Strange vision: ganglion cells as circadian photoreceptors

David M. Berson

Department of Neuroscience, Brown University, Providence, RI 02912, USA

A novel photoreceptor of the mammalian retina has recently been discovered and characterized. The novel cells differ radically from the classical rod and cone photoreceptors. They use a unique photopigment, most probably melanopsin. They have lower sensitivity and spatiotemporal resolution than rods or cones and they seem specialized to encode ambient light intensity. Most surprisingly, they are ganglion cells and, thus, communicate directly with the brain. These intrinsically photosensitive retinal ganglion cells (ipRGCs) help to synchronize circadian rhythms with the solar day. They also contribute to the pupillary light reflex and other behavioral and physiological responses to environmental illumination.

For 150 years, rods and cones have been considered the only photoreceptors of the mammalian eye. For more than a decade, however, evidence has been mounting for the existence of other ocular photoreceptors. A flurry of recent reports has now established the identity of these novel retinal photoreceptors and has begun to delineate their photochemistry, anatomy, functional attributes and roles in behavior. This review surveys the emergent evidence for this remarkable retinal subsystem, and for its roles in synchronizing circadian rhythms and in other physiological responses to environmental illumination.

Synchronization of circadian rhythms

The roots of this discovery lie in the field of circadian physiology. Circadian rhythms are biological cycles that have period of about a day. Body temperature, hormonal levels, sleep, cognitive performance and countless other physiological variables exhibit such daily oscillations. In mammals, a pacemaker in the hypothalamus called the suprachiasmatic nucleus (SCN) drives these rhythms [1]. Lesions of the SCN abolish circadian rhythms and SCN grafts can restore rhythms in arrhythmic hosts. The SCN is a self-sustaining oscillator, able to maintain daily rhythms for weeks when isolated and cultured. The clockwork of SCN neurons consists of interlocking feedback loops of gene expression [2–4].

Because the intrinsic period of the SCN oscillator is not exactly 24 h, it drifts out of phase with the solar day unless synchronized or 'entrained' by sensory inputs. Light is by far the most important entraining cue. When we experience a sudden change in light cycle, as in air travel to a new time zone, we suffer an unpleasant mismatch between our biological rhythms and local solar time ('jet lag'). Normal synchrony is restored over several days as the rising and setting of the sun resets our biological clock [2,5,6].

Behavioral evidence for novel ocular photoreceptors

In mammals, light adjusts circadian phase by activating the retinohypothalamic tract, a direct pathway linking a small population of retinal ganglion cells (RGCs) to the SCN [5,7–12]. In the conventional view of retinal organization, these RGCs, like all others, would derive their visual responsiveness solely from synaptic inputs and, ultimately, from the classical photoreceptors. According to this view, rods and/or cones would be the photoreceptors through which light influenced circadian phase. Beginning in the 1980s, however, behavioral studies, especially those of Foster and colleagues, began to challenge this model [13]. Photic entrainment exhibited high thresholds, low-pass temporal filtering and long-term temporal integration that seemed difficult to reconcile with a mechanism based purely on rods or cones [14–18]. And, remarkably, in mice with severe degeneration of classical photoreceptors, light was as effective as in normal mice at resetting the circadian clock [19–21]. This is also true in certain blind humans [22]. Initial studies left open the possibility that a few surviving rods or cones might have accounted for the persistent photoentrainment, but improved experimental models have since laid that concern to rest [23,24].

Could the photoreceptors supporting photoentrainment in rodless and coneless mice be located outside the eye? A clear precedent for such a mechanism exists in non-mammalian animals, in which light penetrating the brain acts directly on photosensitive circadian pacemaker neurons [25–28]. In mammals, however, this can be discounted because eye removal abolishes photoentrainment [20,23,29–32]. In a recent human study, bright light behind the knee was reported to phase-shift circadian rhythms [33] but these results have not been replicated [20,34–36]. In short, the eyes are necessary for mammalian entrainment but classical photoreceptors are not, strongly implicating a novel ocular photoreceptor in the workings of the retinohypothalamic tract [20,22,37,38].

Melanopsin – a candidate circadian photopigment

A key strategy in the hunt for these enigmatic photoreceptors was to seek candidate photopigments within the inner retina. Attention initially focused on the

Corresponding author: David M. Berson (david_berson@brown.edu).

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cryptochromes, blue-light-absorbing flavoproteins that function as circadian photopigments in invertebrates [38–40]. Despite some evidence supporting an equivalent role in mammals (see following discussion), cryptochromes have been eclipsed, at least momentarily, by melanopsin. This novel vertebrate opsin, discovered by Provencio and colleagues [41,42], gets its name from the cells in which it was first isolated: the dermal melanophores of frog skin. These cells are directly photosensitive, redistributing their pigmented organelles when illuminated. Melanopsin was also found in several other cell types in frogs known or presumed to be intrinsically photosensitive, including myocytes of the iris and cells in hypothalamic regions containing deep-brain photoreceptors. Melanopsin was also detected in certain retinal neurons other than rods and cones [41].

In mammals, melanopsin was found only in the retina and, specifically, in a tiny subset of neurons of the inner retinal layers [42–46]. This distribution was unique among mammalian opsins in matching the presumed inner retinal location of the mysterious circadian photoreceptors. Moreover, the sparseness of the distribution was reminiscent of the RGCs innervating the SCN, leading Provencio and colleagues to propose melanopsin as a circadian photopigment [42]. Support for this hypothesis came swiftly from three studies showing that melanopsin was expressed specifically within retinal ganglion cells of the retinohypothalamic tract [43,45,47]. Apparently, most melanopsin-containing RGCs innervate the SCN and most RGCs innervating the SCN contain melanopsin [43,47].

Intrinsic photosensitivity of ganglion cells innervating the circadian pacemaker

To determine whether ganglion cells innervating the SCN were directly photosensitive, Berson *et al.* [48] made whole-cell recordings from such cells in isolated rat retinas. Light strongly depolarized the cells, triggering sustained spiking. These responses persisted even when rods and cones were severely photobleached and their synaptic influences on ganglion cells were thoroughly blocked. Most tellingly, the cell bodies of these ganglion cells still responded to light when physically dissociated from the retina. Thus, ganglion cells projecting to the circadian pacemaker are indeed photoreceptors, able to convert electromagnetic radiation into transmembrane receptor potentials. For want of a pithier name, these neurons were called 'intrinsically photosensitive retinal ganglion cells' (ipRGCs).

Functional features of ipRGCs

The intrinsic light responses of ipRGCs differ radically from those of rods and cones [48] (Table 1). Light depolarizes ipRGCs but hyperpolarizes rods and cones (Fig. 1a). The ipRGCs are less sensitive than the classical photoreceptors and are far more sluggish, with response latencies as long as one minute (Fig. 1a). Bright continuous illumination evokes a remarkably sustained depolarization in ipRGCs that faithfully encodes stimulus energy. This sets these cells apart from essentially all other mammalian RGCs, which cannot represent ambient light levels in this way [49]. In their classic work on this topic, Barlow and Levick [49] drew attention to a tiny minority of RGCs that were able to encode irradiance, cells they called 'luminance units'. In retrospect, these seem likely to have been ipRGCs, in which case Barlow and Levick deserve credit for spotlighting these oddities more than three decades before their capacity for phototransduction was recognized.

The action spectrum (or wavelength-sensitivity function) for synaptically isolated ipRGCs is typical of a vitamin-A-based photopigment, as are the action spectra for rods and cones (Fig. 1b). This strongly suggests that the responsible pigment is an opsin. However, ipRGCs are most sensitive at 484 nm, whereas rat rods prefer ~500 nm [50] and rat cones are most sensitive at either 510 nm or 359 nm [51]. Thus, a novel opsin drives ipRGC light responses. Melanopsin, which is present in physiologically identified ipRGCs [45], is by far the most likely candidate, as discussed in a following section.

Congruence of ipRGC light responses with properties of the photoentrainment mechanism

Many of the distinctive features of the light responses of ipRGCs parallel the unusual properties of circadian photoentrainment. By comparison with pattern vision, the photoentrainment mechanism is insensitive and responds poorly to brief stimuli, but is able to integrate photic energy over much longer periods [14,15,18,52]. These characteristics seem likely to reflect in part the high thresholds and sluggish, tonic responses of ipRGCs, although the quantitative discrepancies

Table 1. Contrasting structural and functional features of conventional photoreceptors (rods and cones) and novel ganglion cell photoreceptors (ipRGCs) of the mammalian retina[a]

	Rods and cones	ipRGCs	Refs[b]
Soma location	Outer nuclear layer	Ganglion cell layer (rarely inner nuclear layer)	[42–47]
Output	Retina (bipolar and horizontal cells)	Brain (e.g. SCN and OPN)	[43,45,47,48]
Light response	Fast hyperpolarizing	Slow depolarizing	[48]
Action potentials	No	Yes	[48]
Role of retinal pigment epithelium	Essential for photopigment regeneration	Apparently unnecessary for photopigment regeneration	[48]
Sensitivity	Moderate (cone) or high (rod)	Low	[48]
Receptive field	Very small	Very large	[48]
Photopigment	Rhodopsin and cone opsin	Melanopsin?	[42,43,45–48,53]
Photosensitive elements	Outer segment	Soma and dendrites (and axon?)	[43,45,46,48]

[a]Abbreviations: ipRGCs, intrinsically photosensitive retinal ganglion cells; OPN, olivary pretectal nucleus; SCN, suprachiasmatic nucleus
[b]References are those for data on ipRGCs. The characteristics of rods and cones are well documented; for reviews, see Refs [89,90]

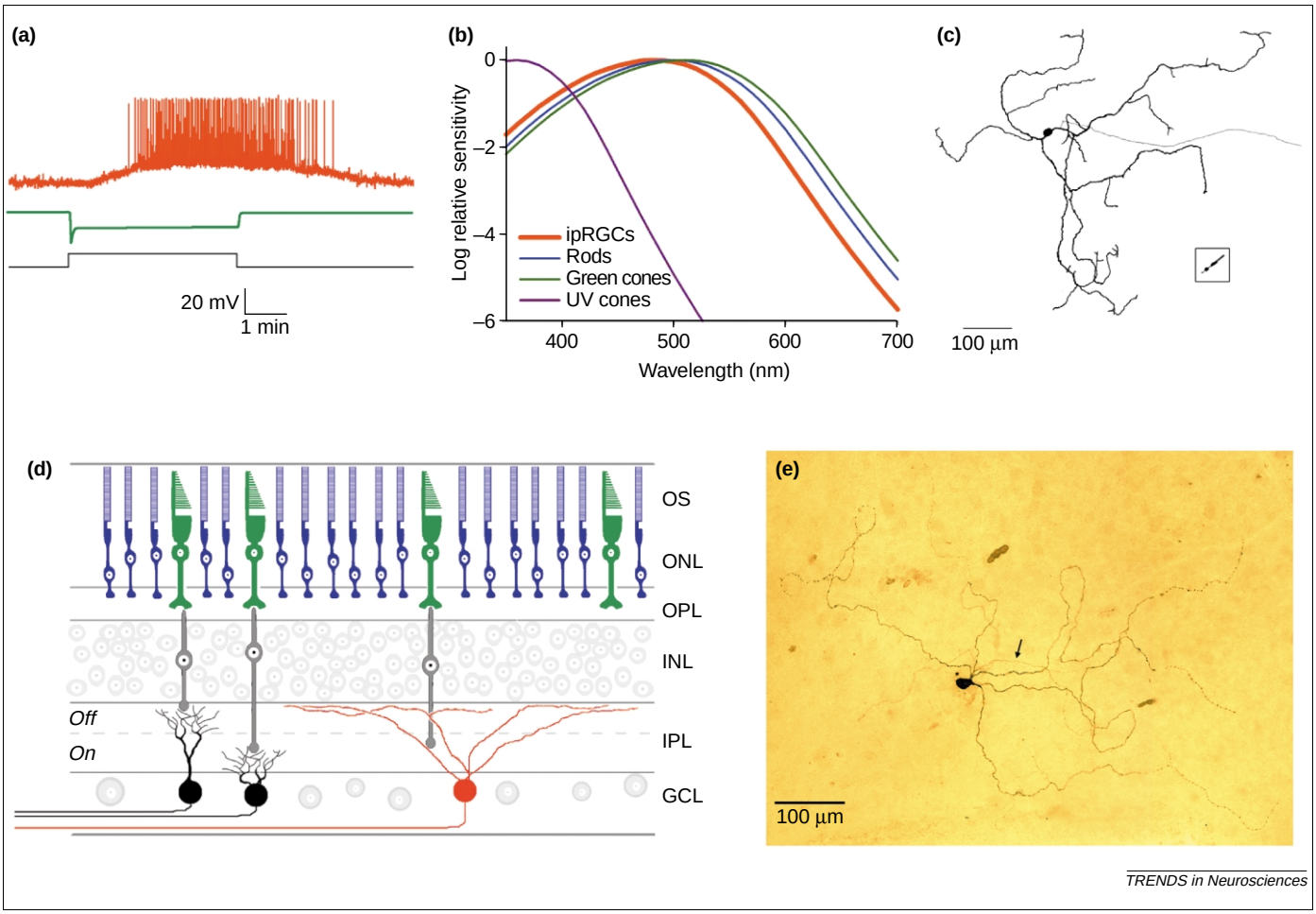

Fig. 1. Functional and structural properties of intrinsically photosensitive retinal ganglion cells (ipRGCs) in relation to those of conventional rod and cone photoreceptors. (a) Schematic representation of contrasting voltage responses of an ipRGC (red) and a cone (green) to a step increase in illumination (black). In the ipRGC, the light response is delayed, depolarizing and includes fast action potentials. In the cone, the response is rapid, hyperpolarizing and lacks spikes. (b) Comparison of action spectra for photoreceptors of the rat retina. The similarity in the forms of the curves indicates that each is based on a photopigment using a vitamin A derivative as the chromophore. Relative displacements of the curves on the wavelength axis reflect differences in the protein (opsin) component of the photopigment. Optimal wavelengths: ipRGC (red), 484 nm; green cone (green), 510 nm; ultraviolet cone (purple), 359 nm; rod (blue), 500 nm. (c) Tracing of a rat ipRGC as viewed in the flat-mounted retina; the axon is shown in gray. The inset shows a rod photoreceptor drawn to scale. (d) Schematic vertical section of retina showing interrelationships between ipRGCs and other photoreceptors (shown in color), other retinal cells (shown in gray or black) and retinal layers. The ipRGC (red) has a cell body in the ganglion cell layer (GCL), whereas rods (blue) and cones (green) have cell bodies in the outer nuclear layer (ONL). The ipRGC has an axon that leaves the eye to communicate with the brain, whereas rods and cones communicate only with other retinal cells through synapses in the outer plexiform layer (OPL). Rods and cones drive conventional ganglion cells (black) by way of bipolar cells (gray), which have their cell bodies in the inner nuclear layer (INL). For clarity, only the cone pathway is shown. This vertical pathway might also influence ipRGCs. Dendrites of ipRGCs stratify in the upper part ('off sublayer') of the inner plexiform layer (IPL). This is surprising because it is typical of retinal ganglion cells that are hyperpolarized by light but not of those, such as the ipRGCs, that are depolarized by light. The dendrites of ipRGCs are photosensitive and spread far more widely in the plane of the retina than do the outer segments (OS) of the classical photoreceptors, permitting greater spatial integration. (e) Photomicrograph of a rat ipRGC, filled by intracellular dye during recording and viewed in the flat-mounted retina after histochemical processing. The axon is indicated by the arrow.

between behavioral and cellular thresholds complicate this picture [17,48,53].

The action spectrum for circadian phase-shifting implicates an opsin-based photopigment. In wild-type rodents, sensitivity peaks near 500 nm [16,54,55], closer to the optimal wavelength for rods and longer-wavelength ('green') cones than to that of ipRGCs (Fig. 1b). In at least one strain of retinal degenerate (*rd/rd*) mice, however, the optimal wavelength shifts to 480 nm [55], closely matching the best wavelength for ipRGCs. These results support the view that ipRGCs are circadian photoreceptors that sustain photoentrainment in rodless and coneless mice. At the same time, they imply that, under normal conditions, classical photoreceptors also help to synchronize the clock. This is consonant with evidence that rods and cones drive SCN neurons [56] and that light continues to affect circadian phase, albeit less effectively, when the direct photosensitivity of ipRGCs is eliminated by knockout of melanopsin [53,57,58].

Is melanopsin the photopigment of intrinsically photosensitive ganglion cells?

At present, melanopsin is by far the best candidate for the ipRGC photopigment. This opsin protein is found within, and perhaps only within, these novel photoreceptors [43,45,47]. It is located not only in their cell bodies but also in their proximal axons and throughout their dendrites [43,45,46]. This satisfies an important criterion for the photopigment in ipRGCs, because their dendrites are independently photosensitive [48]. Perhaps most tellingly, genetic deletion of melanopsin eliminates the intrinsic light response of ipRGCs without altering their structure or projections [53]. Melanopsin knockout also alters behavioral light responses to which ipRGCs are

believed to contribute [53,57,58]. In short, melanopsin is in the right cells, in the right parts of those cells, and has to be there if they are to respond directly to light.

This evidence, although strong, does not conclusively establish melanopsin as the photopigment of ipRGCs. One significant gap in the chain of evidence is the absorption spectrum of melanopsin, which is presently unknown. This must match the action spectrum of ipRGCs if their photopigment is melanopsin. Also lacking is evidence for an intracellular signaling pathway coupling melanopsin to the light-activated ion channels in the ipRGC plasma membrane. Indeed, virtually nothing is yet known about this phototransduction cascade. An invertebrate-like phosphoinositide signaling pathway might be involved because melanopsin structurally resembles invertebrate opsins [41,42], and ipRGCs, like most invertebrate photoreceptors, depolarize in response to light. Alternatively, a cyclic-nucleotide cascade such as that used by vertebrate photoreceptors, or some other signaling pathway, might be operating.

A competing hypothesis is that melanopsin is not a photopigment at all but, instead, is a retinaldehyde photoisomerase. In this view, melanopsin would be essential for regenerating the chromophore of another, unidentified opsin photopigment [59]. As yet, however, there is no evidence that melanopsin has isomerase activity or that ipRGCs contain any other opsin. Only three mammalian opsins have been identified outside the rods and cones: retinal G-protein-coupled receptor (RGR), peropsin and encephalopsin [60–63]. None of these has been identified in ipRGCs or other inner retinal neurons [42,60]. Vertebrate ancient opsin, found in the inner retina of fish [64], has not been identified in mammals.

The direct photosensitivity of ipRGCs, which requires melanopsin, appears sufficient to drive various non-image-forming visual functions in the absence of rods and cones (see following discussion). This should not be taken to mean that melanopsin is required for these functions. Indeed, circadian photoentrainment and phase-shifting, masking and pupillary light reflexes all persist in melanopsin-knockout mice [53,57,58]. Thus, multiple photoreceptor types must contribute to these mechanisms.

Cryptochromes, which are flavoproteins related to the photolyases, have also been proposed as mammalian circadian photopigments [39,40,65]. There is a precedent for such a role in *Drosophila*, where cryptochromes mediate direct photic modulation of the circadian pacemaker [2,38,66,67]. In mammals, there are two cryptochromes. These are widely expressed in the body, including in most cells of the inner retina [65], so they could well be present in ipRGCs. If cryptochromes form functional photopigments in mammals, which remains uncertain, their chromophore would presumably be flavin adenonucleotide and/or a pterin, and not retinaldehyde as in the opsins [40]. Accordingly, cryptochromes have been proposed to support the photic influence on the SCN that persists in the face of severe depletion of retinaldehyde [68]. However, it is doubtful that a flavin-based photopigment could account for the opsin-like action spectrum of ipRGCs [69]. Furthermore, cryptochromes are located mainly within the nucleus, so it is unclear whether they can account for the photosensitivity of ipRGC dendrites. It has proven difficult to determine whether cryptochromes contribute to circadian photoentrainment because they are crucial clock components and knocking them out disrupts circadian rhythmicity [70–72]. Knockout studies have revealed that cryptochrome deletion can disrupt, but does not abolish, photic induction of SCN clock genes [2,71,72] and reduces the sensitivity of pupillary light responses in retinal degenerate mice [73].

Morphology of ipRGCs

In rodents, ~1000–2000 ganglion cells (~1–3% of all ganglion cells) contain melanopsin [45]. Most reside in the ganglion cell layer but a few are displaced to the inner nuclear layer [37,42,45]. Melanopsin-positive RGCs are present throughout the retina, with somewhat higher density superiorly [43,45]. Their dendrites form an extensively overlapping plexus in the inner plexiform layer (IPL) [37,45,46]. Dendritic profiles of individual melanopsin-positive RGCs (or ipRGCs) are large (Fig. 1c and e), spanning ~500 μm or 15° [48]; for comparison, rod and cone outer segments span ~1 μm or <0.05°. The large fields of ipRGCs initiate a process of spatial convergence that culminates in the huge receptive fields of SCN neurons [74].

The dendrites of ipRGCs arborize mainly in the outermost sublayer of the IPL (Fig. 1d), corresponding to the main plexus of melanopsin-immunoreactive dendrites [37,45,46,48]. In mice, however, a second plexus of melanopsin-positive dendrites marks the innermost IPL [46]. It is not known whether this plexus arises from a second population of RGCs and, if so, whether these cells, too, are directly photosensitive. Melanopsin-expressing RGCs also selectively express pituitary-adenylate-cyclase-activating peptide (PACAP), which might participate in retinohypothalamic transmission [37,43].

Intraretinal synaptic modulation: influences of rods and cones

The dendrites of ipRGCs serve, like rod and cone outer segments, as sites of phototransduction. In addition, however, they also play a role more typical of ganglion-cell dendrites, as targets of synaptic input from amacrine and bipolar cells (Fig. 1d). Rods or cones drive brisk, synaptically mediated excitatory ON responses in some ipRGCs when recorded under appropriate conditions (F.A. Dunn and D.M. Berson, unpublished) and melanopsin-immunopositive dendrites receive synaptic contacts from bipolar and amacrine cells [75]. Thus, at least some ipRGC axons carry a hybrid output signal derived from multiple photopigments. Influences of rods and cones upon nuclei innervated by ipRGCs and upon associated behaviors might, therefore, be relayed in part by the ipRGCs themselves [24,53,55–58,76].

Beyond circadian entrainment: other functional roles of ipRGCs

Intrinsically photosensitive RGCs appear to contribute to photic regulation of pineal melatonin release. Light at

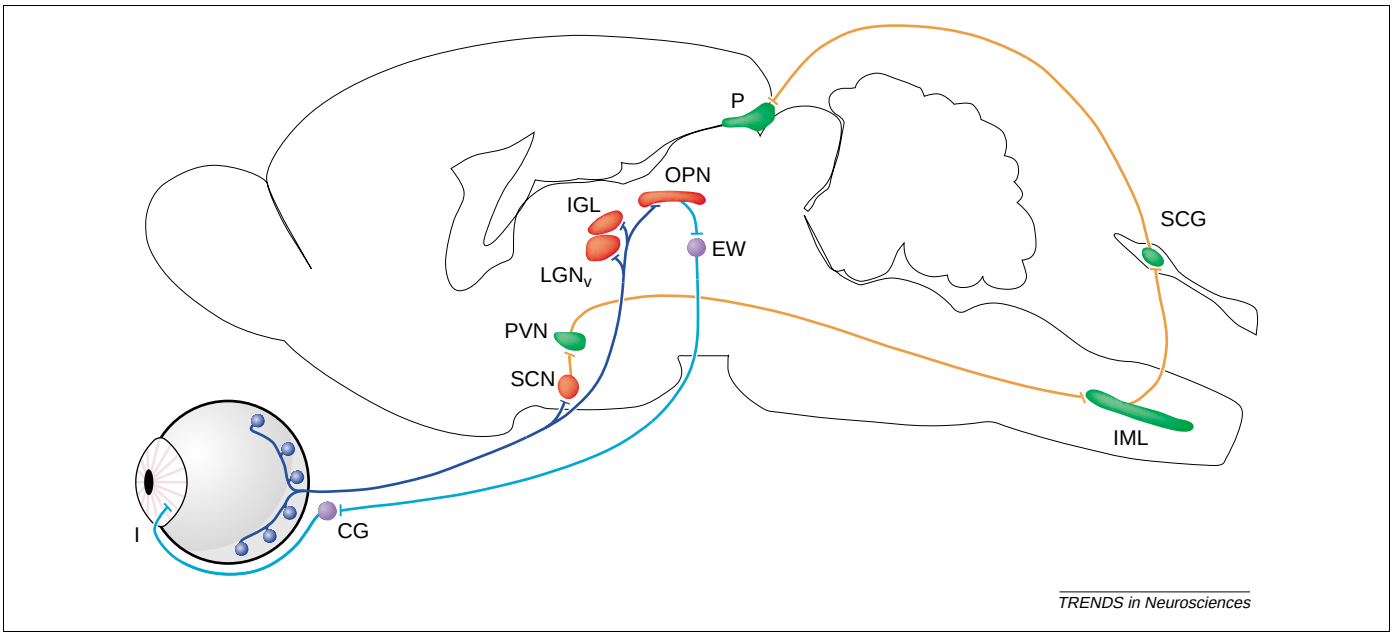

Fig. 2. Schematic summary of brain regions and circuits influenced by intrinsically photosensitive retinal ganglion cells (ipRGCs). The ipRGCs and their axons are shown in dark blue, their principal targets in red. Projections of ipRGCs to the suprachiasmatic nucleus (SCN) form the bulk of the retinohypothalamic tract and contribute to photic entrainment of the circadian clock. The orange pathway with green nuclei shows a polysynaptic circuit that originates in the SCN and photically regulates melatonin release by the pineal gland (P) through its sympathetic innervation. Synaptic links in this pathway include the paraventricular nucleus (PVN) of the hypothalamus, the intermediolateral nucleus (IML) of the spinal cord and the superior cervical ganglion (SCG). Another direct target of ipRGCs is the olivary pretectal nucleus (OPN), a crucial link in the circuit underlying the pupillary light reflex, shown in light blue (fibers) and purple (nuclei). Synapses in this parasympathetic circuit are found at the Edinger–Westphal nucleus (EW), the ciliary ganglion (CG) and the iris muscles (I). Other targets of ipRGCs include two components of the lateral geniculate nucleus of the thalamus, the ventral division (LGN$_v$) and the intergeniculate leaflet (IGL).

night suppresses otherwise high nocturnal plasma melatonin levels through a circuitous pathway originating with the retinohypothalamic tract [77] (Fig. 2). Such photic melatonin suppression persists in rodless and coneless mice and in some blind people [29,78], and its action spectrum bears some resemblance to that of ipRGCs [79,80]. Changes in day length act through this pathway to regulate the melatonin duty cycle and thereby drive seasonal (photoperiodic) changes in reproductive and other physiological functions [81]. In some people, short days precipitate seasonal affective disorder ('winter blues'). The ipRGCs might be key targets of the bright-light therapy used to treat this condition [82].

Light also acutely inhibits night-time locomotor activity in nocturnal rodents ('negative circadian masking'). This photic effect, which is probably mediated by the retinohypothalamic tract, persists in rodless and coneless mice [83,84], presumably by acting through ipRGCs. Masking also persists in melanopsin-knockout mice [57], indicating that multiple photoreceptors must be involved. Other hypothalamic outputs of ipRGCs might contribute to photic effects on sleep, heart rate, cortisol levels and alertness [13].

The pupillary light reflex is also driven in part by ipRGCs. The olivary pretectal nucleus (OPN) is a crucial node in this reflex circuit, linking the retina to the parasympathetic innervation of the iris (Fig. 2). Melanopsin-expressing RGCs project directly to the OPN [45]. This presumably explains why the pupillary light reflex persists in rodless and coneless mice [76,85], with an action spectrum precisely matching that of ipRGCs [76]. Genetic deletion of melanopsin eliminates both the intrinsic sensitivity of ipRGCs and the modulation of pupil size by bright light [53]. The pupillary reflex, like photoentrainment and masking, appears to be mediated by multiple retinal photoreceptor types [53,73,76].

Melanopsin-expressing RGCs in mice innervate several components of the lateral geniculate nucleus [45], including the ventral division and the intergeniculate leaflet (Fig. 2), which has been implicated in circadian entrainment [86]. Their input to the dorsal division of the geniculate nucleus appears weak, however, so ipRGCs might have little direct influence on the murine striate cortex [45]. There is some evidence suggesting a possible role for ipRGCs in intraretinal processing in humans [87] and in gating of an ocular immune response [88].

Concluding remarks

Recent findings have identified a novel photoreceptor of the mammalian retina. The ipRGC is a rare type of ganglion cell with distinctive morphological and functional features. This photoreceptor appears to sacrifice spatial and temporal resolution so as to encode faithfully the intensity of bright environmental illumination. It plays a key role in diverse physiological responses to daylight, including setting the biological clock, regulating activity and melatonin levels, and adjusting pupil diameter. It can maintain these responses when classical photoreceptors are lost. Melanopsin is very probably the photopigment for this novel photoreceptor, although gaps in the chain of evidence remain. This system appears highly conserved evolutionarily and is clearly present in humans.

Almost nothing is known about the signaling cascade that couples photopigment activation to the voltage response, and this will be a major research focus in the future. It will also be important to explore in far greater

detail the light responses of these cells under physiological conditions in which interactions with rods and cones are preserved. A fuller account of the central projections and influences of these ganglion cells is of key importance in understanding the functional roles of this highly specialized and idiosyncratic component of the mammalian visual system.

Acknowledgements

I am grateful to many colleagues for helpful discussions, especially to Felice Dunn, Motoharu Takao, Ignacio Provencio, Mark Rollag, King-Wai Yau, Samer Hattar and Russell Van Gelder. I thank Russell Van Gelder and anonymous referees for their critiques of the manuscript. The intracellular fill illustrated in Fig. 1(e) was generated by Felice Dunn. Supported by NIH grant R01 EY12793.

References

1 Klein, D.C. *et al.* (1991) *Suprachiasmatic Nucleus: The Mind's Clock*, Oxford University Press
2 Devlin, P.F. and Kay, S.A. (2001) Circadian photoperception. *Annu. Rev. Physiol.* 63, 677–694
3 King, D.P. and Takahashi, J.S. (2000) Molecular genetics of circadian rhythms in mammals. *Annu. Rev. Neurosci.* 23, 713–742
4 Reppert, S.M. and Weaver, D.R. (2002) Coordination of circadian timing in mammals. *Nature* 418, 935–941
5 Rea, M.A. (1998) Photic entrainment of circadian rhythms in rodents. *Chronobiol. Int.* 15, 395–423
6 Roenneberg, T. and Foster, R.G. (1997) Twilight times: light and the circadian system. *Photochem. Photobiol.* 66, 549–561
7 Johnson, R.F. *et al.* (1988) Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Res.* 460, 297–313
8 Moore, R.Y. and Lenn, N.J. (1972) A retinohypothalamic projection in the rat. *J. Comp. Neurol.* 146, 1–14
9 Moore, R.Y. *et al.* (1995) The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. *J. Comp. Neurol.* 352, 351–366
10 Morin, L.P. (1994) The circadian visual system. *Brain Res. Brain Res. Rev.* 19, 102–127
11 Pickard, G.E. (1980) Morphological characteristics of retinal ganglion cells projecting to the suprachiasmatic nucleus: a horseradish peroxidase study. *Brain Res.* 183, 458–465
12 Sousa-Pinto, A. and Castro-Correia, J. (1970) Light microscopic observations on the possible retinohypothalamic projection in the rat. *Exp. Brain Res.* 11, 515–527
13 Foster, R.G. (2002) Keeping an eye on the time: the Cogan Lecture. *Invest. Ophthalmol. Vis. Sci.* 43, 1286–1298
14 Nelson, D.E. and Takahashi, J.S. (1991) Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *J. Physiol. (Lond.)* 439, 115–145
15 Nelson, D.E. and Takahashi, J.S. (1991) Comparison of visual sensitivity for suppression of pineal melatonin and circadian phase-shifting in the golden hamster. *Brain Res.* 554, 272–277
16 Takahashi, J.S. *et al.* (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308, 186–188
17 Dkhissi-Benyahya, O. *et al.* (2000) Effects of irradiance and stimulus duration on early gene expression (Fos) in the suprachiasmatic nucleus: temporal summation and reciprocity. *J. Neurosci.* 20, 7790–7797
18 Meijer, J.H. *et al.* (1986) Luminance coding in a circadian pacemaker: the suprachiasmatic nucleus of the rat and the hamster. *Brain Res.* 382, 109–118
19 Ebihara, S. and Tsuji, K. (1980) Entrainment of the circadian activity rhythm to the light cycle: effective light intensity for a Zeitgeber in the retinal degenerate C3H mouse and the normal C57BL mouse. *Physiol. Behav.* 24, 523–527
20 Foster, R.G. *et al.* (1991) Circadian photoreception in the retinally degenerate mouse (rd/rd). *J. Comp. Physiol. [A]* 169, 39–50
21 Foster, R.G. (1998) Shedding light on the biological clock. *Neuron* 20, 829–832
22 Klerman, E.B. *et al.* (2002) Photic resetting of the human circadian pacemaker in the absence of conscious vision. *J. Biol. Rhythms* 17, 548–555
23 Freedman, M.S. *et al.* (1999) Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 284, 502–504
24 Lucas, R.J. *et al.* (2001) Identifying the photoreceptive inputs to the mammalian circadian system using transgenic and retinally degenerate mice. *Behav. Brain Res.* 125, 97–102
25 Helfrich-Forster, C. *et al.* (2001) The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30, 249–261
26 Shand, J. and Foster, R.G. (1999) The extraretinal photoreceptors of non-mammalian vertebrates. In *Adaptive Mechanisms in the Ecology of Vision* (Archer, S.N., ed.), pp. 197–222, Kluwer
27 Vigh, B. *et al.* (2002) Nonvisual photoreceptors of the deep brain, pineal organs and retina. *Histol. Histopathol.* 17, 555–590
28 Zivkovic, B.D. *et al.* (2000) Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *J. Biol. Rhythms* 15, 172–183
29 Czeisler, C.A. *et al.* (1995) Suppression of melatonin secretion in some blind patients by exposure to bright light. *N. Engl. J. Med.* 332, 6–11
30 Lockley, S.W. *et al.* (1997) Relationship between melatonin rhythms and visual loss in the blind. *J. Clin. Endocrinol. Metab.* 82, 3763–3770
31 Nelson, R.J. and Zucker, I. (1981) Absence of extra-ocular photoreception in diurnal and nocturnal rodents exposed to direct sunlight. *Comp. Biochem. Physiol. A* 69, 145–148
32 Yamazaki, S. *et al.* (1999) No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *J. Biol. Rhythms* 14, 197–201
33 Campbell, S.S. and Murphy, P.J. (1998) Extraocular circadian phototransduction in humans. *Science* 279, 396–399
34 Eastman, C.I. *et al.* (2000) Failure of extraocular light to facilitate circadian rhythm reentrainment in humans. *Chronobiol. Int.* 17, 807–826
35 Lindblom, N. *et al.* (2000) No evidence for extraocular light induced phase shifting of human melatonin, cortisol and thyrotropin rhythms. *NeuroReport* 11, 713–717
36 Wright, K.P. Jr and Czeisler, C.A. (2002) Absence of circadian phase resetting in response to bright light behind the knees. *Science* 297, 571
37 Hannibal, J. and Fahrenkrug, J. (2002) Melanopsin: a novel photopigment involved in the photoentrainment of the brain's biological clock? *Ann. Med.* 34, 401–407
38 Van Gelder, R.N. (2002) Tales from the crypt(ochromes). *J. Biol. Rhythms* 17, 110–120
39 Sancar, A. (2000) Cryptochrome: the second photoactive pigment in the eye and its role in circadian photoreception. *Annu. Rev. Biochem.* 69, 31–67
40 Thompson, C.L. and Sancar, A. (2002) Photolyase/cryptochrome blue-light photoreceptors use photon energy to repair DNA and reset the circadian clock. *Oncogene* 21, 9043–9056
41 Provencio, I. *et al.* (1998) Melanopsin: An opsin in melanophores, brain, and eye. *Proc. Natl. Acad. Sci. U. S. A.* 95, 340–345
42 Provencio, I. *et al.* (2000) A novel human opsin in the inner retina. *J. Neurosci.* 20, 600–605
43 Hannibal, J. *et al.* (2002) The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. *J. Neurosci.* 22, RC191
44 Hannibal, J. *et al.* (2002) The circadian photopigment melanopsin is expressed in the blind subterranean mole rat, *Spalax*. *NeuroReport* 13, 1411–1414
45 Hattar, S. *et al.* (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* 295, 1065–1070
46 Provencio, I. *et al.* (2002) Photoreceptive net in the mammalian retina. *Nature* 415, 493
47 Gooley, J.J. *et al.* (2001) Melanopsin in cells of origin of the retinohypothalamic tract. *Nat. Neurosci.* 4, 1165
48 Berson, D.M. *et al.* (2002) Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295, 1070–1073
49 Barlow, H.B. and Levick, W.R. (1969) Changes in the maintained

discharge with adaptation level in the cat retina. *J. Physiol.* 202, 699–718
50 Bridges, C.D.B. (1959) Visual pigments of some common laboratory mammals. *Nature* 184, 1727–1728
51 Jacobs, G.H. *et al.* (2001) Cone-based vision of rats for ultraviolet and visible lights. *J. Exp. Biol.* 204, 2439–2446
52 Nelson, D.E. and Takahashi, J.S. (1999) Integration and saturation within the circadian photic entrainment pathway of hamsters. *Am. J. Physiol.* 277, R1351–R1361
53 Lucas, R.J. *et al.* (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* 299, 245–247
54 Provencio, I. and Foster, R.G. (1995) Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. *Brain Res.* 694, 183–190
55 Yoshimura, T. and Ebihara, S. (1996) Spectral sensitivity of photoreceptors mediating phase-shifts of circadian rhythms in retinally degenerate CBA/J (rd/rd) and normal CBA/N (+/+) mice. *J. Comp. Physiol. [A]* 178, 797–802
56 Aggelopoulos, N.C. and Meissl, H. (2000) Responses of neurones of the rat suprachiasmatic nucleus to retinal illumination under photopic and scotopic conditions. *J. Physiol.* 523, 211–222
57 Panda, S. *et al.* (2002) Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* 298, 2213–2216
58 Ruby, N.F. *et al.* (2002) Role of melanopsin in circadian responses to light. *Science* 298, 2211–2213
59 Bellingham, J. and Foster, R.G. (2002) Opsins and mammalian photoentrainment. *Cell Tissue Res.* 309, 57–71
60 Blackshaw, S. and Snyder, S.H. (1999) Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain. *J. Neurosci.* 19, 3681–3690
61 Halford, S. *et al.* (2001) Characterization of a novel human opsin gene with wide tissue expression and identification of embedded and flanking genes on chromosome 1q43. *Genomics* 72, 203–208
62 Jiang, M. *et al.* (1993) An opsin homologue in the retina and pigment epithelium. *Invest. Ophthalmol. Vis. Sci.* 34, 3669–3678
63 Sun, H. *et al.* (1997) Peropsin, a novel visual pigment-like protein located in the apical microvilli of the retinal pigment epithelium. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9893–9898
64 Soni, B.G. *et al.* (1998) Novel retinal photoreceptors. *Nature* 394, 27–28
65 Miyamoto, Y. and Sancar, A. (1998) Vitamin B2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6097–6102
66 Ceriani, M.F. *et al.* (1999) Light-dependent sequestration of timeless by cryptochrome. *Science* 285, 553–556
67 Hall, J.C. (2000) Cryptochromes: sensory reception, transduction, and clock functions subserving circadian systems. *Curr. Opin. Neurobiol.* 10, 456–466
68 Thompson, C.L. *et al.* (2001) Preservation of light signaling to the suprachiasmatic nucleus in vitamin A-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11708–11713
69 Hsu, D.S. *et al.* (1996) Putative human blue-light photoreceptors hCRY1 and hCRY2 are flavoproteins. *Biochemistry* 35, 13871–13877
70 Griffin, E.A. Jr *et al.* (1999) Light-independent role of CRY1 and CRY2 in the mammalian circadian clock. *Science* 286, 768–771
71 Okamura, H. *et al.* (1999) Photic induction of mPer1 and mPer2 in cry-deficient mice lacking a biological clock. *Science* 286, 2531–2534
72 Vitaterna, M.H. *et al.* (1999) Differential regulation of mammalian period genes and circadian rhythmicity by cryptochromes 1 and 2. *Proc. Natl. Acad. Sci. U. S. A.* 96, 12114–12119
73 Van Gelder, R.N. *et al.* (2003) Reduced pupillary light responses in mice lacking cryptochromes. *Science* 299, 222
74 Groos, G.A. and Mason, R. (1980) The visual properties of rat and cat suprachiasmatic neurones. *J. Comp. Physiol.* 135, 349–356
75 Belenky, M.A. *et al.* Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *J. Comp. Neurol.* (in press)
76 Lucas, R.J. *et al.* (2001) Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat. Neurosci.* 4, 621–626
77 Moore, R.Y. (1996) Neural control of the pineal gland. *Behav. Brain Res.* 73, 125–130
78 Lucas, R.J. *et al.* (1999) Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* 284, 505–507
79 Brainard, G.C. *et al.* (2001) Human melatonin regulation is not mediated by the three cone photopic visual system. *J. Clin. Endocrinol. Metab.* 86, 433–436
80 Thapan, K. *et al.* (2001) An action spectrum for melatonin suppression: evidence for a novel non-rod, non-cone photoreceptor system in humans. *J. Physiol.* 535, 261–267
81 Malpaux, B. *et al.* (2001) Biology of mammalian photoperiodism and the critical role of the pineal gland and melatonin. *J. Biol. Rhythms* 16, 336–347
82 Avery, D.H. (1998) A turning point for seasonal affective disorder and light therapy research? *Arch. Gen. Psychiatry* 55, 863–864
83 Mrosovsky, N. *et al.* (1999) Thresholds for masking responses to light in three strains of retinally degenerate mice. *J. Comp. Physiol. [A]* 184, 423–428
84 Mrosovsky, N. *et al.* (2001) Persistence of masking responses to light in mice lacking rods and cones. *J. Biol. Rhythms* 16, 585–588
85 Keeler, C.E. (1927) Iris movements in blind mice. *Am. J. Physiol.* 81, 107–112
86 Harrington, M.E. (1997) The ventral lateral geniculate nucleus and the intergeniculate leaflet: interrelated structures in the visual and circadian systems. *Neurosci. Biobehav. Rev.* 21, 705–727
87 Hankins, M.W. and Lucas, R.J. (2002) The primary visual pathway in humans is regulated according to long- term light exposure through the action of a nonclassical photopigment. *Curr. Biol.* 12, 191–198
88 Van Gelder, R.N. (2001) Non-visual ocular photoreception. *Ophthalmic Genet.* 22, 195–205
89 Dowling, J.E. (1997) *The Retina: An Approachable Part of the Brain*, Belknap Press, Harvard University Press
90 Rodieck, R.W. (1998) *The First Steps in Seeing*, Sinauer Associates

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