

Intrinsically Photosensitive Retinal Ganglion Cells

Gary E. Pickard and Patricia J. Sollars

Abstract Intrinsically photosensitive retinal ganglion cells (ipRGCs) respond to light in the absence of all rod and cone photoreceptor input. The existence of these ganglion cell photoreceptors, although predicted from observations scattered over many decades, was not established until it was shown that a novel photopigment, melanopsin, was expressed in retinal ganglion cells of rodents and primates. Phototransduction in mammalian ipRGCs more closely resembles that of invertebrate than vertebrate photoreceptors and appears to be mediated by transient receptor potential channels. In the retina, ipRGCs provide excitatory drive to dopaminergic amacrine cells and ipRGCs are coupled to GABAergic amacrine cells via gap junctions. Several subtypes of ipRGC have been identified in rodents based on their morphology, physiology and expression of molecular markers. ipRGCs convey irradiance information centrally via the optic nerve to influence several functions including photoentrainment of the biological clock located in the hypothalamus, the pupillary light reflex, sleep and perhaps some aspects of vision. In addition, ipRGCs may also contribute irradiance signals that interface directly with the autonomic nervous system to regulate rhythmic gene activity in major organs of the body. Here we review the early work that provided the motivation for searching for a new mammalian photoreceptor, the ground-breaking discoveries, current progress that continues to reveal the unusual properties of these neuron photoreceptors, and directions for future investigation.

Keywords Melanopsin · Circadian rhythms · Suprachiasmatic nucleus · Retina

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Early Hints of a Third Photoreceptor in the Mammalian Retina

The perception of shapes, color and objects moving in the world begins in the outer retina where light is absorbed by photopigments that are integral membrane apoproteins (opsins) covalently linked to a retinaldehyde chromophore in the rod and cone photoreceptors. The capturing of photons by rod and cone photoreceptors initiates a signaling cascade in which photon capture is converted into an electrical signal. The simplest common pathway these signals take from the eye to the brain is from the photoreceptors to bipolar cells to ganglion cells. Retinal ganglion cells (RGCs) convey the signals centrally as action potentials, transmitted via their axons in the optic nerve, to higher brain regions for integration and the further processing required for conscious visual perception (Fig. 1). Among non-mammalian vertebrates, photoreceptors are also found in locations outside the retina, including the pineal gland and in the brain itself. These extra-ocular photoreceptors mediate tasks not associated directly with visual perception (non-image forming functions such as hormone regulation). However, since the pioneering descriptions of the vertebrate retina by Santiago Ramón y Cajal in the late 1800s, it was believed that the retinal rods and cones were the only photoreceptors in mammals (Cajal 1894).

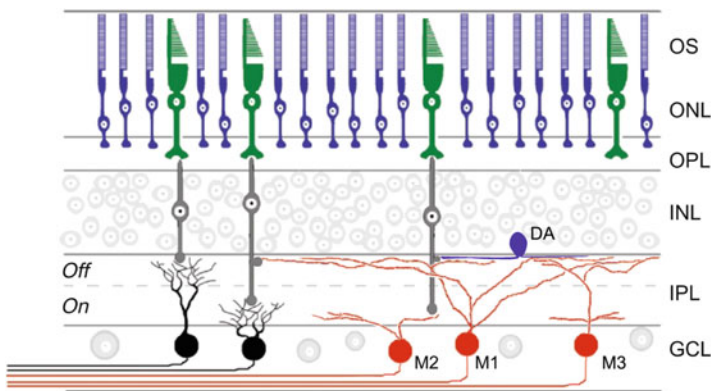


Fig. 1 Schematic vertical section of retina depicting ipRGCs (red) and rod and cone photoreceptors. ipRGCs reside in the ganglion cell layer (GCL) whereas rods and cones have their cells bodies in the outer nuclear layer (ONL). The three morphological types of ipRGC (M1, M2 and M3) are shown. Dendrites 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 (2003) and reprinted with permission from Pickard GE, Sollars PJ (2010) Intrinsically photosensitive retinal ganglion cells. *Science China Life Sciences* 53:58–67

The first suggestion that a non-rod, non-cone photoreceptor might exist in the mammalian retina appeared in 1927. Van Gelder (2008) recalls the story of a young graduate student at Harvard named Clyde E. Keeler, who in 1923 used a wild mouse from several he had caught in his dormitory room, as one of the subjects for an assignment he was given to compare histological sections from the eyes of several different vertebrates. The fact that the histological sections he prepared from the retinas of this mouse were devoid of all rod and cone photoreceptors almost ended his scientific career early, as it was assumed he had done a rather poor job of sectioning the retinal tissue. Keeler, however, convinced that the lack of rods and cones was not a consequence of his histological technique, went on to describe that these mice had actually lost their photoreceptors (Keeler 1924). He next described that, despite having no photoreceptors in the outer retina, the apparently visually blind mice maintained their ability to constrict the iris in response to light stimulation (i.e., the pupillary light reflex) albeit somewhat more slowly than mice with the normal complement of rods and cones. He offered the heretical possibility that “direct stimulation of the internal nuclear or ganglionic cells” by light may have been responsible for the observed behavior of the iris (Keeler 1927). It appeared more likely to others at the time that the mouse iris itself may have been light sensitive and attention to what turns out to have been an important observation, diminished with time.

The Convergence of Retinal Physiology and Internal Time Keeping

Daily rhythms in nature such as the opening and closing of flowers or our patterns of sleep and wakefulness and their association with the perpetual alteration of night and day were perhaps so obvious that their origins were not questioned until the eighteenth century. Jean-Jacques d’Ortous de Marian, a French astronomer, asked if the leaves of the Mimosa plant opened in response to light. He maintained the plants in the dark and noted that the leaves continued to open in the absence of sunlight. Although this is the first description of the endogenous nature of leaf movements, De Marian concluded that the plants must still sense the sun by means other than light (e.g., temperature or humidity) (De Marian 1729). The Swiss botanist Augustin Pyramus de Candolle is credited with the first suggestion of internal timekeeping. He observed that leaf movements persisted under constant light conditions and he concluded from his observations that the rhythm of leaf folding and unfolding must come “from within the plant” (De Candolle 1832). Charles Darwin, in his book on the movements of plants, came to the same conclusion, remarking that “we may conclude that the periodicity of their movements is to a certain extent inherited” (Darwin and Darwin 1880). During the decades that followed the observations made by Keeler, investigators became increasingly aware that not only plants, but all organisms including humans displayed daily rhythms that were generated by an internal time-keeping system or endogenous biological clock (Pittendrigh 1954; Aschoff 1960; Hamner et al. 1962). The first

clear demonstration of this clock-like system in mammals came from a study of the rhythmic behavior of white-footed mice, *Peromyscus leucopus*, housed under constant dim light conditions. Based on his observations, Johnson wrote “this animal has an exceptionally substantial and durable self-winding and self-regulating physiological clock, the mechanism of which remains to be worked out” (Johnson 1939). Richter (1965) sought to determine the clock’s physical location in the body and he concluded, after making lesions throughout the brain and removing all major endocrine organs one at a time, that the biological clock was based in the central nervous system and resided in the hypothalamus.

It had also become clear by this time that retinal projections were required for the entrainment of rhythmic behavior of rodents to the day/night cycle; under constant environmental conditions (i.e., constant light or dark) rhythmic behavior “free-runs,” expressing the species-typical period of the endogenous circadian (circa “about” and dies “day”) clock. After removing the eyes of hundreds of rats, Richter observed that the precise 24 h pattern of rhythmic nocturnal behavior obtained under light:dark conditions free-ran with a circadian period (Richter 1965). The introduction of autoradiographic techniques for demonstrating axonal connections in the central nervous system (Cowan et al. 1972) led to the discovery of a previously unknown retinal projection to the anterior hypothalamus terminating in the suprachiasmatic nucleus (SCN) (Moore and Lenn 1972; Hendrickson et al. 1972). The description of a retinohypothalamic tract (RHT) coupled with the observation that ablation of the SCN abolished behavioral circadian rhythms (Stephan and Zucker 1972) suggested that the SCN was the site of the circadian clock that is normally entrained to the day/night cycle by retinal signals transmitted via the RHT.

Although a direct connection between the retina and the SCN was thus established, it was not known what type of RGC innervated the SCN, whether these cells projected exclusively to the hypothalamus or what their receptive field properties were like. The receptive field properties of SCN-projecting RGCs were determined indirectly by examining the response of SCN neurons to photic stimulation of the retina; SCN neurons had extremely large receptive fields with no “surround” antagonism typical of most RGCs and they responded to stimulus luminance (Groos and Mason 1978, 1980; Meijer et al. 1986). The morphology of SCN-projecting RGCs was first identified by *in vivo* injection of tracer molecules that were retrogradely transported from the SCN to the retina via the RHT. It was estimated that 1–2% of the total RGC population projected to the SCN and these cells appeared to have a very simple dendritic morphology (Pickard 1980). Although the techniques available at the time precluded a complete morphological description, soma size analysis suggested that more than one type of RGC projected to the SCN (Pickard 1982).

It was also not known at this time whether rod and/or cone photoreceptors conveyed signals to SCN-projecting RGCs. The spectral sensitivity of light stimuli that affect circadian rhythms was first assessed using light-induced phase shifts of rodent circadian activity rhythms as an endpoint. The reported spectral sensitivity

curve generated from light-induced phase shifts had a maximum near 500 nm, similar to the absorption spectrum of rhodopsin, implying rod-generated signals. However, the threshold of the response was unusually high for the rod-dominated rodent retina and the temporal integration of the stimulus was also unusual for a rod-mediated response (Takahashi et al. 1984). Ebihara and Tsuji (1980) and later Foster et al. (1991) used *rd* mice to assess whether rod and/or cone photoreceptors conveyed photoentrainment signals to the SCN. Mice carrying the retinal degeneration mutation (*rd*; the mutation identified by Keeler 1924), are virtually devoid of rod and cone photoreceptors by four weeks of age and do not produce recordable electroretinographic responses or visual evoked potentials (Farber et al. 1994). These investigators reported that adult *rd/rd* mice were able to synchronize their circadian activity rhythms to cycles of light and darkness. Similar to Keeler (1927), Ebihara and Tsuji (1980) also suggested that cells other than rods and cones in the retina might be directly light sensitive. However, the universally acknowledged depiction of the organization of the mammalian retina prevailed despite these reports indicating that rodents with severe degeneration of the outer retina remained capable of responding to light (i.e., generating irradiance responses). At the time, the persistent response to light in retinal degenerate mice was widely attributed to the few cone photoreceptor remnants (cone photoreceptors lacking outer segments) that remain in these animals for many months (Dräger and Hubel 1978).

However, many who studied circadian photoentrainment believed that the evidence in support of an unknown “circadian” photoreceptor was compelling and doubted that a few cone photoreceptors lacking outer segments could account for the observed photoentrainment of behavioral rhythms; visual neuroscientists on the other hand, tended to reject the notion that an additional photoreceptor in the mammalian retina had been overlooked throughout 150 years of investigation. To directly test the interpretation that residual cone photoreceptor remnants were responsible for mediating the irradiance responses to light in retinal degenerate animals, transgenic mice were generated lacking all rod and cone photoreceptors. The fact that these animals also retained several irradiance responses including photoentrainment of their circadian locomotor behavior, the pupillary light reflex, and light-induced suppression of nocturnal pineal melatonin secretion provided further strong evidence for the existence of a non-rod, non-cone ocular photoreceptor (Freedman et al. 1999; Lucas et al. 1999) as removal of the eyes eliminates all irradiance responses (Nelson and Zucker 1981). Additional support for a non-rod, non-cone ocular photoreceptor came from reports in rodents and humans describing irradiance responses that had an action spectrum inconsistent with that of any known retinal photoreceptor (Yoshimura and Ebihara 1996; Lucas et al. 2001; Brainard et al. 2001; Thapan et al. 2001). Taken together these data appeared to provide clear evidence for the existence of a non-rod, non-cone photoreceptor in the mammalian retina, although which retinal cells responded to light in the absence of rods and cones and what type of photopigment mediated these responses briefly remained a mystery.

Melanopsin, the Photopigment of Intrinsically Photosensitive Retinal Ganglion Cells

While evidence was accumulating for the existence of a non-rod, non-cone photoreceptor in the mammalian retina, Provencio et al. (1998) were focusing their efforts on identifying the photopigment that might be responsible for the irradiance responses retained in mice lacking rods and cones. But rather than looking in mice or other mammals, these investigators studied frog skin. It was believed that the photoresponse of pigment cells (melanophores) in amphibian skin was mediated by a unique member of the rhodopsin family of G-protein coupled receptors although the exact nature of the opsin in frog skin remained unspecified (Daniolos et al. 1990). Provencio and his co-workers, using cultured dermal melanophores from *Xenopus laevis*, identified the photopigment responsible for the light-induced dispersion of melanosomes. In their ground-breaking paper they described the opsin, named melanopsin, and reported that melanopsin was a member of the opsin family of G-protein coupled receptors sharing the greatest sequence homology to octopus (invertebrate) rhodopsin. Importantly they also reported that melanopsin mRNA was expressed in frog dermal melanophores, in the brain and in the retina, but not in typical retinal photoreceptors (Provencio et al. 1998). These investigators subsequently described the distribution of melanopsin mRNA in the retina of mammals; in both primates and rodents, melanopsin mRNA was expressed not in rod or cone photoreceptors, but rather in the ganglion cell layer, providing the basis for the suggestion that RGCs expressing this novel mammalian opsin were directly photosensitive (Provencio et al. 2000). Gooley et al. (2001) quickly extended these findings by demonstrating that melanopsin was expressed in SCN-projecting RGCs.

SCN-Projecting RGCs Are Intrinsically Photosensitive and Express Melanopsin

The prediction that RGCs were photosensitive was borne out in early 2002 through a set of landmark reports by Berson, Hattar, Yau and colleagues. Berson and coworkers recorded from SCN-projecting RGCs in the rat retina and showed that when these neurons were isolated pharmacologically and physically from all rod and cone synaptic input, they generated action potentials in response to photic stimulation; the ganglion cells were intrinsically photosensitive (Berson et al. 2002). Importantly, they also showed that these intrinsically photosensitive retinal ganglion cells (ipRGCs) expressed melanopsin (Hattar et al. 2002). The discovery of melanopsin by Provencio et al. (1998) and the reports describing ipRGCs in the rodent retina (Berson et al. 2002; Hattar et al. 2002) laid the foundation for what is now an exciting and rapidly growing new subdivision of retinal biology. However, at the time, two key questions remained: (1) was melanopsin truly a photopigment; and (2) was melanopsin required for animals to show irradiance responses such as the pupillary light reflex or entrainment of circadian behavior to the day/night cycle?

The generation of melanopsin knockout mice (*Opn4*^{-/-}) answered the second question first. Mice lacking the melanopsin protein, with the ipRGCs otherwise apparently unaffected, retained the ability to entrain to daily cycles of light and darkness and they generated a pupillary light reflex, although several aspects of these irradiance responses were altered (Panda et al. 2002; Ruby et al. 2002; Lucas et al. 2003). Both acute and chronic effects of light on the circadian system were significantly attenuated in melanopsin-deficient mice (Panda et al. 2002; Ruby et al. 2002) and the pupillary light reflex was described as incomplete at high irradiances (Lucas et al. 2003). These unexpected results indicated that classical rod and/or cone photoreceptors and ipRGCs both contribute to irradiance responses. When melanopsin was knocked out in mice lacking functional rods and cones, all tested responses to light were eliminated, confirming a role for melanopsin in irradiance responses to light and also indicating the unlikelihood that any other photoreceptor in the mammalian retina had remained undetected (Panda et al. 2003).

The observation that melanopsin-deficient mice retain the ability to entrain behavioral circadian rhythms to the day/night cycle suggested either that conventional (non-melanopsin) RGCs send afferent fibers to the SCN or that ipRGCs receive synaptic input and relay rod/cone-driven signals to the SCN integrated with their intrinsic photoresponses. The first electron microscopic examination of melanopsin-expressing RGCs provided morphological evidence that ipRGCs receive synaptic input from amacrine cells and bipolar cells (Belenky et al. 2003); *in vitro* physiological studies of ipRGCs subsequently confirmed that they are driven by rod and/or cone photoreceptor input (Dacey et al. 2005; Perez-Leon et al. 2006; Wong et al. 2007; Schmidt et al. 2008). Moreover, the rod and/or cone photoreceptor-driven input is capable of generating physiological responses in ipRGCs in the absence of melanopsin and the intrinsic photoresponse (Pickard et al. 2009; Schmidt and Kofuji 2010). Thus there is no doubt that ipRGCs are integrated within the conventional signaling pathway in the retina (rod/cone → bipolar cell → RGC). However, the response of ipRGCs to rod/cone generated signals is not homogenous, consistent with the observation that there is considerable variation among ipRGCs in the neurotransmitter receptors they express (see below).

The question of whether conventional RGCs innervate the SCN was addressed initially in double-label studies in rodents. SCN-projecting RGCs were identified using tracer injections into the SCN, and the identified neurons were then assayed by *in situ* hybridization to determine melanopsin mRNA expression or by immunocytochemical staining of melanopsin protein. In the rat (Gooley et al. 2003) and golden hamster (Morin et al. 2003; Sollars et al. 2003), 80–90% of SCN-projecting RGCs were classified as melanopsin-expressing; in the mouse virtually all (>99%) SCN-projecting RGCs express melanopsin (Baver et al. 2008). Genetic or immunotoxin-induced ablation of melanopsin RGCs in the mouse eliminates these animal's ability to entrain to light/dark cycles, confirming that in this species, rod and/or cone influences on circadian entrainment are mediated via melanopsin expressing ipRGCs which act as a conduit for rod/cone signals to reach the SCN (Göz et al. 2008; Göler et al. 2008; Hatori et al. 2008).

In the rodent retina approximately 2–4% of all RGCs express melanopsin (Sollars et al. 2003; Hattar et al. 2006; Baver et al. 2008; Berson et al. 2010). As indicated above, in the mouse it appears that only melanopsin immunoreactive RGCs send afferent fibers to the SCN whereas this does not appear to be the case in the rat and golden hamster. The dissimilarity in findings among these three different rodents may represent true species differences, but it remains to be demonstrated using physiological techniques that the “non-melanopsin” SCN-projecting RGCs identified in the rat and golden hamster are not intrinsically light sensitive. It is possible that these RGCs either express too little melanopsin to be detected (Baver et al. 2008; Ecker et al. 2010) or express a melanopsin isoform not recognized by the antibodies currently available (Torii et al. 2007; Pires et al. 2009; Davies et al. 2011). The number of “non-melanopsin” RGCs sending afferent fibers to the SCN in the golden hamster and rat is small (i.e., 100–200) making the task of recording from these cells difficult. A complete description of the morphology and dendritic arborization pattern of “non-melanopsin” SCN-projecting RGCs would be useful for comparison to SCN-projecting melanopsin RGCs to ascertain if the “non-melanopsin” neurons projecting to the SCN represent a unique RGC type (Wässle 2004). Although genetic mouse models have played an important role in advancing our understanding of ipRGCs, studies are required on additional species before generalizations regarding RGC input to the SCN can be made.

Melanopsin Is a Photopigment

Evidence confirming the identification of melanopsin as a photopigment was derived from the heterologous expression of melanopsin in several different cell lines. The first data came from purified melanopsin protein 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 (Newman et al. 2003), the spectral properties (i.e., maximal absorbance at ≈ 424 nm) were not consistent with the action spectrum observed by Berson and colleagues for melanopsin-expressing RGCs (≈ 484 nm) (Berson et al. 2002). Subsequently, the photopigment properties of melanopsin were confirmed after its expression in HEK cells that also expressed transient receptor potential C3 channels (Qiu et al. 2005), Neuro-2a cells (Melyan et al. 2005), and *Xenopus* oocytes (Panda et al. 2005). Together these studies provided convincing evidence that melanopsin was indeed a photopigment. However, one group again reported a melanopsin absorption maximum ≈ 420 nm (Melyan et al. 2005) whereas the others indicated that expressed melanopsin maximally absorbed light at ≈ 480 nm (Panda et al. 2005; Qiu et al. 2005), matching more closely the spectral tuning of pharmacologically isolated rat (Berson et al. 2002) and primate (Dacey et al. 2005) ipRGCs. It should be noted that non-mammalian melanopsin also shows peak spectral sensitivity ≈ 480 nm, in close agreement with mammalian ipRGCs (Koyanagi et al. 2005; Torii et al. 2007; Davies et al. 2011). Thus, it is now

generally agreed that mammalian melanopsin maximally absorbs light at ≈ 480 nm although direct *in vitro* spectroscopic analysis of purified mammalian melanopsin is still needed (Bailes and Lucas 2010).

ipRGC Physiological Responses to Light

Several characteristics of the ipRGC response to light set these ganglion cell photoreceptors apart from mammalian rod and cone photoreceptors. In particular, ipRGCs are less sensitive to photic stimulation and their response kinetics are extremely slow compared to that of rods and cones. In addition, the melanopsin phototransduction cascade appears similar to that of many invertebrates, with the result that the polarity of the ipRGC response to light is opposite that of rods and cones, resulting in the generation of action potentials.

ipRGC Response Kinetics

ipRGCs are relatively insensitive to light and their response to light stimulation is extremely sluggish compared to conventional RGCs. Response latency is inversely related to stimulus intensity and under dim light conditions ipRGCs can take many seconds to reach a peak response; the response may also persist for minutes after stimulus termination (Berson et al. 2002; Warren et al. 2003). On the other hand, ganglion cell photoreceptors are similar to rods and cones in that they show adaptation by adjusting their sensitivity according to lighting conditions (Wong et al. 2005). While slow to respond to dim light conditions, ipRGCs appear capable of responding to the capture of a single photon of light (Do et al. 2009). Thus the relatively low sensitivity to light does not appear to be the result of inefficient phototransduction but rather of poor photon catch. It has been estimated that the membrane density of melanopsin is about a thousand times lower than that of photopigments in the outer segments of rod and cone photoreceptors; this relatively low density may account for the poor absorption rate of ipRGCs (Brown and Lucas 2009; Do et al. 2009).

The capture of a single photon in an ipRGC generates a large and prolonged membrane current, greater than that recorded in rod photoreceptors but also 20-fold slower (Chen et al. 1999). It has been suggested that 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 (Do et al. 2009). Moreover, since ipRGCs are also synaptically driven by rod and/or cone photoreceptors (Belenky et al. 2003; Dacey et al. 2005; Perez-Leon et al. 2006; Wong et al. 2007; Schmidt et al. 2008), ganglion cell photoreceptors themselves may not require the level of intrinsic sensitivity found in the classic photoreceptors.

It should be noted, however, that brief (1 s) light pulses (DeCoursey 1972; Earnest and Turek 1983) or a short series of extremely brief (2 ms), intense light flashes, which are ineffective as single flashes (van den Pol et al. 1998; Vidal and Morin 2007) can produce significant phase shifts of the circadian clock. Very brief stimuli do not appear to demonstrate the same type of temporal integration associated with light pulses several minutes in duration (Nelson and Takahashi 1999; Vidal and Morin 2007; Morin et al. 2010). The behavioral responses of the circadian system to brief light flashes may represent an integrated response of rod/cone and ganglion cell photoreceptors, although the ability of ipRGCs to generate an intrinsic photoresponse to a 2 ms light flash has not been tested directly. Nevertheless, *rd/rd* mice lacking rod and cone photoreceptors show patterns of locomotor activity suppression similar to wild type mice in response to a series of 2 ms light flashes (Morin and Studholme 2011), suggesting that ipRGCs are indeed capable of generating a photoresponse to very brief, intense light flashes.

Photon Capture in ipRGCs Results in Membrane Depolarization

In response to light stimulation, ipRGCs depolarize and generate action potentials, unlike the hyperpolarizing light responses of mammalian rod and cone photoreceptors (Berson et al. 2002; Warren et al. 2003; Hartwick et al. 2007) but similar to the light-inducing depolarizing responses of *Drosophila* and most other invertebrate photoreceptors (Yau and Hardie 2009). 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 (Isoldi et al. 2005; Nickle and Robinson 2007; Yau and Hardie 2009). Perhaps it should not be a surprise then, that mammalian melanopsin appears to utilize an invertebrate-like phototransduction signaling cascade.

Opsins in mammalian rod and cone ciliary photoreceptors couple to the G_t -protein, transducin, which activates a phosphodiesterase cascade resulting in the closure of cGMP-gated channels and cellular hyperpolarization (Yau and Hardie 2009). Melanopsin in mammalian ipRGCs is believed to be coupled to a G-protein of the G_q family as its cognate G-protein in vivo (Berson 2007; Peirson et al. 2007). In an early study using heterologous expression, melanopsin was shown to activate a G-protein although no further details were provided (Newman et al. 2003). Subsequent studies in other heterologous expression systems suggested a G_q class of G-protein (Panda et al. 2005; Qiu et al. 2005) as did work conducted in *Xenopus* dermal melanophores (Isoldi et al. 2005). Melanopsin and G_q also co-localize in amphioxus rhabdomeric photoreceptors (Koyanagi et al. 2005). However, the identity of the native G-protein employed by mammalian ipRGCs and the downstream cascade triggered by its activation still remain uncertain. There is some evidence supporting a role for $G_{q/11}$ which would activate the effector enzyme phospholipase C, resulting in depolarization, an invertebrate-like phototransduction cascade (Graham et al. 2008).

The membrane channel that carries the initial inward current following the apparent activation of phospholipase C in ipRGCs has also not yet been conclusively identified. Involvement of mammalian homologues of the transient receptor potential (TRP) channels in *Drosophila* photoreceptors, termed TRP canonical (TRPC) channels, 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. Of the seven members of the subfamily of TRPC subunits that combine to form tetrameric channels (Hoffman et al. 2002), TRPC3, TRPC6, and/or TRPC7 have all been implicated as the TRPC channel mediating the initial depolarization in ipRGCs (Warren et al. 2006; Hartwick et al. 2007; Sekaran et al. 2007; Graham et al. 2008). However, in vitro recordings from ipRGCs in mouse lines lacking expression of functional TRPC3, TRPC6 or TRPC7 subunits indicate that light-evoked depolarization persist largely unchanged (Perez-Leighton et al. 2011). These results indicate that TRPC3, TRPC6, or TRPC7 homomeric channels do not mediate melanopsin-evoked depolarization in ipRGCs, but the possibility remains that these subunits may form heteromultimeric assemblies (Perez-Leighton et al. 2011). The demonstration that ectopic expression of melanopsin in conventional RGCs confers intrinsic photosensitivity to these cells (Lin et al. 2008) suggests that the channels gated by melanopsin may be widespread among ganglion cells. In addition, although one study reported that melanopsin activates diacylglycerol-sensitive TRPC channels in ipRGCs (Warren et al. 2006) another study failed to observe reduction of light-evoked currents in ipRGCs exposed to diacylglycerol analogs (Graham et al. 2008). The future development of more selective TRPC channel blockers and knockout mouse lines in which a combination of TRPC channels is eliminated will aid in determining the specific membrane channels mediating phototransduction in mammalian ipRGCs.

In *Drosophila*, light stimulates Ca^{2+} entry via a TRP channel that has an usually high Ca^{2+} selectivity ($P_{\text{Ca}}:P_{\text{Na}} > 50:1$) and there is minimal light-induced Ca^{2+} release from internal stores (Yau and Hardie 2009). As in *Drosophila* rhabdomeric photoreceptors, light stimulates an increase in intracellular calcium in mammalian ipRGCs (Sekaran et al. 2003; Hartwick et al. 2007). A small percentage of the light-evoked rise in somal intracellular Ca^{2+} appears to result from Ca^{2+} entry via the cation channel that carries the initial inward current (e.g., TRPC channel) (Hartwick et al. 2007). In HEK cells expressing human melanopsin, the light-triggered rise in intracellular Ca^{2+} results from the release of Ca^{2+} from intracellular stores (Kumbalasisiri et al. 2007). However in primary cultures of native rat ipRGCs, Ca^{2+} release from internal stores does not significantly contribute to the light-evoked rise in intracellular Ca^{2+} (Hartwick et al. 2007), similar to *Drosophila*. Approximately 90% of the light-triggered rise in intracellular Ca^{2+} in isolated rat ipRGCs maintained in primary culture is associated with the opening of L-type voltage-gated calcium channels and the rise in intracellular Ca^{2+} is highly correlated with action potential firing (Hartwick et al. 2007). The current model of ipRGC phototransduction and the light-evoked rise in intracellular Ca^{2+} is summarized in Fig. 2.

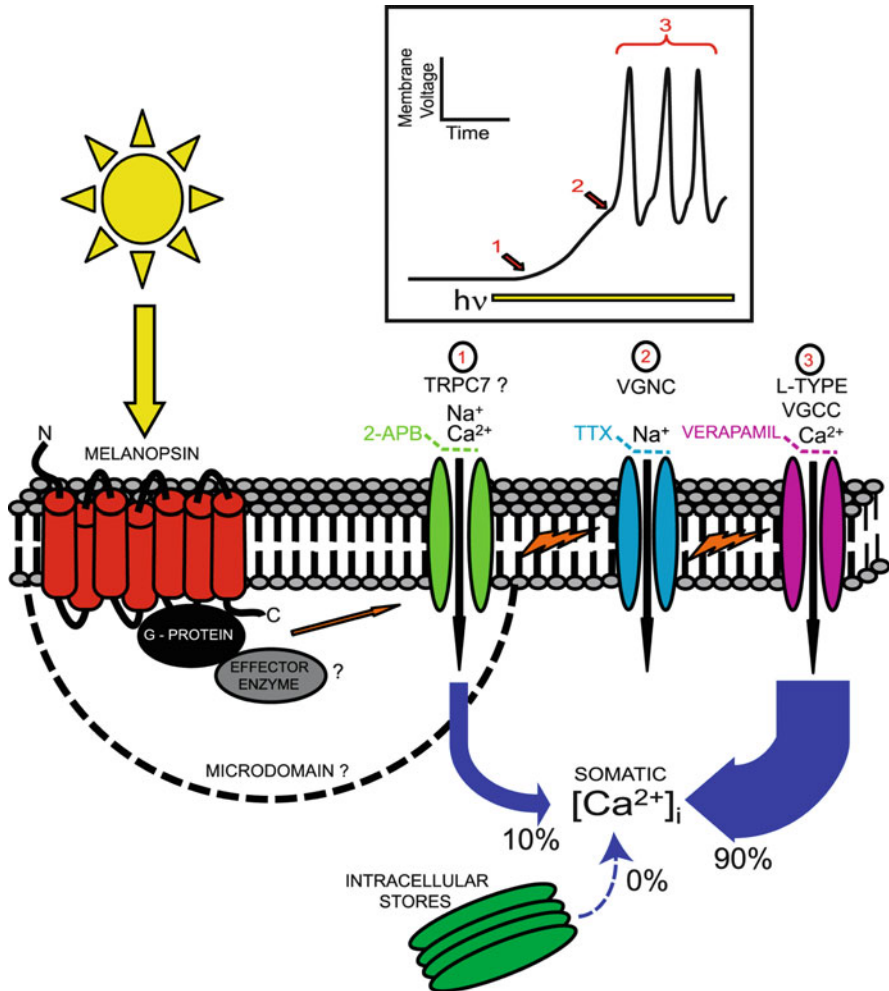


Fig. 2 Schematic overview of phototransduction and light-evoked Ca^{2+} influx in mammalian ipRGCs. After light stimulation of melanopsin photopigment in the plasma membrane, a signaling cascade is initiated that leads to the opening of the light-gated ion channel and membrane depolarization (labeled 1). The details of this cascade remain unclear, although there is evidence that it is G-protein dependent (Warren et al. 2006) with G_q a likely candidate and phospholipase C as the effector enzyme (Graham et al. 2008). The current model suggests a heteromeric TRPC channel is the light-gated ion channel that may include TRPC6 and TRPC7 subunits (Hartwick et al. 2007; Perez-Leighton et al. 2011). The ion flux through this 2-APB-sensitive channel depolarizes (denoted with a lightning bolt in the membrane) the membrane potential, resulting in the activation of voltage-gated Na^+ channels (VGNCs; labeled 2). The Na^+ flux through TTX-sensitive VGNCs during action potential firing further depolarizes the membrane, leading to the activation of verapamil-sensitive L-type voltage-gated calcium channels (VGCCs) (labeled 3). The relative timing for the opening of these three channel types is illustrated in the boxed drawing that depicts the changes in membrane voltage induced by light exposure in a typical ipRGC. Although Ca^{2+} influx through the initial light-gated ion channel contributes to increased somatic

Chromophore Recycling and Bistability

Another aspect of the ipRGC response to light that appears similar to invertebrate rhabdomeric type photoreceptors is that of photopigment regeneration. The visual pigment consists of two components: an apoprotein 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 initiating the phototransduction cascade. After all-*trans*-retinal is reduced to all-*trans*-retinol in mammalian rod and cone photoreceptors, it exits the cell where it is converted in the overlying retinal pigment epithelium (RPE) back to 11-*cis*-retinal by RPE65, a retinyl isomerohydrolase for return to the photoreceptors (Yau and Hardie 2009). Müller cells are intrinsic glia that span the entire width of the mammalian retina and are derived from the same set of progenitors as the retinal neurons. They serve a variety of roles in the mammalian retina, including providing a barrier for substances moving into and out of the retina, and they play a vital role in retinal metabolism. Müller glial cells also recycle 11-*cis*-retinal using a slightly different mechanism than the RPE. These glial cells appear to serve only cone photoreceptors (Wang et al. 2009; Wang and Kefalov 2009) although their role in chromophore recycling from ipRGCs has not been examined.

Unlike vertebrate rod and cone ciliary photoreceptors, the invertebrate rhabdomeric photopigment regenerating system has been considered independent of other cells or tissue. Invertebrate photopigments remain bound to the opsin moiety and are re-isomerized by light of a longer wavelength than that which causes the initial photoactivation; these photopigments are thus considered bistable (Yau and Hardie 2009). It would seem highly unlikely that ipRGCs in the mammalian retina utilize the RPE to recycle 11-*cis*-retinal *in vivo* since ipRGCs are located in the inner retina, quite removed from the RPE. Moreover, native ipRGCs respond to prolonged light exposure when maintained *in vitro* in isolation from other retinal cells including Müller glial cells (Hartwick et al. 2007) suggesting either that ipRGCs can convert all-*trans*-retinal back to 11-*cis*-retinal autonomously or that native ipRGCs contain more 11-*cis*-retinal than can be bleached under those conditions. 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 (Koyanagi et al. 2005). In zebrafish, in which five isoforms of melanopsin are expressed, some forms of melanopsin

← **Fig. 2** (continued) $[Ca^{2+}]_i$ during light stimulation, most of the Ca^{2+} signal is the result of the depolarization-induced opening of VGCCs (Hartwick et al. 2007). The presence of a membrane-associated microdomain that prevents some of the Ca^{2+} flux through the light-gated channel from reaching the somatic cytoplasm is also possible. The contribution of intracellular Ca^{2+} stores to light-evoked elevations in mammalian ipRGC $[Ca^{2+}]_i$ is negligible. Figure reprinted with permission from Hartwick et al. (2007)

display invertebrate-like bistability and remain within the opsin binding pocket, while in other forms of melanopsin, the 11-*cis*-retinal is isomerized and then released from the opsin similar to classic rod and cone photopigments (Davies et al. 2011). It remains to be directly demonstrated that melanopsin in mammalian ipRGCs functions as a bistable photopigment *in vivo*.

This question has been addressed, first by Fu and colleagues using a mouse model deficient in 11-*cis*-retinaldehyde synthesis. 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 (Fu et al. 2005b). Cooper and his coworkers have also 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 pre-stimulation 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 (Mure et al. 2007). 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 (Mure et al. 2009). Surprisingly however, little long-wavelength photic potentiation was observed when mouse ipRGCs were recorded *in vitro* using a multielectrode array (Mawad and Van Gelder 2008). The reasons for these differences are not apparent (Cooper and Mure 2008; Van Gelder and Mawad 2008). 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 (Walker et al. 2008). Similarly, an enzymatic chromophore regeneration mechanism has also been described in *Drosophila* despite the presence of a bistable photopigment (Wang et al. 2010). 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* (Hartwick et al. 2007) may also contribute to determining whether mammalian melanopsin is truly a bistable photopigment.

Multiple ipRGC Subtypes with Widespread Axonal Projections

The existence of a non-rod, non-cone ocular photoreceptor was originally suspected based primarily on the observation that mice lacking rods and cones synchronized their circadian locomotor activity to the day/night cycle by the daily phase resetting of their endogenous circadian clock (Ebihara and Tsuji 1980; Foster et al. 1991). As described above, the SCN circadian oscillator regulates this behavior and the SCN receives direct input from the retina (Moore and Lenn 1972; Hendrickson et al. 1972; Pickard 1982). Thus it was logical to first examine SCN-projecting RGCs for

melanopsin expression (Gooley et al. 2001) and intrinsic photosensitivity (Berson et al. 2002; Hattar et al. 2002). However, in their description of the efferent projections of melanopsin-expressing RGCs, Hattar et al. (2002) indicated that ipRGCs innervated not only the SCN but other brain regions as well (Hattar et al. 2002). Following these initial observations it has become clear that there are multiple ipRGC subtypes that send their axons to many areas in the brain and perhaps even back into the retina.

ipRGCs Targets in the Brain

It was known for some time before the discovery of ipRGCs that RGC axons terminating in the SCN arose as collateral branches of optic axons (Millhouse 1977) that continued on to the intergeniculate leaflet (IGL) of the thalamus (Pickard 1985), that the IGL projected to the SCN via the geniculohypothalamic tract (GHT) (Swanson et al. 1974; Pickard 1982) and that this indirect retinal input to the SCN modulated circadian behavior (Harrington and Rusak 1986; Pickard et al. 1987; Pickard 1989). Using a reporter mouse in which the melanopsin *opn4* gene was replaced with the *tau-lacZ* gene, Hattar and colleagues described melanopsin RGC projections to the IGL in addition to the SCN, consistent with these earlier reports (Hattar et al. 2002). 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 (Hattar et al. 2002). Using this reporter mouse, melanopsin-expressing RGCs were also described projecting to the olivary pretectal nucleus (OPN) (Hattar et al. 2002), the region of the midbrain that regulates the pupillary light reflex, and subsequently these projections were shown also to arise as collateral branches of RGCs innervating the SCN in the rat (Gooley et al. 2003) and golden hamster (Morin et al. 2003). Moreover, melanopsin RGCs were reported to terminate in several sites in the rat hypothalamus in addition to the SCN, including the ventral subparaventricular zone (vSPZ) dorsocaudal to the SCN and the ventrolateral preoptic nucleus (VLPO) lateral to the SCN (Gooley et al. 2003). As more detailed examinations were performed in the mouse, more central targets of melanopsin RGCs were revealed including the medial amygdala, lateral habenula, superior colliculus and periaqueductal gray (Hattar et al. 2006). Melanopsin projections to the superior colliculus were also described in the hamster (Morin et al. 2003) but not in the rat (Gooley et al. 2003). It is interesting to note that the dorsal raphe nucleus in the midbrain receives retinal afferent fibers but these do not appear to originate from ipRGCs (Luan et al. 2011). Figure 3 provides an overview of ipRGC projections.

Conspicuously lacking among 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 the primary visual cortex mediating visual perception

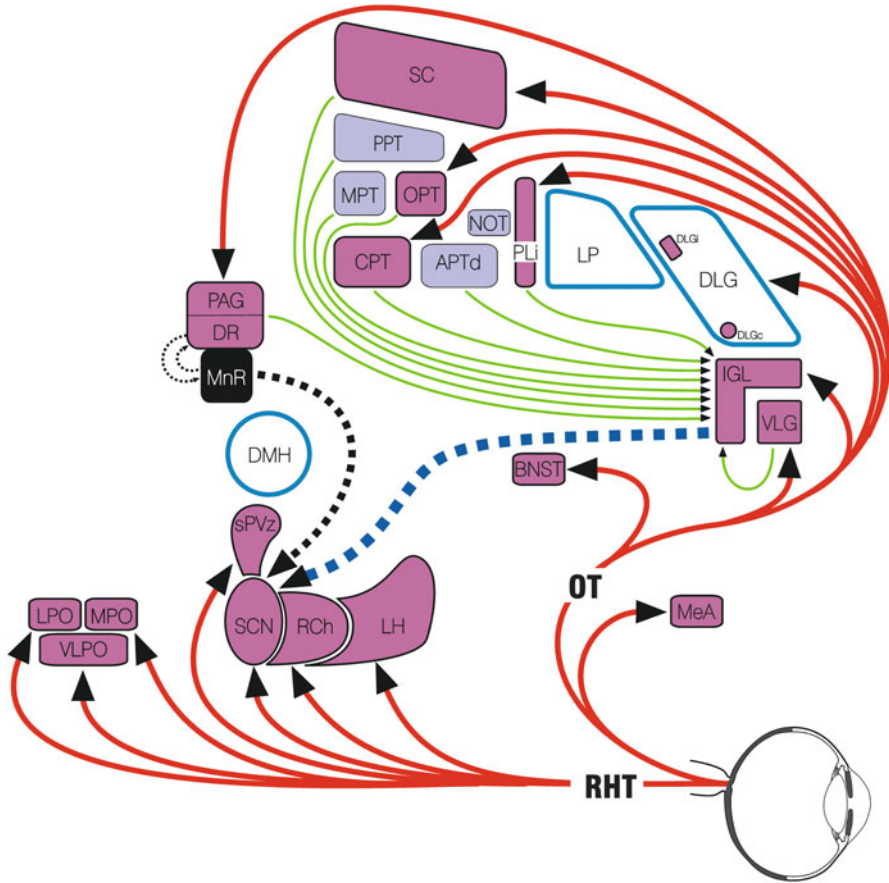


Fig. 3 Schematic representation of fore- and midbrain projections of the rodent retina, with special emphasis on targets of the ipRGCs (*purple regions; thick red lines*). Except for the median raphe nucleus (MnR), all other brain regions illustrated are retinorecipient but not necessarily from ipRGCs. *Thick, broken blue line* = geniculohypothalamic tract (GHT). *Medium, broken black line* = 5HT projection from MnR to SCN. *Thin, solid green lines* = non-visual projections to IGL. *Thin broken black lines* = reciprocal connections between MnR and DR. DLGi and DLGc – ipRGC projections to the ipsilateral and contralateral dorsal lateral geniculate nucleus, respectively. APTd anterior pretectal nucleus dorsal division; BNST bed nucleus of the stria terminalis; CPT commissural pretectal nucleus; DLG dorsal lateral geniculate nucleus; DMH dorsomedial hypothalamic nucleus; DR dorsal raphe nucleus; IGL intergeniculate leaflet; LH lateral hypothalamic area; LPO lateral preoptic area; LP lateral posterior thalamic thalamic nucleus; MeA medial amygdaloid nucleus; MnR median raphe nucleus; MPO medial preoptic area; MPT medial pretectal nucleus; NOT nucleus of the optic tract; OPT olivary pretectal nucleus; OT optic tract; PAG periaqueductal gray; PLi posterior limitans thalamic nucleus; PPT posterior pretectal nucleus; Rch retrochiasmatic area; RHT retinohypothalamic tract; SC superior colliculus; SCN suprachiasmatic nucleus; sPVz subparaventricular zone; VLG ventral lateral geniculate nucleus; VLPO ventral lateral preoptic nucleus. Based on (Morin et al. 1992; Morin and Blanchard 1997, 1998; Hattar et al. 2002; Gooley et al. 2003; Horowitz et al. 2004; Hattar et al. 2006; Ecker et al. 2010). Adapted from Morin LP, (in press) Circadian visual systems of mammals. In: How animals see the world, Lazareva OF, Shimizu T, Wasserman EA (eds), Chapter 21, pp. 389-415, Oxford University Press. Figure generously provided by L.P. Morin (Stony Brook University)

(Hattar et al. 2002, 2006). Although it is understandable that a photoreceptive system conveying irradiance information centrally might not 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 (Dacey et al. 2005). 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 $\approx 50\%$ of the melanopsin RGCs in the adult tau-lacZ mouse retina (Baver et al. 2008). Using a new *Opn4*-reporter mouse line in which all melanopsin RGCs appear to express the reporter protein, Hattar and colleagues have described widespread melanopsin RGC axonal projections that include a substantial innervation in the dLGN (Ecker et al. 2010).

A substantial projection of ipRGCs to the lateral geniculate body 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 (Fu et al. 2005a; Peirson et al. 2009) is perhaps in need of revision, 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 is still unclear although Dacey and colleagues have suggested that dLGN-projecting ipRGCs in the primate might play a role in the conscious perception of brightness (Dacey et al. 2005). This hypothesis is supported by a report describing non-image-forming responses of two visually blind subjects, one a 56 year old female with autosomal-dominant cone-rod dystrophy and the other an 87 year old male patient with retinitis pigmentosa. In each case, the patient’s circadian system appeared to be entrained to the day/night cycle and an intact pupillary light reflex was elicited but only when light exposure was extended to 10 s duration (brief light exposure was ineffective), apparently mediated by ipRGCs (Zaida et al. 2007) and reminiscent of the light-induced iris constriction described by Keeler (1927) in his blind mice. One subject 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 luminance awareness without conscious visual perception was described by the patient as “brightness” (Zaida et al. 2007). These intriguing findings are clearly in need of further investigation.

A role for melanopsin-based phototransduction in pattern vision in the absence of rods and cones was recently reported. Ecker and co-workers (2010) used a mouse strain in which rod and cone phototransduction had been genetically silenced by disruption of the genes encoding rod α transducin (*Gnat1*) and cone-specific $\alpha 3$ cyclic nucleotide gated channel subunit (*Cnga3*) (*Gnat1*^{-/-}; *Cnga3*^{-/-} double knockouts) leaving only ipRGCs as functional photoreceptors. The dKO mice were able to discriminate grating stimuli from equiluminant gray and had measurable visual acuity implying a role for ipRGCs in dLGN-mediated visual perception

(Ecker et al. 2010). However, these findings have come under question because it appears that the method used to silence rod and cone phototransduction in this mouse strain may not have been completely successful; these mice apparently retain significant and widespread outer retinal photoreception based on a Gnat1-independent phototransduction mechanism downstream of rod opsin (Allen et al. 2010). Thus the role of ipRGCs in pattern vision remains unclear.

ipRGC Intraretinal Signaling

ipRGCs send irradiance signals centrally via the optic nerve and in addition, these novel ganglion cell photoreceptors also send signals to other cells in the retina via both synaptic and non-synaptic mechanisms. ipRGCs provide light-evoked excitatory signals to dopaminergic amacrine cells and they are coupled to GABAergic amacrine cells via gap junctions.

ipRGCs Provide Excitatory Drive to Dopaminergic Amacrine Cells

Dopaminergic amacrine cells reside in the INL, 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. However, the mechanism by which light regulates dopaminergic amacrine cell activity has remained poorly understood.

Dopaminergic amacrine cells release dopamine in response to flickering light and steady background illumination, as well as during prolonged darkness (Witkovsky 2004). It was demonstrated that this functional heterogeneity was reflected at the cellular level by physiologically distinct subpopulations of dopaminergic amacrine cells which show transient, sustained, and null responses to light (Zhang et al. 2007). ON-transient dopaminergic amacrine cells receive ON-bipolar cell input, perhaps via ectopic ON-bipolar cell synapses in the outer sublayer of the IPL (Dumitrescu et al. 2009) whereas input from bipolar cells is not required for the excitatory light responses of ON-sustained dopaminergic amacrine cells (Zhang et al. 2008). Surprisingly, ON-sustained dopaminergic amacrine cells receive excitatory (glutamatergic) drive from ipRGCs (Zhang et al. 2008, 2010), perhaps where their processes are in direct contact with ipRGC dendrites in the IPL near the border of the INL. ON-transient dopaminergic amacrine cell responses to light are absent in rd^-/rd^- mice lacking rods and cones, consistent with the interpretation that photoreceptors acting via bipolar cells provide signals to these cells (Zhang et al. 2008). Conversely, when recordings were made in dopaminergic amacrine cells in melanopsin knockout mice, none of the 35 cells examined that responded to light stimulation showed ON-sustained responses, consistent with the previous findings indicating that this subtype of dopaminergic amacrine cell is driven only by ipRGCs (Zhang et al. 2010). This unprecedented ganglion cell signaling within the retina provides a novel basis for light-evoked restructuring of retinal circuits

via dopaminergic amacrine cells and indicates that information flow in the retina is truly bi-directional.

Although dendrites of melanopsin RGCs are in contact with dopaminergic amacrine cell processes in the IPL near the border of the INL (Ostergaard et al. 2007; Viney et al. 2007; Vugler et al. 2007; Zhang et al. 2008), there is no direct evidence that ipRGC signals to dopaminergic amacrine cells occur via their dendrites. Another possible mechanism by which ipRGCs might communicate with dopaminergic neurons is via ipRGC axon collaterals that loop back into the INL to synapse on dopaminergic amacrine cells. RGCs sending axon collaterals into the IPL have been described in cat (Dacey 1985), monkey (Usai et al. 1991) and human retina (Peterson and Dacey 1998); in human retina $\approx 2\%$ the RGCs examined had intraretinal axon collaterals that terminated in the outer half of the IPL and arose from RGCs with sparsely branched monostratified dendritic trees that arborized in the ON sublayer of the IPL (Peterson and Dacey 1998). In the cat retina, a small number of varicose processes in the IPL stain for one of the isoforms of the vesicular glutamate transporter (VGLUT2) and it was suggested that these may represent RGC axon collaterals (Fyk-Kolodziej et al. 2004); ipRGCs use glutamate as a neurotransmitter and express VGLUT2 (Johnson et al. 2007; Englund et al. 2010). A preliminary report indicates that indeed some ipRGCs may send axon collaterals into the IPL (Joo et al. 2011).

ipRGCs Are Coupled to GABAergic Amacrine Cells via Gap Junctions

In addition to regulating the activity of ON-sustained dopaminergic amacrine cells in the retina, it was reported that ipRGCs also influence the activity of other cells in the ganglion cell layer of the retina via electrical synapses or gap junctions (Sekaran et al. 2003, 2005). These investigators examined light-evoked increases in intracellular Ca^{2+} in cells in the ganglion cell layer and concluded that the light-responsive units formed an extensive network that could be uncoupled by application of the gap junction blocker carbenoxolone (Sekaran et al. 2003, 2005). However, Bramley et al. (2011) have reported that carbenoxolone blocks the light-evoked rise in intracellular Ca^{2+} in isolated rat ipRGCs by blocking voltage-gated calcium channels; similar effects of carbenoxolone on voltage-gated calcium channels have been described for isolated amphibian cone photoreceptors (Vessey et al. 2004). The direct action of carbenoxolone on light-evoked Ca^{2+} responses in ipRGCs would have led Sekaran et al. (2003, 2005) to greatly overestimate the extent of coupling between ipRGCs and other cells in the ganglion cell layer of the retina. This is not to say that ipRGCs do not form electrical synapses with other cells. Neurobiotin tracer injections into individual ipRGCs revealed coupling to an average of ≈ 8 widefield GABAergic amacrine cells located in the ganglion cell layer; no homologous tracer coupling to ipRGCs or heterologous coupling to other ganglion cells was observed (Müller et al. 2010). Neither the connexins that mediate coupling between ipRGCs and GABAergic amacrine cells nor the function of this electrical coupling between ipRGCs and displaced amacrine cells is currently known.

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 soma size, the level of dendritic stratification in the inner plexiform layer (IPL), the extent of the dendritic field and the complexity of dendritic branching, conventional ganglion cells have been grouped into many types or clusters (Rockhill et al. 2002; Kong et al. 2005). 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). ON cells have their dendritic processes confined to the lower part of the IPL, the ON stratum, where they typically receive input from ON bipolar cells; OFF cells have their dendrites limited to the upper stratum of the IPL, the OFF sublayer, and receive input from OFF bipolar cells. Ganglion cells with bistratified dendritic arborizations in both the lower and upper layers of the IPL are ON-OFF cells (Famiglietti and Kolb 1976; Nelson et al. 1978).

In their original report of SCN-projecting ipRGCs in the rat, Berson et al. (2002) described these cells as sparsely branching and stratifying almost exclusively in the OFF sublayer of the IPL, near the border of the inner nuclear layer (INL). In the primate retina, two types of monostratified ipRGCs were described that send their dendrites to either the inner or the outer sublayers of the IPL although both types generated sustained ON responses to light stimulation recorded in vitro (Dacey et al. 2005). It was very unusual to find ganglion cells with dendrites in the OFF sublayer of the IPL generating ON responses to light (see below). When Baver et al. (2008) retrogradely labeled SCN-projecting RGCs in the tau-lacZ reporter mouse and examined cells for melanopsin, they found that the vast majority (80%) of SCN-projecting ipRGCs deployed their dendrites to the OFF sublamina of the IPL near the INL border and these cells were termed M1 cells. The remainder of ipRGCs projecting to the SCN sent their dendrites to the ON sublayer of the IPL; these cells were termed M2 (Baver et al. 2008). These two plexuses of melanopsin dendrites in the inner and outer IPL of the mouse had been described previously (Provencio et al. 2002). In addition, the melanopsin immunostaining of M1 ipRGCs usually appeared more intense than the staining of the M2 cells suggesting that M1 ipRGCs expressed more melanopsin protein.

M1 and M2 ipRGCs also project to the OPN in approximately equal proportions, but they terminate in different regions of the OPN; M1 ipRGCs innervate the outer shell region of the OPN where projection neurons that innervate the pre-autonomic Edinger-Westphal nucleus reside (Baver et al. 2008). M2 ipRGCs send their axons to innervate the OPN central core (Baver et al. 2008; Ecker et al. 2010). In addition to sending their dendrites to different regions of the IPL, M2 ipRGCs are generally considered to have a more complex dendritic arborization pattern and are approximately one log unit less sensitive to light compared to M1 cells (Schmidt et al. 2008; Schmidt and Kofuji 2009; Schmidt et al. 2011). The lower intrinsic sensitivity to light would be consistent with the suggested lower level of

melanopsin expression in these cells (Baver et al. 2008). Although M2 ipRGCs may generate a smaller intrinsic response to light, the rod/cone mediated synaptic input plays a greater role in shaping the integrated light-evoked responses and the resting membrane properties of M2 cells than M1 cells (Schmidt and Kofuji 2010).

Both M1 and M2 ipRGCs receive excitatory input via the ON bipolar pathway (Pickard et al. 2009; Schmidt and Kofuji 2010). The anatomical basis for this very unusual ON physiological input to M1 ipRGCs with dendrites in the OFF sublayer of the IPL appears to be mediated by ectopic synapses onto M1 ipRGCs from ON-bipolar cells as their axons pass through the OFF layer of the IPL (Fig. 1) (Dumitrescu et al. 2009; Hoshi et al. 2009). This arrangement has been described as an accessory ON sublayer in the outer IPL (Dumitrescu et al. 2009). In addition to the different morphological and physiological characteristics between M1 and M2 ipRGCs described above, very recently it has been reported that M1 cells can be further discriminated into two subgroups based on a molecular marker, the transcription factor *Brn3b*, with *Brn3b*-negative M1 cells projecting to the SCN and *Brn3b*-positive cells innervating the shell of the OPN (Chen et al. 2011). It remains to be determined if there are also physiological differences between these subgroups of M1 ipRGCs.

In addition to M1 and M2 ipRGCs, an M3 ipRGC has been described with dendrites located in both the ON and OFF sublayers of the IPL (Warren et al. 2003; Viney et al. 2007; Schmidt et al. 2008). M3 ipRGCs form a morphologically heterogeneous population with physiological properties similar to those of M2 cells (Schmidt and Kofuji 2011). It remains unclear whether M3 cells represent a distinct ipRGC subtype (Berson et al. 2010). Using a Cre/loxP system, two additional subtypes of ipRGC have been reported; both cell types (M4 and M5) have dendrites that stratify in the ON sublayer of the IPL (Ecker et al. 2010). The morphology of M4 and M5 cells are distinct from the other ipRGCs and although they display a very weak intrinsic light response, no melanopsin can be detected in these cells using immunocytochemical techniques (Ecker et al. 2010). The central targets of M3 cells are unknown and it has been suggested that M4 and M5 cells project to the dLGN, superior colliculus and OPN core (see Schmidt et al. 2011). The specific physiological roles played by ipRGC subtypes remains to be determined and it is unclear what role the extremely weak intrinsic light response of M4 and M5 ipRGCs plays in the integrated light-evoked response of these cells. It is interesting to note that in the tiger salamander retina, all ipRGCs are ON ganglion cells and all ON RGCs appear to be intrinsically photosensitive (Rajaraman 2012).

ipRGC Input to the Ventrolateral Preoptic Nucleus

A series of papers reported 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 are active during sleep (Gaus et al. 2002). It was previously established that the VLPO receives ipRGC input (Gooley et al. 2003; Hattar et al. 2006) and it was well known that 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 neurons in the VLPO and induces sleep. These effects are still present, albeit reduced, in melanopsin knockout mice, indicating that rods and cones also participate in the effects of light on sleep (Altimus et al. 2008; Lupi et al. 2008; Tsai et al. 2009) although a recent report indicates that some melanopsin KO mice exhibit light-induced photosomnolence whereas other do not (Morin and Studholme 2011). The specific subtype of ipRGC innervating the VLPO is currently unknown.

ipRGC Input to the SCN and Seasonal Affective Disorder

The SCN receives a dense serotonergic input that arises from the mesencephalic median raphe nucleus. Selective destruction of serotonergic input to the SCN amplifies circadian behavioral responses to light (Smale et al. 1990; Morin and Blanchard 1991). One site of serotonin's action in the SCN is at 5-HT_{1B} receptors on ipRGC terminals; activation of these presynaptic inhibitory receptors modulates the response of the SCN to photic input (Pickard et al. 1996, 1999). Moreover, 5-HT_{1B} receptor knockout mice maintained under short-day (winter-like) conditions exhibit a delayed phase relationship to the day/night cycle (Sollars et al. 2006) resembling the phase delay demonstrated by people suffering from recurrent winter depression or seasonal affective disorder (SAD) (Lewy et al. 1987; Terman and Terman 2005). Recently Provencio and colleagues described a missense variant of the melanopsin gene in SAD patients (Roeklein et al. 2009). It is likely that many factors contribute to the etiology of SAD, and these may well include reduced sensitivity to light that may result from abnormalities in phototransduction in ipRGCs (Roeklein et al. 2009) and/or abnormalities in 5-HT neurotransmission in the SCN (Sollars et al. 2006).

Alterations in the phase angle 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; a blunted cortisol rhythm has been reported in SAD patients (Avery et al. 1997). The reduction in corticosterone secretion may result from an altered phase relationship between the adrenal gland's innate circadian rhythm in steroid biosynthesis to the day/night cycle (Oster et al. 2006; Son et al. 2008). Although it is widely accepted that photic input to the hypothalamus is mediated via the RHT to the SCN (see Ishida et al. 2005), it is possible that ipRGC projections to the hypothalamus caudal to the SCN such as the vSPZ (Pickard and Silverman 1981; Gooley et al. 2003; Hattar et al. 2006) may provide direct photic input to hypothalamic neurons that regulate the autonomic outflow to the major organs of the body. Corticosterone is a potent transcriptional regulator and the daily rhythm of corticosterone secretion affects rhythmic gene expression in the brain and many organs of the body (Balsalobre et al. 2000; Lamont et al. 2005). It will be interesting to determine if ipRGCs send photic signals to hypothalamic regions outside the SCN to regulate descending autonomic

circuits that entrain the adrenal clock and thereby contribute to the regulation of corticosterone secretion.

ipRGCs and Retinal Disease

It has been known for several decades that monosodium-L-glutamate (MSG) administered during the neonatal period selectively destroys amacrine, bipolar and ganglion cells in the inner retina resulting in atrophy of the optic nerves and impaired vision in rodents, although the rod and cone photoreceptors are unaffected (Olney 1969). Nemeroff et al. (1977) reported that MSG-treated rats with severe optic nerve atrophy nevertheless showed normal light-induced reduction in serotonin-N-acetyl-transferase activity in the pineal. This result indicated that despite the optic nerve atrophy, sufficient photic signals reached the SCN (or other hypothalamic nuclei) to regulate the well-known descending autonomic circuits to the pineal. Pickard et al. (1982) subsequently examined photic entrainment of circadian activity rhythms and the central projections of the surviving RGCs in MSG-treated golden hamsters and showed that the RHT was affected very little although the primary visual pathways to the thalamus were markedly reduced. These observations suggested that the RGCs that innervated the SCN were resistant to glutamate-induced degeneration (Pickard et al. 1982). It was later estimated that $\approx 90\%$ of all RGCs were destroyed by MSG treatment whereas retinal input to the SCN was reduced by only $\approx 30\%$ (Chambille and Serviere 1993). Among the small subpopulation of RGCs in which the immediate-early gene *c-fos* can be induced by light, only about one third are affected by MSG treatment, whereas $>90\%$ of RGCs in which light does not evoke Fos are destroyed by MSG (Chambille 1998). The vast majority of RGCs expressing Fos after light stimulation project to the SCN (Hannibal et al. 2007) and these would appear to be M1 ipRGCs (Pickard et al. 2009). Collectively these data suggest that a major portion of the RGCs that innervate the SCN survive neonatal glutamate-induced toxicity.

The mechanism(s) whereby M1 ipRGCs might be protected in vivo against glutamate toxicity are unknown although the response of ipRGCs to glutamate application in vitro appears to be more heterogeneous (Pickard, Hartwick and Sollars, unpublished observations) than that described for conventional RGCs in culture (Hartwick et al. 2008). The diverse response of ipRGCs to glutamate application most likely results from the heterogeneous expression of glutamate receptors and their subunits among ipRGCs although little is currently known about the glutamate receptor subtypes expressed by different ipRGCs (Jakobs et al. 2007). Similarly, the responses of cultured ipRGCs to the inhibitory neurotransmitter glycine are not homogenous (Wiles et al. 2011) giving further evidence that different ipRGC subtypes may have distinct neurotransmitter receptor profiles.

Glaucoma, characterized by RGC degeneration and damage to the optic nerves, is a leading cause of blindness worldwide. Several different animal models of glaucoma have been investigated to determine if melanopsin RGCs are injury-resistant.

Melanopsin-expressing RGCs were found to be selectively spared compared to conventional RGCs in a rat model of chronic ocular hypertension induced by laser photocoagulation of the episcleral and limbal veins of the eye with resultant elevated intraocular pressure (IOP) (Li et al. 2006, 2008). In a different rat model of episcleral vein cauterization, melanopsin-expressing RGCs were not apparently spared (Drouyer et al. 2008; Wang et al. 2008) and functional deficits in irradiance responses were reported (Drouyer et al. 2008). In a rat glaucoma model of chronic ocular hypertension induced by weekly injections of chondroitin sulfate into the anterior chamber, melanopsin-expressing RGCs were also found to be equally susceptible to the deleterious effects of ocular hypertension and functional deficits in irradiance responses were also described (de Zavalía et al. 2011). In a model of inherited glaucoma (mouse strain DBA/2J) in which IOP is elevated, melanopsin-expressing RGCs are not selectively spared (Jakobs et al. 2005). Patients with early-stage glaucoma show no deficits in ipRGC function determined by the postillumination pupil response whereas in people with advanced glaucoma, ipRGC function was reduced (Feigl et al. 2011). Conversely, axotomized melanopsin-expressing RGCs show enhanced survival compared to conventional RGCs in the mouse (Robinson and Madison 2004). Melanopsin-expressing RGCs were also substantially unaffected in patients with genetically determined neurodegenerative optic neuropathies that selectively affect RGCs due to mitochondrial dysfunction (i.e., Leber hereditary optic neuropathy and dominant optic atrophy) (La Morgia et al. 2010). Taken together, the data suggest that melanopsin-expressing ipRGCs are relatively resistant to several pathological situations although the mechanism(s) that protect the cells under these particular conditions are unknown.

The Future

Since the discovery of ipRGCs less than a decade ago, a remarkable amount of information has been gathered to document the idiosyncrasies of these neuronal photoreceptors and the diverse array of connections and apparent functions these cells subservise. 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. The development of novel drugs that selectively inhibit melanopsin phototransduction will help answer some of these questions. Novel applications of the melanopsin phototransduction mechanism in cell lines, such as light-inducible transcription for drug delivery (Ye et al. 2011) are sure to become more numerous. Still, as the mere existence of ipRGCs eluded notice during the first century and a half of active retinal research, it is a near certainty that future research on the structure and function of ipRGCs will shed unexpected new light on most currently held notions of retinal organization.

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