UNIVERSITAT DE BARCELONA **FACULTAT DE FARMÀCIA**

BLUE LIGHT AS A DRUG?

TREBALL FI DE GRAU

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Main scope: Physiology and Pathophysiology

Secondary scopes: Biochemistry and Molecular Biology

Nutrition and Food Science

INDEX

I. RESUM

Aquest Treball Fi de Grau es basa en la recerca bibliogràfica i la integració d'articles científics recents, que contenen informació relacionada amb la llum i els seus efectes en l'organisme; i pretén donar a conèixer que els humans necessitem la llum, no només per la visió o funcions conegudes, com la síntesi de Vitamina D, sinó també pels seus efectes no visuals.

La llum és captada i transformada en informació que va directa al cervell per una petita població de cèl·lules ganglionars de la retina que són intrínsecament fotosensibles. Aquesta informació extreta de l'ambient permet a l'organisme regular diverses funcions fisiològiques i de comportament que són essencials per una bona salut i qualitat de vida.

Aquest conjunt de funcions no visuals inclouen la sincronització dels ritmes circadiaris i estacionals, i la regulació del son, l'estat d'alerta, l'humor i el rendiment cognitiu, que estan interconnectades entre elles, i amb altres processos fisiològics i fisiopatològics com l'envelliment, processos metabòlics, desenvolupament de càncer i conseqüències cardiovasculars, per tant, l'alteració d'alguna d'aquestes funcions pot provocar danys en altres sistemes i acabar afectant tot l'organisme.

És molt important per això, controlar l'exposició a la llum en certs moments del dia, ja que no afecta de la mateixa manera la llum al matí que a la nit. Aquesta propietat però permet el seu ús per tractar aquests trastorns, per tant, l'educació en l'estil de vida del pacient i la realització de dissenys arquitectònics més eficaços per entorns d'il·luminació com escoles i treballs són potencialment beneficiosos.

II. ABSTRACT

This Final Project is based on bibliographic research and integration of recent scientific articles that contain information related to light and its effects on the body; and aims to raise awareness that humans need light, not only for vision or known functions such as the synthesis of vitamin D, but also for non-visual effects.

Light is detected and transformed into information that goes direct to the brain through a small population of intrinsically photosensitive retinal ganglion cells. This information obtained from the environment allows the body to regulate several physiological and behavioural functions that are essential for a good health and quality of life.

This set of non-visual functions include the synchronization of circadian and seasonal rhythms and the regulation of sleep, alertness, mood and cognitive performance, which are interconnected with each other and with other physiological and pathophysiological processes such as aging, metabolic processes, development of cancer and cardiovascular consequences, so the alteration of any of these functions can cause damage to other systems and ultimately affect the entire body.

It is important therefore to control light exposure at certain times of day, because light in the morning does not affect the same way than light at night. This property allows its use for treating these disorders; therefore, patient lifestyle education and more effective architectural designs for lighting environments like schools and work are potentially beneficial.

III. BRIEF DISCUSSION OF THE EDUCATIONAL SCOPES' INTEGRATION

This review about light as a medicine encompasses three different educational scopes that are connected at concept level. The core of the work lies in the main scope of Physiology and Pathophysiology, but also includes other educational scopes such as Biochemistry and Molecular Biology, and Nutrition and Food Science.

Regarding the main scope, firstly, there is a brief anatomical and physiological description of the retina, the part of the body responsible for light detection and information transmission to the brain. Then, there is a more detailed description of the parts of the brain that integrate this information for continuously regulating biological rhythms, depending on the presence or absence of light during the day. In addition to the circadian photoentrainment, light also affects sleep, alertness, mood and cognitive performance, therefore, physiology and pathophysiology of these processes, due to changes in light detection and/or transmission, is also explained.

This section of circadian regulation also includes concepts of Biochemistry and Molecular Biology, as it explains the molecular machinery responsible for the synchronization of internal rhythms with day-night cycles, and its operation at gene transcription and protein synthesis levels.

Finally, in the pathophysiological part of the review, which includes issues related to aging, cancer and cardiovascular consequences, there is a point of metabolic disorders related to circadian disruption, such as diabetes and obesity. This point explains that changes in light-dark cycles, like those produced by schedule changes in shift workers, are related to excessive food intake and trend poor nutrition, based in a diet rich in fat and carbohydrates, including thereby concepts of Nutrition and Food Science.

These three scopes are part of a set of scopes that integrate the Bachelor of Pharmacy, and pharmacists have an important role in giving health advices to patients, therefore, we have to be able to raise awareness of the importance of light exposure at certain day times and the need of darkness in others to get a good health and quality of life.

IV. OBJECTIVES

- To analyze the different functions of light on the body, focusing on the non-visual effects triggered by the intrinsically photosensitive retinal ganglion cells (ipRGCs).
- To study the neural pathways of these non-visual effects of light, from the day-night cycle synchronization, or circadian photoentrainment, to the regulation of sleep, alertness, mood and cognitive performance.
- To understand the relationship between the non-visual effects of light and its chronodisruption, and the physiological and pathophysiological processes in the body, such as aging, metabolic processes, cancer and cardiovascular consequences.
- To raise awareness of the importance of exposure to light at certain times of day, and the consequences of alterations in light-dark cycles due to seasonal changes in day length and schedule changes.

V. MATERIALS AND METHODS

The methodology used for conducting this review was based on a bibliographic research of information related to the proposed topic: "Blue light as a drug?"

Firstly, basic general information of the topic was drawn from the book (1) Cambras Riu T, Díez Noguera A. "*Els Ritmes de la vida : Com la cronobiologia ens ajuda a viure millor*", cited in the bibliography.

Then, more specific information was mainly extracted from scientific articles, obtained from searching in the database PUBMED (http://www.ncbi.nlm.nih.gov/pubmed), a platform developed by the National Centre for Biotechnology Information (NCBI) that allows access to most full-text journals.

The other scientific papers were obtained through access provided by the "*Centre de Recursos per a l'Aprenentatge i la Investigació de la Universitat de Barcelona*" (CRAI UB) to several scientific journals.

Many articles related to the topic were found, but only those published from 2008 until now were used to perform this work, despite that some of them are reviews, which include publications of previous years.

VI. INTRODUCTION

The earth rotation on its axis results in periodic changes in the light-dark environment. These predictable changes exert great influence on plants, animals and humans, and allow these organisms to confine their behaviour and physiology to specific times of the day-night cycle. This means that the solar day limits their activities to the correct temporal niche.

To anticipate these light-dark changes, organisms have evolved an internal biological pacemaker, a circadian timing system which acts as an endogenous representation of the external 24h clock. Despite this, the intrinsic oscillation period of this pacemaker is not exactly 24h, in humans it is 24,2h in absence of environmental influences; therefore, this circadian timing system requires daily resynchronization through external time cues, called *zeitgebers*. The major *zeitgeber* is daily retinal illumination through exposure to environmental light, which represents the primary indicator of the time of day (2–5).

Under normal conditions, organisms experience a 24 hour pattern of light-dark (LD), and the circadian system of most of them uses the twilight transitions to align to external time; but also other environmental factors such as temperature, meals, exercise and social cues, including employment, families and alarm clocks, may influence the circadian rhythms. Hence, they can also affect the circadian clock outputs, which include sleep/wake and metabolic cycles as well as hormonal changes. Indeed, circadian rhythm disturbances encompass a number of disorders which can result from either exogenous or endogenous disturbances, or a combination of both (4). So, proper alignment between light, the circadian pacemaker, and output behaviours produces a temporal order in organisms that is essential for survival.

1. Light influences on health

Light has strongly influenced the evolution of life on earth. As widely appreciated, light allows us to generate images of our environment; however, it also lets us the regulation of several behavioural and physiological functions that are essential for our health and quality of life.

For instance, environmental light is the major responsible for vitamin D production, which is very important for calcium homeostasis, indeed, a deficit of vitamin D increases the risk of suffering rickets in children and osteoporosis in adults. Moreover, UV light is really useful for removing the bilirubin excess from skin and mucous of newborns with fetal erythroblastosis, which could cause jaundice or even irreversible damage to the CNS (kernicterus). Furthermore, UV light is also used for other skin treatments in adults such as psoriasis, vitiligo, cutaneous T-cell lymphoma and atopic dermatitis.

Regardless all the possible effects of light on humans' behaviours and health, this project will be focused on light detection through the retina and its most innovative aspects.

2. Light detection

Light detection through the retina not only supports pattern vision, but also has profound effects on many aspects of human physiology and behaviour that are independent of image formation, such as synchronizing and resetting of properties of the endogenous circadian pacemaker, pupillary constriction, heart rate, sleep regulation and alertness, body temperature, brain activity and cognition, hormonal secretion (melatonin) and clock gene expression (2,6–8).

Light exposure at different times of the day can alter circadian rhythms, that is, changes in the timing of the LD cycle (e.g. a nocturnal light exposure) result in shifts in the phase of circadian rhythms; for instance, suprathreshold early morning light advances, while evening light delays rhythms (5,8). So, an adequate light exposure in the correct time is important for the proper maintenance of physiological processes.

The phase-shifting effect of light can only be detected in the longer term (i.e. in the next circadian cycle), but the effects of light are not limited to these long-term effects; the effects of light on circadian physiology can be observed during or immediately after the light exposure. Thus, these effects can be acute (seconds for brain responses)

or extend beyond the light exposure (hours for hormone secretion). Although the acute effects of light were first studied using bright light, it has emerged that they can also be elicited by ordinary room light or even dimmer light (6,8).

The long term and acute effects of light on circadian physiology are referred to as non-visual or non-image forming (NIF) effects of light because they are not directly related to vision and because they present several features that distinguish them from the visual system (8). These NIF effects will be explained later in more detail, but first, it is necessary to introduce the structure and functions of the light detection system in humans.

2.1. The retina

The human retina, located in the back of the eye, is a complex transparent tissue consisting of several layers, only one of which contains light-sensitive photoreceptor cells. So, light must pass through the overlying layers to reach these cells (9).

2.1.1. Photoreceptor cells

A photoreceptor cell is a specialized type of neuron found in the retina that is capable of phototransduction. The great biological importance of photoreceptors is that they transform photon light energy (visible electromagnetic radiation) into electrical signals that can stimulate biological processes in different ways.

A mere decade or so ago, rods and cones were considered the only photoreceptor cells in the mammalian retina. However, the spectral sensitivity of the circadian system provided the initial evidence of an additional photoreceptor system in the retina. Later, this theory was supported when genetically modified mice, lacking rods and cones, were likewise able to detect light for entraining circadian rhythms, as well as other NIF functions (2). Finally, this was confirmed by some studies with totally blind humans, who were unable to form images but were nonetheless capable of detecting light and inducing acute and long-term non-visual responses (i.e. regulation of melatonin secretion), so those people had normally photoentrained behavioural and neuroendocrine rhythms (2,4,8).

The discovery of this non-rod/non-cone novel system, based on the activation of photoreceptor transduction cascades within the mammalian retina, not only revolutionized our understanding of intrinsic retinal biology and physiology, but also defined the important role of light in the non-image forming functions of the retina:

the regulation of circadian rhythms and other NIF effects (2,4), therefore, light is essential for both image and non-image forming functions.

Therefore nowadays, rods, cones and these additional photoreceptor system formed by the intrinsically photosensitive retinal ganglion cells (ipRGCs), which are structurally and functionally different, are all of them considered photoreceptor cells.

2.1.1.1. Rods and cones: the visual part

The classical photoreceptor cells are rods and cones, and both express photopigments in their membranes, rhodopsins (*Opn2*) and photopsins (*Opn1*) respectively, which are responsible for detect and transform light into information that is used by the visual system for image formation and object tracking (2,3).

Rods are exquisitely sensitive and are designed for dim light (scotopic) monochromatic vision, whereas cones are less sensitive and are specialized for bright light (photopic) colour vision and contrast detection. For this, humans have one kind of rods, with a sensitivity peak at 498 nm wavelength (Fig. 1), and three cone types: short wavelength (S-cones), mid wavelength (M-cones) and long wavelength (L-cones) associated with blue, green and red colours, in that order. So, colour vision is mediated by the activation of different cone photoreceptors that express different photopsins, which show sensitivity peaks at different wavelengths (colours) of light, which are 420 nm, 534 nm and 564 nm, respectively (Fig. 1) (2,3,10).

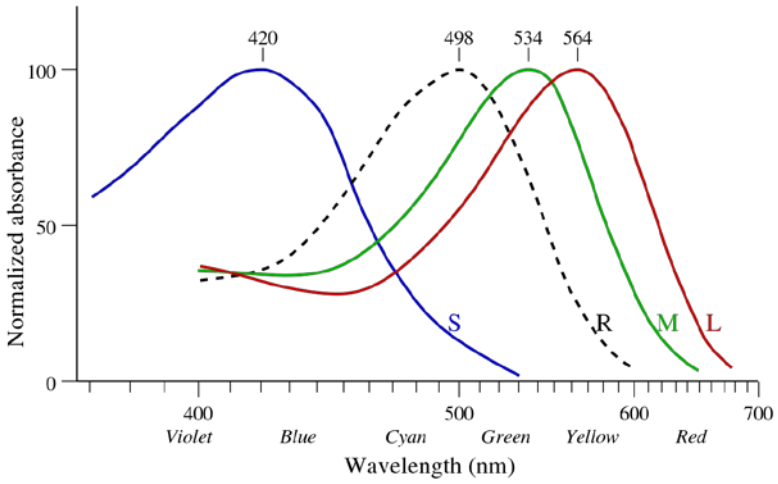

Fig. 1. Spectral absorption curves of the short (S), medium (M) and long (L) wavelength pigments in human cone and rod (R) cells.

Rods and cones relay photic information through multisynaptic pathways to the retinal ganglion cells (RGCs), the output neurons of the retina, which innervate different areas of the brain for complex visual processing (2).

Retina signalling occurs in two directions: vertically and horizontally. The vertical neurotransmission takes place predominantly from photoreceptors to RGCs through bipolar cells by glutamate release; whilst the horizontal neurotransmission occurs through other two types of cells, horizontal and amacrine cells, which facilitate lateral connectivity between the photoreceptors and bipolar cells, and the bipolar cells and RGCs, respectively by GABA and glycine release (10) (Fig. 2).

Then, RGC axons carry the information through the optic nerve to the lateral geniculate nucleus (LGN) of the thalamus, and other sub-cortical targets, where it is integrated and finally leads to vision (10).

Much more information could be given on vision and its mechanisms, this is only a brief touch, but this is not the aim of this project.

2.1.1.2. ipRGCs: the non-visual part

A third class of photoreceptor cells was discovered in vertebrates during the 1990s and termed intrinsically photosensitive retinal ganglion cells (ipRGCs) because it was a subpopulation of RGCs which responded to light intrinsically in absence of rod/cone signalling (2,4). As such, the ipRGCs detect environmental illumination and transform light into information, which permits human physiology to be optimized and aligned with geophysical day-night cycles using neural and hormonal messengers including melatonin (5).

Approximately 3000 ipRGCs are situated in the ganglion cell layer and span the retina with their dendrites overlapping in the inner plexiform layer, thus forming a network and acting as a syncytium which integrates photic energy over long periods (4). Initially, ipRGCs were thought to constitute a uniform population whose predominant role was to influence circadian rhythms. However, depth investigations have revealed the existence of at least 5 subtypes (M1-M5) of ipRGCs, with different morphological and electrophysiological properties, in rodents (11,12). All of them express melanopsin (*Opn4*) in the plasma membrane of their cell bodies, dendrites and axons (4). The distinct ipRGC subtypes express varying levels of melanopsin and have different patterns of dendrite stratification in the inner plexiform layer, indicating that each subtype could play a particular role in detecting light intrinsically and in signalling rod and cone information to the brain (2,12).

Melanopsin is an opsin sparsely distributed in the mammal inner retina, indeed specifically expressed in ipRGCs, which functions as a bistable photopigment able to regenerate its own chromophore using long wavelength light, whereby the light

activated form is capable of absorbing a second wavelength of light and recycling itself back to the inactive form, ready to absorb light and drive more responses; this characteristic of bistability is a melanopsin adaptation. For these reasons, melanopsin was proposed as the circadian responsible photopigment (4,7,13).

The melanopsin expressing RGCs demonstrate a characteristic opsin-like spectral response curve, with peak sensitivity at short wavelengths (446-483 nm). This ipRGC sensitivity peak (480 nm) is also included in blue light wavelength range (450-495 nm); therefore these melanopsin expressing RGCs are most sensitive to short wavelength light of the blue spectrum region (2,4,6). Moreover, sunlight has been the primary stimulus for ipRGCs photoreception throughout human history; in fact, skylight has a dominant wavelength of 477 nm, similar to ipRGCs peak sensitivity (5), but during twilight, the quality of light changes in three important aspects: the amount of light, the spectral composition of light and the source of light or position of the sun. These parameters all change in a systematic way and could be used by the circadian system to detect the phase of twilight and hence time of day. For instance, when the sun is close to the horizon, there is a relative enrichment of blue light in the dome of the sky because of the preferential scattering of short wavelengths of light passing obliquely through the atmosphere (3).

The effectiveness of light exposure for ipRGCs-mediated biological effects depends on its intensity, duration, spectrum wavelength and timing relative to the phase of the circadian rhythm. Thereby, brighter, longer, blue light exposures has been shown to be most effective at inducing a wide range of non-visual responses in humans, including changes in the melatonin rhythm timing, acute suppression of melatonin secretion, phase shifting, photoentrainment, thermoregulation, heart rate variability, improved nocturnal sleep quality, treatment of seasonal/non-seasonal depression, and enhanced mood/well-being, alertness, reaction time, cognitive performance and vigilance. This supremacy of blue light at brain responses was observed as early as 50 seconds after the start of the exposure and persisted for 18 minutes of exposure (5,7,8).

In addition to their intrinsic melanopsin-dependent pathway, the ipRGCs, like all ganglion cells, also receive light information secondarily from rods and cones (Fig. 2), so they partially contribute to non-image forming (NIF) retinal functions. In fact, these melanopsin expressing RGCs are the main link for rod-cone input to circadian timing system daily resynchronization, and both classical photoreceptors and ipRGCs are necessary to observe a complete response to light (2,4,7,8,14,15).

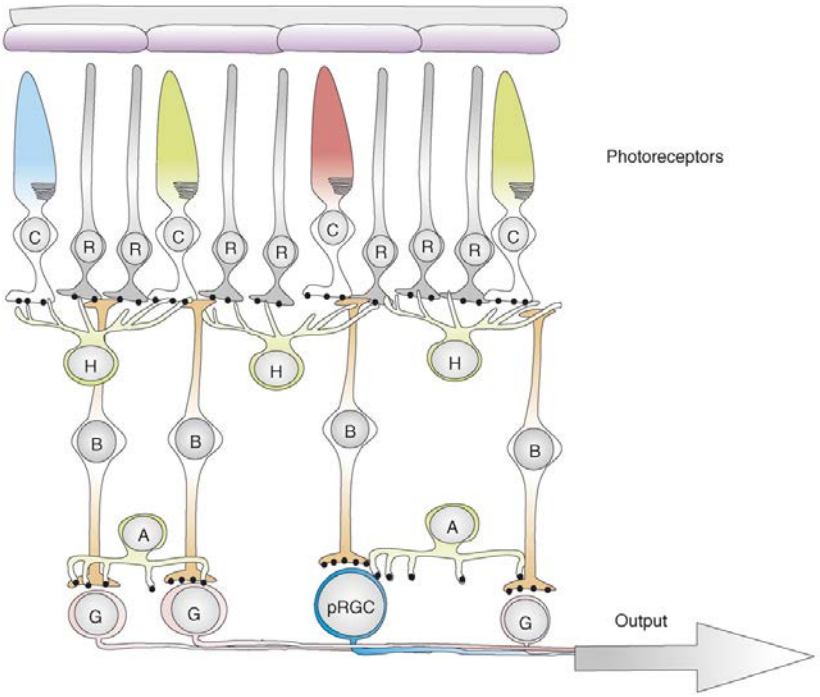

Fig. 2. Light detection in the vertebrate retina. Rods (R) and cones (C) convey visual information to ganglion cells (G) via second-order bipolar cells (B). At the first synaptic layer, horizontal cells (H) facilitate lateral connectivity and feedback to photoreceptors, and at the second synaptic layer in the inner retina, amacrine cells (A) allow lateral connections between bipolar and ganglion cells. The optic nerve is formed from axons of all the ganglion cells. The subset of photosensitive retinal ganglion cells (pRGCs) also detects light directly, using the photopigment melanopsin. Thus, photodetection in the retina occurs both in the outer and inner retina [Modified from REF. (13)]

In the so-called non-image forming pathway, light detection through the ipRGCs is transformed into information, non-visual photic inputs, which are directly transmitted from ipRGCs to non-visual brain centres including the human body's master biological clock, the suprachiasmatic nuclei (SCN), through a monosynaptic pathway that originates in the retina and is called the retinohypothalamic tract (RHT) (4,5) (Fig. 3).

In addition to their projections to the SCN, ipRGCs also show widespread projection patterns throughout the rodent brain, targeting numerous brain regions that have a role in driving light-mediated behaviours, such as the olivary pre-tectal nucleus (OPN) that regulates pupil constriction (7); the subparaventricular zone (SPZ) and intergeniculate leaflet (IGL) of the thalamus, important for the regulation of circadian rhythms; the ventrolateral preoptic area (VLPO) and lateral hypothalamus (LH), related to sleep regulation and alertness; and the limbic regions medial amygdala (MA) and lateral habenula (LHb), implicated in cognitive functions and mood regulation, that includes depression and anxiety states (Fig. 3). Therefore, ipRGCs are the leading candidates for mediating the effects of light on several behaviours, such as circadian rhythms, sleep, alertness and mood (2,8,16).

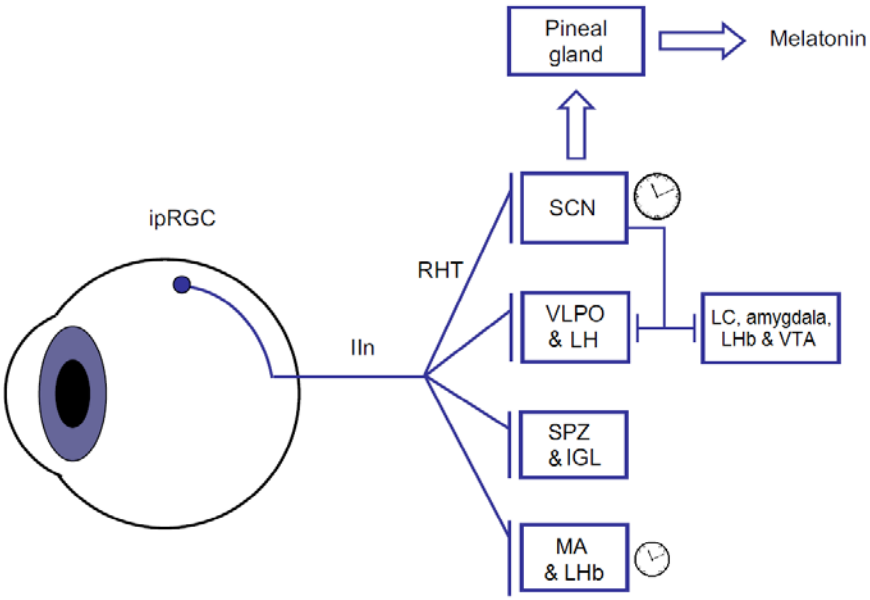

Fig. 3. Schematic representation of the direct ipRGC innervations of the suprachiasmatic nuclei (SCN), ventrolateral preoptic area (VLPO) and lateral hypothalamus (LH), subparaventricular zone (SPZ) and intergeniculate leaflet (IGL), and medial amygdale (MA) and lateral habenula (LHb), via the optic nerve (IIn) and retinohypothalamic tract (RHT); and the polysynaptic pathway link between the SCN and pineal gland for melatonin production, and other SCN outputs innervations such as locus coeruleus (LC) and ventral tegmental area (VTA) [Modified from REF. (4)].

3. Non-image forming functions

In mammals, the eye is the only organ capable of detecting light for non-image forming (NIF) functions, which are light mediated-behaviours that are not involved in detecting contrasts, colour or motion of the visual scene. (2). These NIF functions are mainly led by the, now better known, intrinsically photosensitive retinal ganglion cells (ipRGCs), but also to a lesser extent by rods and cones. Indeed, several studies conducted on one hand with mice lacking rods and reduced number of cones, and the other hand with melanopsin knockout mice, exhibited attenuated NIF responses to light; but further studies performed with triple-knockout mice (lacking rods, cones and melanopsin) revealed a totally unresponsiveness to light, demonstrating that NIF functions in mammals are mediated by rods, cones and the melanopsin expressing RGCs (3,14,15).

These NIF functions include circadian photoentrainment, the tracking of seasonal changes and the regulation of sleep, alertness, mood and learning (2). The circadian system and photoentrainment explained below have been well studied for a long time, but the mechanisms of sleep and mood regulation due to light are still being investigated to get more information, so the explanations given later are based on information available up to now.

3.1. Circadian photoentrainment

3.1.1. The circadian system

3.1.1.1. Suprachiasmatic nuclei (SCN)

The suprachiasmatic nuclei (SCN) of the anterior hypothalamus serve as the body's master biological clock or central circadian pacemaker in animal species that possess complex central nervous system (CNS). As mentioned above, this internal biological clock is capable of maintaining rhythmicity of circadian functions such as sleep, body temperature, hormonal secretion and cognitive performance in the absence of environmental inputs, although it needs synchronization of these functions with the external environmental time (4,17).

The SCN also receives information about internal physiological state. These timed SCN inputs prepare the body for the fluctuating physiological demands of the solar day, such as metabolic, biochemical and physical activities. In the morning, activation of the adenocortical axis and the corresponding catabolic processes prepare the body for the mental and physical demands of awakening; morning exposure to sunlight increases core body temperature, alertness, cognition and brain serotonin levels which enhance mood and vitality. As the day progresses, peak cognition occurs commensurate with maximal core body temperature. By evening, the SCN actively inhibit cortisol secretion and stimulate pineal melatonin secretion, which affects transcription rates in SCN, and other hormones, which decrease core body temperature and reduce alertness, and allow the anabolic functions of growth and repair to predominate (4,5,17).

As noted above, apart from its main activity, that is the circadian timing system daily resynchronization through the external time cues, the SCN have several downstream targets on which have a great influence. These targets include brain regions involved in sleep regulation, such as the VLPO and LH, body temperature regulation, hormone secretion and cognitive performance. But also other brain areas related to mood and motivational states, such as the locus coeruleus (LC), amygdala, LHb and ventral tegmental area (VTA) (2,4) (Fig. 3).

As shown in the figure 3, several of the ipRGC targets also receive innervations from the SCN, raising the possibility that in addition to its pacemaker function, the SCN can also act as a conduit for light information. This means that light can influence these areas through direct projections from ipRGCs and indirectly through the SCN, so, the convergence of environmental light information at specific times of the circadian cycle may influence the physiological functions of these brain regions (2).

3.1.1.1.1. Molecular machinery

The molecular machinery responsible for the generation of circadian rhythms is really complex, with multiple cascades taking place in the nucleus and cytoplasm, which contribute to the expression of circadian oscillations and rhythmicity. Indeed, endogenous circadian rhythms are generated by oscillating gene expression derived from an autoregulatory transcription/post-transcription/translation/post-translation-based feedback loop (18). The transcription, translation and translocation of these clock genes (CGs) vary rhythmically during the day, and these variations are associated with changes in the synthesis, sensitivity, efficacy and concentrations of several neurotransmitters, neuropeptides and hormones throughout the brain and body, which also modulate the molecular machinery of the SCN (17).

The feedback loop, including nuclear transport and cytoplasmic concentration gradients, occurs in neurons; and the system is comprised of two limbs, categorized as either positive or negative (i.e., the presence of the factors increases or decreases the transcription/translation of some product, respectively). The negative component of the feedback loop is dominated by the clock genes Cryptochrome (Cry) and Period (Per), which are typically co-expressed; and the positive component includes the transcription factors CLOCK and BMAL1. In mammals, Per has three isoforms (Per1, Per2 and Per3) and Cry has two (Cry1, Cry2), which are transcribed and translated into PER and CRY proteins. The presence of these protein products within the nucleus inhibits their own transcription by down-regulating CLOCK and BMAL1 (17,19).

Though gene expression is endogenously controlled, and hence intrinsically rhythmic, there are several mechanisms that entrain the system through various inputs or *zeitgebers*, which manipulate the expression of the cascades that can act to stabilize the rhythms, advance, or delay them. For instance, light can influence PER1 and PER2 expression in the SCN in conjunction with endogenously rhythmic transcription factors CLOCK and BMAL1. Light information travelling along the RHT from the ipRGCs activates neurons and subsequently engaged intracellular second messenger signalling cascades, the most important of which involves cyclic AMP response binding element (CREB), whose transcriptional activity acts directly at the PER promoter. As explained above, late-night light exposure increases the expression of PER1, resulting in a phase advance, whereas early-night light exposure increases both PER1 and PER2 expression, which delays the circadian oscillator. The magnitude of the change is in part mediated by the intensity, duration, spectrum wavelength and timing of the light pulse (17).

Not surprising, given their role as intermediaries between inputs and the molecular clock itself, that Per1 and Per2 are among the quickest CGs to adapt to a new circadian period; whilst the role of Per3 is less clearly defined with only mild perturbations observed when its protein product is absent. A length polymorphism of this gene is associated with delayed sleep phase disorder (DSPD), but it is still unclear whether this is due to perturbations of the circadian clockwork or represents some role for PER3 in sleep homeostasis. Adding further complexity to their regulation, PER protein levels are enhanced via Casein Kinase 1E (CK1ε) phosphorylation, a critical agent for the maintenance of normal 24 h rhythms (17,19).

Moreover, Cry1 and Cry2 expression may also be used to quantify circadian phase, because Cry1 shortens the length of the circadian cycle whereas Cry 2 lengthens it; but in the absence of both isoforms, circadian rhythmicity gradually disappears under constant dark conditions.

Furthermore, several neurotransmitters including serotonin (5HT), norepinephrine, glutamate, gamma-aminobutyric acid (GABA) and acetylcholine (ACh) also affect functional activity in the SCN. Molecular communication within the SCN is largely modulated by colocalization of traditional neurotransmitters with various neuromodulators including hormones like melatonin and steroids; neuropeptides such as vasoactive intestinal peptide (VIP), arginine vasopressin (AVP), gastrin releasing peptide (GRP) and substance P; and intracellular mediators of cellular activity such like calbindin, calretinin, pituitary adenyl cyclase-activating peptide (PACAP) and neuropeptide Y (NPY). The colocalization of these numerous molecular agents is a large contributor to the complexity of SCN regulation; and variable concentrations of these molecules alter behavioural state, which can also impact circadian state (17).

3.1.1.2. Peripheral and slave oscillators

In addition to timing cues derived from light, clock genes, and other neurotransmitters, hormones and neuropeptides, many homeostatic processes, such as metabolism (lipids, carbohydrates, proteins and xenobiotic substances), renal (renal plasma flow and urine production) and cardiovascular (blood pressure and heartbeat) functions exhibit their own circadian oscillation that, in turn, may feedback into the SCN to tune the circadian clock (17,18). Therefore, although the SCN is the master pacemaker in mammals, there is a complex network of oscillators in other brain regions and peripheral tissues. Due to their downstream position relative to the SCN and light, the loci responsible for generating these rhythms have been categorized as peripheral oscillators.

The abundance of mediating factors and environmental inputs targeting the SCN potentiate how quickly it is able to adapt to a new circadian phase and CG expression within the SCN (18). SCN is the master pacemaker because there is a high degree of synchronicity between SCN neurons due to this coupling, which means that rhythmicity is maintained in the SCN under conditions that would abolish rhythmicity in peripheral oscillators (20). Indeed, due to the hierarchical nature of the circadian system, except for a few regions, the rhythmicity of peripheral oscillators depends on the SCN; this means that they fail to generate circadian output in the absence of input from the SCN, so they are known as slave oscillators. As a result, these oscillators are potentially slower to adapt to new schedules than the SCN because they do not possess abundant modulatory *zeitgeber* inputs (17).

The SCN as the master oscillator and the periphery together form a resonant network, which communication occurs via direct neuronal efferents, neuropeptide secretion and humoral signals (e.g., glucocorticoids) (18,20,21). Loss of SCN input leads to desynchronization of peripheral cell oscillations, decoupling of the circadian physiology of cells and organs, and increased risk of disease. Some health problems associated with chronic circadian disruption, such as reduced cognitive function and poor health outcomes, are likely a product of desynchronization across the central and slave oscillators for extended periods of time (22). Furthermore, a lack of coherence in the oscillation of individual cells within each system may further contribute to the negative outcomes following circadian disruption (4,7,17).

Moreover, circadian rhythms in clock gene expression, body temperature, feeding and activity can entrain peripheral oscillators indirectly (18,20,21). Indeed, some brain areas such as the medial amygdale (MA) and the lateral habenula (LHb) can be categorized as semi-autonomous oscillators because they are capable of circadian rhythm generation in the absence of the SCN, due to the direct ipRGCs innervations (Fig. 3). Furthermore, the olfactory bulb has all the characteristics of a master pacemaker and appears to be a fully autonomous oscillator such as the SCN (2,4,17). So, not all peripheral oscillators need the SCN for circadian rhythm generation, but most of them require SCN for external synchronization.

3.1.1.3. Pineal gland and melatonin

In mammals, the pineal gland expresses many elements of the phototransduction cascade but lacks photosensitivity so appears exclusively secretory, in fact, it is the main source of the neurohormone melatonin (3), but not the only one; melatonin synthesis also occurs in a variety of tissues ranging from the retina to the gut (17).

Several multisynaptic signals from the SCN are conveyed to the pineal gland and modulate (suppress or stimulate) melatonin synthesis and release into the bloodstream (Fig. 3), this makes melatonin the most closely associated hormone to SCN functions (4,5). But melatonin is not only involved in circadian modulation, but also is implicated in several metabolic disorders, the majority of which (e.g. obesity and diabetes) also have strong ties with abnormal circadian function (17).

Melatonin production is highly rhythmic, in fact, its rhythm is easily reproducible in an individual and, unlike other circadian rhythms, such as core body temperature or cortisol levels, it is minimally masked by sleep, activity or stress (4). Melatonin synthesis and secretion levels are usually characterized by high night-time levels and low daytime levels. Indeed, up-regulation of the rate-limiting enzyme in melatonin synthesis (N-acetyltransferase) is directly and immediately suppressed by the SCN in response to light; that is, darkness permits pineal melatonin production during the proper phase of the SCN cycle, so melatonin levels signal the time of day (5).

The major metabolite of melatonin is 6-sulphametoxymelatonin (aMT6s), an endogenous marker of the circadian clock, whose pattern of production in plasma, urine and saliva has been shown to reflect that of melatonin (4). This pattern is attenuated under constant light conditions such as modern living conditions (i.e., light persisting past sunset), which have dramatically impacted 24h melatonin profiles. Melatonin is a potent antioxidant that contributes to the elimination of free oxygen radicals and nitric oxide, and also provides numerous other beneficial effects, therefore, this attenuation of melatonin levels generates potential health risks (5,17).

At the SCN, melatonin modulates gene expression derived by the transcription-translation feedback loop that supports the 24h circadian cycle. The influence of melatonin within the SCN takes place at specific melatonin receptors (Mel1 and Mel2), and the expression of these receptors oscillates over the 24h cycle (17). So, not only the concentration of melatonin fluctuates across the cycle, but also the ability of melatonin to influence the SCN varies across circadian time.

3.1.2. Photoentrainment

Photoentrainment is the main factor responsible for synchronising the endogenous circadian rhythms to the 24h day, thus serving to optimise the timing of the body's physiology and behaviour with the environment (4,7).

In mammals, the retina is solely responsible for photoentrainment, which linked to melanopsin, differs from image detection, produced by rod and cone opsins, because it

requires higher threshold light intensities (up to 200 times greater than that required to elicit a visual response) over a long period of time. This happens because ipRGCs are relatively insensitive to short duration stimuli, that is, less sensitive and slower to respond to light stimulus than rods and cones, which exhibit a rapid, heightened response to the onset of a bright pulse of light, followed by a slow decay, even after the stimulus is extinguished (2,4,5,14).

Thereby, non-visual retinal ganglion photoreceptor responses to properly timed bright light exposures help assure effective circadian photoentrainment and optimal diurnal physiological processes (5), but also a train of bright, short wavelength light flashes, that predominately stimulate S-cones, could provide photic information to the SCN without necessarily evoking a direct, high-threshold and slow-responding phototransducing response from these ipRGCs (14).

3.2. Regulation of sleep and alertness

Impaired sleep is a common problem, especially among older adults, and is associated with reduced daytime function, in addition to increased falls and injuries, which raise health care costs and reduce quality of life (23). Sleep timing, depth and duration are regulated by homeostatic and circadian factors. So, proper alignment between the circadian and homeostatic mechanisms improves the quality of sleep; and the amount and quality of light striking the retina is really important for this improvement. Light influences sleep through changes in circadian photoentrainment, but also directly affects sleep onset and homeostatic sleep drive (2,4,6). In this way, light, the circadian clock and sleep may closely interact to let organisms to adapt to their environments.

Sleep and wake are modulated by interplay between the light and dark signalling through the rod-cone and melanopsin-based photoreceptive pathways. Despite this, some studies revealed a significant suppression of nocturnal melatonin and delayed sleep onset following exposure to blue-enriched light, suggesting that these effects were mediated presumably by the melanopsin rather than the photic system (4,6). Nonetheless, humans with deficits in either retinal pathway could be particularly vulnerable to acute effects of light and dark on sleep and wakefulness. Indeed, some sleep disorders result from defective light processing, owing to ipRGC malfunction, or due to inadequate light entering the eye to stimulate opsins (4,5).

Sleep disorders specifically related to ipRGC depletion, which include interrupted sleep, increased sleep latency, short sleep duration, daytime sleepiness, and reduced alertness and performance during waking are those observed in blind individuals; and the severity and prevalence of sleep disturbances is thought to be higher in those with

no perception of light. For instance, free-running circadian disorder (FRD) in blind people without ipRGC photoreception is most likely the direct result of ipRGC loss, although inadequate environmental light exposure can also cause free-running circadian rhythms in sighted people (4,5).

FRD is characterized by a progressively deviation of the phase of physiological cycles and the return to environmental day-night cycles after days or months. This happens because of the repetitive cycling without daily synchronization owing to the absence of environmental timing cues that makes the SCN cycle daily at their own intrinsic period, independently of geophysical day-night cycles. Related to this, most totally blind individuals have abnormal or free-running circadian rhythms, although recently, dim light melatonin onset (DLMO) and core body temperature (CBT) have been proven useful in diagnosing and assessing FRD of these individuals, who then typically entrain with daily exogenous melatonin, which can improve their quality of life and possibly reduce otherwise increased early mortality risks (4,5).

Other disorders like FRD in sighted individuals, advanced or delayed sleep phase disorder (ASPD or DSPD), shift-work disorder (SWD), jet lag disorder (JLD) and irregular sleep-wake rhythm (ISWR) are due to light stimulating melanopsin at 'wrong times' during the circadian cycle. Therefore, both changes in the light environment and poor sleep are associated with general alterations in health including mental health issues, such as seasonal affective disorder (SAD), depression and cognitive dysfunction, which reciprocally can cause disturbed sleep (2,4,17,23).

Particular attention has been given to understanding the role of sleep deprivation in the genesis of these health problems. Sleep deprivation has been shown to result in cognitive function deficits, including negative effects on learning and memory, alertness and concentration. The sleep disruptions that precipitate an episode of depression have been well characterized; interestingly, acute sleep deprivation has a robust antidepressant effect; however, the chronic sleep deprivation, such as that associated with shift work, leads to mood disturbances (2).

Most of the disorders listed above are complex and likely to result from a combination of endogenous variation in the circadian oscillator, age, gender, morningness or eveningness preference, genotype, behaviours and response to *zeitgebers* (4).

3.3. Mood and cognitive performance

Ambient light and its physical characteristics emerge as important modulators of brain function and cognition. Initially, it was thought that mood and cognitive disorders associated with changes in light exposure were caused by disruptions in circadian rhythms and sleep. Indeed, in humans, circadian rhythmicity influences numerous cognitive processes including attention, executive functions and memory. Accordingly, the effects of light on the circadian system have been thoroughly studied, with a focus on how changes in the light environment lead to changes in circadian rhythms that, in turn, influence sleep and contribute to alterations in mood and cognitive function, essentially suggesting that the effects of light on mood and cognitive functions are secondary. This is known as the indirect pathway by which changes in the light environment cause mood and cognitive alterations (Fig. 4) (2,8,24).

But later studies have revealed that the effects of light on mood are not solely an outcome of circadian rhythm disruption, but also have a direct, clock-independent role in influencing these functions. This is known as the direct pathway by which light produces changes directly in mood and cognition (Fig. 4). In fact, regions of the brain that are involved in attention, alertness, cognitive function and emotional processes respond preferentially to 480 nm blue light, the wavelength that maximally activates melanopsin, even in blind subjects, which provides an additional support for the role of ipRGCs in these and other NIF functions in humans (2,8,24).

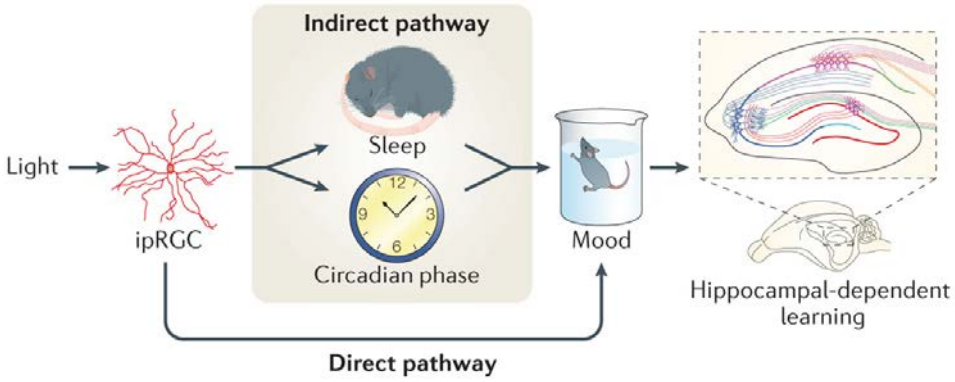

Fig. 4. Schematic representation of the indirect pathway, in which light can regulate mood and learning secondarily to sleep and circadian rhythms modulation, and the direct pathway, in which light can directly affect mood without disrupting sleep or causing circadian arrhythmicity. All of these light effects are mediated by ipRGCs [Modified from REF. (2)]

4. Perturbing NIF functions by light and consequences for health

4.1. Physiological alterations: chronodisruption

4.1.1. Light alterations and schedule changes

Light during the day or light at night affects different on human physiology and behaviour, especially, blue light. Indeed, some disorders are due to light stimulating melanopsin at 'wrong times' during the circadian cycle (4,6). The artificial lighting has enabled us to use more hours of the day, but it has initiated the restructuring of the workday and the shift away from activity in alignment with the solar day. Although this has been thought to increase productivity, it comes at a considerable cost, which is irregular night time light exposure. Furthermore, the impact that light has on physiological functions makes irregular light exposure potentially detrimental to health and wellness. In fact, inadequate environmental light, and/or ganglion photoreception, can be implicated in the manifestation of health problems observed, for example, in shift workers. These range from cardiovascular disease to mood disorders, going through circadian disruption, insomnia, depression, systemic disorders and possibly early mortality (2,5). Irregular light exposure thus becomes an important risk factor for developing health problems.

This risk has the potential to be exacerbated in the presence of additional factors, such as chronic stress or genetic susceptibility factors. Indeed, several genetic factors have been implicated as the underlying cause of psychiatric diseases, and chronic stress is known to influence circadian rhythms and sleep, in addition to have deleterious effects on health. Hence, compounded with the presence of irregular light exposure, these factors could interact and lead to a higher incidence of depression and other neurological disorders that are prevalent in society. For instance, morning bright light shortens sleep duration, whilst evening light decreases sleep propensity. Therefore, prolonged nightlight exposure such as that obtained by artificial lighting can cause circadian arrhytmicity, disrupt sleep-wake cycles and increase depression-related behaviours and deficits in learning and memory (2,4,6). Some of these disorders also appear due to seasonal changes in day length, shift work and transmeridian travels.

4.1.1.1. Seasonal changes in day length

Sunlight's importance is highlighted by seasonal and weather-related neuro-psychological disorders that would not occur if indoor lighting were sufficient for all neurobiological needs. Sunrise occurring later during the winter months causes a delay

in circadian rhythmicity; this seems to lead to dissociation between sleep-wake cycles and other peripheral circadian rhythms that are more tightly coupled to the central circadian oscillator (2). Indeed, reduced sunlight exposure in sighted individuals can cause insomnia, free-running rhythms, extreme flattening of hormonal profiles, depression and cognitive difficulties, which are reversible with restoration of adequate sunshine. Therefore, environmental illumination is inversely correlated with insomnia and depression, both of which increase with aging (5).

Strong support for this delayed phase shift hypothesis lies in the antidepressant effects of morning exposure to bright light, which causes an advance in the phase of the clock. Despite that depression occurs secondarily to changes in sleep, it is also a direct manifestation of reduced light levels, or short photoperiods, owing to the shorter day length, as those experienced during winter months, that induce hippocampal learning deficits and depression-related behaviours, such as the seasonal affective disorder (SAD), a form of depression in which the onset of symptoms, like disabling depression, hypersomnolence and weight gain, is coincident with decreasing day length during autumn and winter months. The prevalence of SAD is greater in populations living at higher latitudes, where the seasonal changes in day length are more extreme. Furthermore, polymorphisms in the gene encoding melanopsin (Opn4) and decreased retinal sensitivity in the winter months is associated with SAD (2,5,8). Moreover, two major hypotheses have been put forth to explain this seasonal form of depression: alterations in the daily rhythms of melatonin release and circadian phase shift, both related to changes in the usually light detection.

Although phase delays have been observed in most patients with SAD, a minority of them show phase advances, indicating that they should respond better to evening light treatment. Indeed, some studies have shown that evening light treatment is as effective as an antidepressant as morning treatment. Thus, there are perhaps two groups of patients with SAD — phase advanced and phase delayed — that would benefit from light treatment at different times of the day (2,5).

Seasonal mood changes are not restricted to SAD. In fact, seasonal fluctuations in mood have been observed in many patients with bipolar disorder, characterized by a profound switch in mood between periods of mania and depression. Shifts to the depressive phase have been observed to begin in autumn as day length decreases and often persist throughout the winter. By March, when day length begins to increase in the northern hemisphere, manic episodes become more prevalent, a phenomenon nicknamed ‘March madness’. The explanation for these seasonal changes is similar to that suggested for SAD. Strong support for this explanation lies in bright light

treatments, which have therapeutic effects in patients with bipolar disorder who experience seasonal fluctuations. In addition, bright light treatment can have a mania-inducing effect, providing further support for the influence of light on mood (2).

Moreover, non-seasonal depression is also closely associated with reduced light exposure (5), thereby, in addition to be effective and useful in the treatment of SAD and bipolar disorder, bright blue-filtered light treatment, delivered in the morning via light boxes, has resulted in a reduction of depressive symptoms and improved sleep efficiency, in older adults suffering from non-seasonal major depressive disorder (23).

Finally, circadian rhythm disruption also negatively impacts reproduction by altering hormone profiles, reproductive timing and fertility. Unlike humans, this is common in animals, especially in many avian and other species which reproduce seasonally (17).

4.1.1.2. Shift work

As already mentioned, the advent of artificial lighting has enabled us to use more hours of the day and create disruptive schedules such as shift work. Under these conditions, individuals are exposed to excessive night time light and alter their sleep-wake schedule, which causes asynchrony between the circadian and sleep systems. The attempt to remain awake at night and sleep during the day can lead to sleep deprivation, because daytime sleep tends to be more fragmented. The risks associated with these disruptions include safety hazards and extensive health problems, which range from mood disorders to cancer (2).

Particular attention has been given to understanding the role of sleep deprivation in the genesis of these health problems. Indeed, sleep deprivation has been shown to result in cognitive function deficits, including negative effects on learning and memory, alertness and concentration. The sleep disruptions that precipitate an episode of depression have been well characterized. Interestingly, acute sleep deprivation has a robust antidepressant effect; however, the chronic sleep deprivation that is associated with shift work leads to mood disturbances (2,34).

Various lifestyle changes coincide with shift work and, rather than a reflection of poor decision-making, it may be that a maladapted circadian system is triggering behaviour and metabolic changes that culminated in the expression of fatigue or increased preference for fatty foods, thereby exacerbating the development of disease states that involve circadian components such as cancer, diabetes and coronary problems. Cognitively, shift workers exhibit increased rates of irritability, affective disorders and memory impairments. Under such adverse conditions, sleep-wake cycles become

disrupted and time-inappropriate cues such as night-time light exposure and eating create conflicting signals in the SCN and peripheral oscillators, or altered cellular mechanics (17).

On the one hand, night-time light exposure is associated with altered 5-HT processing in the SCN, which affects downstream structures related to cognition as well as hypothalamic nuclei that influence metabolism and peripheral circadian oscillators. In addition, light at night affects secretion and receptor density of melatonin, a potent antioxidant whose decreased efficacy is involved in the development of premature aging, heart disease or cancer. Moreover, working at night also results in night-time eating which is associated with a preference for high sugar and/or fat foods. In addition to the increased consumption of fats/sugars, lipid and glucose metabolism is altered resulting in increased adiposity and insulin insensitivity resulting in increased body mass index (BMI). Increased BMI leads to altered leptin/ghrelin signalling that feeds back to the hypothalamus, further altering metabolism (17).

In short, disrupted circadian rhythmicity could be affecting the whole body through both altered metabolic functions and subsequent behaviour modifications, producing a cycle characterized by altered cellular machinery, subsequent changes in diet choices and exercise, and ill health. When combined, various disease states begin to emerge such as diabetes, obesity, or cardiovascular disease (17).

4.1.1.3. Jet lag / Transmeridian travel

The ability to travel rapidly across time zones has the unfortunate consequence of desynchronizing the circadian system. Many individuals have experienced the general malaise associated with transmeridian travel, which is known as jet lag and includes poor mood and cognitive impairments (2). The involvement of glucocorticoids in resetting the phase of the circadian clock has highlighted their possible contribution to the mechanisms underlying the effects of jet lag (35). Indeed, altered cortisol rhythms have been observed subsequent to transmeridian travel (36). In addition, it was found that chronic transmeridian travel occurring at least once per week for 1 to 4 years resulted in cognitive deficits, which were possibly due to chronic circadian disruption and/or chronically increased cortisol levels (2). Moreover, a study showing that fluoxetine (Prozac) modulates levels of corticosterone to alleviate depression in mice maintained under irregular light schedules, further suggests that this mechanism could underlie the depression associated with jet lag (37).

4.1.2. Alterations in ipRGCs and light perception

As explained above, in humans, melanopsin expressing RGCs are responsible for mediating the effects of light on several behaviours, such as circadian rhythms, sleep, alertness and mood. This happens through complex interactions of several neurological pathways, so may exert influence on both maintaining health and the expression of disease (2,4,5).

These ipRGCs are susceptible to primary dysfunction, but, while these cells do not make a significant contribution to image formation, their loss leads to substantial impairment of non-image forming functions (15). For example, cell depletion, in conditions such as inherited or acquired optic neuropathies like glaucoma, or neurodegenerative conditions like Parkinson disease, have shown alterations in the retinal dopamine-mediated neurotransmission, also involved in transmission across the retinohypothalamic tract (RHT) (4,5).

Furthermore, ipRGCs are inherently photosensitive, with persistent responses when all rods and cones have been photobleached. They respond to light even when physically dissociated from the retina. But when they are subjects of disorders influencing light activation of retinal ganglion photoreceptors, usually secondary to common causes of light transmission reduction, such as ageing and cataract formation, it can result in circadian asynchrony and affects physiological function. In fact, melanopsin depletion at certain times not only could contribute to the development of insomnia, yet also could cause depression and cognitive decline, and affect other systems leading to gastrointestinal and cardiovascular diseases, diabetes and cancer (4,5), between others (Fig. 5).

Therefore, circadian rhythms influence metabolic cascades in numerous domains and links occur between several diseases and circadian rhythm disruption. Indeed, changes in metabolic functions in many disease states are related to the molecular machinery that underlies the circadian system; and abnormal circadian function is related to diseases with metabolic components. But in addition, circadian dysfunction can be elicited through environmental manipulations such as night light exposures, and not just innate malfunctioning of the clock system (17).

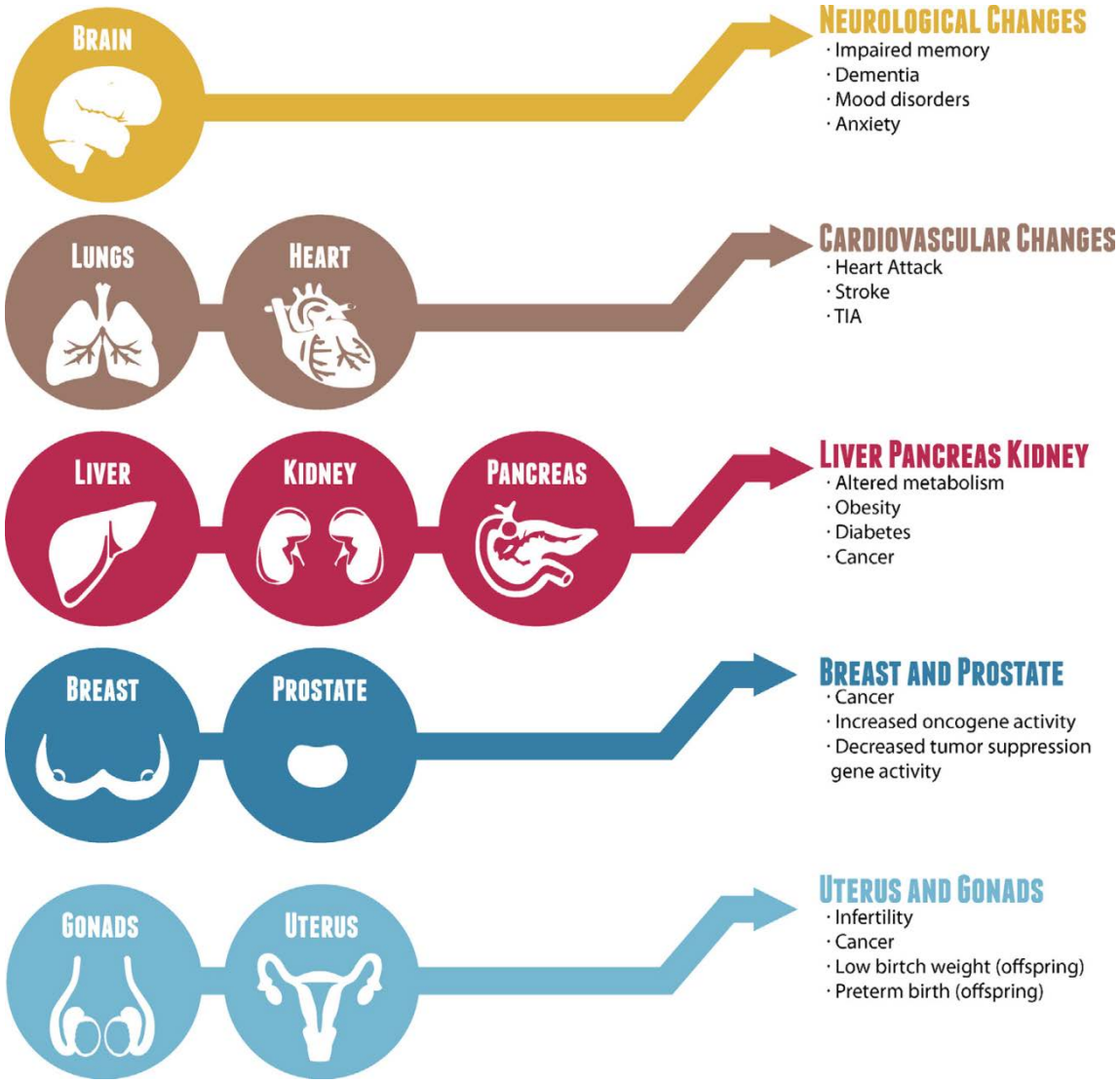

Fig. 5. Diagram with some examples of how circadian disruption negatively impacts the brain and the digestive, cardiovascular and reproductive systems. Though the diagram displays unidirectional affects, there are various feedback loops that exist within the system and interactions that occur between these systems [Modified from REF. (17)].

4.1.2.1. Parkinson disease

Parkinson disease (PD) is a neurodegenerative disorder with an incidence and prevalence increased with age. The original description of 'the shaking palsy' was focussed on the motor features of the disorder, but later studies revealed that non-motor aspects or additional characteristics including circadian rhythm, sleep and mood disturbances, cognitive decline, dementia, hyposmia and autonomic failure were also related to PD. Furthermore, visual symptoms are common and evidence exists of visual dysfunction at several levels of the visual pathway in this disorder; which includes physiological, electrophysiological and morphological evidence of disruption of retinal

structure and function, in addition to disorders of cortical visual processing. All these features affect sleep and general quality of life of people suffering from this disease.

Parkinson disease is due to the primary degeneration of nigrostriatal dopamine (NSD) pathways, which leads to a lack of dopamine, also involved in transmission across the RHT. So, dopamine replacement might additionally activate the circadian system and thus be used as a treatment for these diseases. Conversely, unopposed or excessive melatonin could contribute to exacerbating motor dysfunction, hypotension and body weight regulation, in addition to cause excessive daytime somnolence. Theoretically, suppressing melatonin production with bright light may have some therapeutic value as adjuvant therapy in PD, although the presence or absence of melatonin could affect differently depending on the characteristics of each patient (4,10).

4.1.2.2. Age-related disorders

Daily light exposures necessary for visual and non-visual photoreception depend on numerous intrinsic and extrinsic factors, and oscillations of various circadian parameters decline with age; these attenuations are observed in circulating levels of plasma cortisol and testosterone in aging men.

Moreover, visual function changes as we age, in part due to age-related diseases of the eye such as cataract formation, macular degeneration, senescent miosis, diabetic retinopathy and glaucoma. These age-related ophthalmological diseases contribute to deteriorate visual acuity, contrast sensitivity, colour vision and dark adaptation, in addition to central dysfunction due to visual cortex pathology, and co-existing cognitive decline (5,10,17).

In addition, ageing is accompanied by many changes within the eye physiology including alterations in pupil size, lens transmission and number of photoreceptors. It has been demonstrated that there is a reduction in the size of the S-cones and melanopsin-RGCs population with age that could diminish the photic signal transmitted to the brain. Alternatively, an increased density and yellowing of ocular lens, common in older people, diminishes the amount of short wavelength blue light reaching the photopigments involved in the non-visual photoreceptor system. This places them at risk for retinal ganglion photoreception deficiency, possibly contributing to age-related insomnia, depression and cognitive decline, thus increasing neurobiological morbidity (4,5,7).

The most notable effect of aging could be its association with increased disturbances in the timing, duration, and quality of sleep, including problems falling asleep, night

awakenings, waking too early and daytime sleepiness, which may reflect changes in the circadian timing system and/or the sleep homeostat. Alterations in this balance can have a substantial impact on their quality of life and daytime functioning. So, proper alignment between the circadian and homeostatic mechanisms is necessary to produce consolidated sleep at appropriate time (5,7).

Further, circadian deficits have long been a hallmark of demented states. Formerly referred as nocturnal delirium, sun-downing, characterized by increased levels of agitation and disorientation at particular times of day, is one of the earliest observed cognitive phenomena related to circadian function. But the age-associated decline in brain function is not restricted to the SCN, these changes are linked to alterations in oscillating levels of melatonin, 5HT, SCN morphology and synchronicity with extra-SCN CG activity in dementia, a heterogeneous group of disorders that can be associated with vascular failure, cell death or the accumulation of plaques, in addition to frequent night-time awakenings characteristics of the sleep profiles of aged individuals (17).

All these problems also could arise from changes in the light input pathway, which synchronizes the circadian clock to the local LD cycle. In fact, clock genes involved in the molecular mechanism that drives circadian oscillations in all cells of the body can have their expression levels modified by exposure to light.

As mentioned above, circadian rhythm disturbances parallel the increased prevalence of sleep disorders in older adults, especially in those with cognitive impairment. Therefore, therapies, especially non-pharmacological treatments, such as light therapies specifically targeting the regulation of the circadian system could be used to alleviate many sleep disorders in elderly. Current recommendations for these light treatments require the patients to sit in front of a bright light box for at least 1 hour daily, which perhaps limits their willingness to comply (23).

Despite this, new methods, like the use of a mask delivering light pulses for stimulating the human circadian system through closed eyelids during sleep, have been studied and not only are efficacious for significantly nocturnal melatonin suppression and of phase delaying dim light melatonin onset (DLMO) in older adults, but also lead to better compliance, because patients receive light treatment while sleeping in a home setting. This method offers great promise for clinical studies aimed at correcting circadian misalignment, because light applied close to the minimum core body temperature (CBT_{min}) maximally shift the timing of the circadian pacemaker, that is, retinal light exposure just prior to CBT_{min} maximally delay the timing of the biological clock, while light exposure just after CBT_{min} maximally advance circadian phase (14,23).

Returning to the clock genes involved in the molecular mechanism that drives circadian oscillations in all cells of the body, specifically in humans it has been demonstrated that blue light (460 nm) is more effective than green light (550 nm) in influencing the expression of PER2 in oral mucosa cells of young individuals (25), but not in older men. In addition, the melatonin suppression and alerting data also demonstrate impairment in the ability of blue light to induce acute responses in older people (26) (Fig. 6).

Otherwise, blue light is able to shift the timing of clock gene expression in peripheral oscillators; this has been demonstrated using PER3 expression in the peripheral leukocyte clock as a molecular marker. Older people often experience an advance in the time that they fall asleep and wake up, and there is also an alteration in the phase relationship between the sleep/wake cycle and both the plasma melatonin and core body temperature rhythms. The timing of the PER3 peak was significantly advanced following blue but not green light in both the younger and older groups, similar to the melatonin rhythm. So, the phase advancing response to blue light is sustained in older individuals (7,27) (Fig. 6).

In summary, these studies have demonstrated that acute responses to blue light (melatonin suppression, increased alertness, increased PER2 expression) are impaired in older individuals whereas the phase advancing effects of blue light (plasma melatonin rhythm, PER3 expression rhythm in leukocytes) are sustained. These differential effects may reflect differences in the detection or processing of the photic signal or be a function of the time-course of the response (Fig. 6) (7).

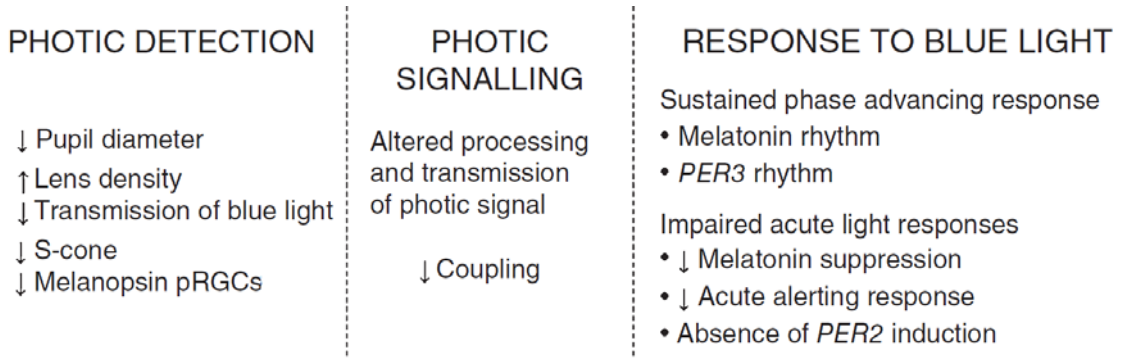

Fig. 6. Summary of age-related changes in ocular physiology and non-visual responses to blue light [Modified from REF. (7)].

The restoring of photoentrainment and neurobiological response might be achieved by lens replacement with a prosthetic intraocular lens (IOL) implant with blue light spectral transmission characteristics, or cataract surgery, which provides older adults with more youthful circadian photoreception. Indeed, patients undergoing IOL and cataract surgery show decreased incidence of insomnia and daytime sleepiness (4,5).

In general, in the reduction of ipRGC functions with aging, environmental lighting might also be modulated to simulate timed exposure levels of more youthful circadian photoreception and in part, reverse the chronic sleep disturbance, depression and cognitive dysfunction among older individuals. Then, another method of improving sleep/activity rhythms in older people may be to boost the amplitude of the circadian clock by strengthening the LD cycle. This can be done by making sure that the light period is as bright as possible and the dark period as dark as possible to provide the best relative contrast and a clear signal to the clock (4,7).

Moreover, a clear link has been established between circadian disruption and dementia and evidence is beginning to accumulate indicating melatonin administration in conjunction with exercise may be neuroprotective, at least in animal models (28). And another link between circadian disruption and cognitive health has also been described whereby circadian therapies (e.g., bright light therapy) may reduce the severity of sun-downing (17,29).

4.1.2.3. Obesity and diabetes

There is considerable epidemiological evidence for a relationship between abnormal circadian rhythms and metabolic changes related to obesity and diabetes in humans. Increased adiposity and altered glucose metabolism create a positive feedback loop that results in more profound adiposity (i.e., obesity) and poorer glucose metabolism that can culminate in diabetes or untimely death. This is due to the extensive connectivity between SCN and various hypothalamic nuclei associated with metabolism that regulate pancreatic, hepatic and adipocyte functions (17,18). Indeed, metabolic demand, such as lipid concentrations in plasma and the accessibility of fat stores, varies across the circadian cycle. Accordingly, as such, organisms have evolved to regulate energy metabolism across their circadian cycle (17,30).

In addition to metabolism, circadian disruption also influences food related behaviours, which is constrained by a complex series of feedback loops that involve several molecules (e.g., ghrelin and leptin). These cascades are one example of endogenous peripheral oscillators that provide signals to the SCN about time of day. However, this feedback is reciprocal, for instance, in addition to circadian disruption possibly contributing to the development of diabetes; diabetes might also disrupt circadian rhythms associated with metabolism (17,22).

Furthermore, circadian disruption also affects gene expression and molecular concentrations of CGs in liver, adipose tissue and muscle, leading to altered experiences of hunger, satiety, restlessness or food preferences. The aberrant CG

expression induced by an episode of circadian disruption, or as a consequence of CG mutations, results in altered glucose/insulin metabolism, hepatic responses and feeding patterns, which could underlie an improper diet and poor food choice. For instance, individuals employed on rotating shift work schedules have higher body mass index (BMI) scores, this happens because of alterations in diet preferences in shift workers, who tend to eat more meals/snacks, or consume carbohydrates and added fat foods. Even sleep deprivation is also associated with alterations to the propensity of humans to overeat (17).

Despite this, in humans, even when food administration is controlled, the highest concentrations of most lipid products peak around midday, which demonstrates that the circadian component of this loop acts independently of sleep, feeding, and fasting schedules (17,30).

4.1.2.4. Cancer

The link between circadian function and cancer is complex. The concentrations of various molecules related to cell proliferation, differentiation, and apoptosis cycle across the circadian period, as do their ability to affect various biological systems (e.g., SCN), are altered in various cancer states. Indeed, disrupted patterns of the underlying molecular oscillations, in addition to sleep disturbances, are observed in cancer patients and are associated with decreased survival rates. In short, cancer's molecular machinery takes advantage of disrupted circadian rhythmicity and then hijacks molecular cascades that result in further disruption the circadian system.

Epidemiological and experimental association between cancer survival and prevalence rates and circadian disruption have both been reported. Circadian disruption is often affected in disease states, but exogenous inputs in our lifestyle choices, such as frequent transmeridian travel, can also affect the system. Shift work is another example of a circadian disrupted lifestyle, indeed, considerable evidence linking shift work to increased prevalence of cancer lead the International Agency for Research on Cancer to classify circadian disruption shift work as a probable human carcinogen; this classification highlights the role of the circadian system on cancer (17).

The link between circadian disruption and cancer has been noted for a range of tissue types (e.g., breast, prostate, pancreas, skin) and various time of day affects in oncogene activity have been observed. Breast cancer alterations may be the result of interactions between circadian gene expression and androgens, but there are likely other systems affected; for instance, a functional CLOCK protein has been implicated with breast carcinogenesis without circadian disruption (17,31).

Another potential mechanism is an alteration in circulating levels of melatonin associated with night-time light exposure and activity in individuals employed on shift work schedules, in fact, light exposure during the dark phase of the circadian cycle is associated with decreased melatonin expression. Furthermore, subjects who do not sleep during the peak hours of melatonin expression have the highest increased incidence of breast cancer; this may occur because melatonin is reported to eliminate nitric oxide and other reactive oxygen species. Even colorectal and prostate cancer tumorigenesis and survival are also associated with sleep/activity cycles (17,32).

4.1.2.5. Cardiovascular consequences

The incidence and severity of cardiovascular events varies across the circadian cycle, indeed, they are more often fatal if they occur early in the morning. The link between circadian rhythms and cardiovascular function is further supported by the examination of heart and renal function in mutant hamsters. The observation of pathology in heart and kidney tissue indicates that the effects of circadian disruption on organ health are pervasive. Furthermore, lesions of SCN ameliorate these changes indicating that it is not circadian rhythm disruption per se that elicits the ill effects, but the desynchronization between central and peripheral oscillators that elicit cardiovascular and renal effects. Despite this, these effects do not appear to be mediated by circadian dysregulation alone, but rather are the product of a lack of coherence between the individual's innate circadian timing system and the world around them (17,33).

VII. CONCLUSIONS

The discovery of the ipRGCs and the role of melanopsin in circadian pathways have contributed to our understanding of light and circadian biology. Light is not only necessary for vision, but also is essential for circadian photoentrainment, sleep and mood regulation, cognition and health.

Circadian function, cognition and health exist as a complex reciprocal network in which all components exhibit multidirectional effects on the other systems. Under healthful conditions, these interactions create a robust, dynamic circuit that allows the organism to adapt well to its environment. But when disease strikes a component of the system, the effects propagate cascade and can lead to a decline in the function of all systems.

The misalignment of these systems can perpetuate disease states that can culminate in damage. For instance, alterations in ipRGCs can result in sleep and mood disorders directly associated with light perception, or secondarily to circadian disruption. In turn, circadian rhythm disturbances, insomnia and depression are significant risk factors for developing some diseases such as obesity, diabetes, cancer, cognitive deficiencies, dementia and cardiovascular diseases, even, premature mortality.

It is important to identify the mechanisms and effects within the system from a genetic level to an epidemiological one; because only with complete understanding will be possible to mitigate the effects of abnormal circadian function on health.

The reliance of the circadian system on environmental inputs makes it possible to manipulate circadian rhythms by altering the timing of relevant events without physical manipulation of the brain, which reduces the surgery-associated risks. All circadian rhythm disturbances are at least partially, if not fully, amenable to planned sleep schedules, timed light exposure and exogenous daily melatonin administration.

Focusing on the effects of light on the body, appropriately timed blue-enriched bright light, such as outdoor daylight, is really useful for photoentrainment and effective in decreasing sleepiness and enhancing performance and cognitive tasks in humans. In addition, blue light is efficient in ipRGC-mediated melatonin suppression, so it could be used as adjuvant therapy in Parkinson disease; it is the treatment of choice of the seasonal affective disorder (SAD) and it is also emerging as a promising treatment for many other psychiatric disorders.

Broadband polychromatic light sources are used to ensure optimal stimulation of the visual system, without concerning the optimizing of light for the non visual system; artificial light sources of indoor environments deprive us of the blue light we need.

In addition, ocular pathology and the resultant reduction in retinal phototransduction lead to circadian disturbances and sleep disorders, with downstream effects on our overall physiological integrity.

Thus patient lifestyle education and more effective architectural designs for lighting environments like schools and work could be potentially beneficial for the population.

As final conclusion, we could say that the knowledge of non-visual effects of light on the body, both at biochemical and physiological levels, allows pharmacists to give advice to patients about the most appropriate behaviour patterns to follow regarding light exposure and nutrition, to get good health and quality of life.

VIII. PERSONAL REFLECTIONS

First, it should be clear that the temporal dynamics of the human circadian system is more poorly understood than its spectral and absolute sensitivities; therefore, more basic research is needed to elucidate the temporal characteristics of human circadian phototransduction.

However, some recommendations could be given to improve the lifestyle of the population in general, such as avoid exposure to permanent dim light, or to bright light at certain times of the day, especially at night; and more specifically to teenagers and college students, who spend many hours at the computer, which can alter their circadian rhythms, mood, and sleep schedules.

Moreover, it would be interesting that this information could reach designers and/or specialists in lighting in schools and workplaces, especially those designed to hold people inside 24 hours a day (e.g., night shift workers or hospitalized patients). This would allow them to make architectural designs more appropriate to people's needs and thus avoid the onset of disorders related to exposure to light at wrong times.

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