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Mammalian Inner Retinal Photoreception

Review

Robert J. Lucas

It is now a decade since the first published reports that a small proportion of mammalian retinal ganglion cells are directly photoresponsive. These cells have been termed intrinsically photosensitive retinal ganglion cells (ipRGCs) and comprise a small proportion of the total population of retinal ganglion cells. The demonstration that these ganglion cells respond to light even when isolated from the rest of the retina established them as potentially autonomous photoreceptors, overturning the dogma that all visual information originates with rods and cones. It also provided a focus for what has developed into a new branch of visual science. Here we place the discovery of ipRGCs into context and review the development of this field over the last decade, with particular emphasis on prospects for practical application.

Introduction

Up until 2002 the vast majority of vision scientists took it as dogma that rods and cones were the retina's only photoreceptors. From this standpoint, the discovery of ipRGCs overturned one of the most fundamental assumptions of how the retina works. However, for another group of researchers, interested in how endogenous circadian clocks are synchronised (entrained) to local time, it validated a conviction that such non-rod, non-cone photoreceptors must exist.

Almost all species exhibit daily cycles in physiology and behaviour generated by endogenous circadian clocks. If these clocks are to provide a selective advantage they must be constantly adjusted to ensure synchrony with external time. The diurnal light:dark cycle is the most reliable environmental representation of local time, and is used as the primary entraining agent for many species. In non-mammalian vertebrates, the photoreceptors responsible for tracking the light:dark cycle and entraining the clock are largely extraocular. They can be found in specialised organs in the central nervous system (pineal, parapineal, parietal eye), in parts of the brain itself, and across many non-neural organs and tissues (see [1] for review). They thus represent a distinct sensory modality, quite separate from what would be conventionally regarded as the visual system.

Mammals, by contrast, rely upon ocular photoreceptors [2] and a distinct component of the retinal projection [3] to entrain the clock. Prior to the discovery of ipRGCs this posed a conundrum: as rods and cones were the only known retinal photoreceptors, it seemed axiomatic that both vision and circadian photoentrainment relied upon these receptors in mammals. But if rods and cones could effectively entrain mammalian clocks, why should non-mammalian vertebrates employ such a wealth of extra-ocular photoreceptors to fulfil this function?

In fact, data which appeared through the 80s and 90s indicated that at some level a similar distinction between pattern vision and circadian photoreception existed also in mammals. A variety of laboratory rodents with progressive outer retinal degeneration were shown to retain circadian photoentrainment even at ages at which conventional visual responses were massively impaired [4–7]. More surprisingly, careful assessments of circadian photosensitivity in these animals revealed that near complete loss of rods and cones had no discernable effect on the clock's sensitivity. Moreover, some profoundly blind human subjects were found to retain circadian light responses [8].

At the time, the most parsimonious explanation for those findings was that the circadian photoentrainment mechanism was constructed in such a way as to extract sufficient visual information from a very few surviving rods or cones even in advanced retinal degeneration. However, this explanation was found wanting in studies using transgenic mice lacking rods and cones. In the first case, these animals were shown to retain circadian entrainment and a variety of other reflex light responses [9–12]. Secondly, a detailed examination of the residual sensory capacity of these rodless+coneless mice revealed characteristics (threshold sensitivity, response kinetics and, above all, spectral sensitivity) quite different from those of retinal rod or cone photoreceptors [12]. It thus became increasingly hard to avoid the conclusion that the retina must contain some new photoreceptor capable of eliciting light responses even in the absence of rods and cones.

Such attempts to understand mammalian photobiology were complemented by parallel studies of the molecular mechanisms of extra-ocular photoreceptors in non-mammals. Several new photopigment proteins were isolated from photoreceptive cells/tissues from a variety of vertebrate species [1]. These showed structural similarity with the well-known rod and cone opsins, and phylogenetic analyses placed them in the opsin family of proteins. Most of the genes encoding such extra-ocular photopigments were subsequently found to have been lost from the mammalian genome. The exception was melanopsin, a pigment initially isolated from the photosensitive dermal melanophores of *Xenopus* [13]. A melanopsin orthologue was found in both mouse and human genomes and *in situ* hybridisation histochemistry showed that it was expressed in a subset of retinal ganglion cells [14]. Retrograde tracing from the suprachiasmatic nucleus (SCN; site of the circadian clock) further revealed that at least some of these melanopsin-expressing ganglion cells provide visual information to the clock [15].

The Discovery of ipRGCs

Two papers published back to back in *Science* in 2002 [16,17] drew these themes together to make the breakthrough discovery of ipRGCs (Figure 1). Berson and his colleagues [16] employed retrograde tracers to label those retinal ganglion cells innervating the master circadian clock in the suprachiasmatic nuclei (SCN) of the rat. They then targeted those cells with recording electrodes in an *ex vivo* retinal prep. They found that SCN-projecting ganglion cells responded to light by depolarising and increasing their firing rate. Amazingly, this occurred when a cocktail of pharmacological agents designed to halt all intercellular communication in the retina was applied and even when these cells were physically isolated from the rest of the tissue. They concluded that, uniquely among mammalian

Faculty of Life Sciences, University of Manchester, Manchester, M13 9PT UK.
E-mail: robert.lucas@manchester.ac.uk

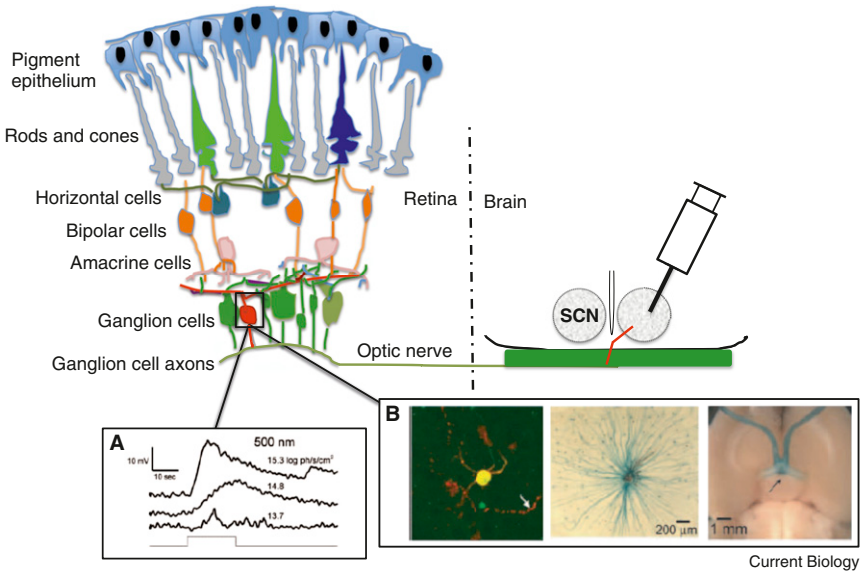

Figure 1. The discovery of intrinsically photosensitive retinal ganglion cells (ipRGCs).

Two papers in 2002 reported the discovery of ipRGCs. Berson *et al*. [16] used retrograde tracing to identify the small number of ganglion cells (shown in red) that project to the SCN (site of the circadian clock) and using recording electrodes found that they are excited by light even when isolated from rod/cone input (Insert A shows depolarising responses from a SCN-projecting neurone when isolated from rod/cone influence and exposed to stimuli of increasing irradiance taken from that paper). Hattar *et al*. [17] showed that these back-labelled cells express melanopsin. Insert B left panel shows a single cell back-labelled from the SCN, filled with dye from a recording electrode (green) and counter stained for melanopsin (red). They also generated a reporter mouse in which melanopsin-expressing cells and axons could be stained blue, revealing that this photopigment is found in a subset of retinal ganglion cells. Insert B middle panel shows an *en face* view of the mouse retina, with blue-stained axons converging on the optic nerve head. This reporter also showed that the melanopsin-expressing ganglion cells strongly innervated the SCN. Insert B right-hand panel shows a ventral view of the mouse brain from a transgenic mouse. Note the strong blue stain in the optic nerves and in the hypothalamus above the optic chiasm. (Panels A and B were reproduced with permission from *Science*.)

retinal ganglion cells, these cells innervating the clock were directly photoreceptive.

Berson *et al.* [16] continued to describe sensory characteristics for the ipRGC's intrinsic light response qualitatively similar to those previously described for behavioural responses in rodless+coneless mice [12]. Thus, when isolated from synaptic input, ipRGCs were most sensitive to light in the blue part of the spectrum ($\lambda_{max} \sim 480$nm). Furthermore, even at these wavelengths, the intrinsic response was elicited only by very bright light (several orders of magnitude above the threshold for cone vision), and had very poor temporal resolution (lagging changes in light intensity by several seconds). On the other hand, Berson *et al.* continued to show that ipRGCs had a good ability to encode background illumination, with steady light exposure inducing a tonic depolarisation, the magnitude of which was dependent upon stimulus irradiance over several decimal orders. In other words, ipRGCs had sensory characteristics well-suited to their proposed role of circadian photoentrainment.

In the associated paper, Hattar and colleagues [17] extended these observations by showing that these light-responsive ganglion cells expressed melanopsin. They did this first by immunocytochemical staining of cells shown, using the techniques outlined in the Berson *et al.* paper, to be intrinsically photoreceptive. They continued to generate a transgenic mouse in which β-galactosidase was localised to the cell body, dendrites, and axons of melanopsin-expressing cells. Histochemical staining of the retina of these transgenic mice revealed an array of melanopsin-expressing ganglion cells whose axons converged on the optic nerve head. Remarkably, however, tracing of those axons along the optic nerve revealed that while they strongly innervated the SCN and some other accessory visual structures, they were largely absent from the major retinal projection to the dorsal lateral geniculate (dLGN). As the dLGN is the origin of thalamocortical projection neurons, it seemed that those ganglion cells expressing melanopsin were excluded from the central pathways responsible for image-forming vision.

The discovery of ipRGCs provided the final proof that some light responses in mammals could originate with non-rod, non-cone photoreceptors. It also represented a breakthrough in our understanding of the retinal circuitry responsible for circadian photoentrainment. The intervening decade has seen this fundamental discovery expand in a number of important directions. It is not possible to cover each of these in detail here, but the major advances fall into a number of broad areas of progress.

What Do ipRGCs Do?

While the attempt to understand circadian photoentrainment was a conspicuous motivation in the discovery of ipRGCs, it has been clear from the start that the influence of ipRGCs extends beyond this single role. The description of a strong pupil light reflex (PLR) in rodless+coneless mice [12] provided part of the justification for seeking new photoreceptors, and it has since become clear that ipRGCs are an important element of the pupilomotor system in both laboratory animals and humans [18]. Similarly, photic inhibition of pineal melatonin production was attributed to non-rod, non-cone photoreceptors prior to the discovery of ipRGCs on the basis of its retention in retinally degenerate rodents [10] and human patients [8], and because of its unusual spectral sensitivity [19,20]. Since their discovery, ipRGCs have been further implicated in providing photic information to sleep/wakefulness systems [21–25], modulating cognitive function [26] (although see also [27]), and to be responsible for at least some aspects of photophobia/photoallodynia [28–30].

The picture that emerges is one in which ipRGCs elicit a range of reflex and sub-conscious light responses. This has given rise to the concept of a 'non image forming' (or NIF) visual system, originating with ipRGCs, and responsible for adjusting multiple aspects of our physiological and behavioural state according to the level of ambient illumination. As we will see later, this idea of ipRGCs as NIF photoreceptors likely underestimates their importance. Nevertheless, it

has proved a useful concept in grouping together some of the major functions of this photoreceptor class.

How Do ipRGCs Work?

Hattar *et al.*'s demonstration that ipRGCs contain melanopsin [17] represented strong circumstantial evidence that this was the photopigment responsible for their photosensitivity. Proof that this was indeed the case came over the following few years with the demonstration that the ipRGCs of melanopsin knockout mice were no longer directly photosensitive [31], and that heterologous expression of melanopsin made other cell types light responsive [32–34]. Melanopsin is therefore necessary and, at least in some cases, sufficient to make cells photoreceptive.

Opsins employ G-protein signalling cascades to translate light absorption into a physiological response. The cognate G-protein of rod and cone opsins is transducin (a member of the Gi class), which activates cGMP phosphodiesterase as its effector enzyme. The resultant light-dependent reduction in local cGMP concentration closes cGMP-gated cation channels and hyperpolarises the photoreceptor [35]. Across the opsin family, however, there is great diversity in the nature of such phototransduction cascades.

When melanopsin was first discovered there was excitement that it shared somewhat greater structural similarity with invertebrate rhodopsins than rod/cone opsins [13,14]. One reason for this interest was that invertebrate visual photoreceptors (like ipRGCs, but unlike rods or cones) depolarise in response to light. Could the sequence of events linking photon absorption to cellular depolarisation in ipRGCs be similar to those of invertebrate photoreceptors? The invertebrate phototransduction cascade is well described and involves a G-protein of the Gq/11 class that activates phospholipase C to produce IP3 and DAG, and results in opening of TRP channels [35]. Physiological and pharmacological data broadly support the view that a similar sequence of events is responsible for the intrinsic light response of ipRGCs (see [36] for an excellent recent review). However, with one or two exceptions, the molecular components of the melanopsin phototransduction pathway remain unknown, and a great deal of work remains before our understanding of this cascade approaches the quantitative sophistication of our knowledge of both rod/cone and invertebrate phototransduction.

One interesting aspect of the melanopsin phototransduction cascade is that it likely has very high gain. Do *et al.* [37] calculated that photoactivation of a single melanopsin photopigment sets in train such a long lasting activation of the phototransduction cascade that the resultant ipRGC depolarisation has a recordable impact on spike firing. This high amplification is thought to compensate for the very small amount of melanopsin in the retina. ipRGCs lack the specialised membraneous discs that rods and cones use to accommodate large amounts of photopigment, and thus must contain proportionally much less opsin. The resultant low probability of photon capture by melanopsin could be important in limiting the potential of ipRGCs to screen rods and cones, which lie further down the light path. However, it means that ipRGCs absorb few photons even under relatively bright illumination, and thus require high signal amplification in order to encode physiologically relevant light intensities.

Another reason that melanopsin's structural similarity with invertebrate rhodopsins provoked such interest is that it provides a potential explanation for the apparent bleach resistance of ipRGCs. In their original description, Berson *et al.* [16] reported that the ipRGC light response persists under bright and/or extended light exposure. As the primary event in photoactivation of opsins is the light-dependent isomerisation of the retinaldehyde chromophore from a *cis*- to all-*trans* conformation, that observation implied the presence of a local and depletion-resistant source of *cis*-retinaldehyde. Invertebrate rhodopsins achieve similar performance by retaining all-*trans* following light absorption and using a second photon to regenerate the *cis* isoform. This light-dependent regeneration mechanism ensures that a portion of the opsin binds *cis*-retinaldehyde, and is able to detect further light exposure, even under the most extended and intense illumination. If melanopsin had similar characteristics, that would explain why ipRGCs were never bleached, irrespective of how much light they were exposed to. Both physiological and spectroscopic evidence in favour of this arrangement has accumulated [32,34,38–40] (although see also [41] for a different interpretation of some of those data), and it now seems most likely that at least a portion of melanopsin's bleach resistance can be explained by such intrinsic light-dependent regeneration. However, there is also good evidence that melanopsin employs a light-independent bleach recovery mechanism [42]. The nature of that latter event is unknown, as is the relative importance of the extrinsic and intrinsic bleach recovery processes *in vivo*.

Mixing It with Rods and Cones

Although the signature feature of ipRGCs is their ability to respond to light even in complete isolation, there is extensive evidence that they also perform the more conventional ganglion cell function of acting as conduits for visual information originating in rods and cones. ipRGCs have extensive dendritic trees in the inner plexiform layer, which function not only as a site for phototransduction, but also as targets for synaptic input from bipolar and amacrine cells [43–47]. The functional significance of this synaptic input is apparent in the phenotype of melanopsin knockout mice. ipRGCs in these mice are no longer directly photoreceptive [31], but these animals retain all of the major NIF responses including photoentrainment [31,48,49]. By contrast, these NIF responses are lost in animals lacking rod+cone+melanopsin photoreception [50,51], and following specific cytotoxic lesion of this ganglion cell class [52–54]. It follows, firstly, that rods and/or cones can support these NIF responses and, secondly, that they do so largely (maybe solely) by influencing the activity of ipRGCs.

If ipRGCs receive visual information from rods and cones why do they also need melanopsin? Or, to avoid an unanswerable 'why' question, what does each photoreceptor class contribute to the visual abilities of ipRGCs and those physiological systems downstream of them? This has been the subject of at least one recent lengthy review [55], and here we do not attempt a comprehensive coverage of a literature in which descriptions of unique rod, cone and melanopsin contributions to visual responses at the level of ipRGCs themselves [56–59], retinorecipient brain nuclei [39,60–62], and integrated behavioural responses [18,63–66] have been made. In brief, those data indicate that melanopsin becomes increasingly important in defining the measured response as stimuli become brighter and more sustained. Put simply, melanopsin phototransduction is a

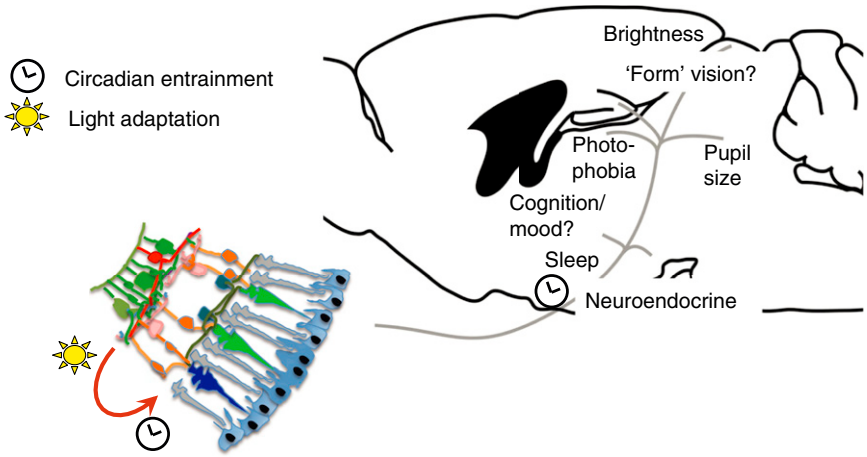

Figure 2. The many functions of ipRGCs.

The number of functions attributed to mammalian ipRGCs has grown progressively in the decade since their discovery. Through their central projection this small fraction of the total ganglion cell population (shown in red) entrain central circadian clocks; modulate the neuroendocrine and sleep/alertness systems; regulate pupil size; explain aspects of photophobia/photoallodynia, and light avoidance behaviours in rodents; and support brightness discrimination. There is also evidence that they influence cognitive function and may even contribute to visual pattern discrimination. Their impact on other aspects of vision remains little explored, but this could be extensive as ipRGCs not only set pupil size but also send signals within the retina that may entrain local circadian clocks and contribute to light adaptation in the retinal network. ipRGCs also influence the activity of many neurons in the thalamocortical projection. See text for further discussion and references.

feature only at relatively high illuminances, under which rods are approaching saturation. At these light levels, ipRGCs rely upon cones to respond to abrupt changes in light intensity while melanopsin plays the predominant role in encoding steady state illuminance. According to this model, then, the task of tracking the diurnal variation in light intensity from sunrise to sunset is assigned mainly to melanopsin.

One note of caution here is that much of the behavioural and electrophysiological data giving rise to this model comes from responses recorded to light pulses applied from darkness. There has been much less investigation of the ability of ipRGCs (or downstream responses) to track modulations in illuminance under light-adapted conditions. Similarly, our picture of melanopsin's sensory capabilities comes mostly from experiments in which rod and cone signalling has first been abolished. However, thanks to its low relative sensitivity, melanopsin will only ever modulate ipRGC activity under conditions in which rod and cone pathways are already providing visual information. Approaches to independently modulate the activity of melanopsin, rod and cone photoreceptors in the intact retina [67] thus hold the promise of providing a picture of melanopsin's behaviour under more naturalistic conditions and revealing complex interactions between the various photoreceptor signals.

Multiple Classes, More Functions

The ipRGCs described by Berson *et al.* [16] and Hattar *et al.* [17] in 2002 appeared to be a morphologically homogeneous class. However, over the intervening years multiple types of ipRGC have been identified (see [68] for a good recent review). The ipRGCs described in 2002 belong to what has since been termed the M1 class. These dominate the retinal projection to the SCN, and have the strongest melanopsin expression. M1 cells have small soma and dendrites that extend into the off-sublamina of the inner-plexiform layer (IPL). Despite this localisation, the M1 dendrites mostly receive synaptic input from en passant depolarising (on) bipolar cells, and only weak input from off bipolar cells. The remaining ipRGC classes have weaker melanopsin expression and, to the extent to which this has been assessed, their intrinsic light response has lower sensitivity. So-called M2 cells have somewhat larger cell bodies and dendritic trees than M1, but are mainly distinguished by their dendritic ramification in the on-sublamina of the IPL. By contrast, M3 cells are bistratified, with dendrites in both on- and off-sublaminae. The remaining two classes (M4 and M5) express so little melanopsin that it is hard to detect with immunocytochemical methods. The M4 cells have very large soma and extensive dendritic fields [69], while dendritic fields of M5 cells are small and bushy. Dendrites for both M4 and M5 cells are found in the on-sublamina of the IPL.

One important reason to keep the diversity of ipRGC classes in mind is that their morphological differences imply diversity in intra-retinal connectivity. The horizontal, amacrine and bipolar cells that lie between rod/cone photoreceptors and ganglion cells perform a number of important computations to extract salient information from the visual scene. As the nature of such computation depends upon the identity of those interneurones, it will be interesting in the future to determine how/whether these differ between ipRGC classes. That in turn will have a big impact on the sensory information that each class conveys.

The discovery of additional classes of ipRGCs has helped to change our understanding of ipRGC function (Figure 2). Hattar *et al.*'s initial report that ipRGCs are excluded from the major retinal projections to the dorsal LGN (dLGN; site of thalamocortical projection neurones) and the superior colliculus (origin of visually guided movements) was influential in the delineation of ipRGCs as 'NIF' photoreceptors [17,70]. It seems true that the M1 class of ipRGC does indeed project exclusively to NIF brain regions, with major innervation of centres involved in circadian entrainment (the SCN, the intergeniculate leaflet and ventral lateral geniculate), and the pupil light reflex (the olivary pretectal nucleus), and sparse projections to other sub-cortical nuclei [70]. However, over the last couple of years, Hattar and others have revisited the question of ipRGC's projection pattern in light of the discovery of additional ipRGC classes [61,71]. They have discovered that inclusion of other ipRGC classes reveals a much more extensive coverage of retinorecipient regions, including the dLGN and, to a lesser extent, the superior colliculus.

'Seeing' with Melanopsin

The recent description of significant ipRGC projections to the dLGN in mice confirms an earlier report that ipRGCs could be back-labelled following injections into this region in the monkey brain [56]. As the dLGN is the relay station for visual input to the cortex, these anatomical data allow the possibility that ipRGCs could contribute quite directly to perceptual vision. In support of this view, electrophysiological recordings reveal a melanopsin component to the response of a large proportion of neurons in the mouse dLGN [61]. The melanopsin-driven light response in this brain region has similar features to that recorded from ipRGCs, with low sensitivity and very poor temporal fidelity. The latter feature especially would appear to preclude melanopsin from contributing to high acuity pattern vision. Indeed, spatial acuity is lost during outer retinal degeneration in laboratory rodents and human subjects even when the retention of NIF responses indicates that ipRGCs are spared. However, some degree of light perception is typically retained even in patients with advanced outer retinal degeneration. In many cases this residual photosensitivity undoubtedly employs surviving rods and/or cones. However, evidence that rodless+coneless mice can distinguish light from dark and even judge the relative brightness of two visual targets confirms that melanopsin can also support some such functions [29,67,72].

If we assume that the ipRGC input to the dLGN did not evolve to support crude light perception in advanced retinal degeneration, then it must perform some other function in animals with an intact visual system. Electrophysiological data indicate that its role is to help the dLGN to encode ambient illumination [61]. The visual significance of this ability is currently under investigation, but recent data indicate that both mice and humans may employ melanopsin to judge spatial brightness [67].

Leaving aside the wide receptive field of most ipRGC classes, it is hard to envisage a photoreceptor with such poor temporal acuity rivalling cones as a source of high acuity spatial information. However, that is not to say that melanopsin does not contribute indirectly to form vision. Indeed, melanopsin has been shown to facilitate pattern discrimination in a mouse model with almost complete loss of rod and cone phototransduction [71,73].

One simple way in which ipRGCs help conventional vision is by regulating pupil size. However, there is growing evidence that they also contribute to diurnal and circadian rhythms in retinal physiology. The first suggestion of such an intra-retinal function for ipRGCs came in 2003, soon after the discovery of ipRGCs, with an electroretinogram assessment of long-term light adaptation in the human cone pathway [74]. A description of the spectral sensitivity of that effect was shown to peak around 480 nm, equivalent to that of ipRGCs but different to any other human photoreceptor. A subsequent study of the mouse electroretinogram indicated that circadian control of retinal physiology in that species is dysfunctional in melanopsin knockout mice [75].

A couple of potential routes via which ipRGCs could regulate retinal physiology have been described. Pharmacological and anatomical data indicate that ipRGCs are coupled to neighbouring GABAergic amacrine cells [76,77], and appear to regulate the activity of dopaminergic amacrine cells (although apparently not dopamine release itself [78]) via a glutamatergic synapse [79,80]. Either of these routes could be used to link ipRGCs to the retinal circadian clock(s) [81] and/or to provide more immediate light adaptation of the retinal circuitry.

Inner Retinal Photoreception in Non-Mammalian Vertebrates

If the inner-retinal connectivity of mammalian ipRGCs makes them well placed to modulate retinal physiology, the capacity of non-rod, non-cone photoreceptors to influence visual processing is even greater in non-mammalian vertebrates. There are two separate branches to the melanopsin gene family across all lower vertebrates with, in some genomes, multiple copies of each [82]. In addition, several of the photopigments associated with extra-ocular photoreception in these species are also expressed in the retina [81]. As a result, the non-mammalian retina is characterised by a great diversity of unconventional photopigments, expressed in a large proportion of inner retinal neurones (and, indeed, conventional photoreceptors) [81–85].

Much basic research on retinal pathways employs non-mammalian species. Sadly, that field has so far taken little notice of the potential for such widespread intrinsic photosensitivity among inner retinal neurons. The most fundamental questions remain largely unaddressed. Thus, although a couple of studies have presented evidence that teleost horizontal cells are intrinsically photosensitive [86,87], we do not know whether this is true for the many other retinal neurons that express non-rod, non-cone photopigments. If some of them are, what sorts of visual stimuli do they respond to? Moreover, what is the nature of their light response — is there a change in membrane potential or is some other aspect of cell physiology altered? Ultimately, of course, we would like to know what impact this putative photosensitivity has on the activity of retinal circuits and the processing of visual information.

Moving to Application

A number of practical applications of the discovery of ipRGCs have been considered over the last decade. There has first been growing awareness among clinicians that, by supporting NIF responses, surviving ipRGCs could make a significant contribution to the quality of life of patients with advanced retinal degeneration. Melanopsin contributions to the pupil light reflex may also be used to aid clinical diagnosis by providing a simple screen for the health of the inner retina [88]. More recently, it has been suggested that the data implicating ipRGCs in photophobia and light exacerbation of headaches could lead to new evidence-based treatments for those conditions. Meanwhile, there has been impressive progress in applying the discovery of melanopsin by using it as an optogenetic tool [89,90].

Perhaps the most significant commercial interest in the discovery of ipRGCs though has come from the lighting industry. Thanks to the development of artificial lighting our pattern of light exposure is increasingly dissociated from the natural day:night cycle. Whereas, through most of human history, people would have been exposed to high levels of light throughout the day and near darkness at night, we now take for granted the use of artificial light at all times of day. This raises two related questions for those behavioural and physiological systems that show diurnal or circadian variations: do built environments provide enough light during the day; and are we experiencing too much light at night?

However you measure it, artificial lighting is much dimmer than natural daytime sunlight. This difference has been

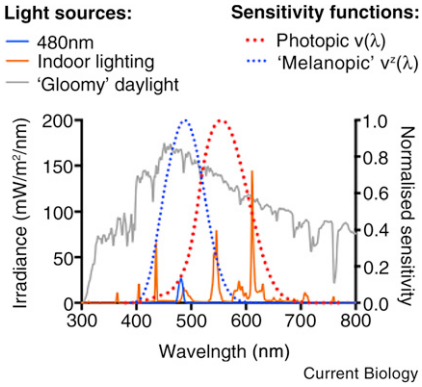

Figure 3. Melanopsin and artificial lighting.

Spectral power distributions for three different light sources: '480 nm' (a near monochromatic light source predicted to be the most efficient for melanopsin), indoor lighting (natural + flourescent lighting in our office in Manchester), and 'gloomy' daylight (direct daylight in Manchester under low, thick cloud). Their divergence not only in total incident power, but also in its distribution across the spectrum highlights the challenge of quantification. Photopic illuminance (measured in 'lux') is calculated by weighting spectral power according to the photopic sensitivity function (V(λ)). However, melanopsin has quite different spectral sensitivity (approximated here by a 'melanopic' function ($V^Z(\lambda)$) after [95]). As a result, if it were possible to increase the output of the indoor and 480 nm lighting such that they matched that of daylight when measured in lux, the effective photon flux for melanopsin would still be 60% lower for the indoor source, but 1000% higher for the 480 nm light.

considered unimportant because it is relatively easy to ensure that light levels are sufficient to support high acuity spatial vision. It is, however, much harder to be sure that artificial lighting provides an effective substitute for sunlight in setting the phase of circadian clocks and eliciting the range of ipRGC-dependent, sub-conscious responses that ensure a 'daytime' physiological state. There is some evidence that it does not. Thus, increasing light levels has been reported to improve productivity in a commercial setting and to help consolidate sleep and even slow cognitive decline in nursing homes for the elderly [91–93]. Moreover, in a large field study across Germany, Roenneberg *et al.* [94] traced a relationship between waking time and longitude. This suggests that, despite the widespread availability of on-demand artificial light, circadian phase is still at least partly set by the timing of sunrise.

Development of electrical lighting over the last 100 years has greatly extended the range of intensities and spectral qualities of light to which we are exposed at night. Typical indoor light levels do appear to lie within the sensitivity range of the circadian clock [95]. It is therefore important to know the extent to which ipRGCs and the NIF system interpret domestic/industrial lighting, and light emitted by TVs/tablet computers etc., as a 'daytime signal'. Perhaps it would be possible to redesign some of these light sources to reduce such biological effects without impacting visual performance?

There has thus been great interest in the possibility that artificial light sources could be redesigned to ensure that they represent a sufficient surrogate for sunlight during the day, while minimally engaging NIF responses at night. The diversity of technologies available for turning electricity into light allows great flexibility in the intensity and spectral composition of artificial lighting. What is lacking at present is an accepted method of predicting the suitability of the various options. At its heart this is a problem of light measurement.

Light sources differ not only in their rate of photon generation, but also in the wavelength(s) over which photons are produced. This characteristic is apparent in the spectral power distribution of several common light sources provided in Figure 3. As photoreceptors are not equally sensitive to light at all wavelengths, this complexity in spectral distribution must be captured in any method of quantification that hopes to predict biological responses.

The accepted solution is to measure the relative sensitivity of the biological process under consideration to different wavelengths. The resultant spectral sensitivity profile can then be used to weight the amount of light produced at each wavelength. Such spectral weighting is routinely applied using a family of spectral sensitivity functions relevant for different aspects of human vision. The most widely used of these spectral sensitivity functions has been codified as V(λ) (Figure 3). V(λ) describes the spectral sensitivity of psychophysical assessments of brightness for a 'standard' observer made under conditions favouring cone-based vision. It provides the spectral correction factor for commonly used light measures including photopic illuminance (units = lux) and luminous flux (units = lumens). These are especially important for the lighting industry with, firstly, the output of light bulbs typically described in lumens and, secondly, drives for efficiency concentrating on maximising lumens produced per Watt of power input and, thirdly, recommended light levels in architectural design described in lux or other derivatives of this system.

Illuminance is often reported in lux in NIF experiments. However, this is invariably incorrect as V(λ) does not provide a reasonable approximation of the spectral sensitivity either of melanopsin or of NIF responses (Figure 1). As a consequence, it is not possible to predict the NIF response to spectrally distinct lights based upon their illuminance in lux. There is thus an urgent need for an alternative to V(λ) that can be used to quantify light with reference to its impact upon ipRGCs and NIF responses. In its absence, comparing data from different laboratories using different light sources, and relating laboratory data to light exposures in the field is all but impossible.

We have proposed using the spectral sensitivity of melanopsin as a method of estimating the effective photon flux for melanopsin phototransduction produced by spectrally distinct light sources [95]. The resultant illuminance measure (termed 'melanopic lux' or m-lux') successfully predicts melanopsin responses under a wide array of conditions. However, it is only a partial solution to the problem, as it does not account for cone contributions to NIF vision, which could dramatically impact spectral sensitivity. The importance of that limitation should become clearer as the conditions under which cones have a significant impact on the ipRGC light response and on aspects of NIF vision become better understood. An optimistic view is that a range of lighting conditions under which NIF responses can be adequately predicted by measuring melanopic lux (or perhaps an alternative based upon the spectral sensitivity of a defined NIF response [96]) will become apparent.

Until a solution to the problem of light measurement is agreed, it will be difficult to use the discovery of ipRGCs to inform lighting design. An internet search reveals large numbers of companies selling 'blue-enriched' sources for

light therapy. These can be defended on the basis that melanopsin, and at least some of the responses driven by ipRGCs, are maximally sensitive to that portion of the spectrum. However, in order to make proper cost:benefit analyses about these, or any other products, a quantitative description of the relative importance of light of different wavelengths needs to be agreed. Without this it is impossible to answer real world questions such as: "is my existing light sufficient"; "which of the available lights is most effective/efficient"; or "should I switch to a 'blue-enriched' source or simply increase the output of my existing lighting?"

The Next Melanopsin(s)?

In addition to rod-, cone- and melan-opsins, a number of other 'opsin-like' genes have been identified in the mammalian genome. Two of these, Opn3 (also called encephalopsin and panopsin [97]) and Opn5 (also called neuropsin [98]), are expressed in the neural retina (albeit along with many other tissues [98–101]). Designated members of the opsin family on the basis of phylogeny and primary structure, until recently there was no indication that either actually acted as a photopigment. That has changed with *in vitro* characterisations of Opn5, initially from birds [102,103], but more recently also from mammals [101]. In each case, heterologously expressed Opn5 was shown to bind retinaldehyde to form a UV-sensitive photopigment, and to couple to a Gi cascade in a light-dependent manner. In birds, Opn5 has been hypothesised to provide extra-ocular photoreception supporting photoperiodism. In mammals, Opn5 remains a photopigment in search of a function. There is no strong evidence that any of the major visual or NIF responses are retained in mice lacking rods, cones and melanopsin. However, this may be because the appropriate lighting conditions have not been employed, or because the appropriate endpoint has not been assessed. Interestingly, there has been a report of light-dependent activation of the thalamus in melanopsin knockout mice at a developmental stage prior to rod/cone development [104].

Could Opn5 be a new photoreceptor in mammals? If the discovery of ipRGCs has taught us one thing it should be to keep an open mind about that possibility. However, a couple of aspects of Opn5 biology suggest caution. Firstly, there is its widespread expression outside of the retina [98,101]. It is exciting to speculate that this could allow extra-ocular photoreception also in mammals, but at present there is little evidence that this occurs [105]. Secondly, there is its UV sensitivity. This seems maladaptive for a photopigment working in the human retina (the human lens filters UV light), or in extraocular tissues (which transmit longer wavelengths much more effectively). It will be very interesting to see how this field develops over the coming years. Will Opn5 (and indeed Opn3) turn out to be the new melanopsin(s) forming the origin of an entirely new sensory modality, or will they be found to perform a quite different function for which their ability to absorb light is incidental?

Conclusions

The decade since the discovery of ipRGCs has seen great progress in understanding this new component of the visual system. We now have a working understanding of how these cells respond to light and the kind of physiological and behavioural responses that they influence. Predictably, as we have learned more about this system additional complexities have become apparent, not least the appreciation that there are multiple ipRGC classes. A related important evolution has been in our view of ipRGC function. Sought initially as a 'circadian' photoreceptor, for most of the last decade ipRGCs have been regarded as the origin of a NIF visual system encompassing a range of sub-conscious and reflex light responses. Growing evidence that ipRGCs project to all major retinorecipient regions, and that their influence extends to aspects of perceptual vision, suggests revising that designation. It now seems more appropriate to consider ipRGCs as the origin of a particular class of visual information (ambient illuminance?) that is available to multiple visual processes.

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