Published in final edited form as:

Prog Retin Eye Res. 2012 July ; 31(4): 287–302. doi:10.1016/j.preteyeres.2012.03.003.

DIVERSE TYPES OF GANGLION CELL PHOTORECEPTORS IN THE MAMMALIAN RETINA

Andrea Sand¹, Tiffany M. Schmidt², and Paulo Kofuji¹

¹Department of Neuroscience, University of Minnesota, Minneapolis, MN

²Biology Department, The Johns Hopkins University, Baltimore, MD

Abstract

Photoreceptors carry out the first step in vision by capturing light and transducing it into electrical signals. Rod and cone photoreceptors efficiently translate photon capture into electrical signals by light activation of opsin-type photopigments. Until recently, the central dogma was that, for mammals, all phototransduction occurred in rods and cones. However, the recent discovery of a novel photoreceptor type in the inner retina has fundamentally challenged this view. These retinal ganglion cells are intrinsically photosensitive and mediate a broad range of physiological responses such as photoentrainment of the circadian clock, light regulation of sleep, pupillary light reflex, and light suppression of melatonin secretion. Intrinsically photosensitive retinal ganglion cells express melanopsin, a novel opsin-based signaling mechanism reminiscent of that found in invertebrate rhabdomic photoreceptors. Melanopsin-expressing retinal ganglion cells convey environmental irradiance information directly to brain centers such as the hypothalamus, preoptic nucleus, and lateral geniculate nucleus. Initial studies suggested that these melanopsin-expressing photoreceptors were an anatomically and functionally homogeneous population. However, over the past decade or so, it has become apparent that these photoreceptors are distinguishable as individual subtypes on the basis of their morphology, molecular markers, functional properties, and efferent projections. These results have provided a novel classification scheme with five melanopsin photoreceptor subtypes in the mammalian retina, each presumably with differential input and output properties. In this review, we summarize the evidence for the structural and functional diversity of melanopsin photoreceptor subtypes and current controversies in the field.

Keywords

Melanopsin; photoreceptors; retina; vision; circadian entrainment; pupillary light reflex

1. INTRODUCTION

Retinal ganglion cells (RGCs) in the mammalian retina comprise at least 10–15 types that are classified according to common structural and functional features (Masland, 2011; Wässle, 2004). The most recently discovered RGC type is the intrinsically photosensitive retinal ganglion cell (ipRGC). These ipRGCs, in addition to acting as a conventional RGC,

© 2012 Elsevier Ltd. All rights reserved

Address for correspondence: Paulo Kofuji Department of Neuroscience, University of Minnesota 6-145 Jackson Hall 321 Church St SE Minneapolis, MN 55455 Phone: 612-625-6457 FAX: 612-626-5009 kofuj001@umn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

are also bona fide photoreceptors that express the photopigment melanopsin (Opn4), which renders these cells directly sensitive to light. Melanopsin shares a higher degree of amino acid sequence homology with invertebrate opsins than vertebrate opsins. Unlike the conventional rod and cone photoreceptors of the mammalian retina, ipRGCs express the melanopsin photopigment diffusely along the dendrites and soma of the cell, and translate photon capture into spike frequency changes. This allows ipRGCs to directly convey ambient irradiance signals to higher brain centers. Genetic and immunological cell ablation experiments in mice have conclusively demonstrated that ipRGCs are obligatory for so called non-image-forming (NIF) visual responses including circadian photoentrainment, pupillary light reflex, and light suppression of locomotor activity. ipRGCs have also been implicated in light exacerbation of migraine, photophobias, seasonal affective disorders, and more recently in pattern vision. Advances have been made in the past few years indicating an unforeseen diversity in the structure and function of these novel photoreceptors. Physiology, molecular biology, and behavioral studies have revealed that distinct ipRGC subtypes in mammals respond differentially to light stimulation, project to distinct higher order visual centers, and possibly evoke diverse behaviors. There are three subpopulations of ipRGC based on the stratification of their dendrites in the inner plexiform layer (IPL), those monostратified in the OFF sublamina (M1), those monostратified in the ON sublamina (M2), and those bistratified in both the ON and OFF sublamina (M3)(Schmidt et al., 2008; Viney et al., 2007; Warren et al., 2003). Moreover, two additional subpopulations of ipRGCs (M4 and M5) with particularly low levels of melanopsin expression and dendrites in the ON sublamina of the IPL have recently been uncovered using novel melanopsin reporter mouse lines (Ecker et al., 2010). Many excellent recent reviews have emphasized the mechanisms of phototransduction in ipRGCs and role of these cells in physiology and disease (Bailes and Lucas, 2010; Do and Yau, 2010; Guler et al., 2007; Hankins et al., 2008; Hatori and Panda, 2010; Pickard and Sollars, 2010, 2011; Schmidt et al., 2011). Our review emphasizes the identification of ipRGC subtypes in the mammalian retina and highlights the current knowledge of the morphological and functional diversity of these novel photoreceptors.

1.1 Behavioral responses to light

Organisms have evolved complex mechanisms for detection and adaptation to ambient light levels. In mammals all light detection is performed by two visual pathways, the image-forming and the NIF systems. In contrast to the image-forming visual system, which processes photic signals related to color, contrast and motion, the NIF visual system is primarily engaged in detection of ambient luminance levels. NIF visual responses include the regulation of the circadian clock, regulation of pupil size, and pineal melatonin secretion. One well characterized NIF visual response is the light regulation of internal circadian clock. All living organisms harbor an internal circadian timing system that adjusts many physiological variables to daily environmental light and dark cycles (Panda et al., 2002a; Takahashi et al., 2008). In mammals, circadian behavior is regulated by a neuronal oscillatory network located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Reppert and Weaver, 2002). Although the SCN intrinsic rhythm exhibits near 24 hour periodicity (circadian), in the absence of environmental cues it gradually becomes out of phase with the external light-dark cycles. In mammals, this circadian rhythm is primarily adjusted (or entrained) by environmental light-dark cycles. Photoentrainment signals travel through a direct pathway from the retina to the SCN, the retinohypothalamic tract (Hendrickson, 1972; Moore and Lenn, 1972; Moore et al., 1995; Pickard and Silverman, 1981). This photoentrainment is driven by a visual stimulus quite distinct from that conveyed by the conventional visual pathways in that it is relatively insensitive to brief light stimulation or to low light levels (Nelson and Takahashi, 1991). During the photoentrainment process, light depolarizes RGCs, which in turn release glutamate and

pituitary adenylate cyclase-activating polypeptide from their axon terminals (Hannibal, 2006). Bilateral enucleation and retinohypothalamic tract lesion studies in rodents have shown that electrical signals transmitted by RGC axons to the SCN are absolutely necessary for circadian photoentrainment (Johnson et al., 1988; Nelson and Zucker, 1981).

Another well-established NIF visual response is the pupillary light reflex (PLR) which regulates the amount of light that reaches the retina across varying levels of environmental illumination. The PLR is the constriction of the sphincter pupillae muscles of the iris in response to an increase in ambient luminance. This light response involves a bilateral projection from the retina to the olivary pretectal nucleus (OPN) which in turn sends fibers to the Edinger-Westphal nucleus. The Edinger-Westphal nucleus contains the autonomic neurons that control pupil size. Furthermore, an autonomous, non-neural PLR component driven by melanopsin phototransduction in the iris muscles of nocturnal rodents has recently been described (Xue et al., 2011). In addition to its roles in photoentrainment and the PLR, light also suppresses pineal melatonin synthesis and secretion in mammals (Lewy et al., 1980; Wurtman et al., 1963), suppresses sleep in diurnal animals and enhances sleep in nocturnal animals (Borbely, 1978; Saper et al., 2005), reduces locomotor activity in nocturnal animals (Borbely, 1978), and enhances activity in diurnal mammals (Redlin, 2001). Finally, NIF visual responses may also be involved in light exacerbation of migraine pain (Nosedá et al.) and photophobias (La Morgia et al., 2011).

1.2 From frog melanophores to melanopsin

Studies of NIF visual responses in mice with severe loss of rod/cone function provided the initial evidence for a third photoreceptor type in the inner retina. Mice were described in the early 1920's with an autosomal recessive mutation leading to “the absence of visual cells (rods), the external nuclear layer, and the external molecular layer” (Keeler, 1924) (Figure 1). Surprisingly, these mice still exhibited PLRs (Keeler, 1927) even in the absence of electroretinogram responses (Keeler et al., 1928). Later, several groups showed that mice homozygous for the *rd* mutation, in which rods and cones degenerate, efficiently phototentrain to light pulses (Ebihara and Tsuji, 1980; Foster et al., 1991). These light-evoked responses in the absence of rods and cones suggested the presence of a novel photoreceptor type unaffected by the *rd* mutation (Foster et al., 1991). An alternate hypothesis was that a small cone photoreceptor population remained in adult *rd/rd* retinas, sufficient to sustain circadian photoentrainment (Foster et al., 1991). However, this hypothesis was later dispelled when it was discovered that circadian photoentrainment was also preserved in *rd/rd* mice in which cone photoreceptors were genetically ablated (*rd/rd cl*) (Freedman et al., 1999). Additionally other NIF visual responses, such as PLR and suppression of melatonin secretion were also retained in the *rd/rd cl* mice (Lucas et al., 2001; Lucas et al., 1999) (Figure 1). Finally, the most compelling evidence for the existence of an additional photoreceptor type in the retina was gathered by measuring the action spectrum for PLRs in *rd/rd cl* mice. The spectral sensitivity of light stimuli for the PLR in *rd/rd cl* mice with a maximum near 480 nm was clearly distinct from that predicted for murine rod (498 nm) and cone opsins (306 nm and 508 nm) (Lucas et al., 2001). NIF visual responses independent of rod and cone photoreceptors were also observed in blind patients. Some patients with severe retinal disease and conscious light perception still exhibited light-induced suppression of melatonin secretion similar to normal subjects (Czeisler et al., 1995). Though not conclusive, these findings suggested a photoreceptive system within the inner retina that relied on a novel photopigment.

The discovery of this novel photopigment came, not from studies in the retina, but from studies on amphibian melanophores. Unlike mammals, many non-mammalian vertebrate and invertebrate species have photosensitive cells in locations outside of the eye. In the skin of several amphibians and fish, photopigments respond to light by dispersing or aggregating

intracellular pigment granules (Oshima, 2001). These melanophores are not unlike photoreceptors in the retina as they display intrinsic photosensitivity with opsin-like spectral properties. Provencio and colleagues screened a *Xenopus* melanophore cDNA library for sequences closely related to rhodopsin and violet opsin (Provencio et al., 1998). Their reasoning was that the light sensitive photopigment in melanophores would harbor a considerable degree of sequence similarity to these known opsins and therefore be amenable to identification upon low stringency screening. Indeed, a photopigment with about 30% amino acid homology to vertebrate opsins was isolated and *in situ* hybridization showed its expression in *Xenopus* melanophores (Provencio et al., 1998). Like other visual pigments, this novel photopigment, termed melanopsin (Opn4), had a predicted topology of seven transmembrane domains and a lysine residue in the seventh transmembrane domain that presumably serves as the site for the Schiff base linkage with the chromophore (see section 2.2). Unexpectedly, sequence similarity analysis showed that melanopsin was more closely related to invertebrate opsins than the typical vertebrate rod or cone opsins. The deduced amino acid sequence of melanopsin shared 39% identity with *Octopus* rhodopsin and only 30% identity with other vertebrate opsins.

Invertebrate opsins are structurally and functionally dissimilar to vertebrate opsins as the chromophore is retained following photoactivation and the interacting G-proteins couple to the phospholipase C pathway (Fain et al., 2010; Provencio et al., 2000). Also surprising was that melanopsin transcripts in *Xenopus* were found not only in dermal melanophores, but also in deep brain structures such as the SCN and ocular sites such as the iris, retinal pigment epithelium, and the inner retina (Provencio et al., 1998). Melanopsin expression in deep brain structures and in the eye led to the suggestion (which turned out to be correct) that this novel photopigment regulates circadian rhythms and PLR (Provencio et al., 1998).

1.3. From melanopsin to NIF visual responses

The molecular cloning of the *Xenopus* melanopsin gene quickly paved the way to the cloning of its orthologs in mammals (Provencio et al., 2000). As in non-mammalian species, the mammalian melanopsin primary sequence was more similar to invertebrate opsins than to other known vertebrate opsins. *In situ* hybridization studies showed that in primate and murine retinas the melanopsin mRNA was restricted to a small subset of neurons in the ganglion cell layer (GCL) (Provencio et al., 2000). This confined expression of melanopsin in the inner retina suggested to these investigators that these cells could harbor intrinsic photosensitivity and perhaps send photoentrainment signals to the SCN (Provencio et al., 2000).

Support to this notion was gathered from the observation that RGCs projecting to the SCN, identified by neuronal retrograde tracing, did indeed express melanopsin (Gooley et al., 2001) and pituitary adenylate cyclase-activating polypeptide (Hannibal et al., 2002). This was then convincingly demonstrated using genetic approaches to visualize axons from RGCs expressing melanopsin. Hattar and colleagues generated mice in which the coding region in the melanopsin gene locus was replaced with a construct encoding tau microtubule-associated protein and β -galactosidase (Hattar et al., 2002). This allowed for direct visualization of the axons of melanopsin-expressing ganglion cells in the mouse brain by histochemical staining for β -galactosidase. Axonal projections from melanopsin expressing RGCs indeed terminate in the SCN and in other brain centers associated with NIF functions (Hattar et al., 2002).

The Berson group provided the breakthrough and conclusive evidence showing that RGCs innervating the SCN are bona fide photoreceptors (Berson et al., 2002). They identified these cells in the retina by introduction of fluorescent microspheres into the rat SCN which retrogradely labeled SCN-projecting RGCs. These investigators then recorded light-evoked

electrophysiological responses from labeled RGCs (Berson et al., 2002). The labeled RGCs responded to light stimulation independently of rods and cones as light responses persisted even upon blockade of synaptic communication with cobalt, which blocks calcium-mediated synaptic release from rods, cones, and other retinal neurons. These intrinsic light responses were rather distinct from those recorded previously in rod and cone photoreceptors. First, light-evoked responses were depolarizing with superimposed fast action potentials, which contrasts with the graded hyperpolarizing responses of rods and cones. Second, the kinetics of light-evoked responses was relatively slow with a time scale of seconds for activation and deactivation, one order of magnitude slower than that observed in rods and cones. Response latency was inversely related to the light stimulus with dim light taking many seconds to evoke a response. Third, the spectral sensitivity of the photoresponses fit an opsin nomogram with peak sensitivity at ~ 480 nm, which was distinct from that predicted for rods and cones, but in excellent agreement for the spectral sensitivity measured for PLR responses in the *rd/rd cl* mice (Lucas et al., 2001). These findings corroborated a novel photoreceptor type in the mammalian inner retina and suggested melanopsin as the photopigment. Another notable feature of the light sensitive RGCs were their large dendritic arbors (~500 μm) with relatively sparse branching in the outer lamina of the IPL. Soon after, the demonstration that the melanopsin underlies that intrinsic photosensitivity of SCN-projecting RGCs was provided by recording from melanopsin knockout mice. In these mice, RGCs innervating the SCN were no longer intrinsically photosensitive although they were morphologically indistinguishable from those recorded in wild-type animals (Lucas et al., 2003).

Paradoxically, several groups found that the melanopsin knockout mice still entrained to light/dark cycles, phase-shifted following light pulses (Panda et al., 2002b; Ruby et al., 2002), and exhibited PLRs (Lucas et al., 2003). However, these NIF visual responses were completely ablated in mice lacking melanopsin and functional rods and cones (Hattar et al., 2003; Panda et al., 2003). These results implied that, just as melanopsin was sufficient to drive NIF visual responses in the absence of classical photoreceptors, the classical outer retina photoreceptors were sufficient to support various NIF visual responses in the absence of melanopsin. Questions remained about how rod/cone signals are relayed to NIF visual centers, either via melanopsin-expressing RGCs or in parallel through other RGCs to mediate these behaviors.

This issue was finally settled by an elegant series of experiments in which ipRGCs were selectively ablated by genetic or immunological means (Goz et al., 2008; Guler et al., 2008; Hatori et al., 2008). Genetic ablation of ipRGCs was achieved by expression of either diphtheria toxin in ipRGCs (Guler et al., 2008) or the diphtheria toxin receptor in these cells followed by diphtheria toxin administration to the mice (Hatori et al., 2008). These ipRGC-ablated mice lacked NIF responses such as circadian photoentrainment or PLR (Guler et al., 2008; Hatori et al., 2008). Targeted ablation of ipRGCs using immunotoxins in the adult mouse also largely rendered the animals non-responsive to light-dark cycles (Goz et al., 2008). However, ablation of ipRGCs did not affect image-forming functions such as optokinetic nystagmus responses, the ability to detect visual cues, or the electroretinograms (Guler et al., 2008; Hatori et al., 2008). Some controversy remains whether regular RGCs also innervate NIF nuclei and regulate NIF visual responses in other species. At least in the rat (Gooley et al., 2003) or in the golden hamster (Morin et al., 2003; Sollars et al., 2003) 10–20% of RGCs innervating the SCN have been reported to be non-melanopsin expressing, while in the mouse almost all RGCs innervating the SCN seemed to express melanopsin (Baver et al., 2008). Thus, at least in the mouse, it appears that both rod/cone and melanopsin signals are relayed to NIF nuclei solely through ipRGCs.

2. Intrinsic phototransduction of ipRGCs

The discovery of this novel photopigment in the mammalian retina raised questions as to whether melanopsin uses an invertebrate-like (rhabdomeric) or vertebrate-like (ciliary) phototransduction signaling cascade. Photoreceptors are usually distinguished by whether the photosensitive membranes are derived from a modified cilium or from microvillar projections (rhabdom) (Lamb et al., 2007; Yau and Hardie, 2009). Ciliary and rhabdomeric photoreceptors are not only morphologically but also functionally dissimilar. Ciliary photoreceptors hyperpolarize to light, whereas rhabdomeric photoreceptors depolarize. Each photoreceptor type employs a distinct complement of G-proteins, signaling enzymes, and ion channels to evoke such photoresponses. Because melanopsin expression is restricted to relatively few cells in the inner retina, knowledge about the melanopsin phototransduction has been rather limited.

2.1 Melanopsin coupling to G-proteins and transducer channels

The prevailing hypothesis is that ipRGCs possess phototransduction machinery more closely related to that found in invertebrate rhabdomeric photoreceptors (Do and Yau, 2010; Hankins et al., 2008)(Figure 2). Phototransduction in mammalian ciliary photoreceptors (rods and cones) proceeds via activation of G-protein transducins, closure of cyclic nucleotide-gated channels, and membrane hyperpolarization (Peirson and Foster, 2006; Yau and Hardie, 2009). ipRGCs, on the other hand, depolarize upon light activation (Berson et al., 2002) and photocurrents exhibit a transient receptor potential (TRP) channel-like pharmacology (Warren et al., 2006), all features found in many rhabdomeric photoreceptors. The invertebrate-like melanopsin phototransduction model was first substantiated in heterologous expression studies. Ectopic expression of the photopigment melanopsin and TRPC3 channel in cell lines or in *Xenopus* oocytes conferred intrinsic photosensitivity to these cells (Panda et al., 2005; Qiu et al., 2005). Likewise, heterologous expression of melanopsin in a mouse neuroblastoma cell line was sufficient to render these cells sensitive to light (Melyan et al., 2005). Light evoked responses resembled those observed in ipRGCs by their sluggish kinetics, sustained membrane depolarizations, and blue light spectral sensitivity (~480 nm). Furthermore, light-evoked responses were strongly attenuated by intracellular application of competitive blockers of the G-protein subunit Gq or by extracellular application of phospholipase C antagonists (Panda et al., 2005; Qiu et al., 2005). Collectively these findings suggested that melanopsin activates the Gq class of G-proteins followed by stimulation of phospholipase C which leads to the opening of cation-selective TRPC channels.

Recording of native ipRGCs in mammalian retinas has in large part supported this working model. For instance, as in heterologous expression studies, phospholipase C antagonists abolished ipRGC light responses (Graham et al., 2008). Pharmacological agents that block TRPC channels prevented light-evoked intracellular calcium increases or membrane depolarization in ipRGCs (Hartwick et al., 2007; Sekaran et al., 2007; Warren et al., 2006). Knockout mouse models of TRPC channels showed impaired melanopsin-evoked photoresponses. Our study showed a diminished ipRGC light response in TRPC6 knockout mice (Perez-Leighton et al., 2011). More recently, Xue and colleagues showed that genetic ablation of both TRPC6 and TRPC7 channels in mouse abolishes the M1 cell light responses. The same result was not observed in single knock-out mice for either TRPC6 or TRPC7, implying that the phototransduction channel is probably a heteromer of these two TRPC channel subunits (Xue et al., 2011). In the same report the requirement of the phospholipase C β 4 for M1 phototransduction was also demonstrated (Xue et al., 2011) (Figure 2).

Another recent report also suggests a potential role of transient receptor potential cation channel subfamily M (TRPM) channels in ipRGC phototransduction (Hughes et al., 2012). TRPM1 channels, also known as melastatin-related TRP channels, function as the transduction channels in ON bipolar retinal cells by coupling to the mGLUR6 signaling cascade and closing in response to receptor activation (Koike et al., 2010). TRPM1 knockout mice have impaired PLRs beyond that expected from ON bipolar cell deficits suggesting the involvement of TRPM1 channels in ipRGC phototransduction (Hughes et al., 2012). However recordings of ipRGCs in TRPM1 knockout mice were not performed so it is unclear whether the effects seen were at the retina level.

2.2. Is melanopsin a bistable photopigment?

Phototransduction is initiated in photoreceptors with the absorption of light by photopigments, which consists of an apoprotein (opsin) and a chromophore (11-cis-retinal), attached to the opsin by a Schiff base bond. The absorption of light by the photopigment causes isomerization of 11-cis retinal to all-trans retinal, which changes opsin's conformation. There is ample evidence that photopigments in invertebrate photoreceptors have two thermostable forms or states that are photointerconvertible in a wavelength-dependent manner (Hardie and Raghu, 2001; Hillman et al., 1983). This bistability confers their ability to regenerate 11-cis retinaldehyde without the need of exogenous isomerases. The stable photoproduct reverts to the dark state by subsequent light absorption of a longer wavelength. By contrast, photopigments expressed in vertebrate rods and cones have only one stable state and regeneration of 11-cis-retinaldehyde depends on accessory enzymes from the retinal pigment epithelium or neighboring glial cells (Wang and Kefalov, 2011). The bistability of melanopsin has been supported by a number of observations: 1) amphioxus melanopsin expressed in cell lines shows two states, one with peak absorption of 480 nm and another orange-shifted of 520 nm which could be photoconverted (Koyanagi et al., 2005); 2) lack of rundown of melanopsin-evoked responses without supplemental retinaldehyde for several hours in heterologous expression systems is consistent with melanopsin functioning without need of exogenous isomerases (Qiu et al., 2005); 3) red shifted light (620 nm) enhances PLRs and pre-exposure to 480 nm light diminishes the PLRs in humans suggesting photoconversion of melanopsin in vivo (Mure et al., 2007). On the other hand, potentiation of ipRGC photoresponses by red-light exposure was not observed in multielectrode array recording of murine retinas (Mawad and Van Gelder, 2008). Mice lacking RPE65, an enzyme in the retinal pigment epithelium required for regeneration of 11-cis retinaldehyde, also showed reduced ipRGC function assessed either by electrophysiological responses of single ipRGCs or behaviorally by measurements of PLRs (Fu et al., 2005). Furthermore, considerable photobleaching of photoresponses in native ipRGCs has also been reported (Do et al., 2009). Finally, in zebrafish multiple melanopsin isoforms have been described (Davies et al., 2011). In heterologous expression systems some isoforms displayed properties consistent with invertebrate-like bistability while other isoforms seemed monostable (Davies et al., 2011). Clearly, more research is needed to ascertain whether melanopsin photopigment regeneration results from photoconversion and/or an exogenous supply of 11-cis-retinaldehyde.

3. Diversity of morphology and molecular markers in ipRGCs

The quest to unravel the neuronal circuits that underlie visual processing in the retina has led to identification of multiple subtypes of neuronal cells that differ in structure and function (Masland, 2011). For RGCs, at least 10–15 subtypes can be identified based on several morphological features such as depth of dendritic arbor stratification in the IPL (ON versus OFF), extent of the dendritic field (narrow versus broad), and density of dendritic branching (bushy versus sparse) (Coombs et al., 2006). Recently molecular markers have also been used as additional means to separate RGC subtypes (Kay et al., 2011). Classification of

ipRGCs, initially considered as one subtype, now includes at least five subtypes of anatomically and molecularly diverse cells.

3.1. Anatomical diversity of ipRGCs: M1, M2, and M3 cells

Melanopsin-containing cells are relatively rare in primate and human retinas (Dacey et al., 2005; Hannibal et al., 2004; Ingham et al., 2009) as revealed by immunocytochemical methods. In the human retina the estimate is that ipRGCs constitute only 0.2% of the total RGC population (Dacey et al., 2005). Similarly, the number of melanopsin-expressing cells in the mouse has been estimated between 500 to 2400 per retina by immunocytochemistry (Berson et al., 2010; Lin et al., 2008; Robinson and Madison, 2004) and between 680 to 780 per retina in the tau-LacZ reporter melanopsin mouse (Hattar et al., 2002). Considering 50000–60000 RGCs in the mouse retina (Dräger and Olsen, 1980), it is estimated that ipRGCs constitute ~1–5% of the total RGC population.

Morphologically, ipRGCs are characterized by their small to medium soma size and very large dendritic fields. The ipRGC dendritic field size has been estimated to range from ~300 to 400 μm in the mouse (Berson et al., 2010; Schmidt and Kofuji, 2009) to ~500 to 600 μm in marmoset retinas and ~400 to 1200 μm in macaque retinas (Dacey et al., 2005). ipRGCs seem to be distributed in a random fashion in cat retinas (Semo et al., 2005) but are somewhat concentrated in the parafoveal regions of the primate retina (Dacey et al., 2005). Immunostaining for melanopsin in macaque retinas shows that ipRGCs are among the largest of any primate RGC type with their processes notably absent in the foveal regions (Figure 3).

Classical structure and function correlation studies have established the relationship between stratification of RGC dendrites within the IPL and the type of RGC light responses (Famiglietti and Kolb, 1976). RGCs with dendrites that stratify within the inner layers of the IPL are activated by light increments (ON RGCs). On the other hand, RGCs with dendrites that stratify in the outer layers of the IPL are inhibited by light (OFF RGCs). The first immunocytochemical studies performed in mouse retinas indicated that ipRGC dendrites branched out to both sublaminae of the IPL (Provencio et al., 2002). Later, ipRGCs were shown to extend dendrites mostly to the OFF sublamina of the IPL (Hattar et al., 2002). We have shown, by employing single cell filling with neuronal tracers in a mouse line in which melanopsin-expressing cells are labeled with EGFP, that three distinct subtypes of ipRGCs coexist in retinas (Figure 4) (Schmidt et al., 2008). The M1 type has dendrites that stratify in the OFF sublamina while the M2 type has dendrites that remain in the ON sublamina. A third subtype of ipRGCs is termed M3, or bistratified, as this cell has dendrites that extend in both sublaminae of the IPL (Figure 4). Similar diversity of ipRGCs was also described in mouse retinas using transsynaptic viral tracing methods as pseudorabies virus preferentially labels ipRGCs, at least during the first wave of infection (Viney et al., 2007). This diversity of ipRGCs in terms of dendritic stratification has also been substantiated in other rodent and primate retinas (Jusuf et al., 2007).

The relative proportion of each ipRGC subtype has been difficult to ascertain as immunostaining techniques tend to overestimate ipRGCs with higher melanopsin content while single cell filling tends to overrepresent cells with larger somas, as they are more accessible for microelectrode impalement. In postnatal P17–P24 mice, using single cell filling, we found that ~22% of cells were M1 type, ~52% of cells were M2 type and ~26% of cells were M3 type (Schmidt et al., 2008). Another group found a greater proportion of M1 (~68%) than M2 (~25%) or M3 (~7%) cells by similar methods (Muller et al., 2010). Equivalent proportion of M1 and M2 cells has been reported using immunocytochemical methods (Baver et al., 2008; Berson et al., 2010). The more unbiased method of labeling ipRGCs using viral tracing techniques yielded ~39% M1 cells, ~40% M2 cells, and ~21%

M3 cells in the adult mouse retina (Viney et al., 2007). Addressing the relative proportions of ipRGC subtypes will have to wait for other methods and similar studies have yet to be carried out in non-rodent models.

Another useful anatomical analysis of RGCs pertains to how their dendrites cover the entire retina. RGCs often extend their dendrites in a regular and territorial manner that minimizes the overlap of their dendritic fields, conferring a “tiling” pattern of their dendritic fields across the retina (Wassle, 2004). The spatial patterning of dendritic arbors is characteristic within particular RGC subtypes in the mammalian retina and it is quantified by the dendritic coverage factor (Cook and Chalupa, 2000; Vaney, 1994; Wassle and Riemann, 1978). The dendritic coverage factor estimates the mean value of how many dendritic fields overlap at a given point on the retina. Berson and colleagues estimated a coverage factor of 3.8 for M1 cells and 4.6 for M2 cells (Berson et al., 2010) indicating broad overlap of dendritic arbors for these cells (Figure 5). The relatively large size and broad overlap of ipRGC dendrites is consistent with their role in NIF functions where environmental light level, not pattern discrimination, is the most relevant sensory signal. In comparison, a RGC type optimized for visual spatial discrimination, such as the midget ganglion cell in the human retina, has a dendritic field size as small as 5 μm and coverage factor close to 1 (Dacey, 1993).

The M1–3 subtypes of ipRGCs have been principally investigated in the mouse retina and other more subtle morphological differences have emerged (Table 1). We observed by neurobiotin filling followed by confocal imaging that M2 cells have significantly larger dendritic field diameters as well as larger overall dendritic length and significantly larger soma diameter in comparison to M1 cells. The dendritic arbors of M2 cells seem more complex and more highly branched than those of M1 cells (Ecker et al., 2010; Muller et al., 2010; Schmidt and Kofuji, 2009). Less information is available for the bistratified M3 cells as they are difficult to separate from the OFF stratifying M1 cells using immunocytochemical procedures. Single cell filling of M3 cells, however, indicate that these cells are morphologically comparable to M2 cells in terms of dendritic field size, soma size, and complexity of their dendritic branching (Schmidt and Kofuji, 2011). However M3 cells have dendritic arbors both in the ON and OFF sublaminae of the IPL, but with surprising variability in the proportion of the arbors stratifying in the ON vs. the OFF sublamina. While some M3 cells have dendritic arbors almost exclusively in the OFF sublamina other M3 cells have dendrites that are mainly localized to the ON sublamina (Figure 6). Curiously, M3 cell dendrites seem to be absent in some areas of the retina (Berson et al., 2010), leaving substantial areas of visual field not “sampled” by these bistratified cells. M3 cells may not be considered a distinct ipRGC subtype given that characteristics defining RGC subtypes include the formation of regular mosaics and that dendritic trees cover the retinal area (Wassle, 2004). However complete coverage of the retina may not be a relevant criterion to define an ipRGC subtype as these cells are more likely to only be involved in irradiance detection rather than image detection (Schmidt and Kofuji, 2011).

An additional poorly described ipRGC subpopulation has cell bodies in the inner nuclear layer instead of the ganglion cell layer (Figure 4). These ipRGCs have their dendritic arbors exclusively in the OFF sublamina of the IPL and therefore have been considered as displaced M1 cells (Dumitrescu et al., 2009). Morphology in terms of dendritic branching and dendritic caliber also resemble regular M1 cells (Berson et al., 2010). Recordings from displaced M1 cells show intrinsic light responses similar to those recorded from regular M1 cells and also a transient ON excitatory response (Dumitrescu et al., 2009). Interestingly, displaced bistratified ipRGCs have also been reported in the mouse retina, and the physiology of these cells is completely unknown (Berson 2010). Extensive characterization

of the physiological properties and projection patterns of these cells has not yet been performed.

3.2. Additional diversity of ipRGCs: M4, M5, and beyond?

Separation of ipRGCs into three subtypes reminiscent of ON, OFF and ON-OFF RGC subtypes may be too simplistic. The use of binary systems to report melanopsin-expressing cells in the mouse has recently expanded the classification of ipRGC subtypes. This approach combines the use of a so-called “driver mouse line” and a “reporter mouse line”. In the driver line, the expression of a site-specific recombinase is placed under the control of a specific genetic locus. The expression of the recombinase is, therefore, restricted to the population of cells in which this locus is actively transcribed. In the reporter line, the site recombination induces the expression of a reporter protein. In this manner, the levels of the reporter protein are uncoupled from the levels of transcription in the targeted gene locus. Ecker and colleagues crossed driver mouse lines expressing Cre recombinase selectively in ipRGCs with reporter mouse lines in which Cre recombination mediates expression of alkaline phosphatase or Enhanced Green Fluorescent Protein (EGFP) (Ecker et al., 2010). Results with these novel reporter mouse lines revealed larger ipRGC numbers than in previous studies (Ecker et al., 2010). About 2000 RGCs showed expression of reporter gene, a value approximately two or three times higher than previously described using other methods of labeling such as immunostaining or expression of lacZ reporter gene (Hattar et al., 2002; Lin et al., 2008; Robinson and Madison, 2004). Part of this discrepancy was accounted for by the discovery of two additional types of ipRGCs that express very low levels of melanopsin. These two additional ipRGC types are termed M4 and M5. M4 cells are identified as large cells with dendritic arbors stratifying in the ON sublamina of the IPL. These cells display the large dendritic field diameters and larger overall dendritic length than the ON-stratifying M2 population, as revealed by tracer filling methods (Table 1). Morphologically, the M4 cells resemble conventional ON alpha RGCs or RGA1 cells (Ecker et al., 2010). M5 cells, on the other hand, have a much more compact dendritic arborization though these cells also have dendritic arbors restricted to the ON sublamina of the IPL. Presumably M4 and M5 cells express very low levels of melanopsin as neither of these two subtypes stain with antibodies raised against melanopsin. Electrophysiological recordings of M4 and M5 cells in the presence of synaptic blockers demonstrate that they are capable of weak intrinsic photoresponses (Ecker et al., 2010).

3.3. Melanopsin density of ipRGC subtypes

Photopigments in rod and cone photoreceptors are packed in their disk membranes at densities as high as $\sim 25,000/\mu\text{m}^2$ (Lamb and Pugh, 2006). High packing densities of photopigments in rods and cones endow these photoreceptors with their extraordinary high light sensitivity. The lack of comparable membrane structures in ipRGCs would suggest lower melanopsin densities, consistent with the lower light sensitivity of ipRGCs (Do et al., 2009). Immunostaining and melanopsin reporter mouse lines also indicate large variability in the levels of melanopsin expression among ipRGC subtypes. M1 cells have the highest levels of expression of melanopsin, M2 and M3 cells intermediate levels, and M4 and M5 cells have the lowest levels of expression (Ecker et al., 2010; Schmidt and Kofuji, 2009).

Measurements of elementary signals in ipRGCs provide a quantitative estimate of the melanopsin membrane densities. These elementary signaling events consist of the concerted opening of many channels and are believed to result from the absorption of a single photon by one molecule of melanopsin (Do et al., 2009). The density of melanopsin estimated by comparing macroscopic and single photon responses provides a pigment density of only $\sim 3/\mu\text{m}^2$ (Do et al., 2009), about 10000 fold lower than that of rod and cone photopigments. These measurements were performed in both dissociated and *in situ* ipRGCs identified by

their expression in the transgenic mice expressing the fluorescent protein tdTomato (Do et al., 2009). As noted, the expression of melanopsin varies among ipRGC subtypes and it is currently unclear whether such estimates are the average among various ipRGC subtypes or are more representative of the melanopsin densities in M1 cells, which are more easily identified in mouse reporter lines (Schmidt and Kofuji, 2009).

3.4. Molecular markers of ipRGC subtypes

Based on the studies summarized above, there is good consensus that ipRGCs are a relatively heterogeneous class of photoreceptors. There is not yet agreement on how many distinct subtypes exist. Investigation of genes differentially expressed among ipRGCs could enhance the understanding of their diversity and ontogeny. Included among known molecules expressed in different neuronal subtypes in the retina are: neurotransmitter receptors, intracellular signal transduction components, and transcription factors. Melanopsin itself, expressed as different isoforms, seems to discriminate ipRGC subtypes. The mouse melanopsin has one long isoform (Opn4L) and another short isoform (Opn4S) that originate from alternative splicing of a single melanopsin gene (Pires et al., 2009). The two variants encode predicted proteins of 521 and 466 amino acids and only differ in their C-terminal tails. Opn4L is predicted to contain a different number of phosphorylation sites than Opn4S. M1 and M3 cells seem to express both Opn4S and Opn4L isoforms while M2 cells express only Opn4L (Pires et al., 2009). Because heterologous expression of Opn4S and Opn4L in cell lines did not reveal any apparent functional differences (Pires et al., 2009), the significance of this diversity is unclear.

Combinations of transcription factors expressed at discrete developmental timepoints dictate the differentiation of the various retinal cell types. The transcription factor family Brn3 (Brn3a, Brn3b, and Brn3c) has a POU-domain and is implicated as a key regulator of RGC differentiation and survival (Gan et al., 1996). Double immunolabeling for Brn3 transcription factors and melanopsin shows that neither Brn3a nor Brn3c are expressed in ipRGCs of the adult mouse retinas. However, Brn3b is expressed in non-M1 cells and a subpopulation of M1 cells (Jain et al., 2011). A larger subpopulation of Brn3b positive (+) M1 cells is also seen using lineage tracing of Brn3b+ ipRGCs in the mouse retina, with just 200 M1 cells that are Brn3b negative (–) (Chen et al., 2011). We described a subpopulation (~10%) of M1 cells with physiological properties similar to those of M2 cells (Schmidt and Kofuji, 2010), also indicating diversity within the M1 population. It is currently unclear whether these physiologically “atypical” M1 cells correspond to the subpopulation of Brn3b + M1 cells. Interestingly, genetic ablation of Brn3b+ ipRGCs in mice leaves just 200 M1 cells in the retina, and results in an impaired PLR but intact circadian photoentrainment (Chen et al., 2011). This indicates that the subpopulation of M1 cells negative for the expression of Brn3b is sufficient for mediating photoentrainment in mice, but not the PLR, through specific projections to the SCN (Chen et al., 2011). Clearly these morphologically and physiologically defined subtypes may show additional diversity at the molecular level, suggesting that ipRGCs arising from distinct molecular lineages might be responsible for driving specific behaviors.

4. Diversity of functional properties in ipRGCs

The initial studies by the Berson group indicated that ipRGCs were able to directly translate ambient light levels into spike discharge (Berson et al., 2002). Those recordings were performed using a fluorescent retrograde tracer transported from the SCN to the ganglion cells, mainly labeling M1 cells (Baver et al., 2008). Thus, the initial recordings from ipRGCs were likely performed in M1 cells which display robust intrinsic photoresponses. The subsequent anatomical evidence that ipRGCs received synaptic inputs (Belenky et al., 2003) and were anatomically diverse, suggested some level of functional heterogeneity.

Recent research has yielded a growing understanding of the surprising diversity of ipRGC light response properties.

4.1. Diversity of intrinsic membrane properties in ipRGCs

Intrinsic membrane properties of neurons such as resting membrane potential, input resistance, and spike properties are established by expression of unique sets of voltage-dependent and -independent conductances. RGC subtypes as identified by their unique morphology in the cat retina show significant differences in their intrinsic membrane properties (O'Brien et al., 2002). We have investigated the overall intrinsic membrane properties of ipRGCs using whole cell patch clamp techniques in the mouse retina. Overall we found that M1 cells have higher input resistance (~710 MΩ) than M2 cells (~216 MΩ). Other noteworthy results revealed from this study were that M1 cells are considerably more depolarized in the dark (-48 mV) than M2 cells (-66 mV) and M1 cells attain lower maximal spiking rates in response to depolarizing current injection (~76 Hz) than M2 cells (~242 Hz)(Figure 7). The differences in input resistance between M1 and M2 cells may arise at least in part by their distinct morphology. M2 cells have larger somas and more complex dendritic arbors than M1 cells (Schmidt and Kofuji, 2009). Although not yet fully investigated, the differences in resting membrane potential in ipRGC subtypes may arise from the differential expression or modulation of membrane channels such as voltage-gated Na⁺ and K⁺ channels, Rat ipRGCs projecting to the suprachiasmatic nucleus (SCN) express a hyperpolarization-activated inwardly-rectifying current (I_h) (Van Hook and Berson, 2010). However I_h seems inactive at rest and thus it is unlikely to participate in setting the resting membrane potential in these cells. Because these studies were performed in ipRGCs labeled with tracers injected in the SCN they likely pertain only to M1 cells. It will be interesting to investigate the role of I_h in M2 cells since they have more depolarized membrane potential that favor the activation of I_h at rest.

M1 cells display high input resistance, and low threshold potential for spiking (Schmidt and Kofuji, 2009). These properties may facilitate the translation of synaptic or melanopsin-evoked signals as small currents should exert relatively large spike output changes. Indeed ipRGCs have an exquisite ability to translate melanopsin phototransduction into spike activity as evidenced by triggering of spike changes through melanopsin-evoked single photon currents (Do et al., 2009). Presumably the single photon responses recorded from ipRGCs were derived from M1 cells as they are more amenable to identification in mouse reporter lines (and cells with the brightest fluorescent signal were targeted). The non-M1 cells may be less efficient in terms of both melanopsin photon capture and melanopsin-evoked spike generation given their intrinsic membrane properties (low input resistance in particular) and apparently lower melanopsin content. We have recently recorded the electrophysiological properties of M3 cells and found that, despite their heterogeneity in dendritic arborization, these cells have uniform intrinsic membrane properties (Schmidt and Kofuji, 2011). In terms of resting membrane potential, input resistance, and spiking properties, the M3 cells resembled M2 cells (Schmidt and Kofuji, 2011).

4.2. Diversity of melanopsin-evoked light responses in ipRGCs

The first hint that melanopsin-evoked phototransduction in ipRGCs is not uniform came from population studies using either multi-electrode array or calcium imaging techniques (Sekaran et al., 2003; Tu et al., 2005). Both studies isolated intrinsic light responses from ipRGCs by bathing retinas with a cocktail of synaptic blockers to impede any influences from the outer retina. Multi electrode array recordings from early postnatal mouse retinas (P8) show light-evoked spike discharge resistant to blockade by a cocktail of glutamatergic and inhibitory synaptic blockers. These cells were presumably ipRGCs and showed a surprising heterogeneity in their photoresponses. These classes of ipRGCs were categorized

as type I with slow onset and sensitive photoresponses, type II with slow onset and relatively insensitive photoresponses and type III with rapid onset and sensitive photoresponses (Tu et al., 2005). Such heterogeneity of intrinsic light responses was replicated in adult mouse *rd/rd* retinas, with responses similar to both the type II and type III classes recorded early in development (Tu et al., 2005). Instead of spike activity, Sekaran and colleagues used elevations of intracellular calcium levels in the *rd/rd cl* retinas to identify ipRGCs (Sekaran et al., 2003). Using this clever approach, three types of light-evoked responses were observed: sustained, transient, and repetitive. Because in these studies the ipRGCs were not individually identified it was unclear whether these classes of ipRGC responses corresponded to the morphological subtypes of ipRGCs. We performed the first experiments investigating the correlation of morphological subtypes of ipRGCs to their intrinsic light responses (Schmidt and Kofuji, 2009). By recording intrinsic light responses in a transgenic mouse line in which EGFP is expressed selectively in ipRGCs, the intrinsic light responses of M1 and M2 cells were compared. M1 cells displayed larger melanopsin-evoked currents or membrane depolarization in comparison to M2 cells (Figure 8). Irradiance-response curves also showed that M1 cells were about 10 fold more sensitive to light than M2 cells (Figure 8). The larger and more sensitive intrinsic photoresponses in M1 cells in comparison to M2 cells seems to originate from differences in their intrinsic membrane properties and melanopsin content (Schmidt and Kofuji, 2009). The melanopsin content in M1 cells is higher than M2 cells as assessed either by anti-melanopsin immunostaining or by using an EGFP reporter line (Schmidt and Kofuji, 2009). On the other side of the spectrum are the newly discovered M4 and M5 cells that are undetectable using conventional immunocytochemical procedures but show weak intrinsic light responses (Ecker et al., 2010).

4.3. Diversity of synaptic-evoked light responses in ipRGCs

Morphological evidence for synaptic inputs to ipRGCs was first provided by immunoelectromicroscopy studies where terminals of bipolar and amacrine cells were shown to synapse onto melanopsin-immunoreactivity dendrites (Belenky et al., 2003). Contacts between PKC-immunopositive bipolar cells or dopaminergic amacrine cells and ipRGCs have been proposed in rat retinas as well (Ostergaard et al., 2007). In primate retinas immunoreactivity for inhibitory and excitatory synapses was observed for both inner and outer stratifying ipRGCs (Jusuf et al., 2007). Inhibitory synapses were identified with antibodies against gephyrin while presumed excitatory synapses were localized with antibodies against CtBP2 or PSD-95 (Jusuf et al., 2007). These results suggested that ipRGCs receive both excitatory inputs via bipolar cells and inhibitory influences presumably from amacrine cells. Quantitative analysis of inhibitory receptor immunoreactivity in macaque retina shows some heterogeneity among ipRGC subtypes (Neumann et al., 2011). M1 cells possess higher densities of inhibitory receptor immunoreactivities (mainly GlyR α_2 and GABA_A α_3 subunits) than M2 cells (mainly GlyR α_3) (Neumann et al., 2011).

The anatomical evidence of synaptic inputs to ipRGCs suggested that rod/cone signaling drives extrinsic, synaptically mediated light responses comparable to conventional RGCs. A large body of work has now established such outer retinal influences on ipRGCs. Single cell recordings from rat ipRGCs show spontaneous excitatory and inhibitory post-synaptic currents that are indicative of functional synaptic connections (Perez-Leon et al., 2006; Wong et al., 2007). In addition, synaptically mediated, sustained ON light responses were recorded in ipRGCs innervating the SCN using either single cell recordings or multi-electrode arrays (Perez-Leon et al., 2006; Wong et al., 2007). Under conditions that promoted ON pathway blockade, a very weak contribution of OFF light responses was also observed in ipRGCs (Wong et al., 2007). Overall the synaptic light responses show higher sensitivity and faster kinetics than the melanopsin-evoked responses (Wong et al., 2007).

Comparable experiments performed in primate retinas demonstrate the strong excitatory input of the ON pathway onto ipRGCs. Primate ipRGCs receive influence from rods and cones allowing irradiance signaling over the full dynamic range of vision (Dacey et al., 2005). As the studies in rodents, synaptic inputs to ipRGCs extend the bandpass of these photoreceptors allowing rapid responses to light stimuli (Dacey et al., 2005). One unique feature so far only described in primate ipRGCs is color opponency, with (L+M) cones providing excitation and S cones evoking inhibition (Dacey et al., 2005).

The next important advancement was to analyze the influence of synaptic inputs to specific ipRGC subtypes. As discussed previously, M1 cells have dendrites largely confined to the OFF sublamina of the IPL while M2 dendrites are ON stratifying. Therefore the initial expectation was that M1 cells would receive synaptic influences from the OFF pathway via OFF bipolar inputs. Conversely, M2 cells were expected to receive inputs exclusively from the ON pathway via ON bipolar cells. However, we found that both M1 and M2 cells receive excitatory synaptic inputs from the ON pathway (Schmidt and Kofuji, 2010). The influence of the ON pathway could be verified by performing recordings in the presence or absence of the pharmacological blocker L-AP4, which inactivates the ON channel signaling in the retina (Slaughter and Miller, 1981). To avoid contamination of light-evoked melanopsin responses we performed recordings in retinas from a mouse line with genetic inactivation of melanopsin. Under these conditions, M2 cells showed much larger synaptic light response than M1 cells (Figure 9). Because we performed the experiments in partially adapted retinas, the synaptically-evoked responses were likely to be mediated by the ON-cone bipolar cells.

Comparable recordings performed in wild-type mice show the interaction of intrinsic (melanopsin-evoked) and extrinsic (synaptically-mediated) influences on the light evoked responses of M1 and M2 cells (Figure 10). We found that the ON pathway forms the dominant synaptic input to both M1 and M2 cells, but is much more influential in shaping the light response of M2 cells. M1 cells, however, show light responses that are unaffected by blockade of synaptic inputs, indicating that these cells have light responses shaped primarily by melanopsin-mediated phototransduction. Thus it seems that the two subtypes of ipRGCs, the M1 and M2 cells, convey photic information by differential reliance on intrinsic and extrinsic mechanisms. The M1 cells respond to light mainly using the melanopsin pathway which confers relatively sustained and sluggish photoresponses (Schmidt and Kofuji, 2010). Thus M1 cells are well suited for signaling over long timescales and high light intensities. Whereas in M2 cells the contribution of intrinsic phototransduction is relatively minor and the ipRGCs display synaptic responses with relatively fast onset and offset comparable to conventional RGCs.

Similar experiments performed in M3 cells show that this class of ipRGCs more closely resembles M2 cells in terms of their synaptic and intrinsic signaling (Schmidt and Kofuji, 2011). Overall the contribution of the synaptic-evoked light responses was much larger than those mediated by melanopsin in M3 cells. Curiously M3 cells have relatively homogeneous photoresponses driven by the ON pathway despite their large variability in proportion of dendrites stratifying in the ON or OFF sublaminae (Schmidt and Kofuji, 2011). Brisk, synaptically-evoked ON responses were also reported in M4 cells (Ecker et al., 2010).

Physiological results demonstrating the ON pathway influences in both M1 and M2 cells fits nicely with recent anatomical data showing that the OFF-stratifying M1 cells receive *en passant* synapses in the IPL (Dumitrescu et al., 2009; Hoshi et al., 2009). On bipolar cells make synaptic contacts in the OFF sublamina of the IPL onto M1 cell dendrites (Dumitrescu et al., 2009). These unconventional ectopic ribbon synapses have been observed in rabbit

and mouse retinas and are likely to provide the functional ON response seen in M1 cells (Schmidt and Kofuji, 2010).

Interestingly, centrifugal modulation of dopaminergic amacrine (DA) cells by ipRGCs has also been proposed (Zhang et al., 2008). DA cells modulate a wide range of processes in the retina for light adaptation and the transition from scotopic to photopic visual function (Witkovsky, 2004). DA cells are located within the INL and receive synaptic input in the outermost stratum of the IPL. Dopamine is released in response to light from physiologically diverse DA cells. DA cells depolarize transiently to light stimulation, and the response is driven by rod-cone photoreceptors via On bipolar cells as it is blocked with L-AP4. However some DA cells which respond to light with sustained depolarization, the responses are L-AP4 resistant implying a pathway independent from On bipolar cells (Zhang et al., 2008). Instead many characteristics of the sustained light response in DA cells are reminiscent of melanopsin-evoked responses, i.e. sluggish kinetics, peak spectral sensitivity of 478 nm, and persistence in retinas from mice with rod/cone degeneration (Zhang et al., 2008). This putative signaling from the ipRGCs to DA cells is highly unusual as it proceeds from the inner retina to the outer retina. So far it is unclear which subtype of ipRGC signals to DA cells but the inner stratifying M1 and M3 cells are the most likely candidates.

5. Diversity of central projections of ipRGC subtypes

Despite the relative scarcity of ipRGCs in the mammalian retina, their influence is widespread in the mammalian brain as ipRGC axons project to numerous brain areas. Using mouse lines in which ipRGC axons are labeled with markers such as β -galactosidase, Hattar and colleagues were able to directly visualize the central projections of ipRGCs in the mouse brain (Hattar et al., 2006). Brain nuclei such as the SCN, OPN, intergeniculate leaflet, and the ventral division of the lateral geniculate nucleus (vLGN) were labeled, as expected, since these brain areas have been linked with NIF vision processing. Other structures such as the medial amygdala, lateral habenula, and periaqueductal gray were also labeled indicating a broader role of ipRGCs in physiology than previously suspected (Hattar et al., 2006). One potential limitation from this study was that only M1 cells are labeled with the β -galactosidase marker in this mouse line, so that in this study M2 and other ipRGC types were not represented (Baver et al., 2008). The first evidence that distinct ipRGC subtypes project to specific brain centers was derived from tracer studies combined with immunostaining (Baver et al., 2008). Tracer injections into two brain centers, the SCN and OPN revealed distinct proportions of labeled ipRGC subtypes. About 80% of ipRGCs that project to the SCN were considered M1 cells (Baver et al., 2008). By contrast M1 and M2 cells project to the OPN in similar proportions (Baver et al., 2008). However M1 cells preferentially innervate the periphery (shell) of the OPN while the M2 cells primarily innervate the core of the OPN.

More recently, studies using a more sensitive axonal marker have revealed additional ipRGC target areas in the mouse brain (Brown et al., 2010; Ecker et al., 2010). In addition to the previously labeled areas, these novel reporter mouse lines revealed more extensive labeling of projections to the dorsal and ventral aspects of the LGN, the core of the OPN, the posterior pretectal nucleus, and the superior colliculus. The discovery that ipRGCs innervate the superior colliculus and dorsal LGN raised the possibility that ipRGCs could also relay signals for discrimination of objects, since these brain centers are associated with the process of image-forming vision. Indeed Ecker and colleagues were able to demonstrate that mice with presumably genetic silencing of functional phototransduction in rods and cones (Gnat1^{-/-}, Cnga3^{-/-} mice) but not in ipRGCs retain the ability to discriminate high-contrast, sinusoidally modulated gratings from uniform gray stimuli (Ecker et al., 2010). It should be acknowledged, however, that Gnat1^{-/-}, Cnga3^{-/-}, Opn4^{-/-} mice still show

electroretinogram responses consistent with some retention of rod and cone phototransduction (Allen et al., 2010). Thus the functional significance of ipRGCs for image-forming vision remains unclear.

The above-mentioned heterogeneity of ipRGC central projections suggests differential roles for ipRGC subtypes in eliciting NIF responses. Recent evidence provides support for this idea. For example, it has now been shown that the SCN receives inputs predominantly from M1 cells and in particular from a subset of M1 cells lacking expression of Brn3b (Chen et al., 2011). The core of the OPN receives axons mostly from non-M1 cells while the outer shell seems to be innervated by the Brn3b+ M1 cells. Selective cell ablation of Brn3b+ ipRGCs impairs PLR but not circadian photoentrainment (Chen et al., 2011). These results show that M1 cells without this transcriptional factor mediate circadian photoentrainment but not PLR.

6. Conclusions and future directions

Since the first discovery of melanopsin in frog melanophores (Provencio et al., 1998) and subsequent description of ipRGCs in mammalian retinas, a remarkable body of work has uncovered surprising diversity of ipRGC morphology and function. As discussed, we now know that distinct subpopulations of ipRGCs express different levels of the photopigment melanopsin, establish different synaptic connections within the retina, and project to distinct brain centers. Future studies will need to address how these morphologically distinct subpopulations of ipRGCs signal photic information integrating their intrinsic and extrinsic phototransduction systems. In particular, the recent demonstration of the distinct contributions of rod, cone, and melanopsin photoreceptors (Altimus et al., 2008; Lall et al., 2010) in driving aspects of NIF vision behaviors raises important questions about how ipRGC subtypes signal scotopic and photopic light information to higher visual centers. Does one subtype have privileged access to the high sensitivity rod pathway? Do ipRGCs in non-primate retinas show cone mediated color-opponent receptive systems? How do distinct ipRGC subtypes adjust light sensitivity in response to diverse lighting conditions? What is the contribution of each ipRGC subtype to specific NIF and image-forming visual behaviors?

Undoubtedly, the central quest is to find molecular markers that discriminate ipRGC subtypes. One drawback to date has been the use of a limited set of morphological and functional criteria to categorize ipRGCs in subtypes. The observation that the apparently morphologically homogeneous M1 cell population can be further subdivided based on the expression of the transcription factor Brn3b (Chen et al., 2011) is a clear example of the present limitations of using a classification system based purely on anatomical characteristics of ipRGCs. Improvements in mouse genetics and single cell transcript profiling techniques make it probable that future research will identify a more complete set of criteria to separate functional classes of ipRGCs. One promising approach is to combine the use of mouse reporter transgenic lines and gene expression profiling using microarrays to identify a set of markers specific to different ipRGC subtypes. This approach has recently been used to identify molecular markers that distinguish RGCs with distinct direction preferences albeit with similar morphology (Kay et al., 2011).

Generation of mouse lines with cell type specific markers would allow for the study of their function at the single cell level, establishment of their relationship with other neuronal cell types and circuits within the retina, and describe their patterns of efferent projections to various brain centers engaged in NIF or image-forming visual processing. Mouse lines with gain or loss of function (e.g. in melanopsin phototransduction, neurotransmitter release, spike generation) in specific ipRGC subtypes could also provide insights to the function of

each subtype at the level of the entire visual system. Functional imaging and in vivo multi-unit recording could provide an important avenue to unlock the roles of these three photoreceptor systems in the primate visual system.

Available evidence implicates ipRGC dysfunction in circadian disorders, sleep malfunctions, photophobias, and possibly mood disorders. An important target for clinical research will be the development of therapeutic agents that modulate melanopsin function or expression. Inducers or blockers of phototransduction in specific ipRGC subtypes may represent a possible strategy to treat specific facets of these disorders. However, at this time these possibilities remain speculative and may depend on a better understanding of ipRGC diversity in rodent and non-rodent retinas.

It is clear that, despite its relatively recent discovery, the ipRGC has now been implicated in a wide range of physiological and pathophysiological processes. Although our understanding of ipRGCs has dramatically expanded in recent years, much remains to be discovered regarding ipRGC function and physiological influences. This task is still in its infancy.

Acknowledgments

This work was supported in part by grants from the NIH R01EY012949, R21-EY018885, and T32 EY0707133

Abbreviations

DA	Dopaminergic amacrine
EGFP	enhanced green fluorescent protein
DAG	diacylglycerol
GCL	ganglion cell layer
Ih	hyperpolarization-activated current
L-AP4	L-2-amino-4-phosphono-butyric acid
IPL	inner plexiform layer
ipRGCs	intrinsically photosensitive retinal ganglion cells
LGN	lateral geniculate nucleus
NIF	non-image-forming
OPN	olivary pretectal nucleus
Opn4	Opsin 4
PLR	pupillary light reflex
PKC	protein kinase C
RGC	retinal ganglion cell
SCN	suprachiasmatic nucleus
TRP	transient receptor potential channel
TRPM	transient receptor potential channel subfamily M
RPE65	RPE specific protein 65 kDa

References

- Allen AE, Cameron MA, Brown TM, Vugler AA, Lucas RJ. Visual responses in mice lacking critical components of all known retinal phototransduction cascades. *PloS one*. 2010; 5:e15063. [PubMed: 21124780]
- Altimus CM, Guler AD, Villa KL, McNeill DS, Legates TA, Hattar S. Rods-cones and melanopsin detect light and dark to modulate sleep independent of image formation. *Proc Natl Acad Sci U S A*. 2008; 105:19998–20003. [PubMed: 19060203]
- Bailes HJ, Lucas RJ. Melanopsin and inner retinal photoreception. *Cellular and molecular life sciences : CMLS*. 2010; 67:99–111. [PubMed: 19865798]
- Baver SB, Pickard GE, Sollars PJ. Two types of melanopsin retinal ganglion cell differentially innervate the hypothalamic suprachiasmatic nucleus and the olivary pretectal nucleus. *Eur J Neurosci*. 2008; 27:1763–1770. [PubMed: 18371076]
- Belenky MA, Smeraski CA, Provencio I, Sollars PJ, Pickard GE. Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *J Comp Neurol*. 2003; 460:380–393. [PubMed: 12692856]
- Berson DM, Castrucci AM, Provencio I. Morphology and mosaics of melanopsin-expressing retinal ganglion cell types in mice. *J Comp Neurol*. 2010; 518:2405–2422. [PubMed: 20503419]
- Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. 2002; 295:1070–1073. [PubMed: 11834835]
- Borbely AA. Effects of light on sleep and activity rhythms. *Progress in neurobiology*. 1978; 10:1–31. [PubMed: 213814]
- Brown TM, Gias C, Hatori M, Keding SR, Semo M, Coffey PJ, Gigg J, Piggins HD, Panda S, Lucas RJ. Melanopsin contributions to irradiance coding in the thalamo-cortical visual system. *PLoS biology*. 2010; 8:e1000558. [PubMed: 21151887]
- Chen SK, Badea TC, Hattar S. Photoentrainment and pupillary light reflex are mediated by distinct populations of ipRGCs. *Nature*. 2011; 476:92–95. [PubMed: 21765429]
- Cook JE, Chalupa LM. Retinal mosaics: new insights into an old concept. *Trends in neurosciences*. 2000; 23:26–34. [PubMed: 10631786]
- Coombs J, van der List D, Wang GY, Chalupa LM. Morphological properties of mouse retinal ganglion cells. *Neuroscience*. 2006; 140:123–136. [PubMed: 16626866]
- Czeisler CA, Shanahan TL, Klerman EB, Martens H, Brotman DJ, Emens JS, Klein T, Rizzo JF 3rd. Suppression of melatonin secretion in some blind patients by exposure to bright light. *N Engl J Med*. 1995; 332:6–11. [PubMed: 7990870]
- Dacey DM. The mosaic of midget ganglion cells in the human retina. *J Neurosci*. 1993; 13:5334–5355. [PubMed: 8254378]
- Dacey DM, Liao HW, Peterson BB, Robinson FR, Smith VC, Pokorny J, Yau KW, Gamlin PD. Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature*. 2005; 433:749–754. [PubMed: 15716953]
- Davies WI, Zheng L, Hughes S, Tamai TK, Turton M, Halford S, Foster RG, Whitmore D, Hankins MW. Functional diversity of melanopsins and their global expression in the teleost retina. *Cellular and molecular life sciences : CMLS*. 2011; 68:4115–4132. [PubMed: 21833582]
- Do MT, Kang SH, Xue T, Zhong H, Liao HW, Bergles DE, Yau KW. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature*. 2009; 457:281–287. [PubMed: 19118382]
- Do MT, Yau KW. Intrinsically photosensitive retinal ganglion cells. *Physiological reviews*. 2010; 90:1547–1581. [PubMed: 20959623]
- Drager UC, Olsen JF. Origins of crossed and uncrossed retinal projections in pigmented and albino mice. *J Comp Neurol*. 1980; 191:383–412. [PubMed: 7410600]
- Dumitrescu ON, Pucci FG, Wong KY, Berson DM. Ectopic retinal ON bipolar cell synapses in the OFF inner plexiform layer: contacts with dopaminergic amacrine cells and melanopsin ganglion cells. *J Comp Neurol*. 2009; 517:226–244. [PubMed: 19731338]
- Ebihara S, Tsuji K. Entrainment of the circadian activity rhythm to the light cycle: effective light intensity for a Zeitgeber in the retinal degenerate C3H mouse and the normal C57BL mouse. *Physiology & behavior*. 1980; 24:523–527. [PubMed: 7375573]

- Ecker JL, Dumitrescu ON, Wong KY, Alam NM, Chen SK, LeGates T, Renna JM, Prusky GT, Berson DM, Hattar S. Melanopsin-expressing retinal ganglion-cell photoreceptors: cellular diversity and role in pattern vision. *Neuron*. 2010; 67:49–60. [PubMed: 20624591]
- Fain GL, Hardie R, Laughlin SB. Phototransduction and the evolution of photoreceptors. *Curr Biol*. 2010; 20:R114–124. [PubMed: 20144772]
- Famiglietti EV Jr, Kolb H. Structural basis for ON-and OFF-center responses in retinal ganglion cells. *Science*. 1976; 194:193–195. [PubMed: 959847]
- Foster RG, Provencio I, Hudson D, Fiske S, De Grip W, Menaker M. Circadian photoreception in the retinally degenerate mouse (rd/rd). *J Comp Physiol A*. 1991; 169:39–50. [PubMed: 1941717]
- Freedman MS, Lucas RJ, Soni B, von Schantz M, Munoz M, David-Gray Z, Foster R. Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science*. 1999; 284:502–504. [PubMed: 10205061]
- Fu Y, Zhong H, Wang MH, Luo DG, Liao HW, Maeda H, Hattar S, Frishman LJ, Yau KW. Intrinsically photosensitive retinal ganglion cells detect light with a vitamin A-based photopigment, melanopsin. *Proc Natl Acad Sci U S A*. 2005; 102:10339–10344. [PubMed: 16014418]
- Gan L, Xiang M, Zhou L, Wagner DS, Klein WH, Nathans J. POU domain factor Brn-3b is required for the development of a large set of retinal ganglion cells. *Proc Natl Acad Sci U S A*. 1996; 93:3920–3925. [PubMed: 8632990]
- Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat Neurosci*. 2001; 4:1165. [PubMed: 11713469]
- Gooley JJ, Lu J, Fischer D, Saper CB. A broad role for melanopsin in nonvisual photoreception. *J Neurosci*. 2003; 23:7093–7106. [PubMed: 12904470]
- Goz D, Studholme K, Lappi DA, Rollag MD, Provencio I, Morin LP. Targeted destruction of photosensitive retinal ganglion cells with a saporin conjugate alters the effects of light on mouse circadian rhythms. *PloS one*. 2008; 3:e3153. [PubMed: 18773079]
- Graham DM, Wong KY, Shapiro P, Frederick C, Pattabiraman K, Berson DM. Melanopsin ganglion cells use a membrane-associated rhabdomic phototransduction cascade. *J Neurophysiol*. 2008; 99:2522–2532. [PubMed: 18305089]
- Guler AD, Altimus CM, Ecker JL, Hattar S. Multiple photoreceptors contribute to nonimage-forming visual functions predominantly through melanopsin-containing retinal ganglion cells. *Cold Spring Harbor symposia on quantitative biology*. 2007; 72:509–515.
- Guler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, Barnard AR, Cahill H, Badea TC, Zhao H, Hankins MW, Berson DM, Lucas RJ, Yau KW, Hattar S. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature*. 2008; 453:102–105. [PubMed: 18432195]
- Hankins MW, Peirson SN, Foster RG. Melanopsin: an exciting photopigment. *Trends in neurosciences*. 2008; 31:27–36. [PubMed: 18054803]
- Hannibal J. Roles of PACAP-containing retinal ganglion cells in circadian timing. *Int Rev Cytol*. 2006; 251:1–39. [PubMed: 16939776]
- Hannibal J, Hindersson P, Knudsen SM, Georg B, Fahrenkrug J. The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. *J Neurosci*. 2002; 22:RC191. [PubMed: 11756521]
- Hannibal J, Hindersson P, Ostergaard J, Georg B, Heegaard S, Larsen PJ, Fahrenkrug J. Melanopsin is expressed in PACAP-containing retinal ganglion cells of the human retinohypothalamic tract. *Investigative ophthalmology & visual science*. 2004; 45:4202–4209. [PubMed: 15505076]
- Hardie RC, Raghu P. Visual transduction in *Drosophila*. *Nature*. 2001; 413:186–193. [PubMed: 11557987]
- Hartwick AT, Bramley JR, Yu J, Stevens KT, Allen CN, Baldrige WH, Sollars PJ, Pickard GE. Light-evoked calcium responses of isolated melanopsin-expressing retinal ganglion cells. *J Neurosci*. 2007; 27:13468–13480. [PubMed: 18057205]
- Hatori M, Le H, Vollmers C, Keding SR, Tanaka N, Buch T, Waisman A, Schmedt C, Jegla T, Panda S. Inducible ablation of melanopsin-expressing retinal ganglion cells reveals their central role in non-image forming visual responses. *PloS one*. 2008; 3:e2451. [PubMed: 18545654]

- Hatori M, Panda S. The emerging roles of melanopsin in behavioral adaptation to light. *Trends in molecular medicine*. 2010; 16:435–446. [PubMed: 20810319]
- Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, Berson DM. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. 2006; 497:326–349. [PubMed: 16736474]
- Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. 2002; 295:1065–1070. [PubMed: 11834834]
- Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M, Hofmann F, Foster RG, Yau KW. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*. 2003; 424:76–81. [PubMed: 12808468]
- Hendrickson AE. Electron microscopic distribution of axoplasmic transport. *J Comp Neurol*. 1972; 144:381–397.
- Hillman P, Hochstein S, Minke B. Transduction in invertebrate photoreceptors: role of pigment bistability. *Physiological reviews*. 1983; 63:668–772. [PubMed: 6340134]
- Hoshi H, Liu WL, Massey SC, Mills SL. ON inputs to the OFF layer: bipolar cells that break the stratification rules of the retina. *J Neurosci*. 2009; 29:8875–8883. [PubMed: 19605625]
- Hughes S, Potheary CA, Jagannath A, Foster RG, Hankins MW, Peirson SN. Profound defects in pupillary responses to light in TRPM-channel null mice: a role for TRPM channels in non-image-forming photoreception. *Eur J Neurosci*. 2012; 35:34–43. [PubMed: 22211741]
- Ingham ES, Gunhan E, Fuller PM, Fuller CA. Immunotoxin-induced ablation of melanopsin retinal ganglion cells in a non-murine mammalian model. *J Comp Neurol*. 2009; 516:125–140. [PubMed: 19575450]
- Jain V, Ravindran E, Dhingra NK. Differential expression of Brn3 transcription factors in intrinsically-photosensitive retinal ganglion cells in mouse. *J Comp Neurol*. 2011
- Johnson RF, Moore RY, Morin LP. Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain research*. 1988; 460:297–313. [PubMed: 2465060]
- Jusuf PR, Lee SC, Hannibal J, Grunert U. Characterization and synaptic connectivity of melanopsin-containing ganglion cells in the primate retina. *Eur J Neurosci*. 2007; 26:2906–2921. [PubMed: 18001286]
- Kay JN, De la Huerta I, Kim IJ, Zhang Y, Yamagata M, Chu MW, Meister M, Sanes JR. Retinal ganglion cells with distinct directional preferences differ in molecular identity, structure, and central projections. *J Neurosci*. 2011; 31:7753–7762. [PubMed: 21613488]
- Keeler CE. The Inheritance of a Retinal Abnormality in White Mice. *Proc Natl Acad Sci U S A*. 1924; 10:329–333. [PubMed: 16576828]
- Keeler CE. Iris movements in blind mice. *Am. J. Physiol*. 1927; 81:107–112.
- Keeler CE, Sutcliffe E, Chaffee EL. Normal and “Rodless” Retinae of the House Mouse with Respect to the Electromotive Force Generated through Stimulation by Light. *Proc Natl Acad Sci U S A*. 1928; 14:477–484. [PubMed: 16577134]
- Koike C, Numata T, Ueda H, Mori Y, Furukawa T. TRPM1: a vertebrate TRP channel responsible for retinal ON bipolar function. *Cell calcium*. 2010; 48:95–101. [PubMed: 20846719]
- Koyanagi M, Kubokawa K, Tsukamoto H, Shichida Y, Terakita A. Cephalochordate melanopsin: evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. *Curr Biol*. 2005; 15:1065–1069. [PubMed: 15936279]
- La Morgia C, Ross-Cisneros FN, Hannibal J, Montagna P, Sadun AA, Carelli V. Melanopsin-expressing retinal ganglion cells: implications for human diseases. *Vision Res*. 2011; 51:296–302. [PubMed: 20691201]
- Lall GS, Revell VL, Momiji H, Al Enezi J, Altimus CM, Guler AD, Aguilar C, Cameron MA, Allender S, Hankins MW, Lucas RJ. Distinct contributions of rod, cone, and melanopsin photoreceptors to encoding irradiance. *Neuron*. 2010; 66:417–428. [PubMed: 20471354]
- Lamb TD, Collin SP, Pugh EN Jr. Evolution of the vertebrate eye: opsins, photoreceptors, retina and eye cup. *Nat Rev Neurosci*. 2007; 8:960–976. [PubMed: 18026166]
- Lamb TD, Pugh EN Jr. Phototransduction, dark adaptation, and rhodopsin regeneration the proctor lecture. *Investigative ophthalmology & visual science*. 2006; 47:5137–5152. [PubMed: 17122096]

- Lewy AJ, Wehr TA, Goodwin FK, Newsome DA, Markey SP. Light suppresses melatonin secretion in humans. *Science*. 1980; 210:1267–1269. [PubMed: 7434030]
- Lin B, Koizumi A, Tanaka N, Panda S, Masland RH. Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. *Proc Natl Acad Sci U S A*. 2008; 105:16009–16014. [PubMed: 18836071]
- Lucas RJ, Douglas RH, Foster RG. Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat Neurosci*. 2001; 4:621–626. [PubMed: 11369943]
- Lucas RJ, Freedman MS, Munoz M, Garcia-Fernandez JM, Foster RG. Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science*. 1999; 284:505–507. [PubMed: 10205062]
- Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, Yau KW. Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science*. 2003; 299:245–247. [PubMed: 12522249]
- Masland RH. Cell populations of the retina: the Proctor lecture. *Investigative ophthalmology & visual science*. 2011; 52:4581–4591. [PubMed: 21712414]
- Mawad K, Van Gelder RN. Absence of long-wavelength photic potentiation of murine intrinsically photosensitive retinal ganglion cell firing in vitro. *Journal of biological rhythms*. 2008; 23:387–391. [PubMed: 18838602]
- Melyan Z, Tartelin EE, Bellingham J, Lucas RJ, Hankins MW. Addition of human melanopsin renders mammalian cells photoresponsive. *Nature*. 2005; 433:741–745. [PubMed: 15674244]
- Moore RY, Lenn NJ. A retinohypothalamic projection in the rat. *J Comp Neurol*. 1972; 146:1–14. [PubMed: 4116104]
- Moore RY, Speh JC, Card JP. The retinohypothalamic tract originates from a distinct subset of retinal ganglion cells. *J Comp Neurol*. 1995; 352:351–366. [PubMed: 7706557]
- Morin LP, Blanchard JH, Provencio I. Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: bifurcation and melanopsin immunoreactivity. *J Comp Neurol*. 2003; 465:401–416. [PubMed: 12966564]
- Muller LP, Do MT, Yau KW, He S, Baldrige WH. Tracer coupling of intrinsically photosensitive retinal ganglion cells to amacrine cells in the mouse retina. *J Comp Neurol*. 2010; 518:4813–4824. [PubMed: 20963830]
- Mure LS, Rieux C, Hattar S, Cooper HM. Melanopsin-dependent nonvisual responses: evidence for photopigment bistability in vivo. *Journal of biological rhythms*. 2007; 22:411–424. [PubMed: 17876062]
- Nelson DE, Takahashi JS. Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *The Journal of physiology*. 1991; 439:115–145. [PubMed: 1895235]
- Nelson RJ, Zucker I. Absence of extraocular photoreception in diurnal and nocturnal rodents exposed to direct sunlight. *Comp Biochem Physiol [A]*. 1981; 69:145–148.
- Neumann S, Haverkamp S, Auferkorte ON. Intrinsically photosensitive ganglion cells of the primate retina express distinct combinations of inhibitory neurotransmitter receptors. *Neuroscience*. 2011; 199:24–31. [PubMed: 22044923]
- Nosedá R, Kainz V, Jakubowski M, Gooley JJ, Saper CB, Digre K, Burstein R. A neural mechanism for exacerbation of headache by light. *Nat Neurosci*. 13:239–245. [PubMed: 20062053]
- O'Brien BJ, Isayama T, Richardson R, Berson DM. Intrinsic physiological properties of cat retinal ganglion cells. *The Journal of physiology*. 2002; 538:787–802. [PubMed: 11826165]
- Oshima N. Direct reception of light by chromatophores of lower vertebrates. *Pigment Cell Res*. 2001; 14:312–319. [PubMed: 11601652]
- Ostergaard J, Hannibal J, Fahrenkrug J. Synaptic contact between melanopsin-containing retinal ganglion cells and rod bipolar cells. *Investigative ophthalmology & visual science*. 2007; 48:3812–3820. [PubMed: 17652756]
- Panda S, Hogenesch JB, Kay SA. Circadian rhythms from flies to human. *Nature*. 2002a; 417:329–335. [PubMed: 12015613]
- Panda S, Nayak SK, Campo B, Walker JR, Hogenesch JB, Jegla T. Illumination of the melanopsin signaling pathway. *Science*. 2005; 307:600–604. [PubMed: 15681390]

- Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, Pletcher MT, Sato TK, Wiltshire T, Andahazy M, Kay SA, Van Gelder RN, Hogenesch JB. Melanopsin is required for non-image-forming photic responses in blind mice. *Science*. 2003; 301:525–527. [PubMed: 12829787]
- Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenesch JB, Provencio I, Kay SA. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science*. 2002b; 298:2213–2216. [PubMed: 12481141]
- Peirson S, Foster RG. Melanopsin: another way of signaling light. *Neuron*. 2006; 49:331–339. [PubMed: 16446137]
- Perez-Leighton CE, Schmidt TM, Abramowitz J, Birnbaumer L, Kofuji P. Intrinsic phototransduction persists in melanopsin-expressing ganglion cells lacking diacylglycerol-sensitive TRPC subunits. *Eur J Neurosci*. 2011; 33:856–867. [PubMed: 21261756]
- Perez-Leon JA, Warren EJ, Allen CN, Robinson DW, Lane Brown R. Synaptic inputs to retinal ganglion cells that set the circadian clock. *Eur J Neurosci*. 2006; 24:1117–1123. [PubMed: 16930437]
- Pickard GE, Silverman AJ. Direct retinal projections to the hypothalamus, piriform cortex, and accessory optic nuclei in the golden hamster as demonstrated by a sensitive anterograde horseradish peroxidase technique. *J Comp Neurol*. 1981; 196:155–172. [PubMed: 7204664]
- Pickard GE, Sollars PJ. Intrinsically photosensitive retinal ganglion cells. *Science China. Life sciences*. 2010; 53:58–67. [PubMed: 20596956]
- Pickard GE, Sollars PJ. Intrinsically Photosensitive Retinal Ganglion Cells. *Reviews of physiology, biochemistry and pharmacology*. 2011
- Pires SS, Hughes S, Turton M, Melyan Z, Peirson SN, Zheng L, Kosmaoglou M, Bellingham J, Cheetham ME, Lucas RJ, Foster RG, Hankins MW, Halford S. Differential expression of two distinct functional isoforms of melanopsin (Opn4) in the mammalian retina. *J Neurosci*. 2009; 29:12332–12342. [PubMed: 19793992]
- Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: An opsin in melanophores, brain, and eye. *Proc Natl Acad Sci U S A*. 1998; 95:340–345. [PubMed: 9419377]
- Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. *J Neurosci*. 2000; 20:600–605. [PubMed: 10632589]
- Provencio I, Rollag MD, Castrucci AM. Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. *Nature*. 2002; 415:493. [PubMed: 11823848]
- Qiu X, Kumbalasiri T, Carlson SM, Wong KY, Krishna V, Provencio I, Berson DM. Induction of photosensitivity by heterologous expression of melanopsin. *Nature*. 2005; 433:745–749. [PubMed: 15674243]
- Redlin U. Neural basis and biological function of masking by light in mammals: suppression of melatonin and locomotor activity. *Chronobiology international*. 2001; 18:737–758. [PubMed: 11763983]
- Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature*. 2002; 418:935–941. [PubMed: 12198538]
- Robinson GA, Madison RD. Axotomized mouse retinal ganglion cells containing melanopsin show enhanced survival, but not enhanced axon regrowth into a peripheral nerve graft. *Vision Res*. 2004; 44:2667–2674. [PubMed: 15358062]
- Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, O'Hara BF. Role of melanopsin in circadian responses to light. *Science*. 2002; 298:2211–2213. [PubMed: 12481140]
- Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature*. 2005; 437:1257–1263. [PubMed: 16251950]
- Schmidt TM, Chen SK, Hattar S. Intrinsically photosensitive retinal ganglion cells: many subtypes, diverse functions. *Trends in neurosciences*. 2011
- Schmidt TM, Kofuji P. Functional and morphological differences among intrinsically photosensitive retinal ganglion cells. *J Neurosci*. 2009; 29:476–482. [PubMed: 19144848]
- Schmidt TM, Kofuji P. Differential cone pathway influence on intrinsically photosensitive retinal ganglion cell subtypes. *J Neurosci*. 2010; 30:16262–16271. [PubMed: 21123572]

- Schmidt TM, Kofuji P. Structure and function of bistratified intrinsically photosensitive retinal ganglion cells in the mouse. *J Comp Neurol.* 2011; 519:1492–1504. [PubMed: 21452206]
- Schmidt TM, Taniguchi K, Kofuji P. Intrinsic and extrinsic light responses in melanopsin-expressing ganglion cells during mouse development. *J Neurophysiol.* 2008; 100:371–384. [PubMed: 18480363]
- Sekaran S, Foster RG, Lucas RJ, Hankins MW. Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Curr Biol.* 2003; 13:1290–1298. [PubMed: 12906788]
- Sekaran S, Lall GS, Ralphs KL, Wolstenholme AJ, Lucas RJ, Foster RG, Hankins MW. 2-Aminoethoxydiphenylborane is an acute inhibitor of directly photosensitive retinal ganglion cell activity in vitro and in vivo. *J Neurosci.* 2007; 27:3981–3986. [PubMed: 17428972]
- Semo M, Munoz Llamas M, Foster RG, Jeffery G. Melanopsin (Opn4) positive cells in the cat retina are randomly distributed across the ganglion cell layer. *Vis Neurosci.* 2005; 22:111–116. [PubMed: 15842746]
- Slaughter MM, Miller RF. 2-amino-4-phosphonobutyric acid: a new pharmacological tool for retina research. *Science.* 1981; 211:182–185. [PubMed: 6255566]
- Sollars PJ, Smeraski CA, Kaufman JD, Ogilvie MD, Provencio I, Pickard GE. Melanopsin and non-melanopsin expressing retinal ganglion cells innervate the hypothalamic suprachiasmatic nucleus. *Vis Neurosci.* 2003; 20:601–610. [PubMed: 15088713]
- Takahashi JS, Hong HK, Ko CH, McDearmon EL. The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nature reviews. Genetics.* 2008; 9:764–775. [PubMed: 18802415]
- Tu DC, Zhang D, Demas J, Slutsky EB, Provencio I, Holy TE, Van Gelder RN. Physiologic diversity and development of intrinsically photosensitive retinal ganglion cells. *Neuron.* 2005; 48:987–999. [PubMed: 16364902]
- Van Hook MJ, Berson DM. Hyperpolarization-activated current (I_h) in ganglion-cell photoreceptors. *PloS one.* 2010; 5:e15344. [PubMed: 21187958]
- Vaney DI. Territorial organization of direction-selective ganglion cells in rabbit retina. *J Neurosci.* 1994; 14:6301–6316. [PubMed: 7965037]
- Viney TJ, Balint K, Hillier D, Siegert S, Boldogkoi Z, Enquist LW, Meister M, Cepko CL, Roska B. Local retinal circuits of melanopsin-containing ganglion cells identified by transsynaptic viral tracing. *Curr Biol.* 2007; 17:981–988. [PubMed: 17524644]
- Wang JS, Kefalov VJ. The cone-specific visual cycle. *Progress in retinal and eye research.* 2011; 30:115–128. [PubMed: 21111842]
- Warren EJ, Allen CN, Brown RL, Robinson DW. Intrinsic light responses of retinal ganglion cells projecting to the circadian system. *Eur J Neurosci.* 2003; 17:1727–1735. [PubMed: 12752771]
- Warren EJ, Allen CN, Brown RL, Robinson DW. The light-activated signaling pathway in SCN-projecting rat retinal ganglion cells. *Eur J Neurosci.* 2006; 23:2477–2487. [PubMed: 16706854]
- Wassle H. Parallel processing in the mammalian retina. *Nat Rev Neurosci.* 2004; 5:747–757. [PubMed: 15378035]
- Wassle H, Riemann HJ. The mosaic of nerve cells in the mammalian retina. *Proc R Soc Lond B Biol Sci.* 1978; 200:441–461. [PubMed: 26058]
- Witkovsky P. Dopamine and retinal function. *Documenta ophthalmologica. Advances in ophthalmology.* 2004; 108:17–40. [PubMed: 15104164]
- Wong KY, Dunn FA, Graham DM, Berson DM. Synaptic influences on rat ganglion-cell photoreceptors. *The Journal of physiology.* 2007; 582:279–296. [PubMed: 17510182]
- Wurtman RJ, Axelrod J, Phillips LS. Melatonin Synthesis in the Pineal Gland: Control by Light. *Science.* 1963; 142:1071–1073. [PubMed: 14068225]
- Xue T, Do MT, Riccio A, Jiang Z, Hsieh J, Wang HC, Merbs SL, Welsbie DS, Yoshioka T, Weissgerber P, Stolz S, Flockerzi V, Freichel M, Simon MI, Clapham DE, Yau KW. Melanopsin signalling in mammalian iris and retina. *Nature.* 2011; 479:67–73. [PubMed: 22051675]
- Yau KW, Hardie RC. Phototransduction motifs and variations. *Cell.* 2009; 139:246–264. [PubMed: 19837030]

Zhang DQ, Wong KY, Sollars PJ, Berson DM, Pickard GE, McMahon DG. Intraretinal signaling by ganglion cell photoreceptors to dopaminergic amacrine neurons. *Proc Natl Acad Sci U S A*. 2008; 105:14181–14186. [PubMed: 18779590]

Figure 1. Mutant mouse with photoreceptor degeneration provided the first evidence of a third photoreceptor type in the mammalian retina

(A) Mutant mouse with photoreceptor degeneration described by Keeler in 1924. Drawings of retinal sections of a wild-type and mutant mouse depicting the retinal layers. In the mutant mouse (later named *rd* mouse) there is selective loss of photoreceptors. ONL, Outer Nuclear Layer; INL, Inner Nuclear Layer; GCL, Ganglion Cell Layer. Adapted from Keeler (1924). (B) Preservation of PLR in the *rd/rd cl* mouse. Wild-type and *rd/rd cl* mice were exposed to bright white light. Modified from Lucas et. al. (2001),

Figure 2. Working model for melanopsin phototransduction in mammalian retinas

(A) Light-activation of melanopsin is thought to engage G α T ϵ D q/11 subunits followed by activation of downstream signaling components such as phospholipase C beta 4 (PLC β 4), diacylglycerol (DAG), and protein kinase C (PKC). The role of inositol triphosphates (IP3) remains undefined. Recent evidence suggests the TRPC6/7 channels as the effector channels. (B) Melanopsin-evoked light response in a mouse ipRGC. Melanopsin-evoked depolarization was elicited by a 5 s bright, full-field, white-light stimulus in the presence of synaptic blockers to prevent influences from rods and cones. Notice the slow depolarizing responses with spiking even following the termination of the light stimulus. Modified from Schmidt and Kofuji, 2010.

Figure 3. Distribution and morphology of ipRGCs in the macaque retina

(A) Distribution of ipRGCs in a macaque retina. T, temporal retina; N, nasal retina; S, superior retina; I, inferior retina. (B) Morphology of ipRGCs in peripheral and foveal regions of a macaque retina. Upper panels show ipRGCs in peripheral retina (left) and morphology of an ipRGC in comparison to a parasol and a midget cell in the same region. Lower panels show ipRGCs in the foveal region of the retina with dendrites encircling the fovea. Tracings of two ipRGCs and a parasol and midget cell in the foveal region. Modified from Dacey et.al. (2005).

Figure 4. M1, M2, and M3 ipRGC subtypes in the mouse retina

(A) Whole-mount retinas of ipRGCs filled with biocytin/neurobiotin. Side view of filled cells (green) and cholinergic amacrine cells (red). M1 cell dendrites stratify in the outer portions (OFF sublamina) of the inner plexiform layer (IPL) in proximity to the inner nuclear layer (INL). M2 cell dendrites stratify in the inner portions (ON sublamina) of the IPL near the ganglion cell layer (GCL). M3 cells have a bistratified dendritic arborization. (B) Diagram depicting the M1, M2 and M3 subtypes. Displaced M1 cells (M1*) have cell bodies in the INL. Modified from Schmidt et al. (2008). Scale bar = 50 μ m.

Figure 5. Dendritic territories of ipRGCs in the mouse retina

A. Tracing of M1 cells in a region of a mouse retina showing the mosaic distribution of M1 cell somas and dendrites. Notice the large size and extensive overlapping of M1 cell dendrites. Asterisks denote displaced M1 cells. Modified from Berson et.al. 2010. Scale bar = 50 μm .

Figure 6. Schematic representation of dendritic stratification of M3 cells in the IPL

Traced dendritic arbors of neurobiotin filled M3 cells. Red-colored arbors represent those dendrites terminating in the OFF sublamina of the inner plexiform layer. Gray processes represent dendrites terminating in the ON sublamina. (A) M3 cells with dendrites confined mainly to the OFF sublamina. (B) M3 cell with dendrites confined mainly to the ON sublamina. (C) M3 cells with substantial dendritic stratification in both the ON and the OFF sublaminae. Scale bar = 100 μ m. Modified from Schmidt and Kofuji (2011).

Figure 7. Distinct intrinsic membrane properties of M1 and M2 cells

(A) M1 cells have a more depolarized resting membrane potential and higher input resistance than M2 cells. (B) M2 cells reach higher spiking rates than M1 cells in response to depolarizing current pulses. Modified from Schmidt and Kofuji, 2009.

Figure 8. Intrinsic light responses of M1 and M2 cells

All light responses recorded in the presence of synaptic blockers and TTX. (A,B) (left panels), EGFP signal (top) under epifluorescent illumination of M1 and M2 cell targeted for dual whole cell current clamp recordings (bottom). (A,B) (right panel), Responses in current clamp mode (A) or voltage clamp (B) of M1 and M2 cells shown in left panel to a 30 s white light stimulus. (C) Maximum depolarization measured in current clamp mode of M1 and M2 cells. (D) Maximum current measured in voltage clamp mode of M1 and M2 cells. (E) Brightness (in arbitrary units, AUs) of epifluorescence of EGFP signals for pairs containing one M1 and one M2 cell. (F) Irradiance response curves for M1 and M2 generated by stimulating cells with increasing intensities of a 5 s 480 nm light stimulus. Adapted from Schmidt et al. 2009.

Figure 9. ON pathway evoked currents in M1 and M2 ipRGC subtypes

Light evoked, synaptic responses were measured in the melanopsin knockout mouse in the absence or presence of the 100 μ M L-AP4. Modified from Schmidt and Kofuji (2010).

Figure 10. Contribution of ON channel input to light response of M1 and M2 ipRGCs in wild-type mouse

(A) M1 cell response in current clamp mode to 5 s bright, full-field, white-light stimulus in control solution (top panel), in the presence of 100 μ M L-AP4 (middle panel) and after washout (bottom panel). (B) M2 cell response in current-clamp mode to 5 s bright, full-field, white-light stimulus in control solution (top panel), with 100 μ M L-AP4 in the bath (middle panel) and after washout (bottom panel). (C) Diagram depicting the influence of the ON (ON CBC) and OFF (OFF CBC) cone bipolar cells on conventional ON and OFF RGCs. (D) Representation of the ON pathway influence on M1 and M2 ipRGCs. Modified from Schmidt and Kofuji (2010).

TABLE 1

Morphological diversity of ipRGCs subtypes in the murine retina

CELL TYPE	DENDRITIC STRATIFICATION IN THE IPL	MELANOPSIN CONTENT	DENDRITIC FIELD DIAMETER (μM)	DENDRITIC COMPLEXITY	CELL BODY DIAMETER (μM)
M1	Off	+++++	275 ^a 350 ^b 314 ^c 365 ^d 377 ^e	++	13 ^a 16 ^b 17 ^c 16 ^d 17 ^e
M2	On	+++	310 ^a 325 ^b 422 ^c 425 ^d 403 ^e	+++	15 ^a 17 ^b 22 ^c 19 ^d 19 ^e
M3	On	+++	477 ^d 449 ^e	+++	18 ^d 17 ^e
M4	On	+	302–444 ^b	++++	17–22 ^b
M5	On	+	149–217 ^b	+++++	?

^a (Berson et al., 2010)

^b (Ecker et al., 2010)

^c (Schmidt and Kofuji, 2009)

^d (Schmidt and Kofuji, 2011)

^e (Muller et al., 2010)