## 1 Introduction

Life has been evolving on this planet for some 3.5 billion years. For a good portion of that time (depending, for example, on atmospheric conditions), life has been exposed to the regular and alternating pattern of light and dark caused by the Earth's 24-hour rotation on its axis as it orbits the sun. It is perhaps unsurprising then that light is one of the most powerful drivers of behaviour – light influences the way that we think, feel, and act.

The study of these effects of light has a long and rich history that is rooted in medicine. The ancient Greek physician Hippocrates built a solarium and prescribed sunbaths to manage a variety of disorders. The Roman scholar Aulus Cornelius Celsus recommended that sufferers of sickness or melancholy (depression) live in light-filled houses, especially in winter. More recently, Florence Nightingale argued that 'Where there is sun, there is thought', and that hospital wards should be brightly lit, ideally by sunlight. Contemporary medicine now recommends light exposure as a first-line treatment against both seasonal and non-seasonal depressions.

Our understanding of the detection of light is often discussed in relation to an aspect of perception known as 'image-forming' vision mediated via the rods and three cone photoreceptor classes and their classical post-receptoral pathways. Image-forming vision includes the sensory and perceptual aspects of visual experience such as colour, form, or motion, usually discussed in the context of the neurotypical individual. However, lighting also drives diverse aspects of the human experience through setting physiology, arousal, cognition, and mood; responses that are classified as 'non-image-forming'. While these non-image-forming pathways can drive conscious awareness, many of these responses occur over timescales that are much longer than the momentary changes to which our visual perceptual awareness is tuned. This requires a mechanism with a fundamentally distinct temporal tuning to that of the classical visual pathways.

The modern study of non-image-forming vision is grounded in the scientific method and draws strongly from the fields of neuroscience, sleep and circadian sciences, and experimental and applied psychology. Its study has undergone a recent renaissance, where modern psychophysical and neuroscience methods have converged to identify the specialized visual circuits that serve non-image-forming vision and that originate in the retina of the eye. This fifth human photoreceptor class is located in the inner retina and termed the intrinsically photosensitive retinal ganglion cells (ipRGCs). Phototransduction initiated by the intrinsic melanopsin photopigment expressed by ipRGCs was initially shown to have a unique, characteristic temporal response: a slow onset followed

by a sustained depolarization that is maintained even after the stimulating light is switched off. In addition to their unique intrinsic photoresponse, ipRGCs extrinsically mediate signals originating in outer retinal rod and cone photoreceptors. The ipRGCs therefore possess temporal characteristics suited to sensing both transient changes in light but also day-length changes.

These non-image-forming pathways project to over a dozen diverse efferent brain targets, and in this Element we evaluate the current state of knowledge for these functional melanopsin pathways that set pupil size, perceptual vision, circadian rhythms and sleep/wake transitions, and arousal, mood, and cognition. We focus on delineating findings in primates (including humans) from those of other model organisms. Indeed, these non-image-forming signals appear fundamentally entwined with the human condition and we discuss lightscapes that not only serve image-forming vision, but that target non-image-forming physiology to positively modify health and behaviour. Physiologically targeted electric light sources have future applications as 'photoceuticals', with therapeutic effects analogous to those of pharmaceuticals and designed with similar considerations concerning disease specificity, dosage, and timing. Given the new developments in the understanding of ipRGCs and their image-forming and non-image-forming projections, we provide a contemporary account of the importance of light and melanopsin function for brain, mind, and behaviour.

## 2 Evidence for the Non-image-Forming Pathways and Novel Retinal Photoreceptors

The non-image-forming pathways are a relatively new discovery, and were initially a contentious one at that, because the visual pathways have long been studied. For some 150 years, vision scientists had modelled human visual perception by the rod and cone photoreceptor classes (Maxwell 1855, König and Dieterici 1893, von Helmholtz 1896, Schrödinger 1925). As early as the start of the twentieth century, however, evidence was mounting for a non-image-forming visual pathway that was at least partially independent from rod and cone photoreception. In the 1920s, a graduate student named Clyde Keeler was working with mice that were severely degenerate in their outer retina, lacking rod and cone photoreceptors, making these mice functionally blind (Keeler et al. 1928). Despite this, the mice still demonstrated robust and repeatable pupillary light constrictions (Keeler 1927). Potentially, another class of photoreceptors could be present in the retina, one that was necessarily able to survive outer retinal degeneration and that projected to the pupil control pathway. On the other hand, it was possible that the outer retinal degeneration was simply incomplete, leaving a small but functionally

significant population of rod or cone photoreceptors that could still drive pupillary responses to light. This was the most parsimonious explanation at the time, and it was not until many decades later that concerted and compelling evidence was presented for the non-image-forming pathways.

In mammals, light detected by the eye is the primary time cue that synchronizes the circadian rhythms of activity and rest – a process termed photoentrainment. The twilight transitions of light that occur at dawn and dusk play a key role, adjusting the phase of the master circadian clock in the hypothalamic suprachiasmatic nuclei (SCN) (Roenneberg and Foster 1997, Hughes et al. 2015, Walmsley et al. 2015). Evening light exposure results in a phase delay in the circadian clock, whereas light exposure in the morning produces phase advances. In this way, light adjusts the phase of the internal circadian clock to the external light/dark (LD) environment.

Research during the 1990s on the non-image-forming effects of light provided important clues that the mammalian eye may contain an additional photoreceptor. The evidence came from studies on retinally degenerate mice, in which rods and most of the cones were lost. Even though these animals were visually blind, their circadian phase-shifting responses to light persisted (Provencio and Foster 1995, Yoshimura and Ebihara 1996), commensurate with Keeler's observations many years earlier permissive of an additional photoreceptive mechanism. When exposed to a brief light pulse (~15 mins) in the early night, mice delay their activity onset the following day. This response is intensity dependent, enabling an irradiance-response curve (IRC) to be constructed (Figure 1), in a similar manner to a drug dose-response curve. Such curves have a characteristic sigmoid shape, which moves to the left when sensitivity increases and to the right when sensitivity declines, so that a different dose of light is required to evoke an equivalent biological response (see the caption of Figure 1) (Peirson et al. 2005). When studied in this manner, the blind mice showed circadian responses, but with a spectral sensitivity shifted to shorter wavelengths and a reduced sensitivity to irradiance (Yoshimura and Ebihara 1996). The photoreceptors mediating circadian entrainment were certainly ocular, as loss of the eye abolished all responses to light (Nelson and Zucker 1981, Foster et al. 1991). However, as with Keeler, a potential explanation for these findings was that these circadian responses could be driven by the few remaining cones that survived.

Subsequent studies in retinally degenerate mice in which cones were also genetically lesioned demonstrated that both circadian phase shifting and melatonin suppression were retained in the absence of rods and cones (Freedman et al. 1999). Moreover, an action spectrum on the pupillary light response in these mice demonstrated that this was driven by a photopigment with a peak

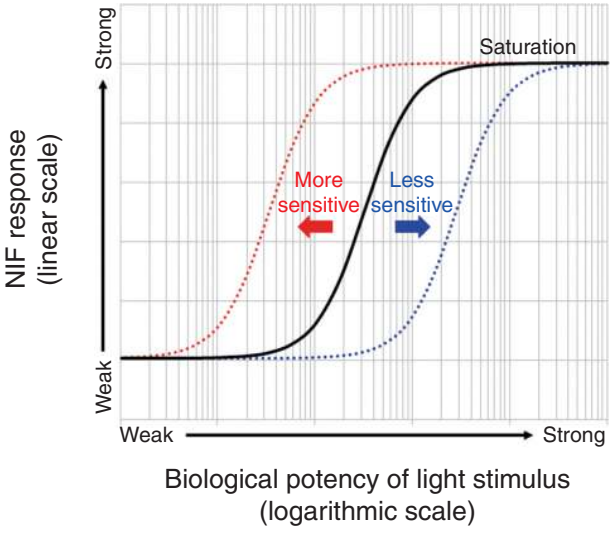


**Figure 1** Example IRC. A log-linear relationship typically exists between light stimuli and non-image-forming responses, such as the suppression by light of the circadian hormone melatonin in humans (Zeitzer et al. 2000) (solid curve). In practice, a complex interplay between stimulus parameters, intra-individual and inter-individual factors result in non-image-forming IRCs that are not static: the physical parameters of the light (intensity, duration, spectral power distribution (SPD)), the individual's light exposure history, and the timing of the light exposure relative to circadian phase can all impact the IRC. When the sensitivity of the system increases, the sigmoid curve shifts to the left wherein the same light stimulus becomes more effective (or more biologically potent) in eliciting a non-image-forming response. When the sensitivity of the system decreases, the sigmoid shifts to the right and the biological potency of the stimulus is reduced. Response threshold (below which no response occurs), saturation (above which the response does not increase in magnitude), and slope of the relationship (determining the magnitude of response change to a unit change in stimulus) may also vary (not shown).

sensitivity ($\lambda_{max}$) of 479 nm, which corresponded to none of the known mouse visual pigments (Lucas et al. 2001). Together, these studies provided the key evidence for a novel retinal photoreceptor in mammals.

## 3 Intrinsically Photosensitive Retinal Ganglion Cells

There are some 30 types of ganglion cells identified in mammals (Sanes and Masland 2015). They relay signals that originate in the photoreceptors in the retina to higher brain centres via their axons that form the optic nerve that

attaches the eye to the brain. Ganglion cells were not known to be photosensitive and so it was remarkable that the novel photoreceptor system identified in mice consisted of a subset of ganglion cells that uniquely express the photopigment melanopsin (OPN4), now known as intrinsically photosensitive retinal ganglion cells (Figures 2 and 3) (Provencio et al. 2000, 2002, Hattar et al. 2002). Melanopsin is named because it was initially isolated from melanophores in amphibian skin, and is an opsin-vitamin A type photopigment that shares many characteristics with invertebrate visual pigments (Provencio et al. 1998b). In the mammalian retina, ipRGCs form a syncytium or photoreceptive net across the retina (Provencio et al. 2000, 2002). These ipRGCs project directly to the rodent SCN and other brain regions associated with non-image-forming responses and have a peak response to light at ~480 nm that appears blueish-cyanish (Berson et al. 2002, Hattar et al. 2003). In the literature, these cells have also been referred to as photosensitive retinal ganglion cells (pRGCs) or melanopsin retinal ganglion cells (mRGCs).

Following the identification of ipRGCs, it was initially thought that the image-forming effects of light were independently mediated by rods/cones while the

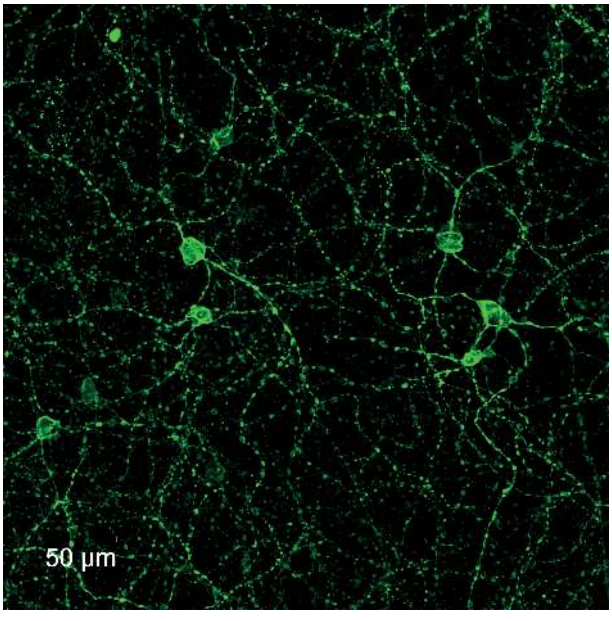


**Figure 2** Intrinsically photosensitive retinal ganglion cells. Intrinsically photosensitive retinal ganglion cells form a syncytium or photoreceptive net within the mammalian retina. Flatmount image of mouse retina immunostained for melanopsin. Image courtesy of Steven Hughes.

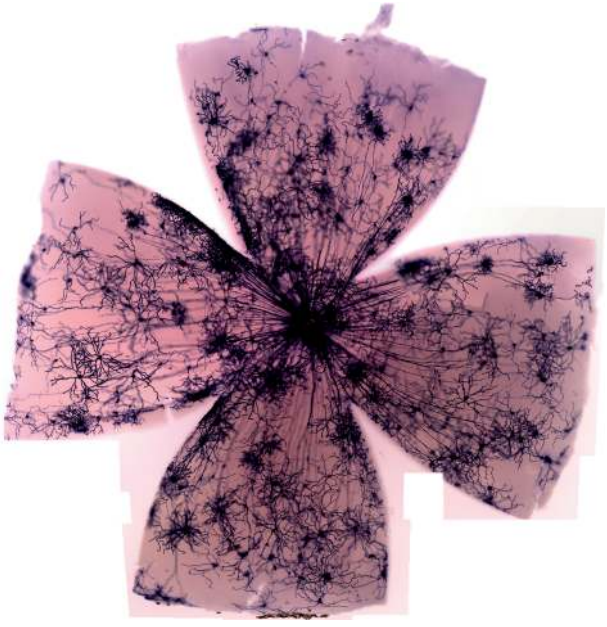

**Figure 3** Stitched micrograph labelling melanopsin-expressing cells of the mouse retina, focussed at the OFF layer of the inner plexiform layer (IPL). Mice were a cross between the Opn4-driven tamoxifen-inducible Cre mouse line (Opn4 $^{CreERT2}$) and the Z/AP reporter line, allowing controlled expression of AP on the plasma membrane of melanopsin-expressing cells (Joo et al. 2013). Image courtesy of Shih-Kuo Alen Chen.

non-image-forming effects of light were mediated by melanopsin. However, studies of transgenic mice that lacked melanopsin found that the mice could still entrain their circadian rhythms, had only mild deficits in circadian phase shifting, and still retained pupillary responses to bright light (Panda et al. 2002, Ruby et al. 2002, Lucas et al. 2003). Therefore, extrinsic rod and cone inputs to ipRGCs were able to drive these responses even in the absence of melanopsin. When the melanopsin ipRGCs are lesioned, non-visual responses no longer occur, demonstrating that ipRGCs provide the primary conduit for this pathway in mice (Guler et al. 2008) and in non-human primates (Ostrin et al. 2018). Moreover, ipRGCs have been shown to mediate visual responses independent of the rod and cone pathways in mice (Ecker et al. 2010, Schmidt et al. 2014) and in humans (Zele et al. 2018c, Allen et al. 2019b). As a result of these and a range of electrophysiological studies on the responses of melanopsin ipRGCs both with and without rod or cone input (Dacey et al. 2005), it is now clear that the response of ipRGCs depends upon both their intrinsic melanopsin-driven photoresponses and extrinsic rod/cone input (Figure 4) (Markwell et al. 2010, Lucas et al. 2014).

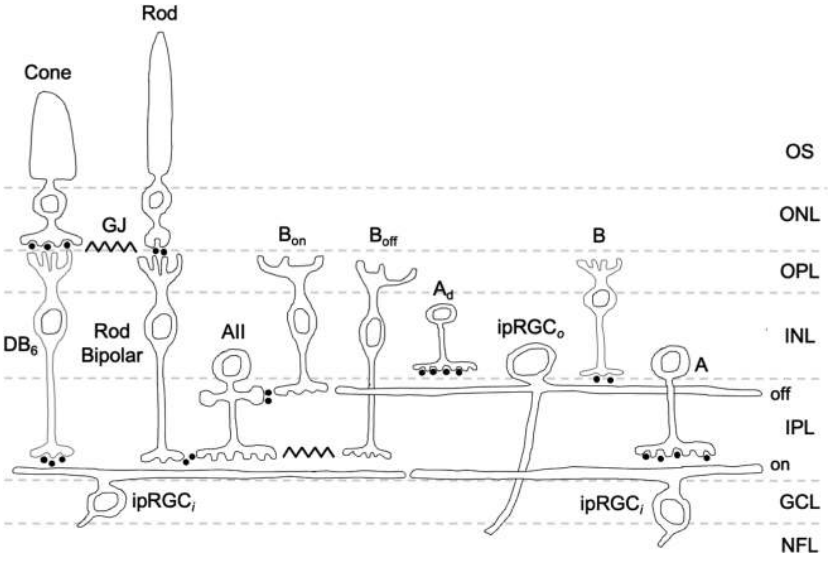


**Figure 4** Intrinsically photosensitive retinal ganglion cell retinal circuits. Inner stratifying photosensitive ganglion cell bodies ($ipRGC_i$s) are located in the ganglion cell layer (GCL) with their dendrites stratifying along the extreme inner strata of the inner plexiform layer (IPL). Outer stratifying photosensitive ganglion cell bodies ($ipRGC_o$s) are co-located in the GCL and the inner nuclear layer (INL) with their dendrites in the extreme outer strata of the IPL. Cone signals are transmitted to ipRGCs via $DB_6$ cone bipolar cells. Synaptic contact also occurs between ipRGCs and dopaminergic amacrine ($A_d$), bipolar (B), and amacrine cells (A), including within an S-cone circuit in primate retina. Rod input to ipRGCs may be transmitted via rod–cone gap junctions (GJs) and the $DB_6$ bipolar cells; extrinsic rod inputs via the ON rod bipolar, AII amacrine cells, and ON ($B_{on}$) and OFF ($B_{off}$) cone bipolars is yet to be determined in primates, although synaptic contact has been shown between rod bipolars and $ipRGC_i$ in rats. Abbreviations: nerve fibre layer (NFL); outer nuclear layer (ONL); outer plexiform layer (OPL); outer segment (OS). Figure from Markwell et al. (2010), copyright © 2022 Optometry Australia, reprinted by permission of Taylor & Francis Ltd, www.tandfonline.com on behalf of 2022 Optometry Australia.

## 3.1 Intrinsically Photosensitive Retinal Ganglion Cell Diversity and Projections in Rodents

Rather than just a single class of circadian photoreceptor, the ipRGC system has remarkable complexity. Electrophysiological responses of mouse ipRGCs to light reveal transient, sustained, and repeatable responses to the same stimulus