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Intrinsically photosensitive retinal ganglion cells

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A new mammalian photoreceptor was recently discovered to reside in the ganglion cell layer of the inner retina. These intrinsically photosensitive retinal ganglion cells (ipRGCs) express a photopigment, melanopsin, that confers upon them the ability to respond to light in the absence of all rod and cone photoreceptor input. Although relatively few in number, ipRGCs extend their dendrites across large expanses of the retina making them ideally suited to function as irradiance detectors to assess changes in ambient light levels. Phototransduction in ipRGCs appears to be mediated by transient receptor potential channels more closely resembling the phototransduction cascade of invertebrate rather than vertebrate photoreceptors. ipRGCs convey irradiance information centrally via the optic nerve to influence several functions. ipRGCs are the primary retinal input to the hypothalamic suprachiasmatic nucleus (SCN), a circadian oscillator and biological clock, and this input entrains the SCN to the day/night cycle. ipRGCs contribute irradiance signals that regulate pupil size and they also provide signals that interface with the autonomic nervous system to regulate rhythmic gene activity in major organs of the body. ipRGCs also provide excitatory drive to dopaminergic amacrine cells in the retina, providing a novel basis for the restructuring of retinal circuits by light. Here we review the ground-breaking discoveries, current progress and directions for future investigation.

melanopsin, circadian rhythms, suprachiasmatic nucleus, retina, pupillary light reflex

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1 The long road to intrinsically photosensitive retinal ganglion cells

Visual perception begins in the outer retina where light is absorbed by the rod and cone photoreceptors and converted into an electrical signal. These signals are relayed to bipolar cells and then to the output cells of the retina, the ganglion cells. Retinal ganglion cell (RGC) signals are conveyed by their axons via the optic nerve to higher centers in the brain for further processing required for conscious visual perception (Figure 1). Although extra-ocular photoreceptors are common among non-mammalian vertebrates, it was believed since the time of the pioneering descriptions of Ramón y Cajal in the late 1800s that retinal rods and cones were the only photoreceptors in mammals [1].

This depiction of the organization of the mammalian retina prevailed despite occasional reports dating as far back as 1927, indicating that rodents with severe degeneration of the outer retina remained capable of responding to light (i.e., irradiance responses). Mice carrying the retinal degeneration mutation (*rd*) are virtually devoid of rod and cone photoreceptors by 4 weeks of age and these animals do not produce recordable electroretinographic responses or visual evoked potentials [2]. Yet Keeler reported that these mice were capable of generating a pupillary light reflex (i.e., *in vivo* contraction of the iris in response to light stimulation) [3]. Others later reported that *rd/rd* mice were able to synchronize their daily activity rhythms to cycles of light and darkness suggesting that cells other than rods and cones in the retina might be light sensitive [4,5]. However, it seemed

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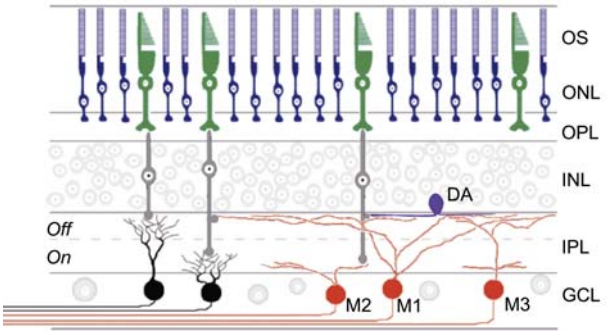

Figure 1 Schematic vertical section of the retina depicting ipRGCs (red) and rod and cone photoreceptors. ipRGCs reside in the ganglion cell layer (GCL) whereas rods and cones have their cell bodies in the outer nuclear layer (ONL). The three morphological types of ipRGC (M1, M2 and M3) are shown. Dendrites of M1 ipRGCs stratify in the distal inner plexiform layer (IPL), near the border of the inner nuclear layer (INL) in the traditional 'OFF' sublamina of the IPL. Dendrites of M2 ipRGCs are confined to the proximal 'ON' sublayer of the IPL whereas M3 ipRGCs are bistratified. Conventional RGCs (black) receive signals from rods and cones via input from bipolar cells located in the INL. M1 ipRGCs and dopaminergic amacrine cells (DA) receive ON bipolar input in the 'OFF' sublamina of the IPL via ectopic synapses of ON bipolar cell axons as they pass through the IPL. Conventional RGCs and ipRGCs send axons from the eye to communicate with the brain. M1 ipRGCs also drive excitatory responses in DA presumably by their dendrites that co-stratify in the IPL near the border of the INL. OS, outer segment layer; OPL, outer plexiform layer. Adapted from Berson, TINS, 2003, 26: 314–320.

unreasonable to most visual neuroscientists at the time that a photoreceptor had been missed despite study of the retina for over 150 years. The persistent response to light in retinal degenerate mice was widely attributed to the few cone photoreceptor remnants that remain in these animals [6], as removal of the eyes of rodents eliminates all forms of light detection [7].

To directly test the interpretation that residual cone photoreceptors mediate the irradiance responses to light in retinal degenerate animals, transgenic mice were generated lacking all rod and cone photoreceptors. Surprisingly these animals retained several irradiance responses including entrainment of their locomotor behavior to a light: dark cycle, the pupillary light reflex, and light-induced suppression of nocturnal pineal melatonin secretion [8,9]. Additional support for a non-rod, non-cone ocular photoreceptor came from reports in rodents and humans describing photic responses that had an action spectrum inconsistent with that of any known retinal photoreceptor [10–13]. Taken together these data provided clear evidence of the existence of a non-rod, non-cone photoreceptor in the mammalian retina.

2 Melanopsin and intrinsically photosensitive retinal ganglion cells

In 1998, while strong evidence was beginning to mount for a non-rod, non-cone photoreceptor in the mammalian retina, Provencio and his colleagues identified a novel opsin in photosensitive dermal melanophores of *Xenopus laevis*. They named the opsin melanopsin based on its isolation from a melanophore cDNA library and they demonstrated that it was a member of the opsin family of G-protein coupled receptors [14]. Provencio and co-workers in 2000 described the expression of melanopsin in cells of the inner retina of both primates and rodents, providing a basis for the suggestion that RGCs expressing this novel mammalian opsin were directly photosensitive [15]. This prediction was borne out in early 2002 in a set of landmark reports, when Berson, Hattar, Yau and colleagues recorded from RGCs that give rise to the retinohypothalamic tract (RHT) and terminate in the suprachiasmatic nucleus (SCN), a circadian oscillator responsible for the generation of rhythmic behavior. These investigators showed that these RGCs, when isolated from all rod and cone synaptic input, generated action potentials in response to photic stimulation [16]. Importantly, they also showed that these intrinsically photosensitive retinal ganglion cells (ipRGCs) expressed melanopsin [17]. These reports laid the foundation for a now rapidly growing new subdivision of retinal biology. However, two key questions remained after the discovery of ipRGCs: was melanopsin truly a photopigment and was it required for animals to show irradiance responses such as the pupillary light reflex or entrainment of circadian behavior to the light: dark cycle?

The second question was the first to be answered unambiguously with the generation of melanopsin knockout mice. These animals retain the ability to entrain to daily cycles of light and darkness and they generate a pupillary light reflex, although several aspects of these responses to light are altered [18–20]. Both acute and chronic effects of light on the circadian system were significantly attenuated in melanopsin-deficient mice [18,19] and the pupillary light reflex was described as incomplete at high irradiances [20] indicating that both the classical rod/cone photoreceptors and ipRGCs contribute to irradiance responses. When melanopsin was knocked out in mice lacking functional rods and cones, all tested responses to light were lost, confirming a role for melanopsin in irradiance responses to light and also indicating the unlikelihood that any other photoreceptor in the mammalian retina has remained undetected [21].

The observation that mice lacking the melanopsin protein retain the ability to entrain the SCN circadian pacemaker to the day/night cycle suggests that either conventional RGCs send afferent fibers to the SCN or that ipRGCs receive synaptic input from rods and/or cones and that this input is capable of driving these cells. Indeed, ipRGCs do receive synaptic input from both amacrine and bipolar cells [22–24] and this rod/cone driven input is capable of inducing physiological responses in these cells in the absence of melanopsin [25]. Moreover, retrograde labeling of RGCs afferent to the SCN in the mouse has revealed that virtually all SCN-projecting RGCs express melanopsin [26]. The data of Güler and colleagues [27] are in full agreement with

these findings. These investigators generated mice in which ipRGCs were genetically ablated and these animals lost their ability to entrain to environmental light/dark cycles, confirming that rod/cone influences on circadian entrainment are via melanopsin expressing ipRGCs which act as a conduit for rod/cone signals to reach the SCN.

In the rodent retina approximately 1%–2% of ganglion cells express melanopsin [26]. While in the mouse it appears that only melanopsin immunoreactive RGCs send afferent fibers to the SCN, in other rodents (i.e., the golden hamster and rat) non-melanopsin expressing RGCs have been described projecting to the SCN; these RGCs comprise 10%–20% of the total number of RGCs afferent to the SCN [28–30]. These differences among rodents may represent true species differences, but it remains to be determined whether the small number (i.e., 100–200) 'non-melanopsin' SCN-projecting RGCs identified in the rat and hamster are actually intrinsically light sensitive RGCs that either express too little melanopsin to be detected [26] or express a melanopsin isoform not recognized by the antibodies currently available [31–32].

Evidence confirming the identification of melanopsin as a photopigment came from heterologous expression of melanopsin in several different *in vitro* systems. The first data came from purified melanopsin harvested from melanopsin transfected COS cells. While it was shown that melanopsin was a photopigment that bound retinaldehyde and was capable of activating a G-protein [33], the spectral properties (i.e., maximal absorbance at ≈424 nm) were not consistent with the action spectrum observed by Berson and colleagues for SCN-projecting ipRGCs (≈484 nm) [16]. Subsequently, the photopigment properties of melanopsin were confirmed after expression in HEK cells that also expressed TRPC3 channels [34], Neuro-2a cells [35] and Xenopus oocytes [36]. Together these studies provided overwhelming evidence that melanopsin was indeed a photopigment. However, one study again reported an absorption maximum ≈420 nm [35] whereas the others indicated that expressed melanopsin maximally absorbed light at ≈480 nm [34,36], more closely matching the spectral tuning of pharmacologically isolated rat [16] and primate [37] ipRGCs. Non-mammalian melanopsin also shows peak sensitivity ≈480 nm in close agreement with mammalian ipRGCs [31,38]. Currently it is generally agreed that melanopsin maximally absorbs light at ≈480 nm although direct *in vitro* spectroscopic and biochemical analysis of purified mammalian melanopsin is needed [39].

3 ipRGC physiological responses to light

Several characteristics of ipRGC responsiveness set these ganglion cell photoreceptors apart from mammalian rod and cone photoreceptors. In particular, the stimulus response kinetics of ipRGCs is extremely slow compared to rods and cones. In addition, the melanopsin phototransduction cascade appears similar to that of many invertebrates, resulting in the polarity of the response of ipRGCs to light opposite that of rods and cones.

3.1 ipRGC response kinetics

The response of ipRGCs to light stimulation is extremely sluggish and ipRGCs are relatively insensitive to light. Under dim light conditions these cells can take many seconds to reach a peak response and the response may persist for minutes after stimulus termination [16]. While slow to respond to dim light conditions, it is remarkable that ipRGCs appear capable of responding to the absorption of a single photon of light [40]. Thus the relatively low sensitivity to light does not appear to be the result of inefficient phototransduction but rather of poor photon catch. This may be related to the low membrane density of melanopsin, estimated to be 10^4-fold lower than that of rod and cone photopigments [40,41]. Indeed, single photon capture in an ipRGC generates a large and prolonged membrane current, greater than that recorded in rod photoreceptors but also 20-fold slower [42]. The slow response kinetics of ipRGCs may provide for long temporal integration which may well suit the primary function of these cells, assessing ambient light levels via irradiance detection [40]. Moreover, since ipRGCs are synaptically driven by rods and/or cones [22–24], ganglion cell photoreceptors themselves may not require the level of intrinsic sensitivity noted for the classic photoreceptors. Considering that light passes first through the inner retina before being captured by rods and cones in the outer retina, a low photon capture rate by ipRGCs may also serve visual perception by allowing the vast majority of photons to pass by the inner retina to interact with rods and cones and thereby not degrading the visual image. It should be noted, however, that very brief (2 millisecond), intense light flashes are capable of driving SCN-mediated behavior, though it remains to be determined if melanopsin is required for these responses to very brief light flashes [43].

3.2 Photon capture in ipRGCs results in depolarization

In response to light, ipRGCs depolarize, unlike the hyperpolarizing light responses of mammalian rods and cones but similar to the responses of most invertebrate photoreceptors [44]. Perhaps this invertebrate-like response to light is not surprising since vertebrate melanopsin belongs to the rhabdomeric-opsin subfamily of opsins characteristic of most invertebrates [45,46]. It is equally unsurprising then that melanopsin also appears to utilize an invertebrate-like phototransduction mechanism.

Melanopsin, a member of the G-protein coupled-receptors, utilizes a G-protein to trigger a downstream cascade that results in generation of action potentials. The details of this basic mechanism remain uncertain but are under active

investigation. Opsins in mammalian rods and cones couple to the G_t-protein, transducin, which activates a phosphodiesterase cascade resulting in the closure of cGMP-gated channels and hyperpolarization of the cell. Melanopsin in ipRGCs is believed to be coupled to a G-protein of the G_q family (q, 11, 14 or 15) as its cognate G-protein *in vivo*. There is some evidence supporting a role for $G_{q/11}$ which would activate the effecter enzyme phospholipase C, resulting in depolarization, an invertebrate-like phototransduction cascade [47]. Preliminary data from G_q/G_{11} double knockout mice indicate that these animals have a defective pupillary light reflex at both low and high irradiances but normal entrainment to the day/night cycle and normal light responses in SCN-projecting RGCs [48].

The membrane channel that carries the initial inward current following the apparent activation of phospholipase C has also not yet been conclusively identified. However, the involvement of a canonical transient receptor potential (TRPC) channel, similar to the channels in *Drosophila* photoreceptors, is supported by several lines of evidence including pharmacological blockade of light responses and identification of TRPC channel protein and/or mRNA in RGCs expressing melanopsin [45–51]. Of the subfamily of TRPC channels, TRPC3, TRPC6, and/or TRPC7 have all been implicated as the TRPC channel mediating the initial depolarization in ipRGCs although the TRPC7 channel seems to be the current leading candidate [51,52]. As in invertebrates, light stimulates an increase in intracellular calcium in ipRGCs [53]. Some Ca^{2+} appears to enter after activation of the TRPC channel whereas Hartwick and colleagues have shown that approximately 90% of the light-triggered rise in intracellular Ca^{2+} is associated with the opening of L-type voltage-gated calcium channels and is highly correlated with action potential firing [51]. The pharmacological tools currently available do not adequately distinguish specific TRPC channels as the TRPC channel blockers are non-selective and inhibit many different cation channels [51]. As more selective tools become available to probe specific TRPC channels, a more definitive picture will surely emerge.

Identification of TRPC channel protein or mRNA may also not be definitive. Yau and Hardie [46] point out that the assumption used by many investigators, that "phototransduction proteins in ipRGCs ought to be selectively, or at least predominately, present in ipRGCs and not conventional RGCs" may not be valid based on the recent report from Bin and colleagues [54]. These investigators used a viral vector to express the melanopsin gene in the retinas of retinal degenerate mice and they noted that a wide variety of conventional RGCs became light-responsive, demonstrating that a signaling system that functionally couples melanopsin to membrane depolarization may be widespread in retinal ganglion cells [54]. Could there be an alternative explanation for this finding? Perhaps these 'newly melanopsin-expressing RGCs' expressed melanopsin very early in development and thus already possessed the melanopsin transduction machinery although they lost expression of melanopsin during development. The number of melanopsin-expressing RGCs is much greater early in development, being at least a 5-fold greater at postnatal day 4 compared to adult retina although it has been assumed that this represents overproduction followed by apoptotic cell death [55]. There is strong evidence that melanopsin protein levels are regulated developmentally, on a daily basis, and by environmental lighting conditions [55–57].

3.3 Chromophore recycling and bistability

Another aspect of the ipRGC response to light that appears similar to that of the invertebrate rhabdomeric type photoreceptors is that of photopigment regeneration. The visual pigment consists of two components: a protein moiety, the opsin, and the chromophore, 11-*cis*-retinal, a vitamin A derivative. Light isomerizes 11-*cis*-retinal to all-*trans*-retinal which results in rapid conformational changes in the opsin. After all-*trans*-retinal is reduced to all-*trans*-retinol in rod and cone photoreceptors, it exits the cell where it is converted in the overlying retinal pigment epithelium back to 11-*cis*-retinal by RPE65, a retinyl isomerohydrolase for return to the photoreceptors [46]. Müller glial cells in the mammalian retina also recycle 11-*cis*-retinal using a slightly different mechanism although these glial cells appear to serve only cone photoreceptors.

Unlike vertebrate rods and cones, the invertebrate rhabdomeric photopigment regenerating system is independent of other cells or tissue. Invertebrate photopigments do not dissociate from the opsin moiety but instead are re-isomerized by light of a longer wavelength than that which causes the initial photoactivation; these photopigments are thus considered bistable [46]. It would seem unlikely that ipRGCs utilize the retinal pigment epithelium to recycle 11-*cis*-retinal *in vivo* since ipRGCs are located in the inner retina. Moreover, native ipRGCs respond to prolonged light exposure when maintained *in vitro* in isolation from other retinal cells including Müller glial cells [51] suggesting that ipRGCs can convert all-*trans*-retinal back to 11-*cis*-retinal autonomously. Thus melanopsin would appear to be a prime candidate for a vertebrate bistable photopigment, similar to those of invertebrates. Indeed, using heterologously expressed cephalochordate melanopsin, Koyanagi and colleagues unambiguously demonstrated that melanopsin functions as a bistable pigment *in vitro* acting as both a photopigment and a photoisomerase [58], although it remained to be demonstrated that melanopsin in mammalian ipRGCs also functions as a bistable photopigment *in vivo*.

This question was first addressed by Fu and colleagues using a mouse model deficient in 11-*cis*-retinaldehyde synthesis [59]. These experiments firmly established that melanopsin in mouse ipRGCs detects light with a vitamin A-based chromophore and they also suggested that melanopsin

may be bistable [59]. Cooper and his coworkers addressed the issue of melanopsin's bistability *in vivo* using an indirect approach by recording single-unit activity in the mouse SCN in response to light stimulation of different wavelengths. They observed that prestimulation of the animal with long-wavelength light (e.g., 620 nm) enhanced the responses of SCN neurons to 480 nm light stimulation, consistent with long wavelength light causing re-isomerization and melanopsin being bistable [60]. Similarly, these authors examined the pupillary light reflex in humans and reported that prior exposure to long wavelength light increases while short wavelength light decreases the amplitude of pupil constriction, again consistent with the interpretation of a bistable photopigment [61]. Surprisingly, however, little long-wavelength photic potentiation was observed when mouse ipRGCs were recorded *in vitro* using a multielectrode array [62]. The reasons for these differences are not apparent [63,64]. However, whether or not melanopsin is able to regenerate 11-*cis*-retinal through sequential photon absorption, it has also been reported that melanopsin uses a light-independent retinoid regeneration mechanism [65]. Studies using mice lacking outer retinal function may help to determine if long wavelength enhancement of melanopsin-mediated behaviors *in vivo* is mediated by long wavelength cone input to ipRGCs. Examination of individual native ipRGCs maintained *in vitro* [51] may also contribute to determining whether mammalian melanopsin is truly a bistable photopigment.

4 Central and retinal targets of ipRGCs

The suspicion of the existence of a non-rod, non-cone ocular photoreceptor was originally based primarily on the observation that mice lacking rods and cones synchronized their circadian locomotor activity to the day/night cycle [4,5]. The circadian oscillator that drives this behavior is located in the SCN and the SCN was known to receive direct input from the retina [66], so it was unsurprising that the RGCs projecting to the SCN were found to be intrinsically photosensitive [15].

4.1 ipRGC projections are widespread

It has long been known that RGCs afferent to the SCN have axons that bifurcate, sending collateral branches to the intergeniculate leaflet (IGL) of the thalamus [29,67,68] and the olivary pretectal nucleus (OPN), the region of the midbrain that regulates the pupillary light reflex [29,68]. Consistent with these previously documented divergent axonal branches of RGCs innervating the SCN, Hattar and colleagues described melanopsin RGC projections to the IGL and OPN, in addition to the SCN, using a reporter mouse in which the melanopsin gene *opn4* was replaced with the *tau-lacZ* gene [17]. The *tau-lacZ* gene codes for a protein consisting of the β-galactosidase enzyme fused to a signal sequence from tau to promote axonal transport of the reporter enzyme thus enabling visualization of melanopsin axons throughout the brain [17]. The initial description of melanopsin projections to the SCN, IGL and OPN in the tau-lacZ mouse was followed by a more comprehensive examination of melanopsin axonal projections in the mouse brain. This revealed widespread ipRGC targets that included several other hypothalamic nuclei, the medial amygdala, lateral habenula, superior colliculus and periaqueductal gray [69]. Conspicuously lacking in the central targets of melanopsin-expressing RGCs revealed by β-galactosidase axonal labeling in the tau-lacZ mouse was a significant projection to the dorsal lateral geniculate nucleus (dLGN), the thalamic relay to primary visual cortex [17,69]. Although it might have been explicable for a photoreceptive system conveying irradiance information centrally not to send signals to the primary visual system mediating conscious visual perception, the absence of β-galactosidase labeled axons projecting to the dLGN in the mouse stood in contrast to work in the primate where melanopsin RGCs were retrogradely labeled after tracer injection into the dLGN [37]. The apparent species difference between rodent and primate regarding melanopsin afferents to the dLGN is now recognized to be the result of an under-representation of the melanopsin afferent fibers in the tau-lacZ reporter mouse. For reasons not well understood, the β-galactosidase reporter protein is expressed at detectable levels only in ≈50% of the melanopsin RGCs in the adult tau-lacZ mouse retina [25,26] although all melanopsin RGCs appear to express β-galactosidase in the tau-lacZ neonatal retina up to about postnatal day 7 (Sollars and Pickard, unpublished observations). Using a new reporter mouse line in which all melanopsin RGCs appear to express the reporter protein, Hattar and colleagues recently described in a preliminary report, widespread melanopsin RGC axonal projections that included a "substantial innervation in the dLGN" [70].

A substantial projection of ipRGCs to the thalamic relay of the primary visual system indicates that the apparent dichotomy of 'image-forming' and 'non-image forming' visual systems, often used to contrast ipRGCs from the rod and cone photoreceptors of the primary visual system [71,72] is in need of revision if not rejection, since even at the level of the retina the 'visual' rod and cone photoreceptors communicate with the 'non-visual' ipRGCs. The role of ipRGC input to the dLGN in visual perception is still unclear although Dacey *et al.* have suggested that dLGN-projecting ipRGCs in the primate might play a role in the conscious perception of brightness [37]. This hypothesis is supported by results from a case study of an 87 year old patient with autosomal-dominant cone-rod dystrophy with no apparent outer retinal function. The patient however, did have circadian entrainment and an intact pupil response when light exposure was extended to 10 sec duration (brief light exposure was ineffective), apparently mediated by ipRGCs [73].

She was also reported to be able to correctly identify the presence of a 481 nm test light but was unable to detect light at shorter or longer wavelengths. This unprecedented visual awareness without conscious perception was described by the patient as 'brightness' [73]. These intriguing findings are clearly in need of further investigation.

4.2 Different types of ipRGC have different central targets

There are multiple types of ganglion cell in the mammalian retina. Based on morphological criteria such as the level of dendritic stratification in the inner plexiform layer (IPL), the extent of the dendritic field and the density of dendritic branching, conventional ganglion cells can be grouped into clusters [74]. Physiologically, ganglion cells can be classified simply as belonging to one of three types; those that respond to increments in light (ON cells), those that respond to decrements in light (OFF cells), and those that respond to the initiation and termination of a stimulus (ON-OFF cells) [75,76]. ON cells have their dendritic processes confined to the lower part of the IPL, the ON substratum, whereas OFF cell have their dendrites limited to the upper part of the IPL, the OFF sublayer. Ganglion cells with bistratified dendritic arborizations in both the lower and upper layers of the IPL are ON-OFF cells. It has become apparent that ipRGCs are similarly not a homogenous population but rather can be classified into types based on morphology and physiology.

The first morphological description of ipRGCs in the rat described these SCN-projecting cells as sparsely branching and stratifying almost exclusively in the OFF sublayer of the IPL, near the border of the inner nuclear layer [16,17]. This pattern of dendritic aborization in the IPL is very unusual for ganglion cells that are depolarized by light (i.e., ON cells). Baver and colleagues retrogradely labeled SCN-projecting RGCs in the tau-lacZ mouse and discovered that the vast majority (80%) of SCN-projecting ipRGCs had their dendrites in the OFF sublamina of the IPL and appeared to express a greater level of melanopsin protein based on immunostaining; these ipRGCs were termed M1 cells. The remainder of ipRGCs projecting to the SCN expressed less melanopsin protein and had their dendrites in the proximal or ON sublayer of the IPL; these cells were termed M2 [26]. M1 and M2 ipRGCs were also described projecting to the OPN although in approximately equal proportions [26]. However, M1 ipRGCs innervate the shell region of the OPN whereas M2 ipRGCs send their axons to the central core of the OPN [26,70]. M2 ipRGCs have a more complex dendritic arborization, higher input resistance and a lower light sensitivity compared to M1 cells [77,78], consistent with less melanopsin expression in these cells [26]. The specific physiological roles these two subtypes of ipRGC serve remains to be determined.

As mentioned above, rodent ipRGCs do not appear to maintain the strict anatomically determined differentiation between ON and OFF signaling observed for conventional ganglion cells. Moreover in the primate, two types of monostratified ipRGC have been described that send dendrites to either the inner or the outer stratum of the IPL and both types generate sustained ON responses to light *in vitro* [37]. It was recently shown that ipRGCs with dendrites in the OFF sublamina (i.e., M1 ipRGCs) receive ON-bipolar cell input [25] via ectopic synapses of ON-bipolar cells as their axons pass through the OFF layer of the IPL, thereby establishing a new accessory ON sublayer in the outer IPL [79,80] (Figure1).

4.3 ipRGC input to the ventrolateral preoptic nucleus

ipRGC input to the SCN mediates entrainment of circadian rhythms and ipRGC input to the OPN provides the sensory limb of the pupillary light reflex. Retinal input to the IGL feeds back to the SCN to modulate the effects of light on circadian behavior [81,82]. Recently a series of reports have demonstrated that ipRGC input to the ventral lateral preoptic nucleus (VLPO) can induce sleep.

The VLPO is a region of the hypothalamus involved in sleep homeostasis. VLPO neurons, which are active during sleep [83], receive ipRGC input [68,69] and light can influence sleep, promoting alertness in day-active species and sleep in night-active species. Light presented to mice during the dark period activates the VLPO and induces sleep. These effects are still present, but reduced in melanopsin knockout mice, indicating that rods and cones also participate in the effects of light on sleep [84–86].

4.4 Retinal dopaminergic amacrine cells receive signals from ipRGCs

Retinal dopaminergic amacrine cells play a critical role in reconfiguring retinal function according to prevailing illumination conditions, yet the mechanisms by which light regulates their activity has remained poorly understood. Dopaminergic amacrine cells reside in the inner nuclear layer (INL) of the retina. They receive bipolar cell input and release dopamine through volume transmission, influencing visual signaling by all major classes of retinal neurons, from photoreceptors to ganglion cells [87].

Dopaminergic amacrine cells release dopamine in response to flickering light and steady background illumination, as well as during prolonged darkness. This functional heterogeneity is reflected at the cellular level in retinal dopaminergic neurons as transient, sustained, and null light responses in physiologically distinct neuronal subpopulations [88]. ON-transient dopaminergic amacrine cells receive bipolar cell input, perhaps via ectopic bipolar cell synapses [80] whereas input from bipolar cells is not required for the excitatory light responses of ON-sustained dopaminergic amacrine cells. Surprisingly, these cells receive excitatory drive from ipRGCs [89], most likely from

direct contact of their dendrites with those of ipRGCs co-stratifying in the IPL near the border of the INL (Figure 2). ON-transient dopaminergic amacrine cell responses to light are absent in mice lacking rods and cones. Conversely ON-sustained dopaminergic amacrine cell responses to light are absent in melanopsin knockout mice [89]. This unprecedented centrifugal outflow of ganglion-cell signals within the retina provides a novel basis for the restructuring of retinal circuits by light and indicates that information flow in the retina is truly bi-directional.

5 Serotonergic modulation of ipRGC input to the SCN

The intrinsic response of ipRGCs is modified by rod/cone input in the retina. In addition, the release of the neurotransmitter, glutamate, from their axon terminals in the SCN is modulated by serotonergic signals.

The SCN receives a dense serotonergic input that arises from the median raphe nucleus of the midbrain. Selective destruction of serotonergic input to the SCN amplifies circadian behavioral responses to light [90,91]. A serotonin (5-HT) receptor subtype involved in the effects of 5-HT in the SCN is the 5-HT_{1B} receptor. ipRGCs synthesize 5-HT_{1B} receptors in the retina and ship them to their terminals in the SCN where they function as presynaptic inhibitory receptors; activation of these receptors modulates the response of the SCN to photic input [92,93]. Moreover, 5-HT_{1B} receptor knockout mice display a behavioral phenotype under short-day (winter-like) conditions, a delayed phase relationship to the day/night cycle [94], that resembles people suffering from recurrent winter depression or seasonal affective disorder (SAD) [95,96]. Recently Provencio and colleagues described a missense variant of the melanopsin gene in SAD patients [97]. Several factors surely contribute to the etiology of SAD, and these may well include reduced sensitivity to light that may result from abnormalities in phototransduction in ipRGCs [97] and/or abnormalities in 5-HT neurotransmission in the SCN [94].

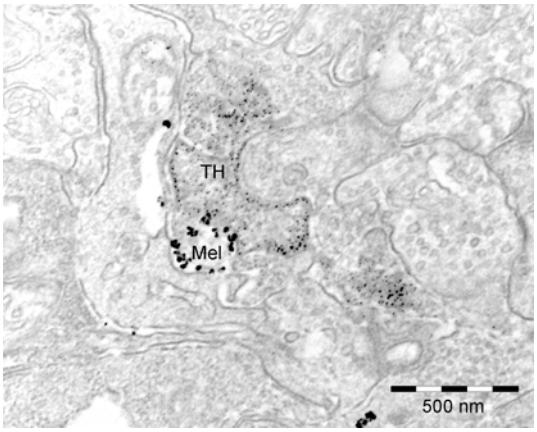

Figure 2 Electron micrograph showing immunolabeling for tyrosine hydroxylase (TH) and melanopsin (Mel) in the innerplexiform layer of the mouse retina. Tissue was double-labeled with a sheep anti-TH antibody and rabbit anti-melanopsin antibody using a pre-embedding protocol and silver intensification of gold particles. TH processes and melanopsin dendrites are in contact although to date no synaptic specializations have been observed. Note that the melanopsin immunoreaction product is located along the plasma membrane whereas TH staining is distributed throughout the cytoplasm as expected. Scale bar=500 nm.

Alterations in the phase of entrainment to the day/night cycle are associated with alterations in the amplitude of the diurnal rhythm of plasma corticosterone secreted from the adrenal cortex; phase delayed entrainment in mice significantly blunts the daily corticosterone rhythm (Sollars and Pickard, unpublished observations). A blunted cortisol rhythm has been reported in SAD patients [98]. The reduction in corticosterone secretion may result from an altered phase relationship between the adrenal gland's innate circadian rhythm in steroid biosynthesis [99] and the availability of adrenocorticotropic hormone (ACTH) released from the anterior pituitary gland [100,101]. It is possible that ipRGC projections to the hypothalamus caudal to the SCN [69,102] may provide a direct input to hypothalamic neurons that regulate the sympathetic outflow to the major organs of the body. Considering that corticosterone is a potent transcription factor and the daily rhythm of corticosterone secretion affects gene expression in the brain and major organs of the body [103,104], this is an area that requires further investigation.

6 The long road ahead

Since the discovery of ipRGCs less than a decade ago, a remarkable literature has arisen documenting the idiosyncrasies of these neuronal photoreceptors and the diverse array of connections and functions these cells subserve. It is clear that much work remains to be done, for although many questions have already been addressed, few answers seem definitive, particularly with regard to the basic phototransduction cascade. Still, after the mere existence of ipRGCs eluded notice during more than a century of active retinal research, it is a near certainty that unraveling the many puzzles that are posed by the structure and function of ipRGCs will shed unexpected new light on most currently held notions of retinal organization.

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