammals also, a notion apparently supported by the finding that the signaling of light to the SCN is preserved even when retinaldehyde is severely depleted by dietary vitamin-A deprivation [40]. The validity of this finding [40], however, has since been questioned by a recent study on a mouse model that lacks 11-*cis*-retinaldehyde production in the retina [24••]. Moreover, a flavin-based photopigment is unlikely to account for the opsin-like action spectrum of ipRGCs [41]. Furthermore, genetic deletion of cryptochromes results in complete circadian arrhythmicity in mice [42–44] but does not abolish light-induced expression of SCN clock genes [42]. Therefore, mammalian cryptochromes appear to have a much more important role in the generation of the circadian oscillation itself than in the presumptive circadian photoresponse. Finally, the mammalian cryptochromes in cultured cells function as light-independent components of the circadian clock [45]. It was reported that the deletion of cryptochromes reduces the sensitivity of the pupillary light reflex in mice with degenerated rods and cones [46], an observation again used for arguing for a role of cryptochromes in non-image-forming photoreception. Nonetheless, this interpretation is complicated by the fact that cryptochrome-knockout mice frequently show ocular inflammation [47], which might cause subtle changes in retinal function. Moreover, parallel experiments on mice lacking cryptochromes but having normal rods, cones and ipRGCs show no diminution in the sensitivity of the pupil reflex [46], a finding difficult to reconcile with the proposal of cryptochromes having a photoreceptive role. The hypothesis that cryptochromes are mammalian circadian photopigments is also inconsistent with the observation mentioned earlier that mice lacking melanopsin and functional rods and cones (but with intact cryptochromes) show no sign of any non-image-forming visual response [31,32]. Thus, taking all the evidence into account, cryptochromes are unlikely to be responsible for non-image-forming ocular photo-reception in mammals. Whether cryptochromes take on such a role in other vertebrate species or contexts, such as in chick iris constriction [48], remains to be examined further.

Any remaining doubt about melanopsin being the signaling photopigment was removed by several recent studies showing that heterologously expressed melanopsin confers photosensitivity to non-photosensitive cell lines [49••–51••]. Supporting evidence also came from experiments showing that exogenous chromophore was unable to restore the photosensitivity of ipRGCs in melanopsin-knockout mice (a result inconsistent with melanopsin acting solely as a photoisomerase), but was able to do so in mice having melanopsin but deficient in 11-*cis*-retinaldehyde [24••].

There is also some evidence to suggest that melanopsin exhibits bistability, that is, the ability to serve the dual function of photopigment and photoisomerase [24••,50••,51••]. To date, bistable pigments have only been found in invertebrates. They have two photoconvertible stable states, bound to 11-*cis*-retinaldehyde or all-*trans*-retinaldehyde. By contrast, vertebrate pigments have only one stable state (the state with 11-*cis*-retinaldehyde bound), and rely on the retinoid cycle in the retinal pigment epithelium for regeneration. A bistable melanopsin would mean that ipRGCs can regenerate their pigment autonomously, possibly explaining why melanopsin appears less susceptible to chromophore-deprivation than rod and cone pigments [24••,40]. It is conceivable that a bistable melanopsin in the ipRGCs would be able to function continuously despite being physically far removed from the retinal pigment epithelium.

Heterologously expressed melanopsin is able to signal through $G_{\alpha q}$ or $G_{\alpha 11}$ and the TRPC3 ion channel ([49••,50••] but see [51••]), analogous to an invertebrate photo-transduction cascade. Likewise, a phospholipase C signaling pathway triggered by light has been demonstrated in cultured *Xenopus* dermal melanophores, which express melanopsin [52]. However, whether these results implicate a G-protein-mediated phospholipase C pathway in ipRGCs remains unclear.

Morphology and axonal projections of melanopsin-expressing retinal ganglion cells

In rodents, roughly 1000–2000 RGCs (~ 1–3% of all RGCs) express melanopsin [26]. The number is about 3000 in primates (0.2% of total RGCs) [17••]. Most melanopsin-expressing RGCs reside in the ganglion cell layer but some are displaced to the inner nuclear layer [17••,26]. The melanopsin-expressing RGCs give rise to large and extensively overlapping dendritic fields, forming a photoreceptive net [17••,26,53]. Individual cells with respect to their dendritic aborizations are principally monostratified, creating two distinct subpopulations that send dendrites to the extreme inner or outer boundaries of the inner plexiform layer (Figure 1) [17••,26]. These RGCs also express PACAP (see earlier), a neuromodulator in retinohypothalamic transmission [27].

Melanopsin-expressing RGCs innervate a variety of brain regions (Table 1). Their projections were most readily visualized in a mouse line harboring the *tau-lacZ* marker gene (which codes for a β-galactosidase fused to a tauprotein sequence for axonal localization) targeted to the melanopsin gene locus [26]. The blue labeling of the β-galactosidase activity provides a vivid image of individual axons in this mouse. Using this and other techniques, investigators found that the majority of melanopsin-expressing RGCs innervate the SCN, and most of the RGCs innervating the SCN express melanopsin [25,54–57]. Melanopsin-expressing RGCs also send dense projections to the intergeniculate leaflet (IGL), which integrates photic and non-photic cues for the control of circadian rhythms, and the olivary pretectal nucleus (OPN), which controls the pupillary light reflex [17••,26,54,56]. Brain regions that receive melanopsin RGC fibers but are more heavily innervated by conventional RGCs include the ventrolateral preoptic nucleus (VLPO), which is implicated in sleep–wake regulation in mammals; the lateral hypothalamus (LH), which is involved in energy homeostasis; and the ventral subparaventricular zone (vSPZ), which is involved in circadian regulation and the acute suppression

of locomotor activity by light [54,56–59]. The functional significance of the various minor melanopsin RGC projections remains to be elucidated.

Rod and cone inputs onto ipRGCs

Mice lacking melanopsin still show pupillary light response and can be photoentrained (although both functions become subnormal), indicating the involvement of rods and cones in these functions [28–30]. The most obvious source of rod and cone inputs for the ipRGCs would be through synapses from amacrine and bipolar cells [60] (Figure 1). The rod–cone influence on the ipRGCs was nicely demonstrated by a recent study in primates, showing that short-wavelength-sensitive cones inhibit, whereas rods and medium- and long- wavelength cones excite, ipRGCs ([17••] see also [61]; Figure 1). Rods and cones can provide the initial sensitivity and speed of the light signals for non-image-forming functions, whereas the intrinsic response of the ipRGCs can provide sustained signals throughout a light stimulus and long temporal integration [17••]. The fact that the ipRGCs in primates also project to the lateral geniculate nucleus, which is the thalamic relay to primary visual cortex, suggests that these cells might contribute to conscious visual perception [17••].

Inner-retina photoreceptors in fish

Compared with the situation with mammals, much less is known about ocular non-rod and non-cone photoreception in lower vertebrates, even though at least one photopigment (VA opsin, for vertebrate ancient opsin) had been found in their inner retinae before melanopsin was discovered [62]. VA opsin was first isolated from the eyes of Atlantic salmon and was localized to a subset of horizontal cells and to cells in the ganglion and amacrine cell layers [62]. When heterologously expressed, this pigment gave an absorption spectrum with λ_{max} at ~460 nm [63]. Subsequent electrophysiological recordings from the intact roach retina suggested the presence of a subclass of intrinsically photosensitive horizontal cells, which gave a very slow, depolarizing light response with an action spectrum peaking at 477 nm [2]. However, blockade of synaptic transmission eliminated these responses [2], making the finding inconclusive. Even if real, the photopigment underlying this ‘intrinsic’ photo-response of horizontal cells remains unclear because both melanopsin and VA opsin were suggested to be present in these cells [2]. Several isoforms of VA opsin were subsequently found [64–66]. So far, VA opsin has only been found in fish.

Conclusions

The past decade has witnessed the fascinating discovery that a subset of inner retinal neurons in mammals is intrinsically photosensitive and is important for non-image-forming visual functions such as pupillary light reflex and circadian photoentrainment. Melanopsin appears to be the photopigment underlying this intrinsic light response. Moreover, melanopsin might be a bistable pigment, a feature found in many invertebrate pigments. If so, this property will benefit the ipRGCs by removing their need to compete with rods and cones for chromophore.

The next challenge will be to understand the phototransduction mechanism in these unusual RGCs, in addition to the detailed interactions between their intrinsic light responses and the rod-cone signals.

Update

After the completion of this review, two interesting studies relating to melanopsin function were published. In one report [76••], a homolog of melanopsin was found to be expressed in rhabdomeric photoreceptor cells of the amphioxus and shown to be a bistable pigment possibly coupled to a Gq-mediated transduction cascade. In the other [77••], mouse ipRGCs were found

to be light-responsive from birth. Thus, this photosensitivity appears much earlier than that of rod and cone photoreceptors that starts at postnatal day 10.

Acknowledgments

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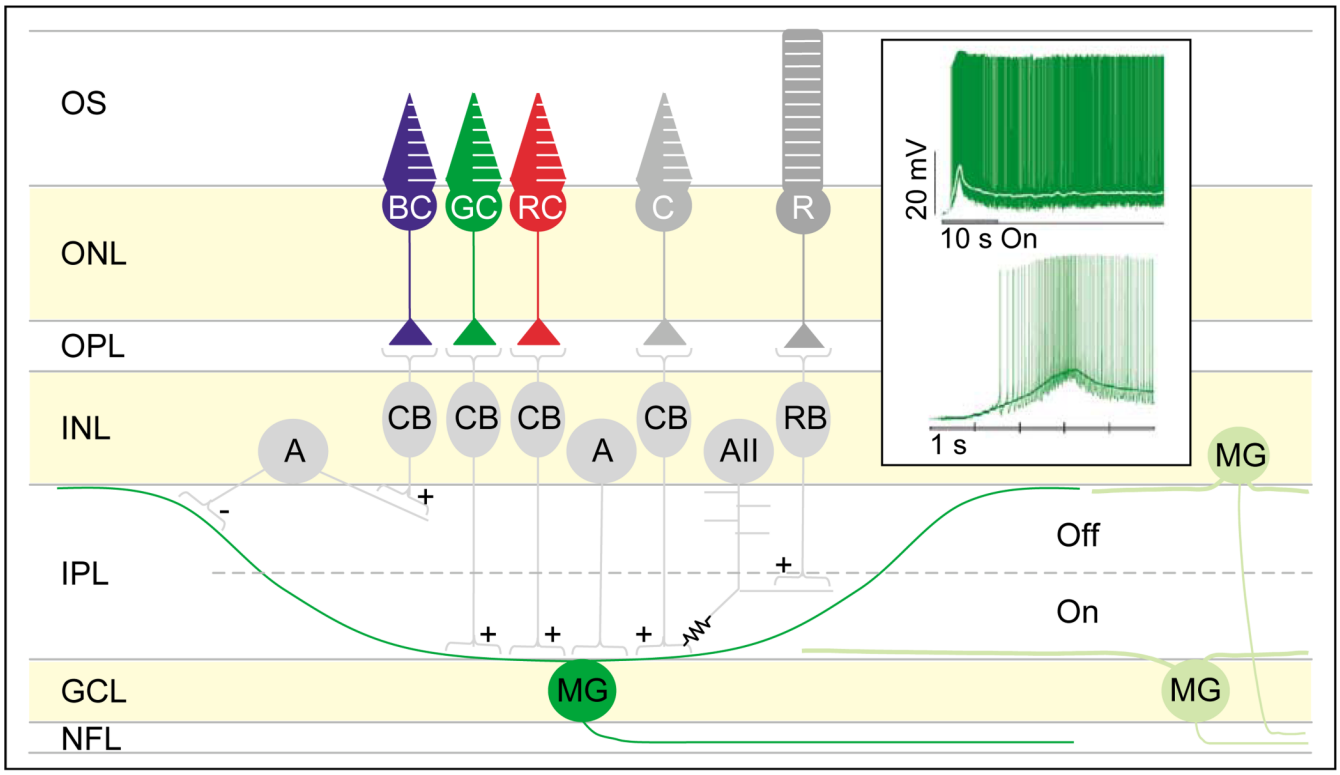

Figure 1.

Schematic showing the synaptic circuitry of primate melanopsin-expressing RGCs in the retina. Melanopsin-expressing RGCs (MGs) are primarily (~95% in rodents, ~60–70% in primates) located in the ganglion cell layer, and the rest (~5% in rodents, ~30–40% in primates) are displaced to the inner nuclear layer (INL) (H-W Liao, unpublished; [17••,26]). MGs have sparse dendrites and extremely large dendritic fields. The dendrites arborize in the inner plexiform layer (IPL), forming a major (both in rodents and in primates) plexus in the outermost boundary of the IPL and a minor (even less prominent in rodents) plexus in the innermost boundary of the IPL. In primates, green and red cones provide excitatory inputs through bipolar cells to MG proximal dendrites [17••,60], and rods provide excitatory inputs through, presumably, rod bipolar cells, AII amacrine cells, and cone bipolar cells successively. Blue cones provide inhibitory inputs [17••], presumably through cone bipolar cells and inhibitory (probably GABAergic) amacrine cells [60,67]. Some amacrine cells of unknown identity also make synaptic contacts with MG somata [60]. Inset (adapted with permission from Nature [17••] copyright 2005 Macmillan Publishers Ltd; http://www.nature.com/): top panel shows the response of a primate MG cell to a 470 nm light pulse. The cell continued to fire action potentials for 30 s after the end of the light stimulus. White line shows membrane potential values averaged over 0.5 s sliding time windows. Bottom panel shows the first 5 s of the response shown on top panel. Abbreviations: +, excitatory input; −, inhibitory input; resistor symbol, electrical coupling; A, amacrine cell; AII, Type II amacrine cell; BC, blue cone; C, cone; CB, cone bipolar cell; GC, green cone; GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; MG, melanopsin ganglion cell; NFL, nerve fiber layer; ONL, outer nuclear layer; OPL, outer plexiform layer; OS, outer segment; R, rod; RB, rod bipolar cell; RC, red cone.

Table 1

Projections of melanopsin retinal ganglion cells.

Melanopsin RGC target	Abbrev.	Function of target	Innervation	Species examined
Highest density of melanopsin fibers				
Suprachiasmatic nucleus	SCN	Master regulation of circadian rhythms [68]	Dominant	Mouse [26,58], rat [25,54,56] and hamster [55,57]
Intergeniculate leaflet	IGL	Integration of photic and non-photic circadian cues [68,69]	Major	Mouse [26,58], rat [54,56] and hamster [57]
Olivary pretectal nucleus	OPN	Pupillary constriction [70]	Major	Mouse [26,58], rat [54,56], hamster [57] and macaque [17••]
Lateral habenula	LHb	Integration of limbic, motor and circadian systems [71]	Undetermined	Mouse [58]
Lower density of melanopsin fibers				
Dorsal lateral geniculate nucleus	dLGN	Image-forming vision [72]	Minor	Mouse [58] and macaque [17••]
Lateral hypothalamus	LH	Energy homeostasis [68]	Minor	Mouse [58] and rat [56]
Lateral posterior thalamic nucleus	LP	Higher-order processing of thalamic, cortical and visual signals [73]	Minor	Mouse [58] and rat [56]
Posterior limitans thalamic nucleus	PLi	Detection of rapid illumination changes for non-imaging vision [74]	Moderate	Rat [56]
Superior colliculus	SC	Integration of multiple modalities for gaze control [75]	Minor	Mouse [58], rat [56]and hamster [55,57]
Ventral lateral geniculate nucleus	vLGN	Visuomotor function [69]	Minor	Mouse [26,58] and rat [56]
Ventral subparaventricular zone	vSPZ	Circadian and direct regulation of locomotion and sleep [68]	Minor	Rat [54,56]
Ventrolateral preoptic nucleus	VLPO	Promotion of sleep [68]	Minor	Mouse [58] and rat [54,56]

Reported central targets of melanopsin RGCs are listed with the general function of those brain regions. Areas receiving the highest absolute density of melanopsin fibers are grouped at the top of the table, and areas receiving a lower density are listed below in alphabetical order. 'Innervation' refers to the density of melanopsin fibers as compared with conventional retinohypothalamic fibers in each region. Melanopsin afferents from the contralateral eye predominate in all regions evaluated except for the SCN, which receives just slightly more fibers from the contralateral eye. This list of targets is not exhaustive, leaving out areas for which melanopsin innervation is suggested but less certain. Moreover, it does not describe the apparent innervation of multiple target regions by single melanopsin RGCs [54,57]. Results from mouse are from [26,58], rat from [54,56], hamster from [57], and primate from [17••].