Melanopsin Ganglion Cells: A Bit of Fly in the Mammalian Eye by Dustin M. Graham

Dustin M. Graham

1. Introduction.

For the greater part of 150 years it was assumed that the mammalian retina contained only two types of photoreceptors; rods and cones. However, a flurry of recent evidence has demonstrated the existence of a third type of mammalian photoreceptor that differs greatly from rods and cones. This type utilizes a different photopigment, is much less sensitive to light, and has far less spatial resolution; characteristics that fit perfectly with this photoreceptor's primary function of signaling changes in ambient light levels to the brain throughout the day. Most surprisingly, these photoreceptors are ganglion cells, and thus, have the unique ability to communicate directly with the brain. These intrinsically photosensitive retinal ganglion cells (ipRGCs) are a rare sub-population of ganglion cells (1-3%) whose primary role is to signal light for unconscious visual reflexes, such as pupillary constriction, and regulating a number of daily behavioral and physiological rhythms, collectively called circadian rhythms. This latter process, which adjusts circadian rhythms to the light/dark cycle of an animal's environment, is known as photoentrainment. The visual behaviors under ipRGC control are remarkably tonic, and require long integration times of ambient light levels. The unique properties of ipRGCs, both functionally and anatomically, make them well suited for regulating such behaviors.

2. History and discovery.

As a graduate student in 1923, Clyde Keeler made a number of interesting observations from mice that had severe outer retinal degeneration (Keeler, 1928; Van Gelder, 2008). Though they lacked most of their rod and cone photoreceptors, and were considered functionally blind, these mice were still able to generate a number of visual reflexes, including constriction of their pupils in response to light (Keeler, 1927). From these observations he deduced that some other photosensitive cell type must be lurking in the retina. Later, using genetically engineered mice with no rods or cones, Foster and colleagues showed that these blind mice also maintained the ability to shift their daily biological rhythms in accordance with shifting light cycles, and suppress pineal activity in response to brief pulses of light (Freedman et al., 1999; Lucas et al., 1999). These processes disappear, however, when animals have their eyes removed, strongly supporting Keeler's prediction of a novel occular photoreceptor (Klein and Weller, 1972). However, it wasn't until the discovery of a new photopigment (or opsin), from an unlikely source, that Keeler's prediction would prove true.

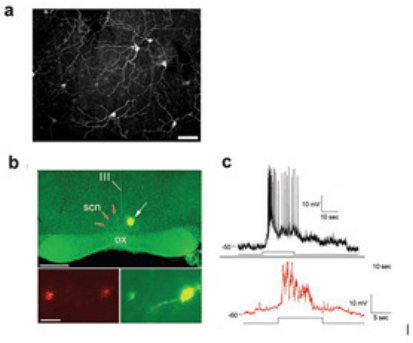

Fig. 1. Discovery of ipRGCs. (a) Melanopsin immunostaining of ganglion cells in the whole-mount retina. (b) Injection of tracer into the SCN above the optic chiasm (ox) (top panel) and subsequent retrolabeling of ipRGCs (red image, lower left panel), which express melanopsin (green image, lower right panel). (c) Whole-cell patch clamp recordings of ipRGCs showing responses to light under synaptic blockade in the whole retina (black trace), and after mechanical isolation (red trace). Adapted from Provencio et al. 2002 and Berson et al. 2002.

Provencio and colleagues, studying the dermal melanophores of frogs, cloned a novel opsin molecule which they found was responsible for redistribution of skin pigment in direct response to light (Provencio et al., 1998). Othologs of this opsin, which they called melanopsin, was also found to be selectively expressed in a small subset of ganglion cells in mouse retina (Fig. 1a) (Provencio et al., 1998; Provencio et al., 2000), and additional work found that these cells send their axons to the suprachiasmatic nucleus (SCN), the site of the mammalian biological clock controlling circadian rhythms (Gooley et al., 2001; Hannibal et al., 2002). All these findings heavily suggested that these ganglion cells were the mysterious third photoreceptor type Keeler had predicted almost 80 years earlier. They express a putative opsin, potentially allowing them to directly respond to light, and they send axon projections to brain targets known to be involved in visual reflexes that rodless/coneless mice were still

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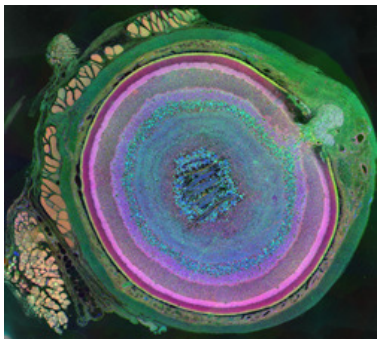

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capable of performing. However, definitive proof that these melanopsin containing ganglion cells could signal light without the function of rods and cones was still lacking. Combining retrograde tracer injections into the SCN with patch clamp electrophysiology, David Berson and colleagues directly tested the hypothesis that melanopsin ganglion cells are intrinsically photosensitive. They performed targeted patch-clamp recordings and found that the retrolabeled ganglion cells (which stained positive for melanopsin using immuno-hisotchemical techniques) were indeed able to respond to light in the presence of a cocktail of pharmacological blockers that eliminate virtually all rod and cone signaling in the retina (Fig. 1b-c) (Berson et, al. 2002; Hattar et al., 2002). Furthermore, even after mechanical isolation, these cells were still intrinsically photosensitive, leaving to rest all doubts that the labeled ganglion cells were true photoreceptors (Berson et al., 2002). Since the discovery of the original mealanopsin ganglion cell (M1 cell) there have been 2 or 3 new varieties of melanopsin ganglion cells described (see later). The description in this chapter is primarily concerned with the M1 cell, which is the best and most in-depth studied (Berson, 2003; 2007; Berson et al., 2002).

3. Melanopsin.

The unique ability of ipRGCs to respond to light is due to their exclusive expression of the photopigment melanopsin. Originally cloned from frog dermal melanophores, the melanopsin gene (OPN4) has orthologs in many mammalian species, including mice, monkeys, and humans (Provencio et al., 2000). Hydrophobicity analysis of melanospin's amino-acid sequence predicts a 7-transmembrane structure, common to all G-protein coupled receptors (Fig. 2a, 2c) (Provencio et al., 1998). Interestingly, melanopsin shares more homology with invertebrate rhabdomeric opsins (r-opsins) than with ciliary opsins of vertebrate species (c-opsins), suggesting that melanopsin may signal light through a different mechanism than that used in vertebrate rods and cones (Fig. 2b) (Provencio et al., 1998).

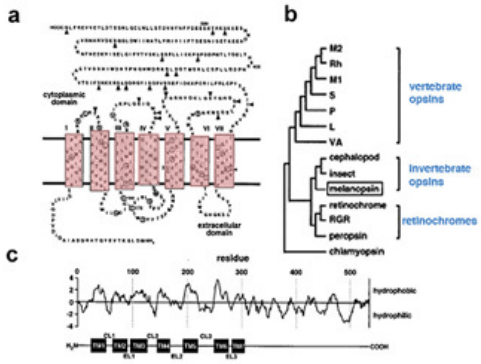

Fig. 2. The melanopsin gene. (a) Deduced amino acid sequence and predicted secondary structure of melanopsin. Shaded region indicates transmembrane domains. (b)) Phylogenetic tree comparing melanopsin to representative opsins of vertebrate and invertebrate species (L, long-wavelength-sensitive opsin; M1, blue-like middle-wavelength-sensitive opsin; M2, green-like middle-wavelength-sensitive opsin; P, pineal opsin; Rh, rhodopsin; S, short-wavelength-sensitive-all from chicken; VA, vertebrate ancient opsin-from Atlantic salmon),. (c) Hydropathy analysis for secondary structure prediction. Adapted from Provencio et al. 1998.

Although the first studies of ipRGCs strongly suggested melanopsin as the photopigment in these cells, a role for a group of blue-light absorbing flavoproteins known as cryptochromes could not be initially ruled out (Berson, 2007). Cryptochromes function as circadian photopigments in invertebrates, and early studies favored their functioning as the mammalian circadian photopigments (Kavakli and Sancar, 2002; Van Gelder et al., 2002). However, there is now overwhelming evidence that melanopsin is the photopigment in ipRGCs, and thus the true circadian photopigment. When the melanopsin gene is deleted via transgenic techniques in mice (knockout mice), retinal ganglion cells labeled from the SCN no longer can signal light (Fig. 3) (Lucas et al., 2003). In addition, animals missing the melanopsin gene show deficiencies in multiple visual reflexes such as pupillary constriction and photoentrainment (Panda et al., 2002; Ruby et al., 2002; Lucas et al., 2003; Panda et al., 2003). As a further test, Tu and coauthors used a multi-electrode array, which is a planar array of electrodes that allows one to record extracellularly from dozens of retinal ganglion cells at once, to isolate light responses from multiple ipRGCs. Retinas from melanopsin knockout mice showed no intrinsic photoresponses from ganglion cells (Tu et al., 2005). Despite this evidence, there were still controversies regarding melanopsin's ability to function as a true photopigment. These were firmly extinguished with a series of elegant experiments whereby the melanopsin gene was expressed in multiple cell types that are normally light insensitive. When made to express the melanopsin gene, these cells are able to robustly respond to light, indicating melanopsin's ability to function as a bonafide photopigment (Fig. 3) (Melyan et al., 2005; Panda et al., 2005; Qiu et al., 2005).

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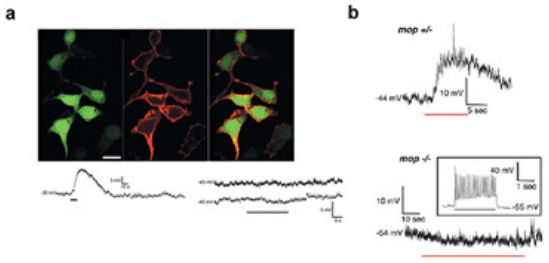

Fig. 3. Evidence for melanopsin as the ipRGC photopigment. (a) Transfection of HEK293 cells (green far left image) with melanopsin (red middle image, overlaid red and green images in far right panel) makes them photosensitive (far left trace). Untransfected controls (far right traces) showed no such response to light at various intensities. (b) Ganglion cells retrolabeled from the SCN in mice with one copy of the melanopsin gene (top trace, mop +/-) have robust intrinsic light responses, whereas labeled ganglion cells from mice completely lacking melanopsin do not show any intrinsic photosensitivity to light (mop -/- bottom trace). Inset in bottom trace shows response to direct injection of current showing labeled cells from mop -/- mice are healthy. Adapted from Lucas et al. 2003 and Qui et al. 2005.

4. ipRGC form and function.

In rodents, ipRGCs make up about 1-3% of the total ganglion cell population, and are distributed throughout the entire retina, at a somewhat higher density superiorly (Fig. 4) (Hattar et al., 2002). Their dendritic profiles are large, spanning roughly 500 µm, and form an extensive overlapping plexus within the retinal inner plexiform layer (IPL) (Hattar et al., 2002; Provencio et al., 2002; Berson, 2003). Owing to their large dendritic spread, ipRGCs initiate a process of spatial convergence the leads to large receptive fields in target structures like the SCN (Groos and Mason 1980). The dendrites of ipRGCs terminate mainly in the outer-most sublayer of the IPL, corresponding to the main plexus of melanopsin-immunoreactive dendrites (Fig. 4) (Berson et al., 2002; Hannibal et al., 2002; Hattar et al., 2002; Provencio et al., 2002), however, in mice there appears to be a second plexus of melanopsin-positive dendrites, suggesting the presence of a possible second population of ipRGCs.

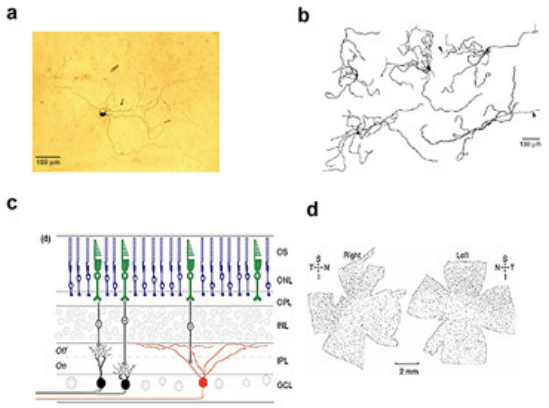

Fig. 4. . Morphological characteristics of ipRGCs. (a) Photomicrograph of a typical ipRGC filled with intracellular dye during patch-clamp recording. (b) Camera lucida drawing of multiple ipRGCs stained with intracellular dye. (c) Schematic drawing comparing spread and termination patterns of ipRGC dendrites with conventional ganglion cells. (d) Whole-mount retinas stained for melanopsin showing overall distribution of ipRGCs throughout the retina. Adapted from Hattar et al. 2002, and Berson 2003.

As seen in Fig. 5, the intrinsic light response differs dramatically from those of rods and cones. Most notable, is the depolarizing light response seen in ipRGCS, as opposed to the hyperpolarizing response of rods and cones. In addition, ipRGCs are much less sensitive to light than classical photoreceptors, and signal with far slower kinetics (Fig. 5) (Berson et al., 2002; Berson, 2003). ipRGCs are also capable of sustained light responses under conditions of bright continuous illumination, faithfully encoding stimulus energy over relatively long periods of time (Fig. 5). These features set ipRGCs apart from all other mammalian retinal ganglion cells, which cannot represent ambient light levels in this fashion (Barlow and Levick, 1969; Berson, 2003). Another remarkable feature of ipRGC physiology is the ability of their dendrites to respond directly to light (Berson et al., 2002). Combined with their large overlapping dendritic fields, this creates what Provencio and colleagues called a “photoreceptive net” (Provencio et al., 2002). These characteristics of ipRGCs are no doubt matched perfectly to their role in signaling diffuse ambient light levels over many hours for tonic behaviors such as photoentrainment and pupillary reflex (Panda et al., 2002; Lucas et al., 2003). A further distinction of ipRGCs is their action spectrum (or wavelength-sensitivity function), due to the utilization of melanopsin as their visual pigment. ipRGCs are most sensitive to light at around 480nm (Fig. 5) (Berson et al., 2002).

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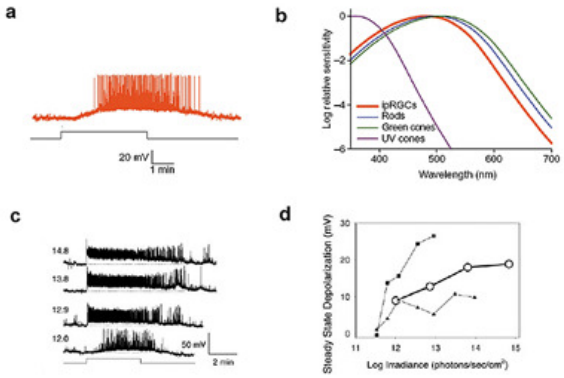

Fig. 5. Physiological characteristics of intrinsic photosensitivity in ipRGCs. (a) Current clamp recording from an ipRGC showing depolarizing response with spiking and slow response kinetics. (b) Comparison of action spectra for rod and cone photoreceptors with that of ipRGCs. (c) Whole-cell recording from an ipRGC showing changes in response amplitude with increasing levels of illumination. (d) Plot of response amplitude versus light intensity for 3 separate ipRGCs. Adapted from Berson et al. 2002 and Berson 2003.

5. Phototransduction: a bit of fly in the mammalian eye.

Animal photoreceptors come in two basic flavors: rhabdomeric (found mainly in invertebrates), and ciliary (vertebrate rods and cones being most notable). A defining feature of rhabdomeric and ciliary photoreceptors is the distinct biochemical cascades both cell types use to transduce light energy into an electrical signal the brain can interpret. Rods and cones use a cascade involving cyclic guanyl monophosphate (cGMP) as a second messenger (Fig. 6) (Arendt, 2003; Fu and Yau, 2007). The response to light is a decrease of cGMP levels which closes cyclic nucleotide gated channels causing the plasma membrane to hyperpolarize. This is in stark contrast to invertebrate rhabdomeric photoreceptors which use a phosphoinositide signaling cascade involving the enzyme phospholipase C (PLC) and breakdown of the membrane lipid phosphotidylinositol bisphosphate (PIP2) (Fig. 6), leading to the opening of cationic channels and membrane depolarization (Hardie, 2001; Hardie and Raghu, 2001). In addition, although both phototransduction cascades are G-protein mediated, the specific G-proteins required by ciliary and rhabdomeric photoreceptors are different. Ciliary photoreceptors require transducin, a member of the Gi/o-family of G-proteins, whereas rhabdomeric photoreceptors use a member of the Gq/11-family of G-proteins (Fig. 6).

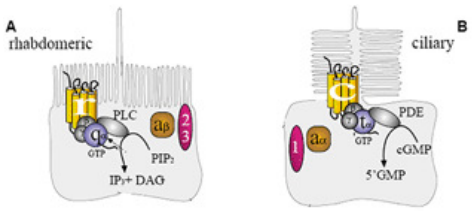

Fig. 6. Schematic representation of the phototransduction cascades in (A) rhabdomeric photoreceptors and (B) ciliary photoreceptors. Adapted from Arendt 2003.

Melanopsin's strong homology with invertebrate opsins and the depolarizing light response of ipRGCs suggests they may use a rhabdomeric phototransduction cascade. However, early patch clamp and pharmacological studies of ipRGCs could not directly confirm this hypothesis. This was most likely due to the whole-mount retina recording configuration often used in studying ganglion cell function. The combination of photosensitive ipRGC dendrites buried deep within the IPL, and a membrane sheath covering the ganglion cell bodies, can create a significant diffusion barrier for pharmacological agents, especially hydrophobic agents commonly used to study transduction mechanisms. To overcome this hurdle, Graham et al. (2008) recorded from dissociated ipRGC cell bodies in culture to study the intracellular phototransduction cascade. Isolated ipRGCs survive remarkably well in culture, generating robust light responses for up to 6 days and allowing for excellent pharmacological manipulation. Using this system, they showed that ipRGC phototransduction follows a rhabdomeric-like phosphoinositide cascade, requiring a member (or possibly members) of the Gq/11 family of G-proteins and the effector enzyme phospholipase C (PLC) (Fig. 7) (Graham et al., 2008). In addition, the presence of specific Gq/11 and PLC isoforms was confirmed in ipRGCs using single-cell RT-PCR and immunocytochemistry, consistent with the pharmacological findings (Fig. 8) (Graham et al., 2008).

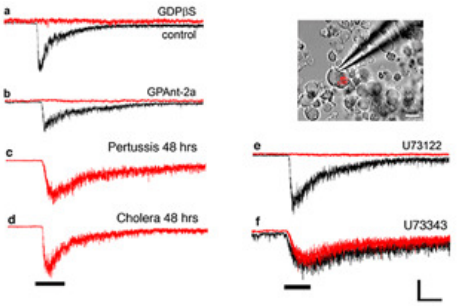

Fig. 7. Invertebrate-like phosphoinositide phototransduction cascade in ipRGCs. (a) GDP_S, a general G-protein blocker, completely abolishes the light response in ipRGCs, indicating a G-protein coupled cascade. (b) The specific Gq/11 family G-protein blocker GPant-2a completely blocks the light response in ipRGCs, while 48 hour incubation in toxins disrupting (c) Gi/o family G-protein signaling (pertussis) and (d) Gs G-protein signaling (Cholera) did not block the light response in ipRGCs. (e) Consistent with a Gq/11 and PLC mediated cascade, the PLC blocker U73122 completely blocks the light response in ipRGCs, while the inactive analog U73343 (f) has no effect, indicating the response to U73122 is specific. Inset is a picture of a labeled isolated ipRGC in culture during a typical patch-clamp recording. Calibration: 5 sec; 40 pA for all traces. Adapted from Graham et al. 2008.

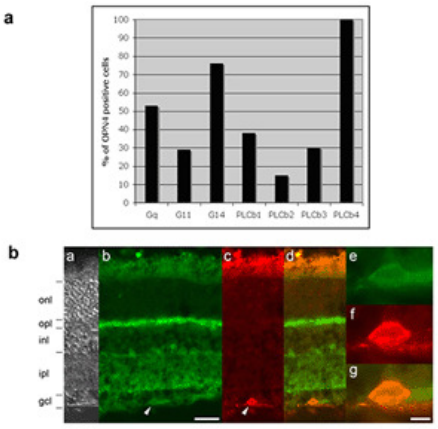

Fig. 8. Molecular evidence for Gq/11 and PLC expression in ipRGCs. (a) Single-cell RT-PCR results from isolated ipRGCs expressing the melanopsin gene (OPN4) showing percentage that expressed various members of the Gq/11 family of G-proteins and members of the PLC_ family. (b) Immunocytochemistry confirming co-expression of PLC_4 (green, sub-panel b) with melanopsin (red, sub-panel c). Sub-panel d is the overlay image of panels b and c, and sub-panels e-g are close-up images of the stained cell near the white arrow in sub-panels b-c. Scale bar in b equals 50 _m for panels a-d; scale bar in g equals 10 _m for e-g. Adapted from Graham et al. 2008.

In rhabdomeric photoreceptors, breakdown of PIP2 by PLC generates two by-products; a membrane-bound component called diacylglycerol (DAG), and inositol-triphosphate (IP3), which is free to move about the cytosol (Hardie, 2001). DAG can be broken down within the membrane into polyunsaturated faty acids (PUFA's), which have been shown to directly open Drosophila TRP channels (Chyb et al., 1999). IP3, on the other hand, can cause release of intracellular calcium from stores within the cell, which can lead to the opening of so called "store-operated channels". In Drosophila photoreceptors the exact downstream mechanism linking PIP2 breakdown to TRP channel opening is still unknown, although a membrane-associated pathway is heavily favored (Acharya et al., 1997). In ipRGCs, data strongly suggest that the cytosolic IP3 pathway is not required for phototransduction, similar to Drosophila photoreceptors (Fig. 9) (Graham et al., 2008). Intracellular application of high concentrations of IP3 neither induces a current, nor does it block light responses in ipRGCs (Fig. 9), although it does modulate the response properties, suggesting a non-essential secondary role for IP3 and intracellular calcium. Likewise, blocking IP3 receptors with intracellular heparin and intracellular calcium store depletion with thapsigargin, both fail to block phototransduction in ipRGCs (Fig. 9). Only after extended exposure to very high (10mM) concentrations of the general calcium chelator BAPTA, does one see significant blockade of phototransduction, although this is most likely a side effect of clamping resting intracellular calcium levels so low that enzymes such as PLC, are no longer able to function (Hardie, 2005; Graham et al., 2008). The most compelling evidence that ipRGC phototransduction does not require the cytosolic IP3 branch is that excised patches of ipRGC membrane (outside-out and inside-out) remain autonomously photosensitive (Fig. 9), ruling out a vital role for highly diffusible cytosolic components. Based on these findings, all the necessary components for ipRGC phototransduction appear to be within, or tightly coupled to, the plasma membrane.

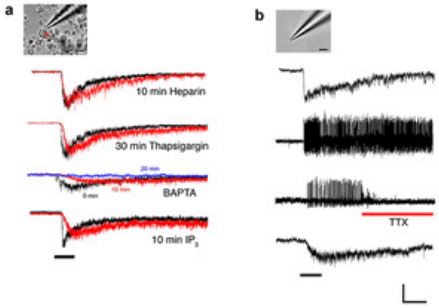

Fig. 9. Evidence for a membrane-associated phototransdcution cascade in ipRGCs. (a) Whole-cell patch-clamp recordings from ipRGCs. Voltage-clamp traces showing control light responses (black traces) and light responses after 10 minutes (red traces) and 20 minutes (blue trace) application of various agents to block the cytosolic IP3-related branch of the phosphoinositide signaling cascade. Onset of light is indicated by black bar. (b) Light responses from excised patches of ipRGC membrane. Top two traces are voltage-clamp (top trace) and current-clamp (second from top trace) recordings from an inside-out patch of ipRGC membrane. The bottom two traces are current-clamp (second from bottom trace) and voltage-clamp (bottom trace) recordings from outside-out patches of ipRGC membrane. Note the cessation of activity in response to applied TTX (second to bottom trace) indicating the spikes are due to voltage-gated sodium channels. Onset of light is indicated by black bar.

Calibration: (a) 10 sec; 100 pA; (b) Calibration 6s and 10 pA for voltage-clamp, and 6s and 0.2 mV for current clamp recordings.

The exact gating mechanism downstream of PLC activation in ipRGC phototransduction is still not clear. Application of both DAG and PUFA's, either to isolated ipRGC cell bodies, or to excised patches of ipRGC membrane, fail to evoke a current or to block light responses (Fig. 10). An alternative hypothesis, and one in-line with a membrane-associated transduction cascade, is that the breakdown of PIP2 itself (as opposed to production of DAG or PUFA's) is the critical signal that opens the light gated channels in ipRGCs. There is evidence that PIP2 can either open or close a variety of ion channels, including the light-gated channels in Drosophila (Hardie 2003; Suh et al., 2006). As a preliminary test of this hypothesis however, Graham and coauthors pharmacologically interfered with PIP2 synthesis using wortmannin. This drug inhibits phosphoinositide 4-kinase (PI4-K), the synthetic enzyme for phosphatidylinositol 4-phosphate (PIP), which is an essential precursor of PIP2. According to the hypothesis, wortmannin should slow the termination of the photocurrent at light offset by delaying the restoration of resting levels of PIP2 and, thus, the closure of the light gated channels. Indeed, when wortmannin was included in the pipette solution, response shutoff was dramatically delayed (Fig. 10). Further and more direct evidence, however, is still necessary to resolve the gating mechanism of the light sensitive channels in ipRGCs.

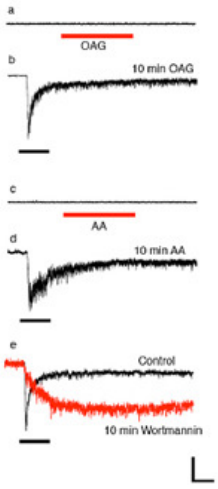

Fig. 10. Possible gating mechanism for the light-sensitive channels in ipRGCs. (a) Puffer pipette application of the DAG analogue OAG does not induce a current in ipRGCs, (b) nor does it block the light response after prolonged bath application at high concentrations. (c) The polyunsaturated fatty acid (PUFA) arachodonic acid (AA) does not induce a current in ipRGCs when applied via puffer pipette, (d), nor does it block the light response when bath applied for prolonged periods. (e) Wortmannin significantly increases the recovery rate in ipRGCs (red trace) after illumination compared with control cells (black trace), suggesting PIP2 breakdown itself may be the gating mechanism of the light-sensitive channels in ipRGCs. Calibration: 50pA and 6s. Adapted from Graham et al. 2008.

The similarity of both the opsin and the signaling cascade in ipRGCs to those in rhabdomeric photoreceptors has encouraged speculation that the light sensitive channels might belong to the TRP family, originally discovered in Drosophila photoreceptors (Montell, 2005). Although definitive evidence is still lacking, most studies suggest that a member (or members) of the TRPC family of TRP channels is involved (Warren et al., 2006; Hartwick et al., 2007). Light induces a current that reverses around 0 mV, suggesting a nonspecific cationic channel, and when melanopsin is expressed in heterologous cell systems, co-expression of TRPC channels allows the cells to transduce light (Qiu et al., 2005). Ion substitution experiments suggest that calcium is a significant charge carrier through the light sensitive channels, consistent with findings of Sekaran et al. who used calcium imaging to measure light responses from ipRGCs in retinas lacking rods and cones (Sekaran et al., 2003; Warren et al., 2006). Likewise, drugs which are known to block TRPC channels are able to block light response in ipRGCs, although none of the drugs used are specific to TRPC channels (Warren et al., 2006; Hartwick et al., 2007). However, in dissociated ipRGCs, application of DAG analogues (drugs known to activate TRPC channels) neither induce a current nor do they block the light response (Graham et al., 2008). A stronger case for TRPC channels awaits better pharmacological tools and genetic/molecular manipulation of ipRGCs. It seems safe to say that uncertainty about the identity of the light gated channels in ipRGCs remains the most glaring gap in our understanding of phototransduction mechanisms in these neurons.

6. Synaptic connectivity with classic rod/cone photoreceptors.

Although ipRGCs can function as photoreceptors, they also receive intraretinal synaptic input from rods and cones. Their photosensitive dendrites terminate in the inner plexiform layer (IPL), the layer of synaptic contact for amacrine and bipolar cells to convey rod and cone signals to ganglion cells (Berson, 2003). Using electron microscopy, Belenky and coauthors (2003) identified synaptic contacts between amacrine cells and presumed ON-bipolar cells with melanopsin-immunopositive processes in the IPL. Also, as demonstrated in both rats and primates, in addition to their intrinsic melanopsin-based light response, ipRGCs respond to light through synaptically mediated input from rods and cones (Dacey et al., 2005; Perez-Leon et al., 2006; Wong et al., 2007). Dacey and coauthors (2005) showed that at light levels too low to activate cones, ipRGCs responded well, indicating rod-based input. In addition, using light of different wavelengths, they showed that ipRGCs display a

color opponency in their responses, indicating that they also receive input from cone photoreceptors (Dacey et al., 2005).

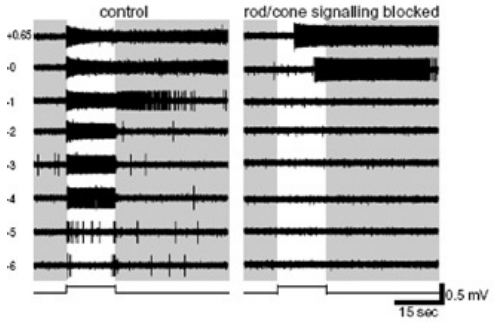

Fig. 11. Extracellular recording of an ipRGC using a mulitelectrode array. Note how the long lasting and relatively insensitive intrinsic melanopsin-driven component becomes obvious at increasing illumination levels (left traces) and can easily be isolated using pharmacological blockade of rod/cone driven input (right traces). Adapted from Wong et al. 2007.

Using pharmacology and a multielectrode array (MEA) to record extracellularly from multiple ipRGCs simultaneously, one can clearly separate the synaptically driven and intrinsic light responses of ipRGCs (Fig.11) (Wong et al., 2007). In addition, the unique response characteristics (insensitive and sluggish) of the intrinsic melanopsin-based signaling becomes quite apparent (Fig. 11).

Using whole-cell voltage clamp recordings combined with pharmacology, investigators demonstrated that ipRGCs have spontaneous inhibitory and excitatory synaptic inputs at rest in the dark , and that they express receptors for glutamate, GABA and glycine (Fig. 12a) (Perez-Leon et al., 2006; Wong et al., 2007). In response to light, ipRGCs receive a mix of synaptic input, arising from amacrine cells and both ON and OFF cone bipolar cells (although the ON signal is much stronger) (Wong et al., 2007; Schmidt and Kofuji, 2010). Under more physiological conditions (recording with the electrode array), the sustained extrinsic response to light appears to be driven only by ON-bipolar cells. This is shown by the fact that the drug L-AP4 abolishes the synaptically mediated rod and cone driven light responses (Wong et al., 2007). Thus, amacrine cells most likely inhibit ipRGCs from firing spontaneously in the dark, and the ON cone bipolar cell input drives them to spike during light stimulation. This was confirmed by Wong et al. (2007) by applying agents to block input from amacrine cells. Under these conditions, ipRGCs begin to fire spontaneously in the dark.

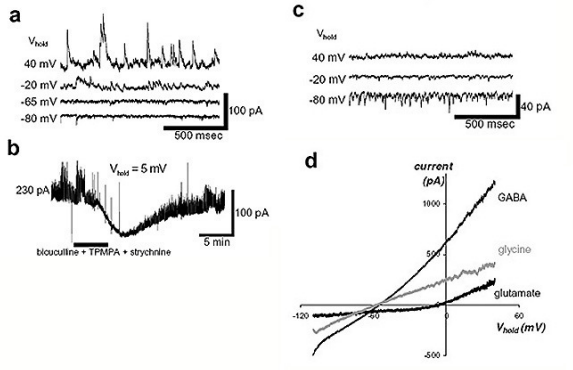

Fig. 12a. ipRGCs at rest show spontaneous inhibitory and excitatory synaptic inputs. (a) Synaptic currents in control ringers solution are driven primarily through presumed GABA or glycine receptors. (b) Application of drugs that block GABA and glycine receptors suppresses synaptic input when the ipRGC is held at the reversal potential for glutamate driven currents. (c) Blockade of amacrine cells reveals spontaneous excitatory inputs to ipRGCs. (d) Puffer pipette application of GABA, glycine and glutamate during voltage-ramps reveals receptors for all three transmitters are expressed in ipRGCs. Adapted from Wong et al. 2007.

Fig. 12. ipRGCs at rest show spontaneous inhibitory and excitatory synaptic inputs. (a) Synaptic currents in control ringers solution are driven primarily through presumed GABA or glycine receptors. (b) Application of drugs that block GABA and glycine receptors suppresses synaptic input when the ipRGC is held at the reversal potential for glutamate driven currents. (c) Blockade of amacrine cells reveals spontaneous excitatory inputs to ipRGCs. (d) Puffer pipette application of GABA, glycine and glutamate during voltage-ramps reveals receptors are for all three transmitters are expressed in ipRGCs. (e) Presumptive synaptic connection scheme for ipRGCs. '+' and '-' symbols represent excitatory and inhibitory receptors, respectively. ONL, outer nuclear layer; OPL, outer

plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFF BC, OFF bipolar cell; ON BC, ON bipolar cell; AC, amacrine cell; ipRGC, intrinsically photosensitive retinal ganglion cell. Adapted from Wong et al. 2007

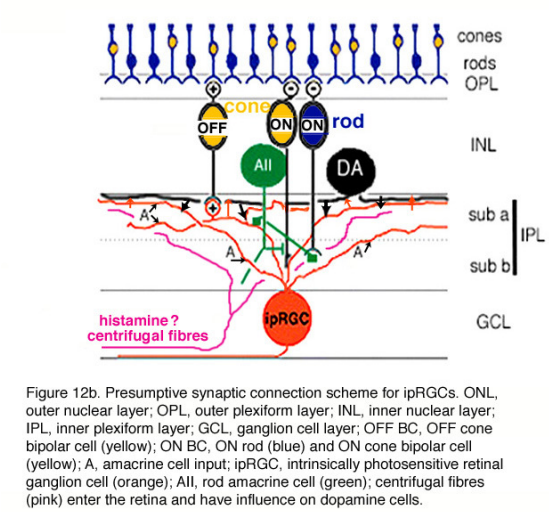

Figure 12b. Presumptive synaptic connection scheme for ipRGCs. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFF BC, OFF cone bipolar cell (yellow); ON BC, ON rod (blue) and ON cone bipolar cell (yellow); A, amacrine cell input; ipRGC, intrinsically photosensitive retinal ganglion cell (orange); AII, rod amacrine cell (green); centrifugal fibres (pink) enter the retina and have influence on dopamine cells.

Figure 12b. Presumptive synaptic connection scheme for ipRGCs. ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; OFF BC, OFF cone bipolar cell (yellow); ON BC, ON rod (blue) and ON cone bipolar cell (yellow); A, amacrine cell input; ipRGC, intrinsically photosensitive retinal ganglion cell (orange); AII, rod amacrine cell (green); centrifugal fibers (pink) enter the retina and have influence on dopamine cells.

If the input in the light comes from ON cone bipolar pathways, the input in the dark must come from a rod photoreceptor pathways. Thus in the wiring diagram of Fig. 12b we suggest that as well as ON cone bipolar input, the ipRGCs receive ON rod bipolar input through AII amacrine cells making gap juctions to ON cone bipolars and then to the ipRGC cell route. A paper from Ostergaard and coauthors suggests that, in dystrophic retinas, where remodeling is taking place, the rod bipolar cells seem to have direct synapses upon the cell bodies of ipRGCs and their proximal dendrites (Ostergaard et al., 2007), again suggesting that a rod pathway input to ipRGCs is necessary for their signaling function. In addition it is now known that ipRGCs receive synaptic input and feed-back to dopamine amacrine cells (Fig.12b, DA cell) (Vuglar et al., 2007; Zhang et al., 2008; McMahon et al., 2010). See further details in a later section in this chapter on intraretinal signaling involving dopamine cells. Another putative pathway that affects ipRGCs may be added by centrifugal fibres entering the retina (Fig. 12b). These centrifugal fibers are known to be histaminergic (Gastinger et al., 1999) and prove to have an affect on dopamine cells in mouse retina through H1 receptors (Frazao et al., 2011) (Fig. 12b).

7. Central Projections.

Using a transgenic line of mice where retinal ganglion cells, normally expressing melanopsin, instead express the marker enzyme _-galactosidase, Hattar et. al characterized the central projections of ipRGCs throughout the brain (Hattar et al., 2006). This method provides a very sensitive means to mark cell bodies, and more importantly, axonal projections. As expected for cells that regulate non-visual photic behaviors, such as circadian rhythms and pupillary constriction, they found ipRGCs sent dense projections to the suprachiasmatic nucleus (SCN), the intergeniculate leaflet (IGL), and the olivary pretectal nucleus (OPN) (Fig. 13-14) (Berson, 2003; Hattar et al., 2006). Thence through the Edinger-Westfal nucleus (EW) to the ciliary ganglion (CG) to innervate the iris (blue pathway in Fig. 13). In addition to these expected sites, a number of other central targets are seen. Within the hypothalamus, ipRGCs send axonal fibers to the ventral portion of the subparaventricular zone – a region controlling the autonomic nervous system (Hattar et al., 2006). Rostrolateral to the SCN, a number of scattered fibers reach the lateral and ventrolateral preoptic areas, which influence release of reproductive hormones from the pituitary (P, Fig.13) (Hattar et al., 2006) (yellow pathways in Fig. 13). Thus, ipRGCs most likely play a critical role in light regulation of a diverse array of non-image forming visual functions.

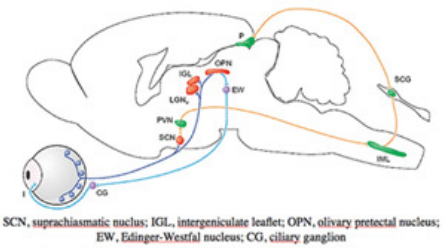

Fig. 13. Schematic representation of central targets of ipRGCs. From Berson 2003.

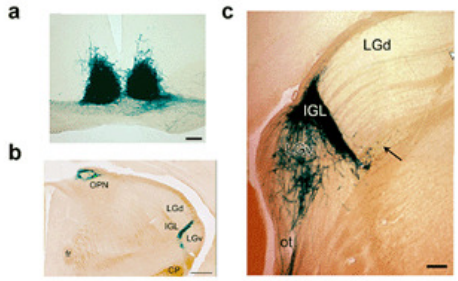

Fig. 14. X-gal staining of axonal projections from ipRGCs in tau-LacZ mouse. (a) ipRGCs send a very dense projection to the suprahciasmatic nuclei (SCN) located in the hypothalamus, consistent with their shown role in regulating photoentrainment of the biological clock, located in the SCN. (b) Staining showing ipRGC projections to the intergeniculate leaflet (IGL) and the olivary pretectal nucleus (OPN). Cerebral peduncle (CP), fasciculus retrofelxus (fr) and the optic tract (ot) are also shown. (c) Close-up view of staining in the IGL, the ventral region of the lateral geniculate nucleus (vLG), and a few stained fibers in the dorsal lateral geniculate nucleus (dLG). Adapted from Hattar et al. 2006.

In brains optimally stained for X-galactosidase activity, a thin sheet of tortuous fibers with apparent terminal swellings can be detected in the dorsal division of the lateral geniculate nucleus (LGN), the thalamic relay site of retinal input to the visual cortex (Fig.14) (Hattar et al., 2006). In addition, the superior colliculus (SC) is a significant target of labeled axon. These findings suggest that in addition to their known role in regulating visual reflexes, ipRGCs may play an as yet unknown role in shaping conscious visual perception. Interestingly, Dennis Dacey and colleagues found that in primates, ipRGCs could be retrolabeled by injecting fluorescent tracers into the LGN, and that these “giant” melanopsin-expressing ganglion cells display a color-opponent receptive field (Dacey et al., 2005).

8. Behavioral aspects of ipRGC function.

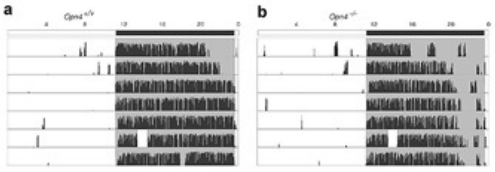

Fig. 15. Melanopsin knockout mice (b) show no significant difference compared to wild type animals (a) in their ability to photoentrain. Wheel running activity is represented as black tick marks during the day (white area) and night (shaded regions). Adapted from Panda et al. 2002.

Daily rhythms in mammalian physiology and behavior, collectively called circadian rhythms, are controlled by a tiny cluster of cells in the suprachiasmatic nuclei (SCN) of the ventral hypothalamus. SCN neurons posses a unique molecular clock that allows them to autonomously regulate activity patterns in near 24-hour rhythms, which ultimately leads to daily changes in animal behaviors such as sleep/wake cycles. However, the clocks are not tuned perfectly to 24 hours, and in order to adjust to changes in the animals environmental light/dark phases, the SCN must be entrained (or reset). Light is by far the most potent entrainment cue, and ipRGCs are the primary cells that carry this daily signal. Melanopsin knockout animals have relatively normal circadian rhythms, and do not display any overt dysfunction in their ability to entrain to light (Fig. 15) (Panda et al., 2002; Ruby et al., 2002). Measured both behaviorally and using cFos staining (a measure of activity in neurons) in the SCN, these animals show no significant difference to wild type animals (Panda et al., 2002; Ruby et al., 2002). However, they display a significantly attenuated phase shifting response (Fig. 16) to brief flashes of light, indicating a crucial role for ipRGCs and melanopsin signaling (Panda et al., 2002; Ruby et al., 2002). The insignificant change in photoentrainment of melanopsin knockout animals is most likely explained by the rod and cone input to ipRGCs, adding a redundant source of photic input to the SCN. This idea was tested directly by doing behavioral analysis on melanopsin knockout mice that lack rods and cones (Fig.17) (Hattar et al., 2003; Panda et al., 2003). In addition, Samer Hattar and colleagues developed an ingenious mouse line where genes encoding key enzymes in rod and cone signaling, as well as the melanopsin gene, were all deleted, leaving the cellular architecture of the retina completely intact, but devoid of all phototransducing capabilities (Hattar et al., 2003). Much like the melanopsin knockout mice without rods and cones, these “triple knockout” mice could neither phase-shift their circadian rhythms to brief pulses of light, nor could they photoentrain to light/dark cycles (Hattar et al., 2003).

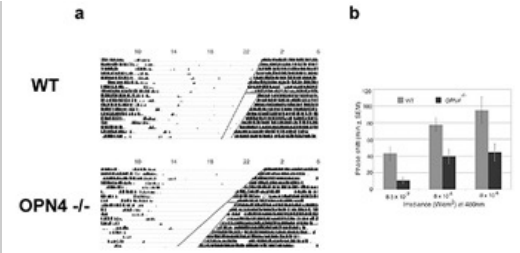

Fig. 16. (a) Wild-type animals (WT) are more sensitive to phase-shifting light stimuli than melanopsin knockout animals (OPN4 -/-). In this experiment, mice were kept in sound and light-proof boxes with no outside indication of time of day. The tick marks indicate rotations of a running wheel present in the cages, and the animals are said to be awake and active during persistent times of wheel running activity. Under these conditions the animals are "free running", which means there rhythm of running and sleeping is determined solely by their internal biological clock, and not outside cues such as light. The magnitude of phase-shifting is measured as the shift in the rhythm of wheel running activity in the days following a pulse of light (grey circles) during what is called the "subjective" night phase (peak of wheel running activity). A comparison of the grey horizontal bars in panel (a), representing the degree of change in the wheel running rhythm, demonstrate wild-type animal's wheel running rhythm is shifted further to the right (longer grey horizontal bar) in the days following a light pulse during the dark phase than is melanopsin knockout animal's wheel running rhythm. A comparison of the degree of the phase-shifting for various intensities of light for both wild-type and melanopsin knockout animals (OPN4 -/-) is given in (b). Adapted from Panda et al. 2002.

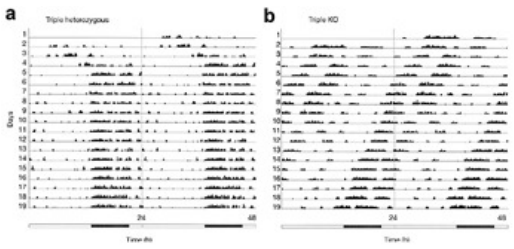

Fig. 17. Mice lacking both rod/cone function and the melanopsin gene (b) loose all ability to entrain their wheel running activity (indicated by black tick marks) to their environmental light/dark cycle, compared to litter mate control animals (a), indicating a role for both melanopsin-based signaling in ipRGCs and signaling via synaptic input from rods and cones in regulating circadian rhythms. Adapted from Hattar et al. 2003.

In addition to their role in regulating photoentrainment of circadian rhythms, ipRGCs are responsible for constriction of the pupil in response to light (Fig. 18) (Hattar et al., 2003; Lucas et al., 2003; Panda et al., 2003). Projections of ipRGC axons to the olivary pretectal nucleus (OPN), the retino-recipient site responsible for the pupillary light reflex, make them likely candidates for regulating this response. Indeed, melanopsin knockout animals have diminished pupillary light reflexes at high irradiance levels (Fig. 18) (Lucas et al., 2003). In addition, melanopsin knockout mice without rods and cones, as well as triple knockout animals, are completely unable to constrict their pupils in response to light (Fig. 18) (Hattar et al., 2003; Panda et al., 2003).

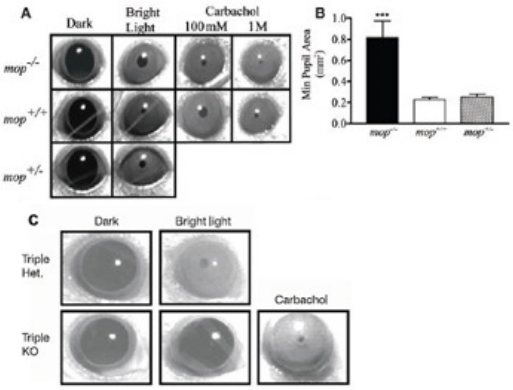

Fig. 18. ipRGCs are involved in regulating pupillary constriction. (a) Melanopsin knockout animals (mop -/-) show a defect in their ability to fully constrict their pupils in response to bright white light compared to wild-type animals (mo +/+), while the effect of the drug carbachol (which acts directly on the muscles involved in constricting the pupil) has the same effect in both melanopsin knockout and wile-type animals suggesting the defect is due to ipRGC signaling. (b) Summary data on pupil constriction for all melanopsin knockout and wild-type animals in response to bright light. (c) Animals with no functioning rod or cones, and missing the melanopsin gene completely loose the ability to constrict their pupil in response to light, indicating the role for both melanopsin-based signaling in ipRGCs, as well as signaling via synaptic input from rods and cones. Adapted from Lucas et al. 2003 and Hattar et al. 2003.

9. Adaptation.

The mammalian image-forming visual system is capable of operating over a wide spectrum of ambient light intensities covering more than ten orders of magnitude. While much of this range is due to the operation of two separate receptor systems (rods and cones) that operate over different light intensity ranges, some of the capacity is due to the ability of both rods and cones to change their sensitivity to light, a process called adaptation (Dowling, 1987; Rodieck, 1998). When placed in constant background light, rods and cones can generate an initial peak response which relaxes during the steady illumination, indicating desensitization to the light over time. This desensitization allows the photoreceptors to increase their dynamic range, allowing them to respond to light intensities that would otherwise be saturating. This is termed “light adaptation”. Conversely, when placed back into a dark environment, rods and cones are able to regain their sensitivity, a process called “dark adaptation”. Both light and dark adaptation are a direct consequence of changes in the biochemical transduction cascades operating in the receptors. While rods have a limited ability to adapt to background illumination, saturating at high light levels, cones exhibit an almost infinite capacity to adapt to light levels (Fain et al., 2001; Knox and Solessio, 2006).

Due to their role in stable representations of absolute light levels, and unusually tonic response properties, it was not clear whether ipRGCs would display similar adaptation mechanisms as rods and cones. To address this issue, Wong and colleagues performed recordings from ipRGCs under luminance conditions to test for both light and dark adaptation. They found that when exposed to a near saturating background light, ipRGCs gradually became desensitized, and responses to a brighter pulse of light on top of the background light became larger over time, a hallmark of light adaptation (Fig. 19) (Wong et al., 2005). Furthermore, when ipRGCs were left in the dark for increasing periods of time, their responses to the same light stimulus intensity increased as well, a clear indication that in addition to adapting to light, ipRGCs are capable of dark adaptation as well Fig. 20 (Wong et al., 2005).

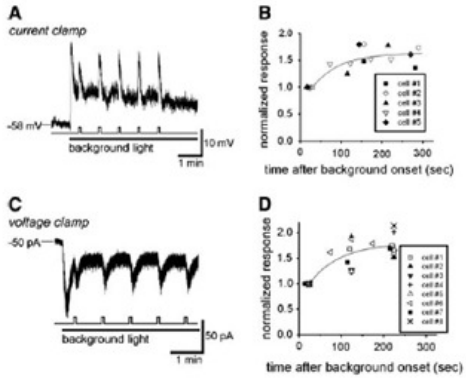

Fig. 19. Evidence for light adaptation in ipRGCs. (a) Current-clamp recording from an ipRGC with test pulses flashed on top of a background light. The cell decreases its sensitivity to the background light over time and is able to generate a larger response to the test pulse towards the end of the recording compared to the response to the same test pulse right after onset of the background light. (b) Summary data for current clamp recordings from five ipRGCs. (c) Voltage-clamp recording using same conditions as (a) showing similar adaptation to the background light. (d) Summary data for voltage-clamp recordings from five ipRGCs. Adapted from Wong et al. 2005.

To track the duration of dark adaptation, the authors used a variation of the patch clamp technique, known as “cell-attached” recording. In this configuration, the patch pipette is sealed onto the cell without breaking in, allowing one to record extracellular activity (in this case action potentials) over long durations without washing out vital intracellular components to cell health and function. Using this technique, the authors found that dark adaptation in ipRGCs is much slower than in rods and cones. Even after 1 hour, ipRGCs appear to continue to dark adapt, increasing their response amplitude to the same light intensity (Wong et al., 2005). Although an exact time point for full dark adaptation is not feasible with electrophysiological approaches used in their study, the authors estimate the time course to be at least several hours (Wong et al., 2005).

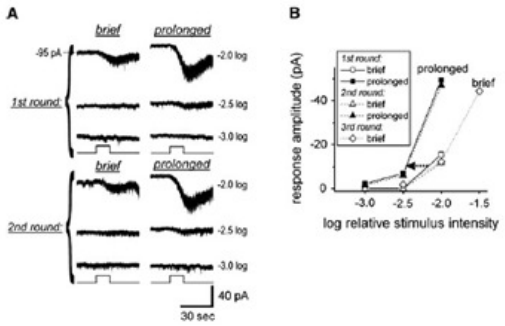

Fig. 20. Evidence for dark adaptation in ipRGCs. (a) Voltage-clamp recordings from an ipRGC responding to different illuminance intensities after multiple rounds of brief and long periods of dark adaptation. Notice the cell consistently generates a larger response to the intensities after longer periods of being in the dark. (b) Summary

data for response amplitudes to intensities for all rounds of brief and prolonged dark adaptation. Adapted from Wong et al. 2005.

10. Intra-retinal signaling.

Dopamine is a neurotransmitter known to play many roles in modulation of neural circuits, and in the retina it is released by a sub-population of amacrine cells (Dowling 1987). These dopaminergic amacrine neurons (DA neurons) play a major role in reshaping the functional properties of retinal circuits according to current lighting conditions, and thus allow the retina to be a more dynamic system. DA neurons come in multiple flavors showing transient, sustained and null responses to light (Zhang, et al., 2007). Interestingly, the sustained responding DA neurons are unaffected by drugs blocking ON-bipolar cell input (L-APB4) (Fig. 21) (Zhang et al., 2007; Zhang et al., 2008). Where do these sustained responses come from if not from bipolar cells? The sustained response to light in DA neurons peaks at a wavelength near 480nm, matching very well with a melanopsin-based input (Zhang et al., 2008). It is now thought that ipRGCs may be providing the synaptic input to these cells (Zhang et al., 2008). The sustained 480nm signals are absent in sustained dopamine cells in melanopsin gene knockout mice (McMahon et al., 2010) concluding again that the ipRGCs have input to the sustained dopamine cells at least.

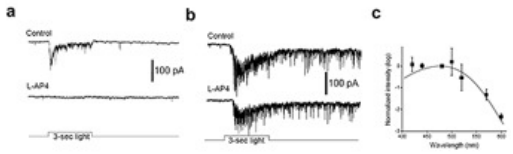

Fig. 21. Physiological evidence for a melanopsin-based input to dopaminergic amacrine neurons (DA neurons). Whole-cell voltage clamp recordings from a transient DA neuron (a) and a sustained DA neuron (b). Notice how the application of L-AP4, an agent used to block responses from rods and cones, completely eliminates the light response from the transient DA neuron (a), whereas it has almost no effect of the sustained DA neuron (b). Panel (c) shows a fitted plot of responses to varying wavelengths of light, showing a peak near 480nm.

In addition, staining for cFos, an immediate early gene used to mark activated neurons, showed that roughly the same percentage of DA neurons are activated by sustained light in retinas without rods and cones (rd/rd animals) as retinas with ON-bipolar cells pharmacologically blocked using the drug L-AP4 (Fig. 22) (Zhang et al. In press). Presumably in both situations the only remaining photosensitivity in the retina would be from ipRGCs. Therefore, the evidence suggests that ipRGCs are communicating back to DA neurons through their dendrites, although no evidence of release sites or vesicles in melanopsin-positive dendrites have been seen. However, a number of recent studies have shown that ipRGC dendrites and DA neuron dendrites co-stratify and have synaptic communication (as shown in Fig. 12b) (Belenky et al., 2003; Viney et al., 2007; Vugler et al., 2007; Zhang et al., 2008; McMahon et al., 2010).

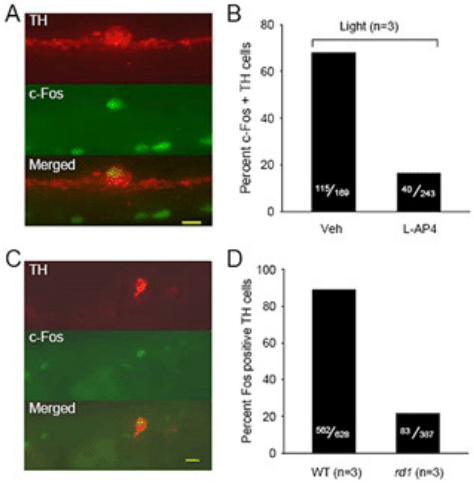

Fig. 22. c-Fos staining in retinas with rod/cone input blocked (a-b), and in retinas with no rods or cones (c-d). Example stainings are shown in the left panels, with red representing the dopaminergic amacrine neurons (DA neurons) (labeled TH for tyrosine hydroxylase) and green for the immediate early gene cFos, an indicator of neural activity. Summary results for all DA neurons with positive cFos staining under both experimental conditions are shown in the right panels. In retinas from wild-type (WT) animals with the drug L-AP4 injected, roughly 20% of the DA neurons were cFos positive, and therefore presumably activated by light. The control retinas from wild-type animals showed about 70% of DA neurons were activated by light, indicating only a small number of DA neurons are potentially contacted by ipRGCs. Likewise, using a mouse line without rods or cones, roughly 20% of DA neurons stained positive for cFos, matching very well with the L-AP4 experiments. Wild-type animals had roughly 90% of DA neurons with positive cFos staining.

11. Other types of melanopsin ganglion cells

David Berson, who was the original discoverer of the melanopsin type of ganglion cell in the retina, has now concluded that there are at least two other morphologically different types of melanopsin cells in mice retina (Berson et al., 2010). The original one, and the subject of this chapter, called M1, has sparse dendritic trees ramifying in sublamina **a** of the IPL under the amacrine cell bodies (Fig. 23 A. B and C, Fig. 24 H1). M2 cells, on the other hand, are larger-bodied than M1 and stratify in sublamina **b** of the IPL (Fig. 23 D, E and F, Fig. 24, M2 cells). A rare bistratified ipRGC is also seen that has dendrites contributing to both M1 and M2 dendritic layers. A further paper by the Berson's goup (Ecker et al., 2010) notes that 5 types of ipRGCs can be detected in a Cre-based melanopsin reporter mouse line. These cells project to brain areas other than the suprachiasmic nucleus of the M1 type, accessing both lateral geniculate nucleus and superior colliculus (Ecker et al., 2010). Other mammals may have different types of melanopsin ganglion cells from those classically described in mouse. One, recorded from and stained by Dacey and coworkers (2005) in primate retina, is a "giant cell", intrinsically photosensitive, activated by rod and cone pathways and has a blue-OFF center and yellow on surround receptive field.

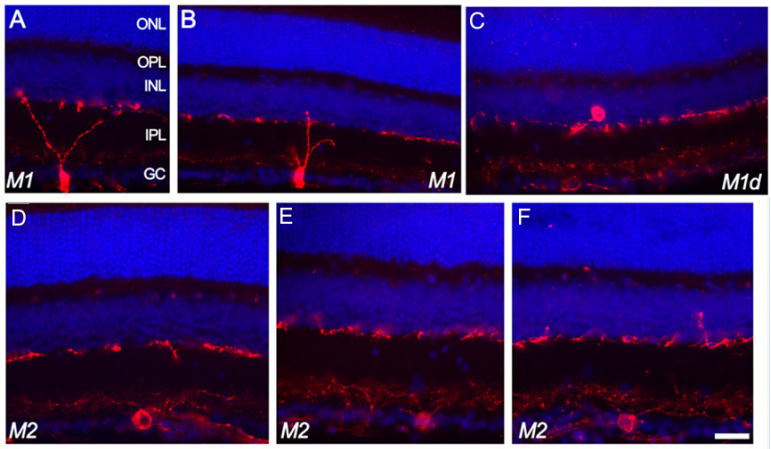

Fig. 23. Ganglion cells of the M1 and M2 types as revealed in vertical sections of the mouse retina immunostained for melanopsin (red). A nuclear counterstain (DAPI; blue) reveals cellular laminae. Top two rows of images (A–C) illustrate melanopsin-immunoreactive cells of the M1 type, characterized by dendritic arborizations in the outer melanopsin immunoreactive plexus lying at the boundary between inner plexiform and inner nuclear layers. Some of these M1 cells were conventionally placed with somata in the ganglion cell layer while others ("M1d") had somata displaced to the inner nuclear layer. The fainter inner (M2) plexus is barely detectable in some of these images. M2 cells (bottom row; D-F) had conventionally placed somata and laterally spreading dendrites entering the inner (M2) plexus of immunopositive dendrites. Scale bar: 30 μm. ONL=outer nuclear layer; OPL=outer plexiform layer; INL=inner nuclear layer; IPL=inner plexiform layer; GC=ganglion cell layer. (From Berson et al., 2010).

Fig. 23. The morphologies of M1 and M2 ganglion cells as seen in vertical sections of immunostained (melanopsin, red; DAPI, blue) mouse retina. (From Berson et al., 2010)

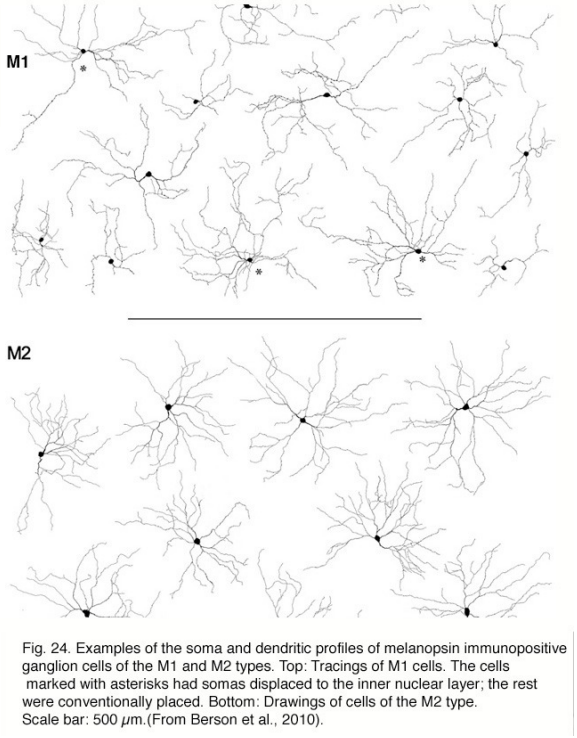

Fig. 24. Examples of the soma and dendritic profiles of melanopsin immunopositive ganglion cells of the M1 and M2 types. Top: Tracings of M1 cells. The cells marked with asterisks had somas displaced to the inner nuclear layer; the rest were conventionally placed. Bottom: Drawings of cells of the M2 type. Scale bar: 500 μm.(From Berson et al., 2010).

Fig. 24. Drawings of immunostained melanopsin ganglion cells in mouse retinal wholemount. M1 and M2 cell morphologies are shown. (From Berson et al., 2010).

ipRGCs are a novel mammalian photoreceptor whose morphological and physiological characteristics seem well suited for their primary role as light detectors for non-image forming visual reflexes. However, many mysteries remain, and an untold number of functions for this rare and special type of ganglion cell should not be overlooked. Their invertebrate-like phototransduction cascade makes them unique among all other known vertebrate photoreceptors, and provide a window into possible mechanisms of the evolution of the retina. In addition to their intrinsic melanopsin-driven photosensitivity, ipRGCs also receive rod and cone synaptic input and thus may provide the brain with different information in series, separated by complex spatial and temporal dynamics. Although they drive a number of tonic behaviors, requiring accurate representation of ambient light levels of long periods of time, ipRGCs have the ability to adapt to both light and darkness, and appear to have an ability to communicate back to the retina, possibly changing the functional properties of retinal circuitry. The functional role these processes have on animal behavior remains to be understood. As the years catch up with this relatively young field of intrinsically photosensitive retinal ganglion cells, new and interesting cell types are being revealed and there is still much to learn in the future about them.

Other mammals may have different types of melanopsin ganglion cells from those described above in mouse. One, recorded from and stained by Dacey and coworkers (2005) in primate retina, is a “giant cell”, intrinsically photosensitive, activated by rod and cone pathways and has a blue-OFF center and yellow on receptive field (Figs 25 and 26). This melanopsin ganglion cell is intrinsically photoreceptive due to the melanopsin photopigment in its membrane but also is driven by both rod and cone inputs in an ON response (Fig. 26, top left). Furthermore the cone input is S-cone (blue) OFF and M- and L-cone ON in response (Fig. 26, top right), with a coextensive receptive field organization (Fig. 26, receptive field difference of Gaussians). The morphology of the “giant” ganglion cells is shown in Fig. 25. As can be seen this cell type is many times larger in both cell body size and dendritic field diameter than a comparative peripheral midget ganglion cell (Fig. 25e, dendritic tree covers a 1 mm area). Dendrites of the “giant” melanopsin cell stratify in stratum S1 right under the amacrine cell layer. And further dendrites run in S5 above the ganglion cell layer (Fig. 25c). There may be two varieties of this “giant type” GC labeled upper and lower according to where their dendrites run in the IPL and in one case having a displaced cell body (Dacey et al., 2005). Alternatively there is only one type that has dendrites in both strata of the IPL and often exhibits a displaced cell body. The “giant” blue-OFF GCs project to the parvo and magnocellar layers of the lateral geniculate nucleus (Dacey et al., 2005). This suggests that this ganglion cell is involved in color processing vision as well as low level light detection due to the melanopsin photopigment it contains.

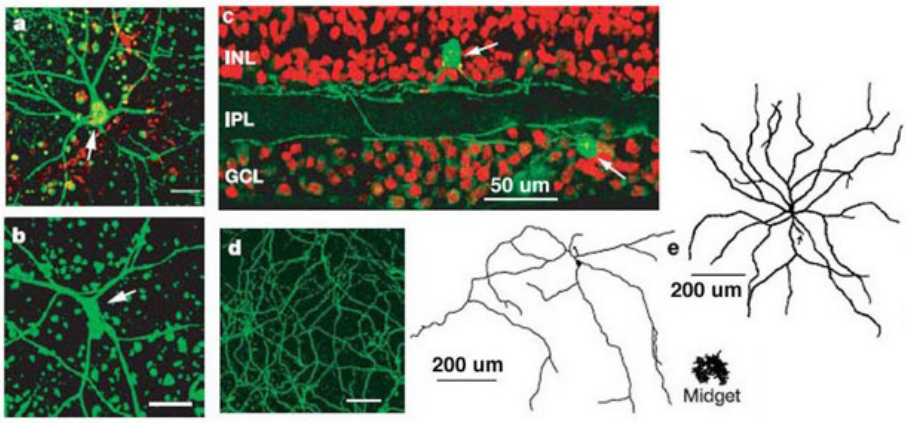

Fig. 25. The morphology of the "Giant" melanopsin ganglion cell in monkey and human retina. a) Melanopsin-IR human ganglion cells (green) as seen in peripheral retina wholemount. b) Macaque melanopsin-IR ganglion cell. Scale bars both 50um. c) Dendrites of melanopsin-IR ganglion cells running in S1 and S5 of the IPL. Vertical sections, stacked confocal images. d) Melanopsin-IR ganglion cells in peripheral retina. Scale bar 200um. E) drawings of "giant" melanopsin-IR ganglion cells. Most right-hand cell is a "giant" cell that was intracellularly recorded and filled with Neurobiotin. A peripheral midget ganglion cell is drawn for comparisons of size in this part of peripheral retina. (Adapted from Dacey et al., 2005).

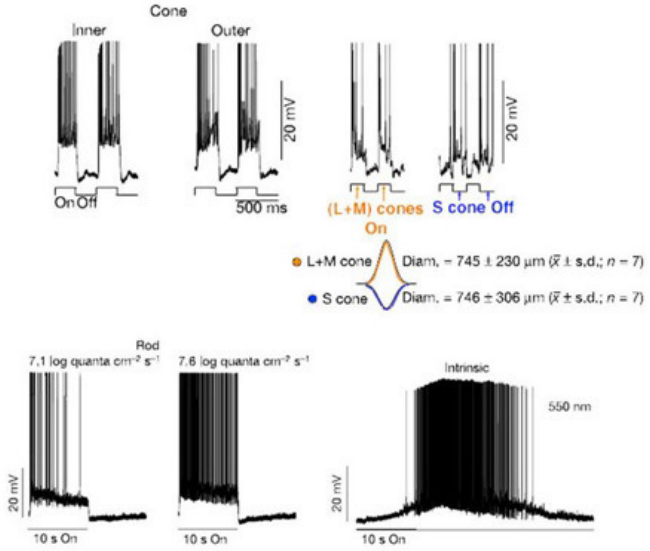

Fig. 26. Intracellular recordings of cells light response to cone stimulating wavelengths show "giant" cells respond with a vigorous ON-response. When the cone responses are narrowed in wavelength to specifically stimulate at S-cone wavelengths, the "giant" cells respond OFF to S-cone (blue) light and ON to S+M=cone stimulating light. The receptive field is blue OFF and yellow ON coextensive with no surround inhibition as shown in the difference of Gaussians plot. The "giant" cell gives a strong ON response to rod stimulating wavelengths. Right, the response to rods and cone stimulations is blocked by L-AP4 and the slow, excitatory, sustained, intrinsic response to light (550 nm) is revealed. (From Dacey et al., 2005).

12. Conclusions

ipRGCs are a novel mammalian photoreceptor whose morphological and physiological characteristics seem well suited for their primary role as light detectors for non-image forming visual reflexes. However, many mysteries remain, and an untold number of functions for this rare and special type of ganglion cell should not be overlooked. Their invertebrate-like phototransduction cascade makes them unique among all other known vertebrate photoreceptors, and provide a window into possible mechanisms of the evolution of the retina. In addition to their intrinsic melanopsin-driven photosensitivity, ipRGCs also receive rod and cone synaptic input and thus may provide the brain with different information in series, separated by complex spatial and temporal dynamics. Although they drive a number of tonic behaviors, requiring accurate representation of ambient light levels of long periods of time, ipRGCs have the ability to adapt to both light and darkness, and appear to have an ability to communicate back to the retina, possibly changing the functional properties of retinal circuitry. As the years catch up with this relatively young field of intrinsically photosensitive retinal ganglion cells, it is beginning to look like many ganglion cell types are melanopsin photoreceptive and have dual roles in both image processing, color processing and subconscious circadian photoentrainment.

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Updated July, 2011

The author

Dr. Dustin M. Graham was born and raised in Pleasanton, California and received his Ph.D. in neuroscience from Brown University. He began his research career at Santa Clara University in the lab of Dr. David Tauck, studying neural pathways of learning and memory in the pond snail Lymnaea stagnalis. His interests turned to the retina while working with Dr. Ralph Nelson at the National Institutes of Health. There he helped develop a rapid labeling technique to delineate morphological subtypes of retinal neurons in zebrafish. As a graduate student, Dustin studied mammalian circadian rhythms and the newly discovered melanopsin ganglion cell activity in Dr. David Berson's lab. He focussed on the phototransduction cascade in ipRGCs, and developed a dissociation and culturing procedure to identify and record light responses from isolated ipRGCs. Dustin is currently a post-doctoral research fellow in the Psychology department at the University of Virginia where he studies development and synaptic mechanisms of the gustatory system in rats.

10 Responses

Comment Feed

Aloha Dustin Graham:

Humans have been wandering over the globe for thousands of years. Experienced hunters seldom get lost. Some humans always seem to know where north is. Wild and semi-wild animals can usually find their way even in blinding conditions. Ancient Polynesians are said to have been able to accurately sail to distant islands, ostensibly by reading ocean swells – perhaps. Or they, the very few, select navigators, could have been using an electro magnetic sense. Grazing, free ranging, cattle typically orient themselves in a magnetic north/south direction. In fact a great many animals seem to know where north is most of the time.

Two recent articles have caught my attention, one in the Jan/Feb, 2012 issue of Discover and the other in the Jan, 2012 issue of Sci. Am. The Sci. Am. Article discusses magnetism in animals and mentions that

cryptochrome is involved. The Discover article mentions melanopsin and how, as it is found in certain ganglion in the eyes, it enables animals to set their biological clocks, even if rods and cones are silenced – blue light seems to be the key. However, the biological signals are disrupted by power lines. The Sci. Am. Article pretty much deals with cryptochrome, but does mention the possibility that humans might have some magnetic sense. I've not noted anything in my organization's publication, Science – there may be some as I haven't done a detailed search.
I did a brief search of other articles and found a couple; 2008, Nature, v.454(7207):1014-1018: 2004, PNAS, v. 101(39):14294-14299; and a sort of update (2011) on cryptochrome in the Theoretical and Computational Biophysics Group, U of Ill. At Urbana-Champaign web page.

Why am I sending this to you? No particular reason other than in my searches I came across your article on Web vision about a "Bit of fly in the mammalian eye" and enjoyed it, at least the parts I understood. I read a lot of science and if I have a scientific skill it appears to be able to see a common thread from different fields. So perhaps here.

If the above is common knowledge, I apologize for taking up your time.

Burt Smith, PhD (retired)
okburt75@yahoo.com

Burt Smith — December 22, 2011 @ 3:52 pm — Reply

Nice piece. However I found that your reference to Keeler is incorrect. I think the paper of him you want to refer to is: Keeler, C.E. (1927). Iris movements in blind mice. Am. J. Physiol. 81, 107–112. It is cited correctly in the Van Gelder 2008 article.

Duco Endeman — July 26, 2013 @ 4:18 am — Reply

> Much obliged Duco. Appreciate the correction.
>
> **Bryan William Jones** — July 26, 2013 @ 9:52 am — Reply

I do not know how Dr. Dustin, among so many papers, missed a unique related paper: 1993: 125, 32-25; the only embryonic study that reported the third type of photoreceptors in inner retina of human embryo and non visual pigments which later they changed to ipRGCs. I invite you also to see IRJBCS VOl(1)1-5 AJan 2013. Thank you for your and every one attention and response

A. A. Kashani M.D. — September 4, 2014 @ 11:48 pm — Reply

> Thank you for pointing out your paper Dr. Kashani.
>
> **Bryan William Jones** — September 5, 2014 @ 8:19 am — Reply

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