THE GOOD BLUE AND CHRONOBIOLOGY: LIGHT AND NON-VISUAL FUNCTIONS

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__ INTRODUCTION

Over the past ten years there has been a wealth of discoveries in the field of chronobiology. Since the discovery of a new retinal photoreceptor in 2002 (melanopsin ganglion cells), shown to be involved in the synchronisation of the circadian clock, it is now clear that the eye is not for seeing only, it is also involved in a range of non-visual functions, directly stimulated by light. The mechanisms involved are mainly yet to be explored but all biological responses to photic stimulus show the way to clinical applications of light in a range of disorders and pathologies, from sleep to alertness, from cognition to memory and mood.

__ LIGHT AND THE CIRCADIAN BIOLOGICAL CLOCK

The link between light and the internal biological clock was discovered in humans in 1980. The circadian clock (from the Latin *circa* "close to" and *dies* "day") is a physiological component that is essential to life since it has been observed in almost all the living organisms that have been studied, from prokaryotes through to humans[4].
Two fundamental properties characterise the circadian clock[4]:
1. Its rhythmic activity is endogenous. Located in the *suprachiasmatic nuclei (SCN)* of the hypothalamus in mammals[7], its circadian electric activity is supported by around ten *clock genes* whose cyclic activity is responsible for the near 24-hour rhythm of each of its neurons[9].
2. Its activity must be synchronised to 24 hours. Its endogenous period is actually close to but slightly different from a 24-hour period. Therefore, the clock has to be synchronised (reset in time) in order to enable its activity to be in phase with the solar day. In mammals, light is the most powerful clock synchroniser, and its effect takes place solely through the eye.

__ FUNCTIONS CONTROLLED BY THE CIRCADIAN CLOCK

Lots of physiological functions work according to circadian rhythm. Figure 1 shows circadian control over several functions in humans. The clock acts like an orchestra conductor, enabling the expression of physiological activities at the right time. Alertness, cognitive performance, memory, body temperature and blood pressure are at their highest during day time (awake). On the contrary, secretion of the hormone melatonin, muscle relaxation and sleep pressure are at their highest during the night (sleep).
Many circadian biological activities have been discovered over the past 30 years, both in the periphery and at central level. Depending on the tissue, between 8 and 20% of the genome is expressed rhythmically via the endogenous clock.
The circadian system is involved in the control of cell division, apoptosis in cancer[10] and in the repair of DNA[11]. Because of this, these results can be used to understand how desynchronisation of the circadian system could be responsible for the increased prevalence of certain cancers in shift work[12].
The importance of the circadian system and its synchronisation therefore appears to be crucial to human health.

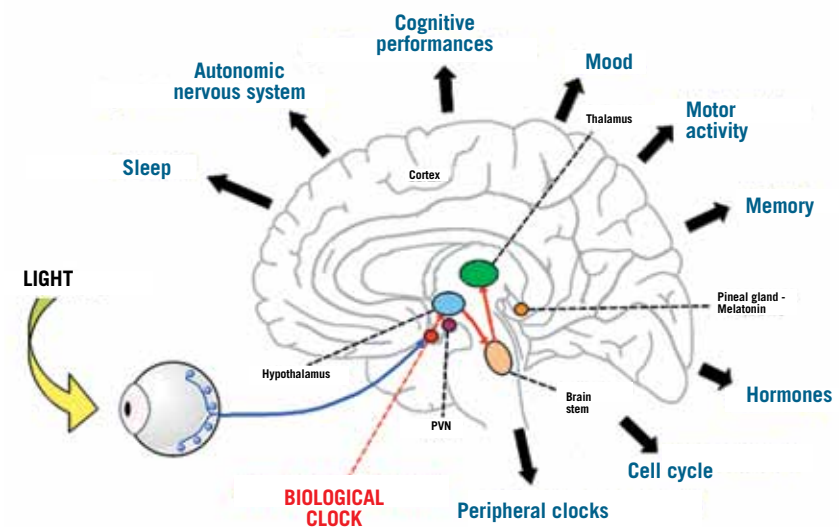


FIG. 1 | Diagram of the biological functions controlled by the circadian biological clock (non exhaustive list).

The structures indicated in colour are respectively in red: the suprachiasmatic nucleus, in orange: the pineal gland, in blue: the hypothalamus (containing the VLPO [ventrolateral preoptic area], known as the *sleep switch*), in beige: the brain stem (containing the ascending activator cortical pathway and the slow wave / paradoxical sleep *sleep switch*), in green: the thalamus (responsible for cortical activation and synchronisation of the EEG. (Modified diagram by Mignot *et al.* Nature 2002 [3] and Gronfier *et al.* 2012 [6]).

__ THE CONSEQUENCES OF CIRCADIAN DESYNCHRONISATION

In humans the importance of synchronisation is clear in symptoms of "jet lag" or in night work (20% of the population in industrialised countries). A lack of synchronisation of the clock is generally translated by a change in numerous physiological functions (sleep, alertness, cognitive performance, cardio-vascular system, immune systems [4,13,14]), the deterioration of neurocognitive processes (cognitive performance, memory) and a disturbance of sleep and alertness[15].
These changes are also found, chronically, in night workers, elderly patients, blind people, in certain psychiatric pathologies and in certain degenerative diseases of the central nervous system (Alzheimer's and Parkinson's disease[16]). Chronobiological disorders associated with these normal or pathological conditions have major socio-economic consequences since they can lead to a fall in the general state of health and to an increase in associated pathological risks.
The French Society of Occupational Medicine has just published a report under the aegis of the High Health Authority (*Haute Autorité de Santé*) on the consequences of shift work, including recommendations for detecting them and ways in which to minimise them[17].

__ ENDOGENIC PROPERTY OF THE CIRCADIAN CLOCK

In light conditions that are unsuitable for the synchronisation of the circadian system, the endogenous clock functions according to a rhythm that is no longer that of a 24 hour day.
In this case it expresses its own endogenic rhythmicity (period). Just like a mechanical clock that has not been adjusted to time regularly, the circadian clock loses time or runs fast, depending on the individual (according to the length of the period of their own clock) in the absence of any synchronisation by the environment.
This phenomenon, known as "free run", is observed in blind people in whom the absence of any light means that the biological clock cannot synchronise to the 24-hour period[18]. This explains why about 75% of blind people complain that their sleep is not of good quality and consult their doctors for recurrent sleep disorders[19].
It should be noted that the length of the clock's period is a highly precise individual characteristic. It does not vary with age in adults[20], but is relatively flexible during childhood and adolescence (lengthening of the period in adolescence could explain in part the late-to-bed factor, or even disorder of the delayed phase type observed in the 15-25 age range[21]).
Thanks to the use of strictly controlled experimental protocols[20], it has been possible to demonstrate that the length of the clock period in humans is very close to 24 hours (24.2 hours on average[20]).

One of the direct impacts of the endogenous period in everyday life is the chronotype. Individuals with a short period (a fast clock) are generally those who go to bed early (morning chronotypes) whereas people who go to bed late (evening chronotypes) have a longer period (a slower clock)[22].

__ SYNCHRONISATION OF THE CLOCK

Because the endogenic period is close to, but not exactly, 24 hours, the circadian clock must be constantly synchronised to 24 hours.
In mammals it is light that is the most powerful synchroniser of the internal clock.
The term *synchronisation* of the biological clock corresponds, just as with a wrist watch, to setting the time, whether the watch is running fast or slow, in order to get it back into phase with the environment. For an "evening" individual, whose endogenic period is 24 hrs and 30 mins, the clock has to be put forward by 30 minutes every day in order to be synchronised to 24 hours, if not it will be another 30 minutes late every day. On the other hand, in a "morning" person, whose period is 23 hrs and 30 mins, the circadian clock has to be delayed by an average of 30 minutes every day.
Animals have different synchronisers, which are less efficient in humans. They are known as "non photic" synchronisers because they do not involve light. Eating and physical exercise have a synchronising effect on the human clock but this is not very strong.
Studies carried out in the fifties had led researchers to believe that social synchronisers were more powerful than light in Humans[23]. We now know that this is not the case.
The best proof that non-photic synchronisers have, if anything, an extremely limited effect, has been obtained from the observation that the vast majority of blind people – with no perception of light – are in a state of non synchronised "free run", despite a social life and activities set out according to the 24-hour period (work, going to bed / rising, eating meals, physical and intellectual activities, etc.).
The hormone melatonin is the only non-photic synchroniser for which the effect on the human circadian clock is without a doubt[24]. It should be considered as a priority approach in the treatment of "free run" in blind people.

__ CIRCADIAN PHOTORECEPTION

Until recently it was accepted that the cones and rods of the external retina were the only photoreceptors responsible for the transduction of light information to the endogenic clock. Studies carried out since the year 2000 in both humans and animals show that two retinal systems are involved in circadian photoreception *(fig. 2)*:

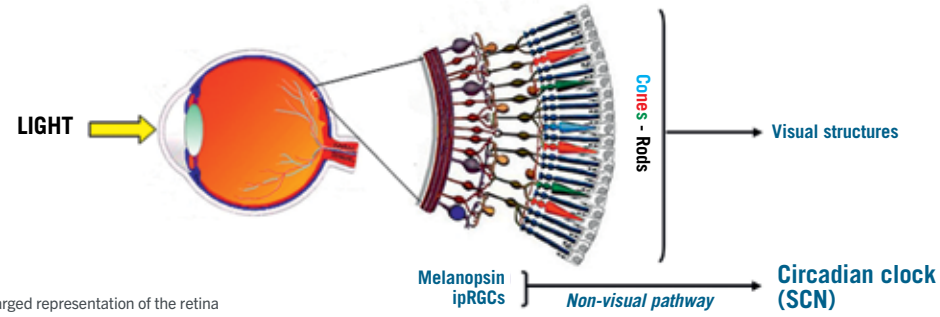


FIG. 2 | Diagram of the eye (section) with an enlarged representation of the retina (in the centre).

Surrounding light is perceived by the retina. The cones and rods project towards visual structures (perceptive vision). Melanopsin ganglion cells are involved in the regulation of biological rhythms via their projection towards the suprachiasmatic nucleus (modified image by webvision and Gronfier *et al.* [1]).

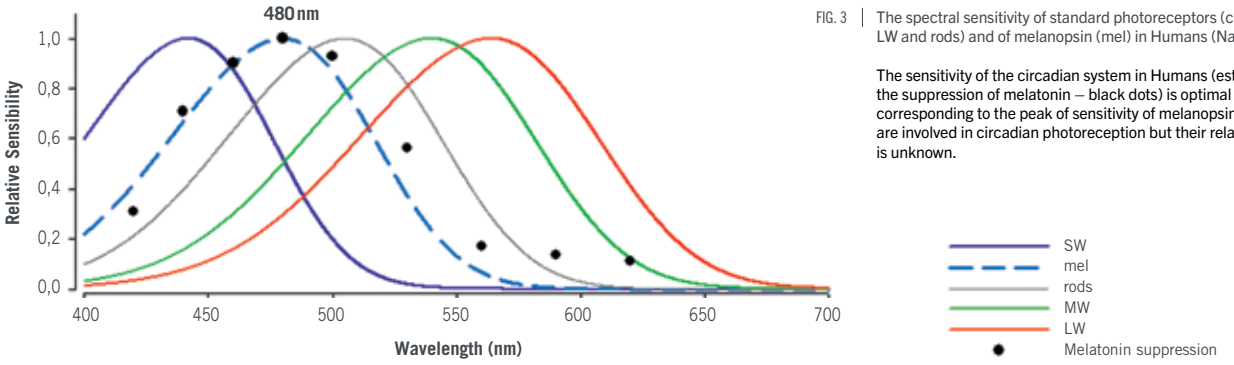


FIG. 3 | The spectral sensitivity of standard photoreceptors (cones SW, MW, LW and rods) and of melanopsin (mel) in Humans (Najjar et al.[2]).

The sensitivity of the circadian system in Humans (estimated by the suppression of melatonin – black dots) is optimal at ~480 nm, corresponding to the peak of sensitivity of melanopsin. Cones and rods are involved in circadian photoreception but their relative contribution is unknown.

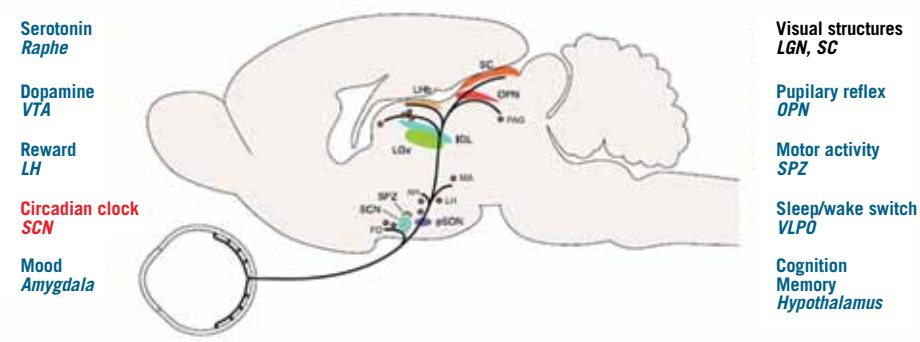
1. The photoreceptors involved in conscious vision (cones and rods).
2. The intrinsically photosensitive retinal ganglion cells: (ipRGC) involved in a large number of non-visual functions[25]. In the absence of these 2 systems, the circadian system is "blind" in rodents and functions in "free run", expressing its endogenic rhythmicity[26]. It is currently thought that the light information responsible for synchronisation of the biological clock passes through the melanopsin ganglion cells, either by stimulating these cells directly or by stimulating them indirectly through cones and rods. Because of this fact, it is now considered that the eye is not used for vision only, but that it possesses both visual and non-visual functions *(fig. 2 and 4)*.
The two types of photoreceptors in the external and internal retina are phylogenetically and functionally different. Unlike cones and rods, melanopsin ganglion cells require high illuminances and show a peak of sensitivity at around 480nm (in all the mammals studied). These rhabdomeric type cells also show the property of bistability, which makes them virtually insensitive to *bleaching*[29].
These photoreceptors are currently the subject of a great deal of research, aimed at developing methods for treating certain chronobiological disorders (including disorders of the circadian rhythms of sleep and seasonal affective disorders), which could be faster and more efficient than the current methods which use fluorescent white lights[29].

The circadian system's response to light depends on photic characteristics. The effect of light on the clock *depends on the intensity of light and how long it lasts.* The more intense the light stimulus[30], and/or the longer it lasts[31], the greater the effect. For example, nocturnal exposure to light lasting for 6.5 hours leads to a delay of more than 2 hours in the melatonin rhythm when intense white light is used (10000 lux)[32]. A stimulus given at the same time for the same length of exposure, with a light intensity of 100 lux, i.e. 10% of the maximum intensity tested, produces a delay of about 1 hour, i.e. 50% of the maximum observed[32]. Recent studies show that the circadian clock is actually particularly sensitive to low light intensities, and that exposure to a LED computer screen (between 40 and 100 lux) for 2 hours partially inhibits melatonin secretion, activates alertness, and delays the biological clock and sleep onset[33].
The effect of the light *depends on its spectrum.* As shown in figure 3, the circadian system is at maximum sensitivity to a coloured light of between 460-480nm[34]. A monochromatic blue light (wavelength 480nm) can be as efficient on the circadian system as a fluorescent white light 100 times more intense (comprising 100 times more photons). This property is based on the sensitivity of melanopsin ganglion cells.
Finally, *the effect of light depends on the time at which it is perceived.* The phase response graph shows that the light to which we are exposed in the evening and at the beginning of the night (on average between 5pm and 5am) has the effect of delaying the clock, whereas light received at the end of the night and in the morning (on average between 5am and 5pm) has the reverse effect of advancing the clock[54]. It is this specific temporal sensitivity that explains the clock's daily synchronisation under normal circumstances and its non-synchronisation in the presence of jet-lag and night work.

__ LIGHT AND NON-VISUAL FUNCTIONS

Since the discovery of melanopsin ganglion cells in the retina 10 years ago, a range of non-visual, light-sensitive functions have been described. These functions involve anatomical pathways and cerebral structures

FIG. 4 | The retinal melanopsin ganglion cells project towards a range of structures involved in the regulation of the circadian system (SCN), pupil reflex (OPN), motor activity (vSPZ, IGL), sleep (VLPO) and alertness (LC). These projection pathways are the non-visual pathways of light. Modified diagram by [5,8].



that are different to those involved in vision, and do not lead to the formation of images *(fig. 4)*. Studies in animals[35] show projections of melanopsin ganglion cells towards structures involved in the regulation of biological rhythms, the regulation of alertness and sleep states, the regulation of locomotor activity, the pupil reflex, etc.
In humans, studies show that melanopsin ganglion cells, via non-visual pathways, are involved in the effect of light on the resetting of melatonin phase[36], the increase in alertness, body temperature and heart rate[37], expression of the PER2 gene[38], resetting of the rhythm of the PER3 gene[39], the increase in psychomotor performances and EEG activity[40], sleep structure[41], and activation of cerebral structures involved in memory and mood regulation[42-51]. Light, via non-visual retinal projections, will therefore directly stimulate the cerebral structures involved in the control of alertness, sleep, mood and cognitive and psychomotor performances.
Before the identification of two anatomical pathways (visual and non-visual), it has been known since 1995 that some blind people who do not have any conscious visual perception can have a light-sensitive circadian system[52]. The visual system of these patients is blind, but their non-visual functions (including their circadian clock) are not blind and receive photic information. These cases are probably rare (very few individuals have been studied worldwide) and the majority of patients with ocular pathologies leading to partial or total privation of photic information have an increased prevalence of sleep and biological rhythm disorders (their circadian rhythms are most often expressed through “free run” and this clinical condition is associated with sleep disorders in over 75% of cases[19]). Nevertheless, ophthalmologists should be aware of the eye‘s non-visual function and its importance in the synchronisation of the circadian system. In view of the risk of adding a blind circadian system (and the free-run symptoms with their associated treatments) to a defective vision, the non-visual sensitivity to light should be evaluated prior to enucleation of a blind patient.

__ CONCLUSIONS

In view of the importance of the circadian system synchronisation and the nature of the non-visual functions, light appears to be a biological requirement essential to health. It is predictable that light will be used in the future in the treatment of numerous normal or pathological conditions, in which a physiological malfunction will be corrected through activation of the eye‘s non-visual functions. •

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