A Light for Time, and a Time for Light

Light as a clock: retinal cell significance and function

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As everybody knows, our eyes are the sensory organs that allow us to see the world around us. Light enters the eye through the pupil and strikes the photosensitive retina at the back to commence a complex biochemical and physiological process that we know as vision. This ability is vital to recognize friends and family, avoid danger, and find food and shelter. Loss of vision through blindness is a major handicap, and unfortunately the numerous causes which can lead to sight loss are on an upward trend. Yet, a generally less appre ciated fact is that the eyes serve a second, distinctly different purpose to that of classical "vision". They also function as detectors of the general level of light intensity, akin to a photometer. This second function does not provide form vision (see below), but in fact allows us to position ourselves with respect to solar time. In other words, during the transition from night to day (in a natural world without electric lighting), the retinas in our eyes register the increase in light intensity as a signal that dawn is here. This light information is hence interpreted as time, which is of utmost importance in synchronizing the internal, central circadian clock located in the brain (see q&more issue number 02.11, page 24) with the real time of the outside world.

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Discovery of a totally different photoreceptor in the eye

Just how this process of "non-image forming" (NIF) vision is accomplished has long been a subject of controversy and it is only in the past ten years or so that many pieces of the puzzle have fallen into place. Most importantly, the cells that communicate this special information to the central clock have been identified and it transpires that they are completely different from any other retinal cells. History provides an edifying lesson in breaking down scientific dogma in that the basic anatomy of the retina was already known by the 19th century and seen to be made up of three cell layers: the outermost layer of rod and cone photoreceptors, found to be responsible for detecting light; a middle layer made up of several types of relay neurons; and an inner layer formed by ganglion cells that collect and integrate visual information generated by the other retinal cells and send it down their axons along the optic nerve and eventually to the visual cortex (see Fig. 1). The vast numbers of studies performed over the decades revealed that the rod and cone photoreceptors were the only cells in the retina capable of being stimulated directly by light and that the degeneration and death of these cells, as happens in many debilitating diseases, leads inexorably to visual impairment and blindness. Yet contradictions to this idea were apparent as early as 1923, when it was shown that blind mice (lacking rods and cones) were still able to react to light in as much as their pupils would constrict when exposed to bright illumination. However, such observations were dismissed without further thought and, ultimately, it was only in the late 1990s that studies conclusively proved that such blind mice could indeed still detect light. It was not that the mice could "see" in any formal way, but they were able to coordinate their activity with ambient lighting conditions. This phenomenon, known as photoentrainment, is the underlying factor in the sensation of jetlag one experiences when changing time zones by air travel. As explained in the article by Eckert and Brown (q&more 02.11), all organisms possess an internal biological clock that drives many basic physiological processes such as the sleep/wake cycle, hormone secretion, body temperature etc. In mammals, including humans, this central clock is located in a part of the brain known as the ventral hypothalamus. The periodicity of this internal (or circadian) clock is close to but not exactly 24 hours (hence the name "circadian", from "circa" (about) and "dien" (day)). When animals or people are placed in continual darkness (such as when speleologists descend into deep subterranean caves), this difference between "inner time" and external solar time leads to progressive misalignment between the two, given that relative to every real 24 hour period, the circadian clock will be running either slightly faster or slightly slower. Jetlag occurs when there is a sudden mismatch between the "inner" clock time and "outer" solar time; as would happen if you were to fly to New York from Paris, leaving at mid-day – on arrival it would be around 4 p.m. local east coast time, but your body would still be on "French time", namely 10 p.m. It usually takes three or four days for the inner clock to realign itself with the local solar time (the experience of jetlag), and this synchronization of rhythmic behaviors (the sleep/wake cycle for example) with external time (as indicated by the sun) is known as "photoentrainment". To come back to mice, we can replicate jetlag in the laboratory by simply changing the time at which the lights come on. Mice are nocturnal creatures, which in an undisturbed animal room, sleep when it is light and are active at night. So if the lights are on from 8 a.m. until 8 p.m., mice will sleep during these hours and then start to run around when it goes dark at 8 p.m. (Fig. 2). If we turn the lights on at 2 p.m. instead of 8 a.m. (hence a 6 hour delay), mice will take a few days to adapt to the new scheme (i.e. they experience jetlag), but will soon become active at the onset of the new night-time, namely 2 a.m. Finally, if the lights are left off permanently, the mice will not run around for 24 hours, but rather only during the period they anticipate as night-time, whether apparent or subjective night-time. As explained above, this sense of inner time, driven by their circadian clock, is not exactly 24 hours. Consequently, the activity patterns slowly start to drift, a phenomenon called free-run (Fig. 2). Thus, in the case of blind mice, because rods and cones were thought to be responsible for photoentrainment, it was fully expected that such animals would not be able to readjust their sleep/wake cycles to timed changes in lighting and would demonstrate free-run, even in an alternating light/dark cycle. However, the blind mice reacted in exactly the same way as normal sighted mice. Conversely, if the optic nerve was cut so there was no longer any connection between the eye and the brain, all of the mice adopted the free-run activity. In other words, although difficult to believe at the time, it seemed the retina contained photosensitive cells other than rods and cones. These cells, which have been named "intrinsically photosensitive retinal ganglion cells", or ipRGC, were finally discovered and characterized in the early 2000s and are remarkable in many ways. Firstly, it is not surprising they escaped attention for so long, because there are very few of them: around 1 – 2 % of the total RGC population, which itself is only about 1 % of the total

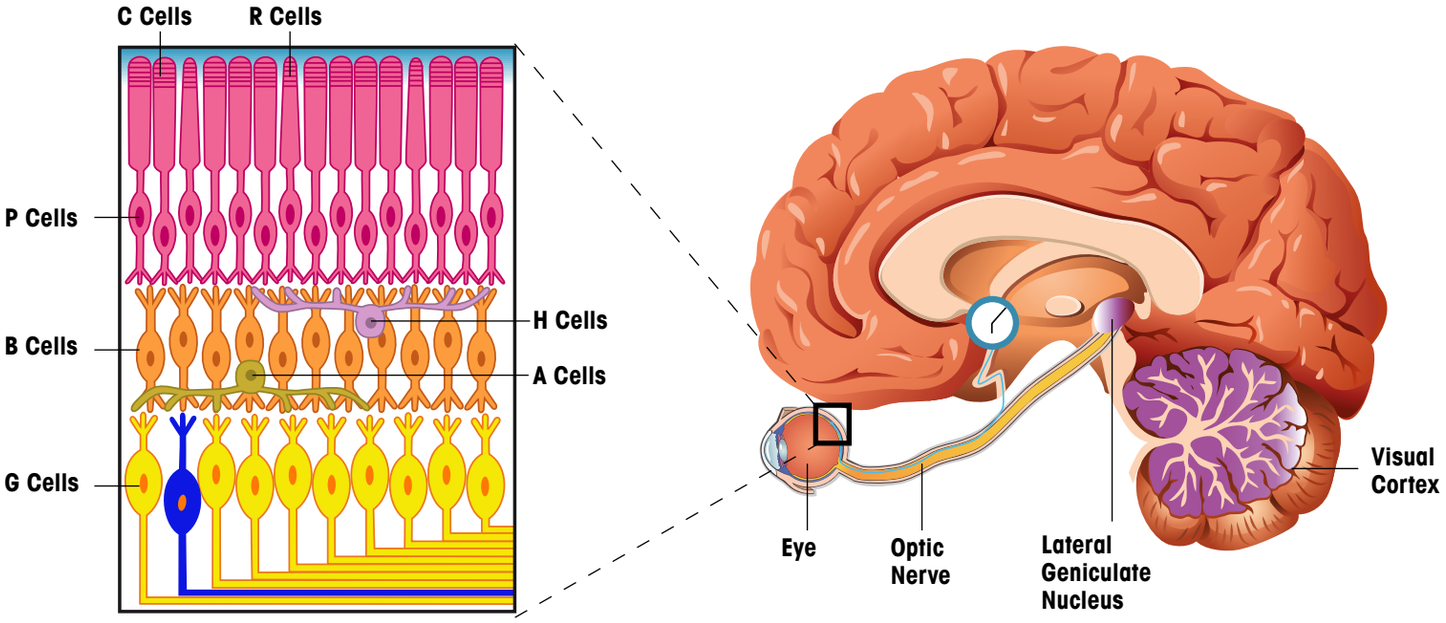


Fig. 1 The visual and „non-visual" photoreceptive systems. The retina (shown as a blow-up on the left) covers the back of the eye and is composed of a highly organised succession of cell layers, each projecting to the next. The rod and cone photoreceptors (red cells at top) were previously thought to be the only cells in the retina that could be directly stimulated by incoming light. They contact neurons in the intermediate layer – horizontal, bipolar and amacrine cells – which modify the signals and transmit them to the output neurons of the retina, the ganglion cells (yellow cells at bottom). But it was recently discovered that a small subset of these ganglion cells (coloured in blue) were also able to detect light directly. All ganglion cells send their axons down the optic nerve, but whereas the "classical" ones project to the lateral geniculate nucleus and thence to the visual cortex, the axons of the "intrinsically photosensitive ganglion cells" (shown as a blue trace) exit the optic nerve earlier, connecting with several centres in the brain, among which the suprachiasmatic nucleus in the ventral hypothalamus, home of the circadian clock (represented by a clock).

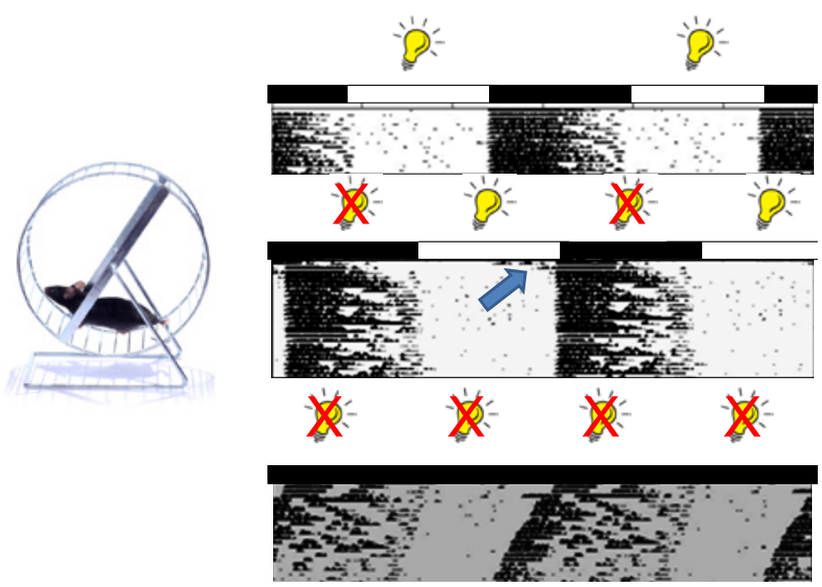


Fig. 2 Mice get jetlag just like humans! Mice are nocturnal animals, being active mostly at night. When kept in a room with artificial lights providing 12 hours light and 12 hours dark, mice run in wheels (top) principally during the night time. This is depicted in the actograms in the panels, the black and white bar at the top of each actogram represents the day-night cycle and each line represents a new day. Each wheel revolution makes a small dot, and it is clear that nearly all black dots are aligned within the night periods. If the room lights are now switched on 6 hours later (second panel), the mice take a few days to adjust to the new time schedule (shown as the drifting spots by the blue arrow – the mouse equivalent of jetlag) but eventually re-align their wheel-running activity with the new night. If the mice are kept in total darkness throughout 24 hours, their biological clock "remembers" the time corresponding to night ("subjective night") and they continue to run at the same time. However, internal clock time is slightly shorter (or longer) than the real day-night cycle, and so the activity period slowly shifts in relation to the outside. This is known as free run, seen in the third panel as the diagonal leftward drift in activity.

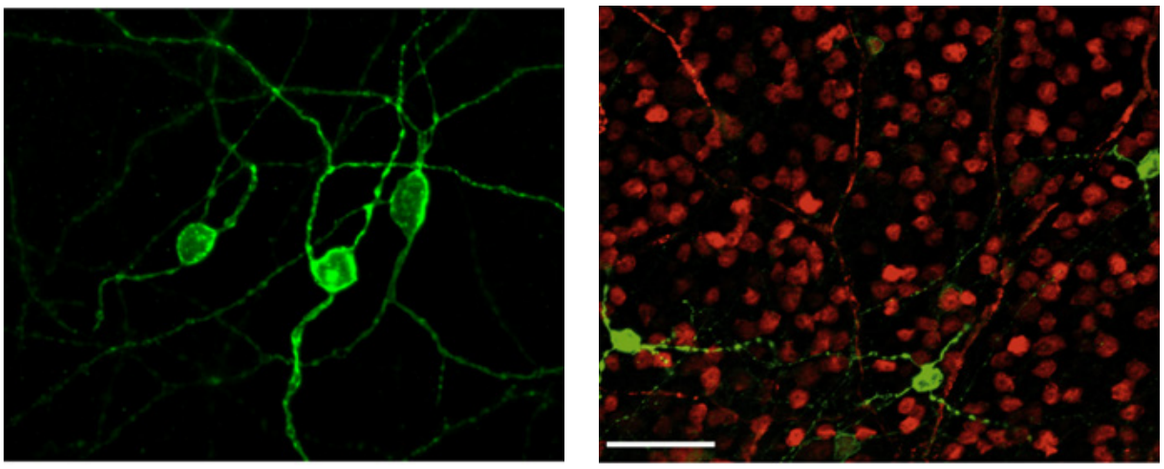


Fig. 3 The intrinsically photosensitive retinal ganglion cells can be visualized by their expression of melanopsin, the visual pigment they use to detect light. In the left panel, three such cells are shown at high magnification, visible as round cell bodies and long branching processes extending across the retinal surface. In the right panel, these cells are seen against a background of red circles, corresponding to the „classic" ganglion cells. The small percentage of melanopsin-containing cells can be appreicated, only 2–3 % of the total. The white scale bar equals 50 µm for the left panel, 100 µm for the right one.

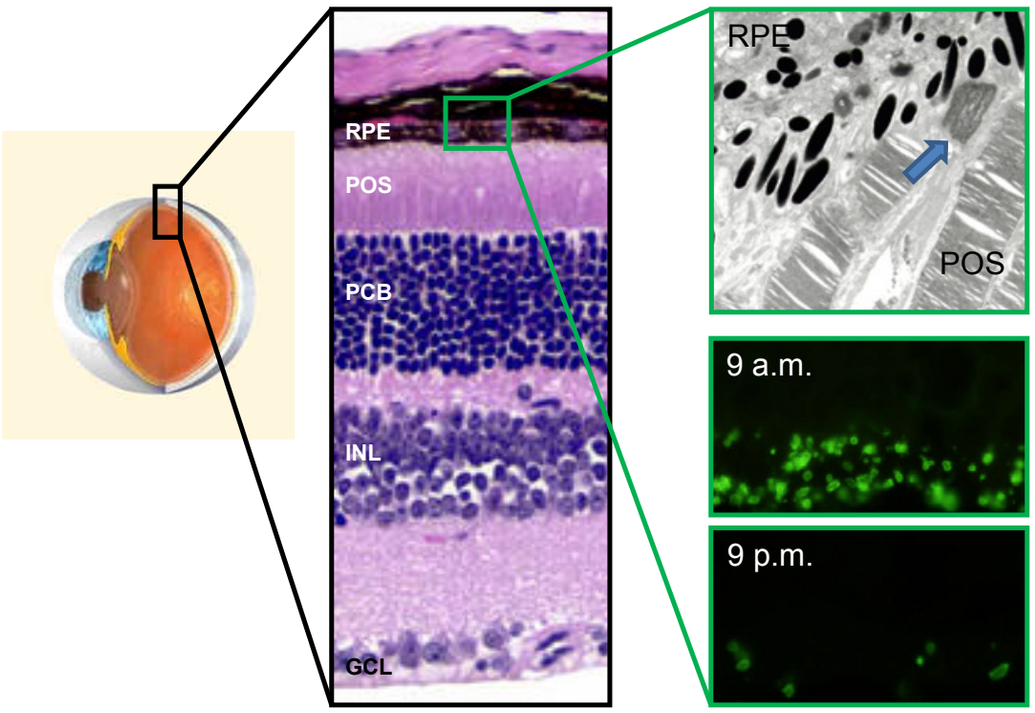
retinal cell population. Secondly, in comparison to rods and cones, ipRGC use a completely different molecular and biochemical strategy to detect light in that they contain a distinct visual pigment called melanopsin (Fig. 1 and 3), meaning the cells use a different intracellular biochemical cascade to signal light activation, and they depolarize instead of hyperpolarizing. Perhaps the most intriguing aspect is that, in terms of molecular composition and physiology, they are much closer to the photoreceptors present in insects than to vertebrate rods and cones! To sum up a great deal of research and add some speculation, these ipRGC may correspond to an ancestral form of photoreceptor that appeared long ago in the evolution timeline and served the purpose of distinguishing light from darkness.

There are many "non-visual" light-driven processes

The last ten years have seen intensive research on these novel photoreceptors and it is now known that ipRGC underlie many "NIF" functions – they are responsible for constriction of the pupils in response to bright light, control of sacchades (tracking movements of the eye), synchronization of the circadian clock with solar time, and probably additional roles that we are only just beginning to unravel, such as direct control of sleep and involvement in migraine and depression. It should be noted that the rods and cones are also important in NIF vision, since they are connected indirectly to ipRGC through their specific circuitry; hence, it seems that in a normal retina all three photosensitive systems – rods, cones and ipRGC – operate together to produce a coherent message to send to the brain. Also worth mentioning is an aspect that often occurs in science: as more and more information on ipRGC accumulates, the frontier between "NIF" and regular vision is becoming blurred as these cells may actually also be involved in some form of visual perception as well.

A clock in the eye…

It should be apparent from this article that the retina forms an interface between the environment and the brain - detecting, filtering, and processing light information that is then delivered to the appropriate brain region. However, the retina also contains its own endogenous circadian clock, separate from that of the hypothalamus. Indeed, the retina was the first tissue outside the hypothalamus in which the existence of an autonomous clock was demonstrated. A study by Tosini and Menaker in 1996 showed that when it was isolated and maintained in culture, removed from all neural input, the retina was able to rhythmically synthesize melatonin according to a



POS (Photoreceptor Outer Segment
PCB (Photoreceptor Cell Bodies)
INL (Inner Nuclear Layer)
GCL (Ganglion Cell Layer)

Fig. 4 Many processes in the eye are rhythmic. In the example shown, as seen before the retina is made up of highly stratified cell layers, the outermost of which is formed by the rod and cone photoreceptors. These cells interdigitate with an overlying single cell layer known as the retinal pigmented epithelium. This RPE is critical for the proper function and survival of photoreceptors, one of its most important roles being as an efficient "garbage disposal system". Photoreceptors undergo intense recycling, incorporating new membrane and shedding old membranes at a very high rate. The old membranes are eaten by the RPE (shown by the blue arrow in the upper right panel). This process of internalization and degradation is very rhythmic, since at 9 am the RPE is full of shed membrane (seen as all the green dots in the middle panel) whereas at 9 pm it is virtually empty (bottom panel). The scales of the photographs are very different: top panel is x15000; middle and lower panels x1000.

regular 24-hour cycle, with low levels of synthesis in the day and high levels of synthesis at night. But why should the retina need a clock? We think the reason is because this sensory tissue has to cope with a huge range of light intensity, changing every 24 hours by more than a million-fold between a dim moonlit night and the mid-day sun. During the course of evolution, the retina has acquired the capacity to anticipate these changes and adapt its biochemistry and physiology accordingly. In simple terms, when there is very little light available (at night-time), the retina becomes maximally light sensitive because it has to detect the slightest trace of illumination. Conversely, during the day, the intensity of the electromagnetic radiation is so strong that the retina has to ramp down its detection systems to minimize damage. Many of the biological processes inherent to the eye – synthesis of melatonin, synthesis of dopamine, synthesis of visual pigments, ion channel sensitivity, blood flow, etc. – exhibit cyclical variations over the 24-hour period.

Photoreceptor repair is controlled by a clock

One such process that deserves special mention is photoreceptor turnover: these cells are extremely vulnerable to oxidative damage, not only due to the high energy in electromagnetic radiation, but also the high oxygen levels found in the retina and the elevated content in poly-unsaturated fatty acids. Collectively, all of these create a cellular environment that is susceptible to damage by reactive free radicals. To counteract damage of this nature, rods and cones have developed the ability to undergo balanced repair by synthesizing and incorporating new molecules at the same time as eliminating old ones, effectively ensuring the cell remains at a stable constant length. In all, this occurs at an astonishing rate and constitutes one of the most active metabolic processes in the body. This is accomplished by another cell type that lies closely adjacent to the photoreceptors, the retinal pigmented epithelium or RPE. The RPE has an incredible

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capacity to phagocytose the shed membranous debris from the photoreceptors, ingesting and degrading the material to recycle essential components back to the retina. It is estimated that in an 80-year-old person, every single RPE cell will have ingested some 100 million membrane "packets", an immense burden which contributes to part of the visual decline observed during aging. Even more amazing, this process of phagocytosis is controlled by the retinal circadian clock, since the number of these packets of membranous debris (called "phagosomes") changes across the course of 24 hours – in every species so far examined, rod phagosomes show a large peak of activity in the early morning, whereas cones seem to be less strict in their timing (Fig. 4). It is not known why such metabolic processes need to be controlled in such a tight temporal manner; however, in terms of energy expenditure, it may be more economical for the cell to simultaneously bring together all the complex molecular machinery required to recognize, bind, internalize, digest, and recycle such large amounts of membrane, rather than spreading it out across the entire day. Some very recent evidence indicates that if this phagocytic activity is no longer synchronized correctly, the RPE become full of incompletely digested membranes, which contribute to the formation of age-related debris and potentially to failing vision.

Consequences of artificial lighting for vision and clocks?

As a conclusion to this short description of retina and circadian rhythms, one should wonder about the possible consequences of "inappropriate" lighting. We live in a modern world of artificial illumination, both during the day inside offices and factories, and at night in homes and under street lights (and for the many people doing shift work, in work places such as factories, bakeries and hospitals). The recent publication by NASA of high-resolution images of the earth's surface photographed at night shows just how little "true" night remains in many industrial nations [1]. The long-term effects of such unnatural conditions are unknown, but it is likely that the finely tuned regulation of the circadian system, both within the retina itself and in the central clock in the brain, is being perturbed. Our laboratory is very interested in the impact of lighting regimes upon the circadian clocks and, through the influence of these clocks, upon so many aspects of biology. Night illumination is beginning to adversely affect animal movements and behavior, since many species active at night shun away from lit areas. In humans, as the extent of nocturnal lighting has increased over the last 20 years, so has the occurrence of depression. In addition, we have lost about an hour of sleep compared to 20 years ago. Nobody knows what the consequences will be for the eyes themselves. Maybe it is time to turn the lights down a little … [2].

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References
[1] (http://www.nasa.gov/mission_pages/NPP/news/earth-at-night.html)
[2] Readers interested in the biological effects of nocturnal lighting are referred to ANPCEN (only in French) for further information: www.anpcen.fr