Invited Review

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Circadian mechanisms in the regulation of melatonin synthesis: disruption with light at night and the pathophysiological consequences

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Abstract

In the past two decades, the results of a number of epidemiological studies have uncovered an association between excessive light exposure at night and the prevalence of cancer. Whereas the evidence supporting this link is strongest between nighttime light and female breast and male prostate cancer, the frequency of other tumor types may also be elevated. Individuals who have the highest reported increase in cancer are chronic night shift workers and flight attendants who routinely fly across numerous time zones.

There are at least two obvious physiological consequences of nighttime light exposure, i.e., a reduction in circulating melatonin levels and disruption of the circadian system (chronodisruption). Both these perturbations in experimental animals aggravate tumor growth. Melatonin has a long investigative history in terms of its ability to stymie the growth of many tumor types. Likewise, in the last decade chronodisruption has been unequivocally linked to a variety of abnormal metabolic conditions including excessive tumor growth.

This brief review summarizes the processes by which light after darkness onset impedes melatonin production and disturbs circadian rhythms. The survey also reviews the evidence associating the ostensible danger of excessive nighttime light pollution to cancer risk. If an elevated tumor frequency is definitively proven to be a consequence of light at night and/or chronodisruption, it seems likely that cancer will not be the exclusive pathophysiological change associated with the rampant light pollution characteristic of modern societies.

Key words:
Biological clock; Cancer; Circadian rhythm; Light pollution; Melatonin

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Introduction

A biological clock exists in the brain of all mammals including man. This clock, referred to as the suprachiasmatic nuclei (SCN), provides circadian information to all cells in the body thereby allowing animals to adjust their physiology according to the time of the day [1, 2]. This endogenous timing mechanism ensures that the appropriate cellular physiology and overt behavior is synchronized with the external environment including the light:dark cycle, changes in ambient temperature and even food ingestion. This system of precise timing is required for the optimal function of organisms and disturbances of these regular fluctuations lead to improper physiological responses and, in the worst cases, to pathologies [3, 4].

The SCN, also known as the master pacemaker, is represented in the brain by a pair of small nuclei (roughly 20,000 neurons) in the anterior hypothalamus immediately above the optic chiasm [5]. This strategic location allows the neurons in these nuclei to readily receive electrical messages from the retinas via a specialized group of axons (the retinohypothalamic tract) embedded in the optic nerve [6]. The neurons in the SCN exhibit an intrinsic circadian rhythm which is not precisely 24 hours in duration; in most cases this endogenous cycle is closer to 25 hours [7]. Thus, under conditions of constant darkness the cycles of the SCN are free running. It is a function of the prevailing light:dark cycle to synchronize the activity of the SCN to a 24-hour rhythm. Since the master oscillator subsequently conveys, via neural and humoral means, this circadian information to peripheral clocks in individual cells throughout the body [8], cells and organs with no apparent connection to the external photoperiodic

environment can respond to changes in the light:dark cycle.

The environmental factors that cause misalignment of internal physiology with external environment are referred to circadian disruptors [9]. The consequences of disturbances of the biological clock and the down stream cellular/organ physiology are classified as circadian disruption or chronodisruption [10]. The resulting physiological turmoil is manifested as unusual cellular physiology which can, on occasion, be physiologically experienced by the intact organism, e.g., jet lag [11].

The most obvious and perhaps most potent entrainer of the activity of the master clock is light. In mammals, light detection is exclusively a function of the retinas [12]. While this is virtually universally accepted, there was a report more than a decade ago claiming that, in humans, light perception may be achieved by extraocular structures [13]. This finding, however, has never been confirmed and is disavowed by the vast majority of scientists working in this field [14].

A major recipient of light:dark information relayed to the organism by the SCN is the pineal gland [15]. The pineal, an outgrowth of the posterior dorsal diencephalon, is highly metabolically cyclic and produces its humoral product, melatonin, almost exclusively at night in darkness [16]. Since melatonin after its synthesis is quickly released into the blood and cerebrospinal fluid (CSF), it is a major means by which cells receive information about the photoperiodic state. Given that melatonin, under normal conditions, is exclusively elevated in bodily fluids at night, it is referred to as the chemical expression of darkness [17].

Retinas and the circadian System

The point has already been made that the retinohypothalamic tract (RHT) transfers electrical signals from the eyes to the biological clock, the SCN. Classically, the only known receptors capable of light perception in the retina were the rods and cones. Recently, however, a third photoreceptor has been identified in the retinas; these are the intrinsically photosensitive retinal ganglion cells (*ip*RGCs) [18]. The function of the *ip*RGCs is to perceive light that cues the circadian system via the SCN. The axons of the *ip*RGCs project directly or indirectly, via the RHT and other pathways, to the SCN. Conversely, the rods and cones subserve vision and have no direct association with the biological clock. Thus, the retinas of mammals have a dual visual system. These are referred to as "visual vision" (or conscious vision), mediated by classical retinal rods and cones, and "circadian vision" (or unconscious vision) mediated by *ip*RGCs [19]. The latter is also identified as non-image forming vision.

While circadian vision relies on the *ip*RGCs, only 1-2% of all ganglion cells are actually capable of responding to light because of the presence of a specialized photopigment, melanopsin, that they contain [20, 21]. This highly specialized and cell-limited photopigment is also highly unique in that it is sensitive to only a restricted portion of the usual visible electromagnetic spectrum. Thus, melanopsin responds primarily to wavelengths in the range of 460-480 nm (blue light) [22, 23]. Wavelengths outside of this range, although they influence the rods and cones, are minimally or non-functional in terms of the *ip*RGGs. Thus, only a limited portion of the visual electromagnetic spectrum is capable of determining the function of the circadian visual system, i.e., regulating the biological clock and endogenous circadian rhythms.

To illustrate the selective independence of the *ip*RGCs from the remainder of the retina, loss of the retinal rods and cones, e.g., due to their degeneration, minimally alters the ability of light to modulate the SCN and circadian physiology [24] despite the fact that the quantity of melanopsin is dramatically reduced [25]. Hence, an individual can be visually blind (due to loss of the rods and cones) while circadian vision remains intact and functional. Interestingly, however, in melanopsin knock-out mice, the effects of light on the circadian system, based on behavioral parameters, although reduced are not completely lost [26].

The RHT, axons specifically derived from the *ip*RGCs, project to the SCN and thereby adjust the circadian activity of the master oscillator. The neurotransmitters in the SCN as well as the molecular mechanism regulating the circadian machinery are, in part, identified [27]. The terminals of *ip*RGCs in the SCN are not uniformly distributed within these nuclei with the organization exhibiting a remarkable degree of heterogeneity and complexity.

The neural projections from the SCN are highly complex with axons of clock neurons terminating in many areas of the diencephalon. In specific relation to the current review, those axons projecting to the paraventricular nuclei (PVN) of the anterior hypothalamus are of particular interest, given that they are relay nuclei for the photic information being transferred to the pineal gland and

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determining cyclic melatonin production. The PVN axons project down the brain stem via a non-descript pathway and eventually terminate on preganglionic sympathetic neurons of the intermediolateral cell column of the upper one or two thoracic segments of the spinal cord [28]. This long descending neural pathway is necessary to link the visual system and the SCN to the peripheral sympathetic outflow of the spinal cord since organs outside the central nervous system that are influenced by the sympathetic division of the autonomic nervous system must receive information from the intermediolateral cell column which is exclusively located in the thoracic and upper lumbar spinal cord. The axons of the preganglionic neurons located in the intermediolateral cell column exist the thoracic cord in the ventral root, ascend in the sympathetic trunk and synapse on postganglionic sympathetic nerve cells in the superior cervical ganglia (SCG). These ganglia are located at the division of the common carotid into the internal and external carotid arteries. Axons of SCG neurons then accompany the internal carotid arteries and their branches to the tentorium cerebelli where they form rather discrete bundles, the nervi conarii [29], which penetrate the pineal gland and terminate on pinealocytes, the functional endocrine units of the gland [30]. Surgical removal of the SCG bilaterally sympathetically denervates the pineal gland and renders it incapable of producing melatonin in response to darkness [31] and non-functional in terms of its endocrine actions [32].

Photoperiodic regulation of the melatonin rhythm

In all mammals, the daily dark period is associated with a rise in pineal melatonin synthesis (Fig.1). Once produced, melatonin is quickly released from pinealocytes such that the nocturnal rise in circulating melatonin concentrations is a reflection of its degree of synthesis within the pineal at essentially the same time [33]. This regularly recurring melatonin rhythm provides essential information regarding the prevailing light:dark environment to all cells in the body that can "read" the melatonin signal. Thus, the day:night melatonin rhythm provides "clock" information [34]. Additionally, however, changing seasonal daylengths alter the duration of nocturnal circulating melatonin levels and, as a result, melatonin also provides "calendar", i.e., seasonal, information [35]. Indeed, it is now well known that the seasonally-adjusted melatonin cycle drives annual changes of reproductive capability in both long day and short day breeding photoperiod-sensitive mammals [34-36]. In non-photoperiodic species such as humans, the melatonin message primarily functions to provide circadian information and is less important in reference to influencing potential annual fluctuations in physiology.

A major discovery, which influenced circadian biology and pineal melatonin production, occurred in 1879. In that year, Thomas Alva Edison invented the light bulb which markedly changed working, sleeping and eating behavior of humans. Since then, light at night has become common place in all developed societies. Light after darkness onset, sometimes referred to as light pollution, can have dramatic effects on circadian rhythms including the melatonin cycle [37]. In all mammals investigated, appropriately-bright light exposure at night suppresses pineal melatonin production and circulating levels of the indoleamine [38]. There seem to be, however, great differences in the ability of light to suppress melatonin among different species. In general, it has been suggested that nocturnally-active species are more sensitive to light at night, in terms of melatonin suppression, than are diurnally-active animals [39, 40]. If this is verified, it implies that at some level in the pathway between the *ip*RGCs and the pineal gland, the signal being transferred is capable of being muted. In humans, eye color and other orbital parameters do not significantly impact the melatonin response to light at night [41].

Light at night has two major effects on the endogenous melatonin rhythm. Thus, the extension of light into the dark phase or light exposure early in the morning truncates the melatonin rhythm at one or both ends and reduces the total amount of melatonin produced per 24-hour period [42]. Similarly, acute exposure to light during the night quickly suppresses pineal melatonin synthesis as evidenced by the rapid drop in circulating melatonin levels [43]. The duration of acute light exposure required to reduce melatonin levels may be very short, i.e., seconds, based on currently available data. In any case, as with extension of the light period into the night, acute light exposure at night reduces the total quantity of melatonin generated over a given 24-hour period. In addition to limiting the amount of melatonin produced, light at night provides misinformation to the biological clock which contributes to chronodisruption. The consequences of limiting the amount of melatonin produced and disrupting the circadian system along

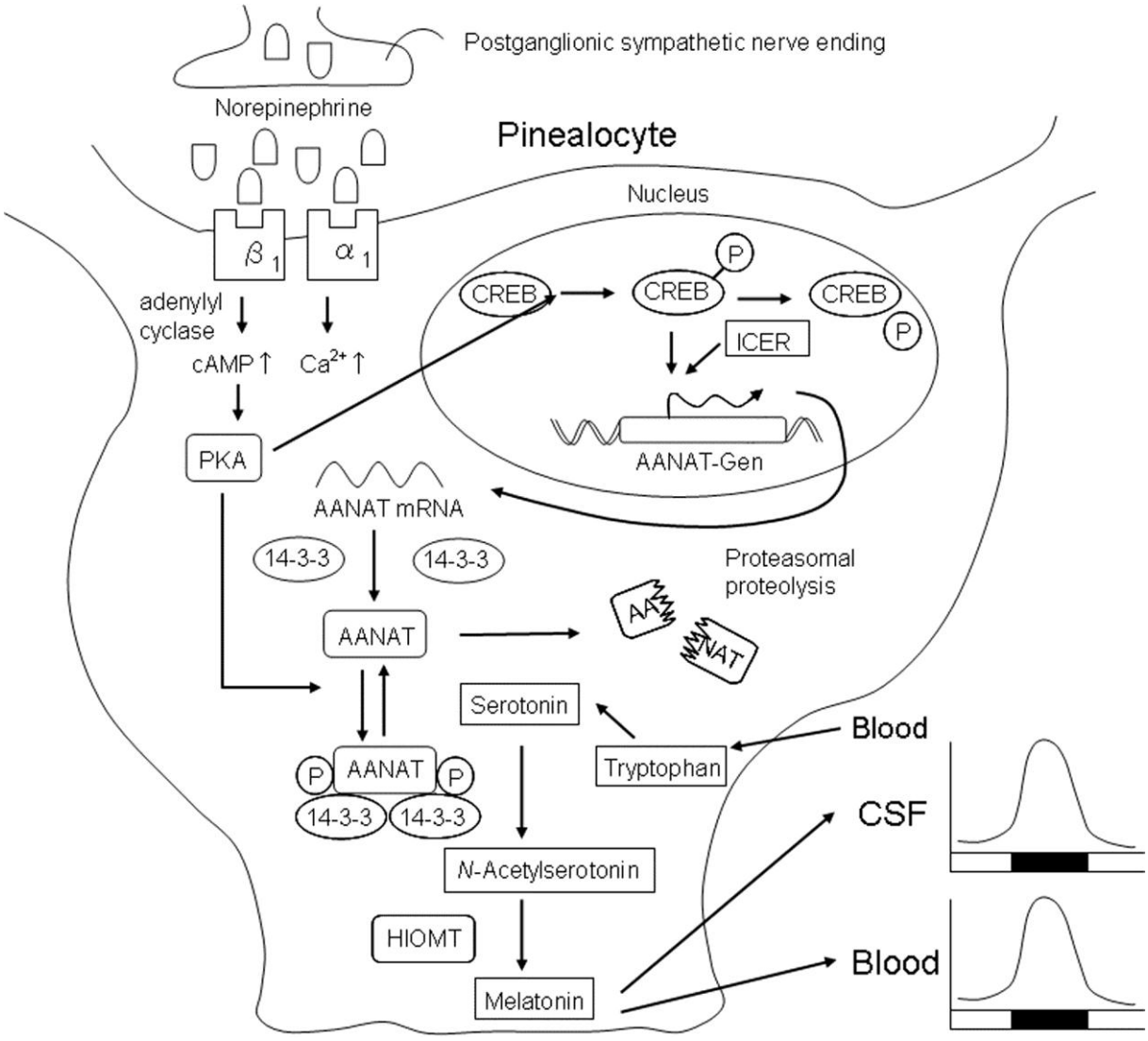

Figure 1. Postganglionic sympathetic fibers, whose cell bodies are in the superior cervical ganglia, release norepinephrine onto the pinealocytes, the functional units of the pineal gland. Norepinephrine is released exclusively at night due to a neural message originating in the biological clock, the suprachiasmatic nuclei. After its release, norepinephrine acts on β_1 and α_1 receptors on the pinealocyte membrane. These actions result in rises in intracellular cyclic AMP (cAMP) levels and Ca^{2+}, thereby stimulating protein kinase A (PKA) which leads to a promotion of the activity of alkylamine-N-acetyltransferase (AANAT), the enzyme which converts serotonin to *N*-acetylserotonin, the immediate precursor of melatonin. *N*-acetylserotonin is converted to *N*-acetyl-5-methoxytryptamine (melatonin) by the activity of hydroxyindole-O-methyltransferase (HIOMT). Once produced, melatonin is quickly released from the pineal gland into the blood and likely also into the cerebrospinal fluid (CSF). Since pineal melatonin is synthesized exclusively at night, both blood and CSF concentrations also rise during the night. This circadian message is essential for the normal physiology of organisms and the disruption of this cyclic signal contributes to pathophysiology. CREB = calcium/cAMP response element binding protein; ICER = inducible cAMP early repressor; P = phosphate.

with other consequences, e.g., sleep deprivation, may be much greater than originally envisioned (see below).

Due to the world wide use of artificial light at night, the amount of true darkness humans, especially those living in urban environments, witness is seriously compromised. Indeed, the dark nights that were common for our ancestors are disappearing. Whereas under carefully controlled conditions in the laboratory setting, light shown directly into the eyes of humans, if sufficiently bright and of the proper wavelength [22], readily

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suppresses endogenous pineal and blood melatonin levels. Whether a similar inhibition of melatonin occurs in a light-polluted night in the urban environment where the radiant energy is not precisely directed into the eyes has not been tested. Thus, whether individuals living in a city and remaining active at night have severely compromised melatonin production remains unknown although this is frequency surmised based on the pathophysiological consequences these individuals experience [44].

A second situation in which the melatonin rhythm is significantly altered and circadian mechanisms disturbed occurs during rapid transmeridian travel over numerous time zones. The resulting phenomenon is referred to as jet lag and is generally worse when individuals are traveling in an easterly direction [45]. This requires the clock to phase advance, a manipulation that is more difficult to achieve than is a phase delay of the clock which occurs when individuals travel westerly. Regardless of the direction of travel, the biological clock and the melatonin rhythm re-adjust and come into synchrony with the prevailing photoperiodic regimen after a period of time determined by the number of time zones crossed.

Negative consequences of light at night

The last two decades has witnessed a sharp rise in the number of studies examining the potential pathophysiological effects of being routinely exposed to light during the normal dark period and/or frequent transmeridian trips across numerous time zones. Epidemiologists were the first to report that cancer incidence was exaggerated in airline hostesses [46] and in women who worked night shifts for prolonged periods [47]. The majority of these observations claimed an elevated incidence of breast cancer in women who commonly disturbed their circadian/melatonin cycles; these findings have been summarized in a number of thorough reviews within the last decade [48-51]. The outcomes of subsequent studies claimed that not only breast cancer in females but likewise prostate cancer was also exaggerated in males experiencing abnormal or irregular photoperiods which curtailed the total amount of melatonin produced and/or frequently caused unusual circadian rhythms [52, 53]. The prevalence of other cancer types, e.g., colorectal cancer, has also been reported to be increased in individuals who live in metropolitan areas where light exposure at night is common. Recently, it was proposed that circadian disruption and melatonin suppression may be associated with a generalized elevation of all cancer subtypes [54]. Indeed, the IARC (International Agency for Cancer Research) of the World Health Organization has classified shift work that involves circadian disruption as a Group 2A carcinogen, i.e., possibly carcinogenic [55]. Despite the substantial body of evidence linking chronodisruption to a higher prevalence of cancer, there are some who feel the hypothesis of light at night, chronodisruption and melatonin suppression as contributing to cancer risk may be an over simplification of what is actually happening [56].

Experimental evidence is compelling that melatonin is an endogenously-produced oncostatic agent [57-61] and as a consequence, its frequent suppression at night as a result of any means including excessive illumination, may increase the possibility of cancer initiation and/or exaggerate the rate of growth of established tumors in humans. A variety of mechanisms have been described by which a reduction in melatonin levels may stimulate tumor growth [62-70]. Moreover, melatonin may also reduce the likelihood of tumor metastases [67] because of its ability to modulate the cellular cytoskeleton [71].

That light suppression of human nocturnal melatonin levels may, in fact, be consequential in supporting the proliferation of cancer cells and tumor growth is suggested by the outcomes of a number of experimental studies. Dauchy and co-workers [72] reported, for example, that when rats bearing Morris hepatoma cells were exposed to a 12:12 light:dark cycle but where the period of darkness was contaminated with low light intensities (to reduce nocturnal melatonin levels) the tumors grew progressively more rapidly as the intensity of the contamination light was increased. Furthermore, the night time melatonin levels were inversely related to the brightness of the light that was causing the contamination. Similar results were obtained in female rats bearing DMBA-induced mammary adenocarcinomas [73]. When these animals were exposed to light at night, especially those under constant dim light during the dark phase, showed, *a)* significantly higher rates of tumor growth and shorter survivals than controls, *b)* higher levels of serum estradiol, and *c)* lower nocturnal exertion of 6-sulfatoxymelatonin with no difference in daytime and nighttime levels.

This was followed by an even more convincing report where human breast cancer xenografts, growing in immunocompromised rats, were strongly inhibited when they were perfused with nighttime blood (containing elevated endogenously-

produced melatonin levels) collected from premenopausal women [74]. When this same group of women was exposed to light at night, a procedure that partially depleted the circulating melatonin levels, and their blood was perfused into the xenografts, it failed to inhibit tumor cell proliferation or any other aspect of cancer cell metabolism. Collectively, the studies of Dauchy *et al* [71], Cos *et al* [73] and Blask and colleagues [74] are consistent with nighttime physiological concentrations of melatonin being sufficient to inhibit tumor growth and that depleting the blood of this oncostatic agent allows the tumor cells to grow at a more rapid rate.

Relative to the role of chronodisruption in contributing to accelerated cancer growth, the studies of Filipski *et al* [75, 76] are highly germane. In these investigations, mice were inoculated subcutaneously with Glasgow osteosarcoma cells and subsequently they were maintained under a stable light:dark cycle of 12:12 or they were kept under a 12:12 cycle that was phase advanced every two days (simulating an eastward transmeridian flight). The repeated phase advances were performed to induce desynchronization of the animals' circadian rhythms (a simulated jet lag). The subcutaneously-growing tumors were regularly monitored. The results documented that tumor progression in the repeatedly phase-advanced animals was accelerated over that in the cancers growing in mice kept in a stable 12:12 light:dark environment. At the conclusion of the study when the tumors were evaluated, the circadian rhythms of their clock genes were obviously altered compared to these cycles in tumors from mice that had been kept in a stable 12:12 cycle. While these findings were interpreted to mean that chronodisruption allows tumors to exhibit an elevated growth rate, it is likely that the melatonin rhythm was also severely disturbed and depressed by the repeated phase advances and, similarly, the sleep:wake cycles of the mice in the repeatedly changing environment were very likely disrupted. These disturbances may also have contributed to the more rapid tumor progression in the jet-lagged animals.

Perhaps the most suggestive evidence documenting the metabolic consequences of frequent eastwardly flights stems from the work of Davidson and colleagues [77]. They subjected aged C57BL/6 mice to chronic jet lag by phase advancing their photoperiodic cycle by 6 hours every 7 days; this treatment caused the mice to die prematurely when compared with mice in a stable photoperiodic environment. Of additional interest is that an equal phase delay every 7 days (as would occur in a westerly flight) was without impact on the survival of the animals. The conclusion is that rapid eastwardly travel across multiple time zones is more detrimental to metabolism than is an equivalent westward journey. This also supports the common observation that feeling of fatigue, disturbed sleep, etc., is more acute after eastward than after westward travel. This is consistent with data documenting that the biological clock can more easily accommodate and adjust to phase delays as opposed to phase advances.

In a recent brief review, we reminded the reader that more rapid tumor growth may only be a convenient endpoint which, in fact, reflects a variety of other metabolic effects of chronodisruption, melatonin suppression and sleep deprivation [78]. Thus, it could reasonably be anticipated that there would be other consequences of chronodisruption and/or melatonin suppression.

This assumption is certainly borne out by a variety of experimental findings. For example, signs of metabolic syndrome [79] and obesity [80] are reportedly more frequent in chronobiologically-disrupted animals. In the report by Turek *et al* [79], they found an association between circadian Clock disturbances and obesity and other metabolic signs in mice. They observed that Clock mutant mice developed hyperphagia and deposited excess abdominal fat. Likewise, Qin and colleagues [80] observed that chronic disturbances in circadian endocrine rhythms in humans were associated with an elevated incidence of obesity and diabetes. The reader is reminded that disturbances of circadian biology are virtually always accompanied by a reduction in the total amount of melatonin produced. Hence, the new discovery that brown adipose tissue (BAT) exists in adult humans [81, 82] has clear implications for the control of body mass. BAT burns large numbers of calories and reduces white fat deposits while melatonin, at least in experimental animals, strongly promotes BAT formation [83, 84]. This being the case, excessive light exposure which reduces endogenous melatonin availability may contribute to white fat deposition without a rise in caloric intake. Obviously if circadian disturbances and a relative melatonin deficiency do have a relationship to obesity, the implications for both type 1 and type 2 diabetes are also clear [85-89].

Ablation of the nocturnal pineal melatonin rise by surgical removal of the pineal gland causes a gradual and sustained rise in blood pressure in rats [90] while melatonin administration reverses

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pinealectomy-mediated hypertension [91]. In humans, nighttime systolic and diastolic blood pressures drop (these individuals are referred to as dippers) commensurate with the nighttime elevation of circulating melatonin [92, 93]. When the nocturnal melatonin increase is attenuated, the magnitude of the drop in blood pressure is reduced (these individuals are referred to as non-dippers) [94]. This seems to have obvious clinical consequences given that dippers typically have a longer survival than do non-dippers [95]. Hence, the nighttime suppression by excessive light pollution may also contribute to diseases of the cardiovascular system [96-98].

In children as well, marked alternations in their photoperiodic environment during the critical developmental period may account for, at least in part, some of the behavioral disturbances that these children experience. Attention deficit hyperactivity disorder and autism are reported to be partially ameliorated by melatonin treatment [99, 100]. This is possibly consistent with these individuals having a deficiency of endogenous melatonin, which could result from excessive light exposure as well. In adults also, a reduced production of melatonin may contribute to a variety of psychological disturbances since melatonin treatment has frequently been reported to improve mental health [101, 102].

Finally, because of the discovery that melatonin, as well as several of its metabolites, are potent free radical scavengers and antioxidants [103-109], a deficiency in melatonin due to frequent light exposure after darkness onset would be expected to increase the amount of oxidative stress people suffer. This is important since many conditions/diseases have, as part of their etiology, elevated oxidative damage [110-113]. Of particular interest are those diseases that involve excessive damage to mitochondria [114, 115].

Concluding remarks

Modern societies are using progressively more lighting during the night for purposes of recreation, work, security, etc., to the extent that in metropolitan areas darkness is essentially disappearing. Humans and other animals have a specialized visual system to detect alterations in the regular light:dark environment and to communicate that information to the biological clock, the suprachiasmatic nuclei. Over hundreds of thousands of years man and his progenitors were exposed to regularly-recurring light and darkness as determined by the rising and setting of the sun. These highly regular alternating periods of day and night were used to adjust organismal physiology over a 24-hour period.

With the advent of artificial light and the extension of the photoperiod into the night and, perhaps worse yet, the acute exposure to light at night, the biological clock receives misinformation and makes adjustments to physiology which are inappropriate for the time of day. When organisms are repeatedly exposed to these inappropriate periods of light, the accumulated time-inappropriate physiological adjustments should be expected to lead to pathologies.

To date, the scientific information in this field is incomplete, but progressively more researchers are becoming concerned about the potential pathophysiological consequences of light at the wrong time. Like most aspects of our environment, humans are clearly polluting the night with light. Only further research will clarify what light pollution means for human physiology; however, it would seem the effects will not turn out to be beneficial.

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