Metabolic and physiologic effects of acute exposure to artificial light at night

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METABOLIC AND PHYSIOLOGIC EFFECTS OF ACUTE EXPOSURE TO ARTIFICIAL LIGHT AT NIGHT.

ACADEMISCH PROEFSCHRIFT

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aan de Universiteit van Amsterdam

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prof. dr. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,
in het openbaar te verdedigen in de Agnietenkapel
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door Anayanci Masís Vargas
geboren te San José, Costa Rica

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Dit proefschrift is tot stand gekomen in het kader van het NeuroTime programma, een Erasmus Mundus Joint Doctorate, met als doel het behalen van een gezamenlijk doctoraat. Het proefschrift is voorbereid in: het Nederlands Herseninstituut en in het Academisch Medisch Centrum (AMC), Faculteit der Geneeskunde, van de Universiteit van Amsterdam; en in het Institut des Neurosciences Cellulaires et Intégratives van de Université de Strasbourg.

This thesis has been written within the framework of the NeuroTime program, an Erasmus Mundus Joint Doctorate, with the purpose of obtaining a joint doctorate degree. The thesis was prepared in: the Netherlands Institute for Neuroscience (NIN) and in the Academic Medical Centre (AMC), Faculty of Medicine at the University of Amsterdam; and in the Institut des Neurosciences Cellulaires et Intégratives of the Université de Strasbourg.

θυμέ, θύμ᾽ ἀμηχάνοισι κήδεσιν κυκώμενε,
ἄνα δέ, δυσμενέων δ᾽ ἀλέξευ προσβαλὼν ἐναντίον
στέρνον, ἐν δοκοῖσιν ἐχθρῶν πλησίον κατασταθείς
ἀσφαλέως· καὶ μήτε νικῶν ἀμφαδὴν ἀγάλλεο
μηδὲ νικηθεὶς ἐν οἴκωι καταπεσὼν ὀδύρεο.
ἀλλὰ χαρτοῖσίν τε χαῖρε καὶ κακοῖσιν ἀσχάλα
μὴ λίην· γίνωσκε δ᾽ οἷος ῥυσμὸς ἀνθρώπους ἔχει.

Ἀρχίλοχος

"My Soul, my Soul, all disturbed by sorrows inconsolable,
Bear up, hold out, meet front-on the many foes that rush on you
Now from this side and now that, enduring all such strife up close,
Never wavering; and should you win, don't openly exult,
Nor, defeated, throw yourself lamenting in a heap at home,
But delight in things that are delightful and, in hard times, grieve
Not too much – appreciate the rhythm that controls men's lives."

- Archilochus.

To all laboratory animals, the heroes of science.

Table of contents

Part I

Introduction and outline

Chapter 1

General introduction and scope of the thesis

General Introduction to circadian rhythms

The rotation of the Earth around its axis determines not only the duration of the day (i.e. 24 hours), but also the alternation between day and night. The exposure to these variations in illuminance (amounts of light, wavelengths, and contrasts) and temperature from the beginning of times has had an enormous impact on the evolution of most life forms on Earth. Amongst others resulting in the development of body clocks that regulate the daily fluctuations of numerous physiological and behavioral processes, which allow organisms to adapt their physiology to the environment with cycles of activity and inactivity. This internal clock oscillates with a period close to, but not exactly 24 hours. Hence the name circadian, from the Latin words circa "about" and dies "day". In order to synchronize that internal circadian clock to the external rhythm of the Earth's rotation, organisms use several environmental cues, such as light, temperature, locomotor activity, and food availability (1). Among those cues of time or *Zeitgebers* (German for time givers) (2), light is the most effective one to entrain vertebrate circadian rhythms.

Over the last 100 years, advances in aviation, industrialization, and urbanization of our society have been beneficial for the progress of most of the world population. Nonetheless, this has led to activities like crossing several time zones when traveling, shift-work, working indoors during the day with no natural illumination, work and recreational activities performed at night under artificial light, and overexposure to screen and light-emitting electronic devices around the clock. All these lead not only to changes in the patterns of light exposure, but also to vast changes in the daily rhythms of sleeping, activity, and eating behavior, which all are activities that can disturb an individual's physiology by disrupting their circadian rhythms. These disruptions of the circadian rhythm can also result in metabolic disorders, including obesity and type 2 diabetes, as clearly shown by mounting evidence from the last thirty years.

The master clock

In the early seventies the hypothalamic suprachiasmatic nucleus (SCN) was identified as the seat of the master clock or circadian pacemaker in the mammalian brain by two independent research groups (3,4). This was later confirmed by others with electrophysiological studies (5), transplantation procedures (6,7) and metabolic experiments (8). These experiments also

showed that the endogenous rhythmicity of the SCN originates at the cellular level and that even when these neurons are isolated, the rhythm still prevails (9–12). The circadian rhythmicity that characterizes each of these SCN neurons is finely regulated by a molecular clock and its transcriptional-translational feedback loops (13–16). Together, these cells make the whole SCN a multi-oscillator system (17).

Peripheral clocks

Around two decades after the discovery of the SCN as the master clock, several research teams observed that those previously described clock genes were also expressed in a rhythmic fashion in peripheral tissues (18–20). Peripheral meaning outside the central nervous system most of the time, but in fact meaning outside the SCN. However, these peripheral oscillators were shown to desynchronize fast when isolated in vitro or in vivo in SCN-lesioned animals (21); suggesting that the SCN must be present to synchronize these peripheral clocks, like for example in hepatocytes, to maintain a coordinated phase (22). It is worth mentioning that nowadays it is known that there are other anatomical regions with semi-autonomous clocks that even though they receive input from the SCN, have been demonstrated to own a circadian rhythm that prevails for longer periods of time even in ex vivo conditions (23–28), hence the use of the term peripheral clock sometimes is used to define those non-SCN areas with rhythmic physiology.

The molecular clock

By now it is well known that the intrinsic rhythmicity of the different circadian clocks are generated by a molecular clock and that the mechanism of this molecular clock consists of a transcriptional feedback loop that involves 10-15 different (clock) genes (Figure 1). The BMAL1:CLOCK or BMAL1:NPAS2 heterodimer activates its target genes by binding to E-box motifs and enhancers regions in their promotor region (29–32). Among these target genes are also *Per1, Per2, Cry1* and *Cry2* that act like the negative feedback loop (29,33,34). The rising levels of PER and CRY proteins form complexes that interact with BMAL1:CLOCK or BMAL1:NPAS2 binding, thereby attenuating their transcriptional activity and allowing the cycle to repeat with lower transcriptional activity (15,35–37). The BMAL1:CLOCK dimer initiates also the transcription of a second feedback loop that involves the orphan nuclear receptor genes *Rev-erb* and *ROR*. Then REV-ERB and ROR proteins compete for RORE binding sites in the *Bmal1* promoter region;

where ROR initiates *Bmal1* transcription while REV-ERB inhibits it (38–40).

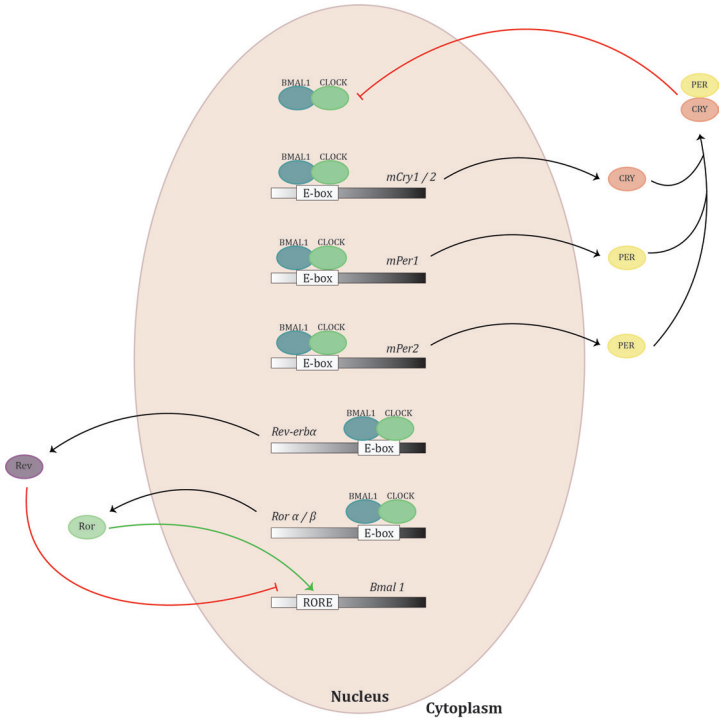

Figure 1. *Simplified schematic representation of the mammalian molecular clock. The CLOCK:BMAL1 heterodimer binds to the E-boxes and initiates the transcription of clock target genes (positive loop). PER and CRY are two of the resulting proteins that form a heterodimer in the cytoplasm and translocate back to the nucleus when sufficiently high concentrations are reached. In the nucleus the PER/CRY heterodimers inhibit CLOCK:BMAL1 from further initiation of the transcription processes, which together constitutes the primary negative feedback loop of the molecular clock. The BMAL1 transcription by Ror, which is inhibited by Rev, constitutes the a second feedback loop. Adapted from Buhr & Takahashi, 2013.*

Entrainment

As described before, body clocks oscillate during periods of approximately, but not exactly, 24 hours. Hence, in order for the endogenous circadian system to be fully synchronized with the external environment. The different clocks are phase-adjusted by different environmental cues such as light and temperature, or behavioral cues like activity patterns or fasting/feeding cycles (41,42). Light is the main *Zeitgeber* for the SCN (43),

while food is thought to be an important *Zeitgeber* for many peripheral tissues (44).

Circadian disruption

Just as many different environmental signals can be used for an optimal synchronization of the different body clocks, these very same signals can cause circadian disruption or asynchrony between the master and the peripheral clocks when received at the wrong time of the day. Unfortunately, our highly industrialized society is an important contributor to this circadian disruption by the increasing number of jobs and industries with shift work, with the amount of environmental artificial light that we receive at night and with the high availability of products that contribute to the consumption of a high fat diet (45–50). All these factors have been shown to induce behavioral, physiological and molecular changes (51–54) that in the long run contribute to the development of metabolic diseases (46,55–62) (Figure 2). In this thesis we will explore further circadian disruption due to acute exposure to light at night. A comprehensive review of the current evidence on the metabolic effects of light, from human as well as animal studies, can be found in Chapter 2.

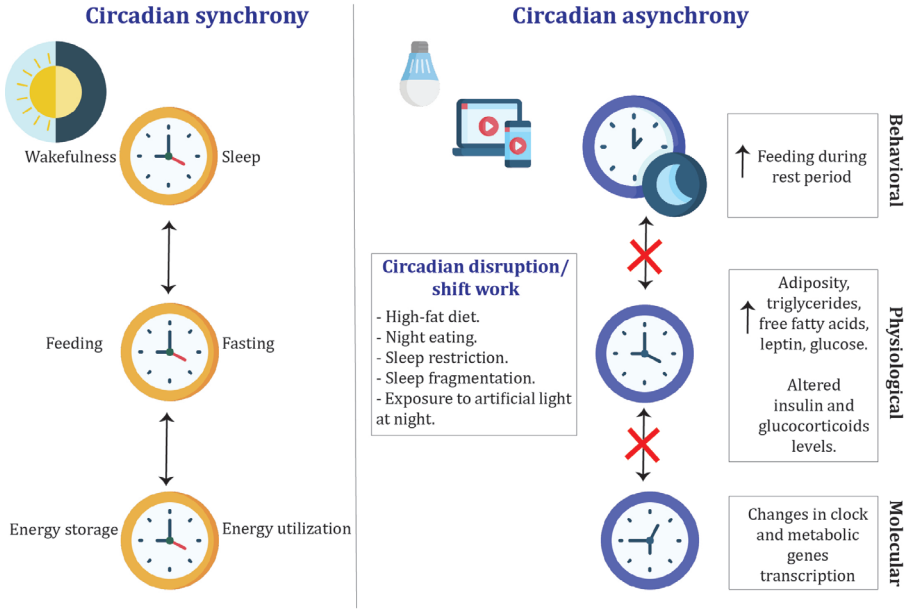

Figure 2. *Circadian asynchrony and metabolic diseases. Lifestyle and behavioral factors are able to disturb the synchrony between of the molecular clock in both the master and peripheral oscillators, which in the long run may result in metabolic diseases. Adapted from Green, Takahashi & Bass, 2008.*

Photoreception and circadian rhythms

In mammals, entrainment due to light (photo-entrainment) starts in the retina and travels via the retinohypothalamic tract to the SCN. Traditionally, the retina was viewed as the anatomical point where the visual process starts with the rod and cone photoreceptors. These cells translate light stimuli into chemical signals, passing first through the retinal circuits and ultimately reaching the retinal ganglion cells (RGC); then these RGC's communicate this information via action potentials down the optic nerve (63). Almost twenty years ago, it was shown that a small portion (4–5%) of these RGC's are photoreceptive themselves, meaning that when isolated from the retina, they are capable of responding to light (64). The capacity of these cells to respond to light is due to melanopsin, an opsin-based photopigment present. Hence they were called intrinsically photosensitive retinal ganglion cells (ipRGCs) (Figure 3) (65–67).

ipRGCs and non-image forming effects of light

Besides its role in the visual process and photo-entrainment, it has been shown that light may also have important direct effects on behavior and physiology. These effects are known as the so-called non-image forming effects of light (NIF) and are mainly driven by changes in the intensity and wavelength composition of the environmental light (68). These NIF effects go from entrainment of circadian rhythms, as mentioned before, to modulation of complex behavioral and metabolic processes like locomotor activity, alertness, mood, anxiety and probably motivated behaviors (67–72), due to the multiple brain regions outside the SCN also reached by the ipRGCs projections (73). As mentioned before, ipRGCs are particularly sensitive to wavelength changes, an although melanopsin can be activated by white polychromatic light, it has a peak of action at around 480 nm of wavelength (68,74,75) (light with blue appearance), which makes the possible NIF effects of blue light not only particularly interesting, but also extremely relevant, taking into account the amount of blue light that we are exposed on a daily basis from light emitting diodes (LEDs) of screens, mobile phones and other light emitting devices. Further details on the projections of the ipRGCs and existing evidence on how light can impact metabolism can be found in Chapter 2.

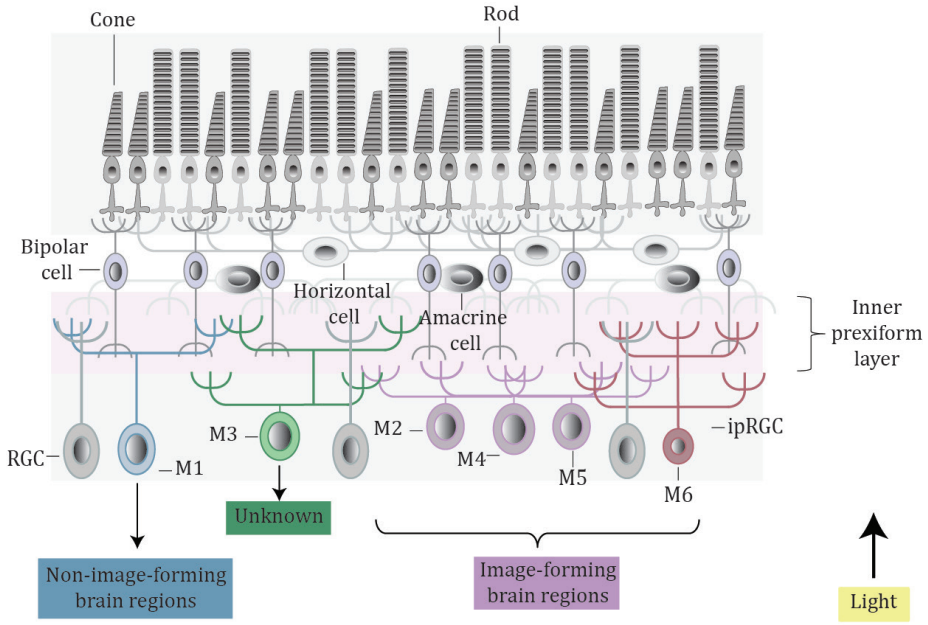

Figure 3. *Schematic view of the retina. Light received by rods and cones is transmitted to the retinal ganglion cells (RGCs) via horizontal, amacrine and bipolar cells. RGCs are responsible for all sensory input from the retina to the brain. Only a small portion of RGCs (4–5%) are intrinsically photosensitive RGCs (ipRGCs). By now at least six different subtypes of ipRGCs (M1–M6) have been described, with different morphological and electrophysiological characteristics. Evidence points out that the M1 ipRGCs are the ones involved in non-image-forming processes. Image adapted from Le Gates, Fernandez & Hattar, 2014.*

Scope of the thesis

This thesis is composed of a series of experiments aimed to study the metabolic and physiological effects of acute exposure to light at night in different species of rodents. **Part I** is comprised of this general introduction to the field of chronobiology research and also summarizes previous evidence that gave rise to our research questions. In **Chapter 1** we introduce the field of circadian biology and the necessary physiological basis to understand our topic of research, together with fundamental physical principles for understanding, using and measuring light and its implications in mammalian 'physiology'.

In **Chapter 2** we provide an extensive compilation of animal and human

studies showing the metabolic implications of artificial light at night, with a focus on the impact of chronic and acute effects of light at night of different wavelengths on locomotor activity, food intake, sleep/wake cycles, body temperature, melatonin, glucocorticoids, and glucose and lipid metabolism.

When translating scientific findings from animal research to human health, preferably the chosen animal model has a physiology as similar as possible to that of humans. Hence in **Part II**, we explored the effects of artificial light at night on food intake and glucose metabolism in the diurnal rodent, Arvicanthis ansorgei. In addition, to better mimic the possibility to choose between a healthy or a palatable diet that we have in our daily lives, we provided these animals with a free choice high-fat high-sucrose diet, which enabled us to study the effects of light exposure at night on the intake of the different diet components. **Chapter 3** is dedicated to exploring the effects of white light, whereas in **Chapter 4** the effects of blue light on glucose metabolism and food intake will be addressed.

Several rodent models have been used to study metabolism and the pathogenesis of several metabolic diseases, as well as the metabolic changes observed in response to different interventions or stimuli. Wistar rats being among the most used animal models, we also wanted to explore the effect of light at night in this nocturnal rodent. In **Part III**, **Chapter 5** we present our findings on the effects of light at night on the energy metabolism of rats and how these effects depend on the wavelength and the time of the exposure.

Over the past decade, numerous new methods and procedures have entered the scientific field, including the development of genetically engineered mice. These mice have increased enormously our ability to study the role of specific genes and proteins in animal physiology. **Part IV** focuses on the effects of artificial light at night on glucose and energy metabolism, including food intake, in wild type mice as well as a couple of transgenic mice models.

As indicated above, melanopsin is the protein present in intrinsically photosensitive retinal ganglion cells that is responsible for the non-image-forming physiological responses to light, therefore in **Chapter 6**, the effects of light on glucose metabolism and food intake were investigated using a combination of C57Bl/6J wild type mice and genetically engineered mice that were melanopsin deficient.

Furthermore, in the last decade mice also many mice with mutations

of core circadian genes have been developed, to study the implications of circadian physiology deregulation. It has been previously shown that *Bmal1* deficient mice have endocrine and metabolic alterations, which makes this animal model even more vulnerable to further circadian disruptions. Consequently, in **Chapter 7** we studied the effects of different wavelengths of light with different times of exposure on the metabolism of C57Bl/6J wild type and *Bmal1* knock out mice.

Lastly, **Chapter 8** takes a retrospective look at all the previous experimental chapters, integrating them with more recent scientific findings and extrapolating the acquired knowledge to improve human health and lifestyle. Also, recommendations for future follow-up experiments and other possible lines of research are discussed in this section.

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Chapter 2

Metabolic implications of exposure to light at night: lessons from animal and human studies

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Abstract

Lately the incidence of overweight, obesity and type 2 diabetes has shown a staggering increase. To prevent and treat these conditions, one must look at their etiology. As life on earth has evolved under the conditions of nature's 24-hour light/dark cycle, it seems likely that exposure to artificial light at night (LAN) would affect physiology. Indeed, ample evidence has shown that LAN impacts many metabolic parameters, at least partly via the biological clock in the suprachiasmatic nucleus of the hypothalamus (SCN). This review focusses on the impact of chronic and acute effects of LAN of different wavelengths on locomotor activity, food intake, the sleep/wake cycle, body temperature, melatonin, glucocorticoids and glucose and lipid metabolism. While chronic LAN disturbs daily rhythms in these parameters, experiments using short-term LAN exposure also show acute negative effects in metabolically active peripheral tissues. Experiments using LAN of different wavelengths indicated an important role for melanopsin, the photopigment found in intrinsically photosensitive retinal ganglion cells (ipRGCs), but also provided evidence that each wavelength may have a specific impact on energy metabolism. Importantly, exposure to LAN has been shown to impact glucose homeostasis also in humans and to be associated with an increased incidence of overweight, obesity and atherosclerosis.

Introduction

In 2016, nearly forty percent of adults worldwide was reported to have overweight and thirteen percent was reported to have obesity (1). Overweight and obesity are associated with type 2 diabetes and cardiovascular disease. As the development of these diseases can in large part be ascribed to lifestyle, it is important to look at societal changes. Over the past century, we have gotten used to abundant food often rich in fat and refined carbohydrates. Cars, public transportation and office jobs have reduced the need for physical activity. Moreover, we have shifted away from nature's 24-hour day/night rhythm, towards a society where people work around the clock, stay out late and have their screens on until the early hours.

Most organisms, including mammals, have developed an endogenous circadian timing system under the earth's natural 24-hour rhythm that is adapted to the regular alternation of light and dark phases. Thus, it seems likely that changing these conditions will impact physiology. The aim of this review is to summarize the current evidence on the impact of exposure to light at night (LAN) on metabolic parameters and present an anatomical framework through which this may happen. Our modern society exposes us to various types of artificial light at night (ALAN). Outdoor ALAN due to street lighting is usually low in intensity and chronically present. Shift work on the other hand exposes us to single nights of bright light. The usage of screens exposes us to light of shorter wavelengths and might thus impact metabolism differently. The aim of this review is to summarize the current evidence of the impact of exposure to LAN on metabolic parameters, as well as an anatomical framework through which this may happen. To properly map the effects of different exposures of LAN, a distinction will be made between chronic and acute LAN exposures and the impact of different wavelengths.

Light's connection to metabolism: an anatomical framework

Both rodents and humans have, apart from rods and cones, a third type of ocular photoreceptor, the so-called intrinsically photosensitive retinal ganglion cells (ipRGCs). These ipRGCs contain the photopigment melanopsin, which is optimally sensitive to light at a wavelength of 484nm in rodents (2). Human ipRGC subtypes have shown peak activity in response to 457, 459 and 470 nm light with a maximal sensitivity at 480 nm (2,3). Rods and cones, with peak sensitivities varying within a range of 440 to 580nm

(4), provide input to the ipRGCs as well, lowering ipRGC response thresholds and increasing their action potential discharge rates (2).

Several brain areas are responsible for coordinating energy homeostasis by regulating locomotor activity, food intake, energy expenditure, hormone levels and activity in metabolic tissues. Some of these areas receive direct input from the intrinsically photosensitive retinal ganglion cells (ipRGCs), which contain the photopigment melanopsin (5,6). One of the areas receiving input from the ipRGCs is the suprachiasmatic nucleus of the hypothalamus (SCN) (6) or master biological clock (7). The molecular mechanism of this biological clock consists of negative transcription and translation feedback loops, causing oscillations in gene and protein expression with a period close to 24 hours (i.e., a circadian rhythm) (8).

Daily alternations of light and dark synchronize the circadian clock in the SCN neurons to the exact 24-hour cycle in our environment. Light information reaching the SCN via the ipRGCs is the most important synchronizer or '*Zeitgeber*' for the SCN neurons. Depending on the timing, light exposure will enhance or dampen the expression of certain clock genes. Light exposure at the end of the night will advance the clock's phase, whereas light at the beginning of the night will delay it (9).

The SCN has reciprocal interactions with the arcuate nucleus of the hypothalamus, which regulates daily rhythms in food intake (10) and locomotor activity (11). The dorsomedial nucleus of the hypothalamus (DMH) receives projections from the SCN and is involved in coordinating daily rhythms of food intake and locomotor activity with the sleep-wake cycle (12). Furthermore, the SCN interacts with the intergeniculate leaflet (IGL), which receives a direct input from the ipRGCs and further helps coordinate circadian rhythms (6). Besides, the SCN projects to the lateral habenula (LHb), a structure involved in several brain functions including reward, memory, learning, mood and sleep (13). The LHb also receives projections from the lateral hypothalamic area (LHA) that regulates feeding and reward (14).

The SCN, the DMH and ipRGCs also project to the paraventricular nucleus of the hypothalamus (PVN) and via this connection transmit the time-of-day signal to other brain areas and periphery. The PVN projects to the intermediolateral column (IML) of the spinal cord, regulating the secretion

of melatonin from the pineal gland (15). It also has sympathetic projections to the adrenal gland, via which it modulates the sensitivity of the adrenal cortex to ACTH (16), and sympathetic and parasympathetic projections to the thyroid gland (17), the pancreas (18), the liver (19) and white adipose tissue (WAT) (20). Furthermore, the PVN controls the activity of the hypothalamo-pituitary-thyroid and hypothalamo-pituitary-adrenal axis via the release of thyrotrophin-releasing hormone (TRH) (21) and corticotrophin-releasing hormone (CRH) (22). Thus, by means of its influence on the autonomic and neuro-endocrine output of the hypothalamus, the SCN's circadian rhythm is transmitted to other brain areas, endocrine glands and peripheral tissues (20). Likewise, peripheral tissues themselves also show a circadian rhythm in clock gene expression. Thus, the molecular clock mechanism is present not just in SCN neurons, but in virtually every cell. As peripheral cells cannot be entrained by light directly, they depend on the SCN to keep their clock synchronized with the environment (23). Additionally, peripheral clocks have been shown to respond to other *Zeitgebers,* including glucocorticoids (24), glucose (25), body temperature (26), melatonin (27), activity rhythms (28), food intake (29) and the microbiome (30). Altogether, the SCN has a vast reach and therefore, through its impact on the SCN, the effects of light are likely to be widespread too (Figure 1).

Moreover, the ipRGCs also project directly to many of the SCN target areas mentioned above. Hence, light exposure could also affect energy metabolism, feeding behavior and reward directly, i.e., independent from the SCN.

Light: definitions and nomenclature

In order to facilitate comparison of the effects of light amongst different studies it is important to report the spectral sensitivity within each retinal photopigment complement using the guidelines proposed by the International Commission on Illumination (31) and the suggestions described extensively elsewhere by Spitschan et al (32). Furthermore, in principle, color terms are not applicable to rodents, but even in humans they are rarely meaningful in the kind of studies described below. For example, both monochromatic 460nm and 495nm light could be described as "blue", as could a cloudless sky, which is not monochromatic. Thus, there are multiple ways to generate 'blue appearing' light (33). However, importantly, the fact that a light appears more or less blue does not uniquely specify its melanopic

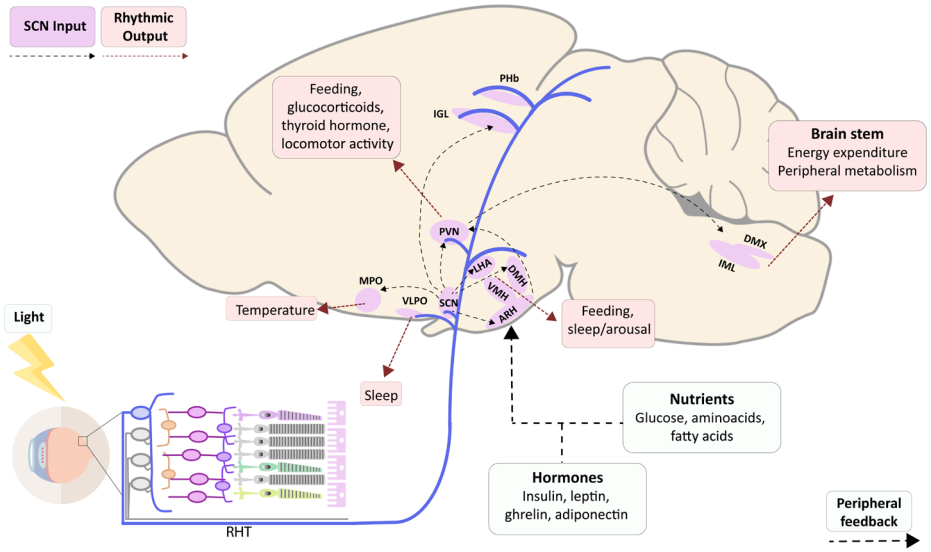

Figure 1. *Schematic overview of brain areas involved in the control of energy metabolism that receive input from the master clock located in the SCN or direct light input via the ipRGCs.*

RHT: retinohypothalamic tract, SCN: suprachiasmatic nucleus, VLPO: ventrolateral preoptic nucleus, MPO: medial preoptic area, ARH: arcuate nucleus of the hypothalamus, VMH: ventromedial nucleus of the hypothalamus, DMH: dorsomedial nucleus of the hypothalamus, LHA: lateral hypothalamic area, PVN: paraventricular nucleus of the hypothalamus, IGL: intergeniculate leaflet, PHb: perihabenular complex, IML: intermediolateral column of the spinal cord, DMX: dorsal motor nucleus of the vagus.

effect. On the other hand, different combinations of light can have a similar effect on melanopsin. In addition, referring to the color of monochromatic light is not useful without intensity information. Because of the principle of uni-variance, i.e. a single photopigment cannot distinguish between a change in wavelength or a change in intensity, different combinations of light (i.e. wavelengths) can elicit the same photoreceptor response (34). Therefore, in principle, the intensity of light of any wavelength can be increased to evoke a response, for example by the method of silent substitution (35). Unfortunately, in most studies, light conditions are only defined by color/wavelength and amount of lux as a measure of intensity. The kind of necessary information detailed above only started to be reported more consistently in the last few

years. In view of this large variation in information provided and in order to maintain readability of this review, we maintained the color information as mentioned in the different manuscripts and provided all the available physical light details in Supplementary Tables. Light information from the references cited in the paragraphs on the chronic effects of bright light and dim light at night is presented in Suppl. Table 1. Suppl. Table 2 contains the information from the references reporting on the acute effects of light and in Table 3 light information from the studies on the LAN effects of different wavelengths is presented.

The impact of exposure to constant bright light on rodent metabolism

Although exposure to constant bright light (LL) is usually not a natural condition, it is an interesting experimental condition to study light effects on clock function. In mice, in vivo recordings of SCN electrical output showed that LL caused an immediate reduction in rhythm amplitude (36). Also, the amplitude of *Per1* expression in the SCN was shown to be blunted and although SCN neurons still oscillated with a phase of roughly 24-h in LL conditions, individual neurons had a different phase in the expression of *Per1* (37). Experiments also showed a loss of daily locomotor activity rhythms (36), with no impact on total daily locomotor activity (38). One experiment showed the disturbance in locomotor activity rhythms to correlate with asynchrony amongst individual SCN neuronal *Per1* rhythms (37). Some mice exhibited activity rhythm splitting. In these mice, neurons in the left and right half of the SCN oscillated in antiphase (37). Multiple experiments in rats showed that circadian rhythms in locomotor activity were abolished by LL (39) and replaced with ultradian rhythms (36,37), with restoration of this rhythm after one week in constant darkness (DD), suggesting clock function might have been suppressed by LL (40).

LL caused the circadian rhythm in body temperature in rats to dampen and be replaced with ultradian rhythms, although the circadian rhythm in body temperature persisted longer than the rhythm in locomotor activity (40). Likewise, one week of DD restored the circadian rhythm of body temperature. This coincided with a restoration of the circadian rhythm and absolute levels of plasma melatonin (40), which were shown to be flattened and decreased in LL conditions (41). In mice and rats, the circadian rhythm in dietary and water intake was shown to be abolished. Total daily food intake

was increased in one experiment in mice (36), whereas in another there was no impact of LL on total food intake (38).

Rats exposed to LL conditions had a disturbed circadian rhythm in plasma glucocorticoid levels and its absolute levels were elevated (41,42). In mice, the rhythm in plasma glucocorticoids was found to be disturbed (38), yet absolute plasma glucocorticoid levels were found to be decreased (38,43). Plasma total fatty acid levels, free fatty acids (FFAs), phospholipids and cholesterol esters, were found to be continuously elevated in one rat study, while circadian rhythms in all plasma fatty acid levels were abolished (41). In both mice and rats, LL was shown to cause weight gain (36,38,39) and increased epididymal fat pad mass (38).

LL rats showed disturbance of the circadian rhythm in basal plasma glucose levels and hyperglycemia, as opposed to nearly constant normoglycemic basal plasma glucose levels in rats in a regular light/dark (LD) cycle (41). Elevated plasma glucose levels were found in mice during an intraperitoneal glucose tolerance test (IPGTT), indicating LL reduced glucose tolerance (38) and in another mice experiment it was found that the diurnal variation in insulin sensitivity was abolished by LL (36). An in vitro study using rat pancreatic islet cells from animals exposed to LL showed reduced glucose sensitivity and reduced total insulin secretion. *Per1* expression in pancreatic islet cells still showed circadian oscillations in LL conditions, with dampened amplitude and individual islets oscillating out of phase (44).

Overall, exposure to constant light caused altered clock function, which translated to a wide array of changes in metabolic parameters. Circadian rhythms in locomotor activity, body temperature, food intake, plasma glucocorticoids, lipids and glucose, and insulin sensitivity were shown to be disturbed and total levels of plasma melatonin, glucocorticoids, fatty acids and glucose were altered. Furthermore, clock gene expression in the pancreas was dampened. Most likely the LL-induced disturbances in the central clock cause perturbed circadian rhythms in brain regions and peripheral organs, resulting in misalignment and altered function amongst organs. Changes in glucose metabolism illustrate particularly well how such a disturbed interplay between different peripheral tissues could cause serious impairment of metabolic efficiency. As peripheral clocks have been shown to rely also on behavioral and metabolic *Zeitgebers*, changes in feeding and locomotor activity and in the output of metabolically active peripheral

tissues will add further to the alterations observed in peripheral clock gene expression. Moreover, recently it was shown that light may also have a direct effect on peripheral clocks, without the need of an oscillating SCN (45).

The impact of exposure to dim light at night on rodent metabolism

Although exposure to dim light at night (dLAN) provides constant exposure to light over the course of the 24-hour day, the difference in light intensity between day and night still provides a temporal cue to discern the 24-hour rhythm of the environment. Therefore, dLAN might affect circadian rhythms to a lesser or different extent than LL. Expression of *Per1, Per2* and *Cry2* in the SCN was altered at multiple time points during dLAN as compared to LD in mice (46). In hamsters, SCN *Per1* expression was altered at the beginning of the dim phase (47), while in rats dLAN decreased diurnal variation in SCN *Per1* and *Arntl* expression (48).

The 24-hour rhythm in locomotor activity was less pronounced in rats in dLAN conditions, although still present. Interestingly, exposure to dLAN gave rise to a second interacting rhythm in locomotor activity, with a period of 25.1 hours (48), possibly reflecting different parts of the SCN expressing their own rhythm. In mice, dLAN did not affect the daily locomotor activity rhythm (38,49). Results on the locomotor activity pattern of hamsters are ambiguous (47,50,51). Total locomotor activity was not affected in either rodent species (38,47–50). In a recent mice study, long term dLAN induced pronounced alterations in sleep architecture, enhancing age-associated changes (52).

dLAN did not affect diurnal variation in plasma glucocorticoid levels in mice (38), while the opposite was observed in hamsters (50). The daily rhythm in plasma glucocorticoids was maintained in rats, but the phase of the rhythm was delayed by 4 hours. Absolute plasma glucocorticoid levels were not affected (41). Interestingly, in mice dLAN advanced the rhythm in body temperature. The amplitude of the rhythm was dampened, with peak temperature being decreased and occurring 3 hours earlier (49). Nocturnal melatonin levels were shown to be suppressed by dLAN in rats (41,53).

Exposure to dLAN induced a shift in the timing of food intake towards daytime in mice and rats (38,48). Total daily food intake was not affected in mice (38,49). In rats there was a small reduction in food intake during dLAN

in one experiment (48), whereas in another there was no difference (54). The importance of the timing of food intake in the metabolic effects of dLAN was demonstrated by Fonken et al., when they showed that restricting food intake to subjective nighttime prevented the dLAN-induced body weight increase in mice (38).

Previously it was shown that dLAN causes an increase in body weight in regular (38,46,55,56) and TALLYHO mice (genetically prone to develop type 2 diabetes) (57), and in hamsters (47), but not in regular (48) or spontaneously hypertensive rats (54). Multiple experiments showed increased epididymal fat pad mass in mice (38,46,55). Interestingly, returning to an LD schedule after dLAN reversed body weight increase in TALLYHO mice (57). An experiment in regular mice found that dLAN induced a shift from lipid to carbohydrate metabolism and reduced energy expenditure without changing locomotor activity (49). In mice, dLAN changed the expression of Rev-erb in white adipose tissue (46).

Spontaneously hypertensive rats showed increased hepatic triglycerides (TG) during dLAN, while there was no effect on basal plasma TG, total and LDL cholesterol or leptin levels. Hepatic expression of *PPARγ* was elevated by dLAN in these rats. Epididymal expression of *PPARγ* and of *PPARα* was also elevated (54). In regular rats, there was no effect of dLAN on absolute values or the daily rhythm in plasma lipid levels (41).

Plasma glucose during an IPGTT was found to be elevated in both TALLYHO (57) and regular mice (38,55). Furthermore, plasma glucose levels during an intraperitoneal insulin tolerance test (IPITT) were elevated in TALLYHO mice in dLAN conditions. Returning to an LD schedule reversed the reduction in insulin sensitivity. Furthermore, basal plasma glucose levels in TALLYHO mice were elevated in dLAN conditions and a larger percentage of dLAN TALLYHO mice acquired diabetes compared to TALLYHO dark controls. Additionally, dLAN TALLYHO mice had a lower survival rate as compared to dark controls (57). In an experiment in regular mice, basal plasma insulin levels were elevated and diurnal variation in plasma insulin levels was abolished (55). Basal plasma glucose levels were not affected in another experiment in regular mice. However, in this experiment liver expression of *Per1, Per2, Cry1, Cry2, Bmal1* and *Rev-erb* was altered (46). In both regular and spontaneously hypertensive rats, there was no effect of dLAN on absolute basal plasma glucose levels (41,54), although dLAN did induce a

shift in the daily rhythm in plasma glucose in regular rats (41). There was no effect on basal plasma insulin levels in spontaneously hypertensive rats. While hepatic expression of *PPARγ* and epididymal expression of *PPARγ* and *PPARα* were increased by dLAN in spontaneously hypertensive rats, cardiac expression of *Glut4* was decreased (54).

Summarizing, dLAN caused shifts in circadian rhythms and extension of circadian periods on multiple parameters, rather than completely abolishing rhythms. It has been established that the ventral SCN region is more susceptible to acute changes in the light/dark cycle as compared to the dorsal SCN (58). Possibly, continuous activity of the ventral SCN region in response to dLAN, combined with a steady generation of a 24-hour rhythm by the dorsal SCN, results in a dampened but intact circadian rhythm in SCN output, which in turn translates to blunted circadian rhythms in locomotor activity, food intake, body temperature and other parameters. On the other hand, in LL conditions both the ventral and dorsal SCN region are disturbed, abolishing all rhythms.

The dLAN-induced increase in body weight could be prevented by restoring the nocturnal rhythm in food intake. Thus, it seems likely that dLAN-induced alteration of circadian rhythms gives rise to metabolic disturbance. Altered expression of proteins like *PPARγ, PPARα* and *Glut4,* however, indicates dLAN also impacts glucose and lipid homeostasis on a tissue-specific level. It is likely that both perturbed behavioral rhythms and tissue-specific changes contribute to the vast range of metabolic disturbances found in dLAN exposed animals. Importantly, metabolic disturbances observed in TALLYHO mice indicate that dLAN might be especially harmful to individuals that have or are prone to develop diabetes.

The effects of chronic exposure to LAN on human metabolism

It has been established that 99% of the population of the EU and the USA lives in areas where night-time illumination is above the threshold for light pollution (59). It is therefore essential to determine the extent to which LAN is a health hazard and how it affects metabolic parameters in humans. Cross-sectional studies have found that high outdoor LAN was associated with disturbances in the daily sleep/wake cycles and in sleep duration (60,61). Obayashi and collaborators performed a cross-sectional study in elderly individuals and measured LAN intensity in people's bedroom (62). They

found that exposure to LAN correlated with elevated plasma triglycerides and LDL, and lowered HDL, although urinary melatonin levels were not affected. In another study this same group found LAN to be associated with subclinical atherosclerosis (63).

Park *et al.* analyzed data of 43.722 women and found that those that slept in light rooms had higher body weight as compared to women sleeping in dark rooms (64). Similarly, a cross-sectional analysis of over 100.000 women in the Breakthrough Generations Study showed that the odds of having overweight and obesity were higher when sleeping in a light room (65). The odds of having an increased waist circumference, waist-hip ratio and waist-height ratio were also higher in people that slept in light rooms (62,64,65). When satellite data of outdoor LAN were matched with data on body weight, it was observed that LAN was a strong positive predictor for overweight and obesity in both men and women (60,66,67).

Several experiments conducted in men living on Antarctica found that in December, when outdoor light exposure is continuous, plasma levels of glucose, insulin and thyroid stimulating hormone were altered as compared to other months (68–71). Unfortunately, in these experiments the indoor lighting regiment was not specified. In addition, variations in temperature, physical activity and food intake between months likely will also have impacted the changes in physiology mentioned above.

Overall, ample evidence suggests that high LAN levels, either outdoor or in the bedroom, correlate with increased body weight and obesity in humans. Furthermore, increased incidence in dyslipidemia, subclinical atherosclerosis and central obesity suggest LAN might be an important risk factor for the development and deterioration of cardiovascular disease.

Acute effects of exposure to LAN on rodent metabolism

Experiments using short-term exposure to LAN in experimental animals are useful to determine whether light has acute effects on energy metabolism and clock function, as it is well known that even very short exposures to nocturnal light will inhibit melatonin release. Many studies have shown acute alterations in clock gene expression and increased c-Fos expression in the SCN upon short term nocturnal light exposure (51,72–74). Experiments in rats have shown nocturnal light pulses to acutely alter *Per1*

and *Per2* expression in the ventrolateral SCN, but not in the dorsomedial SCN. The dorsomedial SCN continued its robust circadian oscillation in clock gene expression (75), indicating that the ventrolateral SCN readily adapts to the environment, whereas the intrinsic core clock rhythmicity is maintained in the dorsomedial SCN. Interestingly, exposure to a light pulse during late subjective night time elicited greater c-Fos expression in the SCN and PVN of hamsters as compared to a pulse early in the subjective night, indicating that SCN sensitivity to light is dependent on its timing within the circadian cycle (76).

Light pulses also affected metabolism in a timing- and intensity dependent manner. For instance, locomotor activity was acutely decreased by a pulse of bright light in rats and mice, but not by a pulse of dim light in rats (77,78). In the rat pineal gland expression of *Per1* and arylalkylamine-N-acetyltransferase (NAT), a key enzyme to produce melatonin, was decreased by a pulse in the early subjective night and by a pulse in the late subjective night. However, expression of *Per2* and *Per3* in the pineal was decreased exclusively by a pulse in the late subjective night (79).

Effects of light on plasma glucocorticoid levels are controversial. In the rat, Cailotto et al. found increased ACTH receptor mRNA expression in the adrenal and increased plasma glucocorticoid levels in the early subjective night, whereas adrenal *Cry2* expression was altered in the late subjective night-time (79). Mohawk and collaborators on the other hand found increased plasma glucocorticoid levels in the middle and end of subjective night-time, along with increased plasma ACTH levels (80). Yet another experiment in rats using light pulses of a lower intensity showed plasma glucocorticoid levels were not affected (78). In mice, a pulse in the middle of the dark phase increased plasma glucocorticoid levels in an intensity dependent manner, as well as increasing *Per1* and *Per2* expression, without alterations in ACTH levels (81,82). Interestingly, the elevated plasma glucocorticoid levels were accompanied by increased activity in the adrenal nerve, suggesting light information was primarily transmitted to the mouse adrenal via the autonomic nervous system (82).

A light pulse in the early dark phase elevated basal plasma glucose levels and decreased expression of *GLUT4* in mice skeletal muscle (72). In rats, plasma glucose levels during an intravenous glucose tolerance test (IVGTT) were elevated by a pulse in the early dark phase, while insulin levels

were not affected. On the other hand, in the late dark phase, no effect of light on plasma glucose levels was found, but insulin levels were elevated. Surprisingly, an intravenous insulin tolerance test (IVITT) showed insulin sensitivity was not affected at either time point. Possibly, elevations in plasma glucose levels were caused by altered insulin-independent glucose uptake or increased glucose production by the liver. The elevation in plasma glucose levels in rats was found as a result of bright light pulses but not dim light pulses (78). In the rat liver, expression of *Per1, Per2, GLUT2*, and *PEPCK* was increased, while glucokinase was decreased by pulses at different time points depending on the specific clock genes and enzymes (83). Interestingly, hepatic denervation prevented light induced changes in clock gene and enzyme expression, supporting the notion that the autonomic nervous system is essential in transmitting these acute effects of light to peripheral tissues (79,84,85).

It appears light has an acute effect on peripheral tissues first via the ventral region of the SCN and then via the autonomic nervous system. As the acute effects of light differ amongst and within peripheral tissues, it is likely that either the transduction of the light signal or the receptivity of the peripheral tissue to light is modulated at a level downstream of the SCN or at the level of the peripheral tissue itself. The effects of LAN on glucose metabolism are ambiguous, as LAN seems to promote both mobilization and conservation of glucose. Possibly, daily variations in the receptivity of the liver, skeletal muscle and the pancreas to light explain the variety of effects LAN has at different circadian time points. Counterintuitively, LAN acutely decreased locomotor activity, yet increased plasma glucocorticoid levels, again suggesting LAN has both an energy conserving and an arousing effect on these nocturnal animals.

Acute effects of exposure to LAN on human metabolism

In an experiment where 48 subjects were exposed to a night of dim light, sleep duration was decreased without affecting salivary melatonin levels (86). In another study in 14 men, salivary cortisol levels were acutely increased by a light pulse in the morning, but not in the evening (87). Similarly, in an experiment involving 17 men, exposure to light acutely increased heart rate in the morning and middle of the night, but not in the evening. Furthermore, the increase in heart rate was intensity-dependent in the morning, but not in the evening (88). Increased heart rate during morning light exposure has

been shown to likely rely on an increase in sympathetic activity (89,90). Interestingly, morning light has also been shown to cause elevated plasma TAG levels in healthy men and elevated plasma glucose levels and plasma TAG levels in men with type 2 diabetes, indicating exposure to light in the morning increases energy availability (89).

A study in 17 subjects found that salivary melatonin levels, as well as evening pre-prandial plasma FFA levels, were decreased in individuals exposed to a night of bright light as compared to a night of dim light. Evening postprandial plasma glucose and insulin levels were elevated in individuals exposed to a night of bright light. Basal TAG levels and basal plasma glucose and insulin levels were similar between lighting conditions (91). Another study in 8 male subjects also found that LAN elevated postprandial plasma insulin levels in the evening. Interestingly, plasma insulin and GLP-1 levels were also elevated after a meal in the morning following LAN exposure. Postprandial glucose levels were not affected at any time point in this study. Plasma glucocorticoid levels were briefly elevated by LAN in the middle of the night. Plasma melatonin levels were decreased (92).

Results of experiments using LAN and light in the morning indicate light has an arousing effect on humans at the onset of the activity period, which is most likely mediated by the autonomic nervous system. Results of LAN exposure on plasma cortisol levels and heart rate at the beginning of the subjective night differed from results found in the morning, indicating the arousing effects of LAN are circadian phase dependent. Contrarily, effects of LAN on postprandial plasma glucose and insulin levels at the beginning of the subjective night and the following morning were similar, suggesting acute effects of LAN on glucose metabolism are more constant. Impairment of glucose metabolism indicates LAN might propose a risk especially to individuals that are prone to or suffer from type 2 diabetes.

The effects of LAN of different wavelengths on rodent metabolism

As multiple photoreceptors contribute to signalling light information to the circadian system, each with their own peak sensitivity. (5,6) it is to be expected that light of different spectral compositions will impact clock function and energy metabolism differently. Indeed, in the hamster SCN a 30-min pulse of dim blue light elicited c-Fos expression, whereas a pulse of dim red light did not. Interestingly, dim blue light also elicited greater c-Fos

induction in the hamster SCN than dim white light (51). Similarly, in the mouse SCN, a 30-min pulse of blue enriched white light caused greater *Per2* expression than a pulse of blue-filtered white light (72). Furthermore, blue light also induced greater c-Fos expression in the mouse SCN as compared to green and violet light. Contrastingly, in the mouse ventrolateral preoptic nucleus (VLPO) green light elicited greater c-Fos expression than blue light, suggesting that especially the SCN is sensitive to blue light. In melanopsin-knockout mice, expression of c-Fos, *Per1* and *Per2* in the SCN in response to blue light was reduced as compared to wild-type mice, but elevated as compared to dark controls, indicating that the melanopsin-containing ipRGCs amplify the effects of blue light in the SCN (93).

In mice, a 1-hour pulse of green light in the early dark phase rapidly induced sleep, whereas blue light induced sleep with a delayed onset. Interestingly, a corticosteroid receptor antagonist advanced sleep onset in blue light conditions, indicating blue light increased arousal whereas green light did not (93). In rats, 2-hour pulses of green and blue light acutely decreased locomotor activity, whereas a pulse of red light did not (78). In hamsters, 8 hours of blue dLAN reduced nocturnal locomotor activity, whereas red dLAN increased both total and nocturnal locomotor activity (51). Constant dim green light disturbed the circadian locomotor activity rhythm in hamsters and lengthened the circadian period by 0.3 hours (94).

In mice, both a 1-hour pulse of green and blue light elevated plasma glucocorticoid levels, although the increase was significantly greater in blue light. Expression of both *Per1* and *Per2* in the adrenal was elevated by blue light but not by green light. The response of both plasma glucocorticoid levels and adrenal *Per1* and *Per2* expression to blue light was decreased in melanopsin-knockout mice as compared to wild-type mice. Contrarily, in green light, the response of plasma glucocorticoid levels was increased in melanopsin-knockout mice, and comparable to plasma glucocorticoid levels in blue light exposed melanopsin-knockout mice. Thus, melanopsin most likely modulated the effects of light, translating into a different impact of green and blue light on the adrenal gland (93).

In *Microtus Socialis*, a nocturnal non-laboratory rodent, 30-min pulses of both blue and yellow light increased urinary corticosteroid levels, with higher levels in blue light, whereas red light did not impact urinary corticosteroid levels (95). In rats, however, plasma glucocorticoid levels

were unaffected by a pulse of either red, green or blue light (78), but when 8 hours of chronic red dLAN was used for 6 weeks , a decrease in total plasma glucocorticoid levels and an 8-hour advance in the phase of its daily rhythm was found (96). In our study with the diurnal rodent *Arvicanthis ansorgei* a 1-hour pulse of blue light reduced plasma glucocorticoid levels in male animals fed a chow diet and increased plasma glucocorticoid levels in female animals fed a high-fat high-sucrose (HFHS) diet (97).

Plasma melatonin levels in hamsters were reduced after 8 hours of dim green light, but not after 2 hours (94). In rats, 4 hours of red dLAN reduced plasma melatonin levels (96). Both a blue and a yellow 30-min light pulse reduced urinary melatonin levels in *M. Socialis* and caused weight loss, whereas red light did not. Interestingly though, daily energy expenditure was reduced in blue light as well as in red light, but not in yellow light, in *M. Socialis* (95). Exposure to 8 hours of red dLAN over the course of 6 weeks did not impact food and water intake or body weight in rats. The daily rhythm in plasma total fatty acid levels in rats persisted during 6 weeks under red dLAN, but the amplitude was dampened with a disturbed daily rhythm. Absolute levels of plasma leptin levels were higher in these conditions (96). Contrastingly, a 1-hour pulse of blue light had no effect on plasma leptin levels in *Arvicanthis ansorgei,* a diurnal rat species (97). In the mouse liver, expression of *Abcg1*, a protein involved in cholesterol and lipid transport, was elevated by a 30-min pulse of white light that contained blue light, but not by blue-filtered white light (72).

Rats showed impaired glucose tolerance during an IVGTT when given a 2-hour pulse of green light, but not by red or blue light. There was no effect of light of any wavelength on plasma insulin levels during the IVGTT, or on basal plasma glucose and insulin levels (78). In *Arvicanthis* a pulse of blue light elevated basal plasma glucose levels in female HFHS animals, but not in male animals. Plasma glucose levels after an OGTT were elevated and basal plasma insulin levels were decreased by blue light in male *Arvicanthis* when fed a chow or HFHS diet, but not in females (97). In rats exposed to chronic red dLAN for 6 weeks, the daily rhythm in basal plasma glucose and insulin levels was maintained, but basal plasma glucose levels were elevated, whereas peak values in basal plasma insulin levels were decreased (96). A 30-min pulse of blue enriched white light increased plasma glucose levels, whereas blue-filtered white light did not have an effect in mice. Expression of *Irs2* was increased by blue-cut white light in mice liver and skeletal muscle,

whereas contrarily *Irs2* was decreased in the liver by blue enriched white light (72).

Thus, both in the SCN and in the adrenal gland, the impact of blue light was stronger than that of green light. It is therefore likely that light of any wavelength travels through the SCN to the adrenal gland but is initially modulated by the ipRGCs. Yet the wavelength effects seem to be species, duration and intensity dependent. Surprisingly, an experiment in rats found that 6 weeks of red dLAN had a wide range of effects on metabolism, a finding that is not in line with the effects of red light on the SCN and seems to contrast with other evidence, i.e., nocturnal rodents being unable to respond to red light (51). However, red light was also found to impact metabolism in other experiments, suggesting chronic exposure to red light perhaps impact clock function or might impact metabolism independent of the SCN. Further research comparing exposure to light of different wavelengths and in different rodent species will be important to elucidate more precisely how LAN impacts metabolism through different pathways. Additionally, research aiming to study the different effects of light should report the spectral sensitivity within each retinal photopigment complement (98) as it facilitates comparisons amongst studies, but also because the importance of non-ipRGCs photoreceptors in circadian entrainment has recently been re-established by Mouland et al. (33).

The effects of exposure to LAN of different wavelengths on human metabolism

Experimental evidence has pointed out that blue light is most potent in suppressing melatonin release in humans (99,100). Both red and green light were shown to suppress melatonin as well, albeit to a lesser extent than blue light (101,102). In an experiment on evening computer use, melatonin levels were decreased in participants that used backlit screens, which emitted high intensity blue light, as compared to participants that used non-backlit screens (103). Comparably, participants that used blue light-enhancing goggles during computer use showed decreased melatonin levels as compared to participants that used the computer without goggles. Interestingly, participants that used a computer without goggles and participants that used blue light-filtering goggles had comparable melatonin levels, suggesting there is an intensity dependent threshold for blue light

to affect melatonin levels (104). Similarly, in an experiment on evening smartphone use, subjects that used a regular smart phone had melatonin levels comparable to subjects that used a smartphone with blue light suppression (105).

Evening exposure to blue light was shown to decrease subjective sleepiness in humans (101,103,105), whereas green light was not (101). Concordantly, EEG power spectra were impacted during evening blue light exposure (102,103,106,107), suggesting blue light might have altered alertness. Interestingly, red light was also shown to impact EEG power spectra (102), whereas green light did not (106). Notably, in one experiment a pulse of evening blue light was shown to impact EEG power spectra the following morning, as well as decrease energy expenditure and reduce the thermic effect of breakfast (107). Core body temperature was shown to be altered by evening blue light pulses, but not by green light pulses (101,106). Contrastingly, smartphone use without blue light suppression did not impact body temperature or plasma cortisol levels, nor did smartphone use with blue light suppression (105). Importantly, an experiment that filtered light of short wavelengths in the evening, as well as 70% of all light, found reduced basal plasma glucose and insulin levels, indicating a reduction in blue light before bedtime may improve insulin sensitivity (72).

Although evidence on light of different wavelengths is scarce, it looks as though blue light increases arousal in humans and impacts energy expenditure and glucose metabolism. As screen use is a frequent source of evening blue light, restricting blue light emission from devices could be an important step in preventing the harmful health consequences of exposure to LAN. Further research will have to indicate to what extent light of short wavelengths is responsible for the deleterious effects of LAN, and whether filtering only blue light is enough of an intervention, since previous human evidence does not support the contribution of the S cones to the neuroendocrine disruptive effects of light (34) and because evidence in animals implies that all light, not just blue, affects metabolism.

Conclusion

The metabolic implications of exposure to chronic and acute LAN and LAN of different wavelengths are summarized in Figure 2. It seems that chronic LAN exposure gives rise to metabolic inefficiency especially by

disturbing daily behavioral rhythms, through its impact on the SCN.

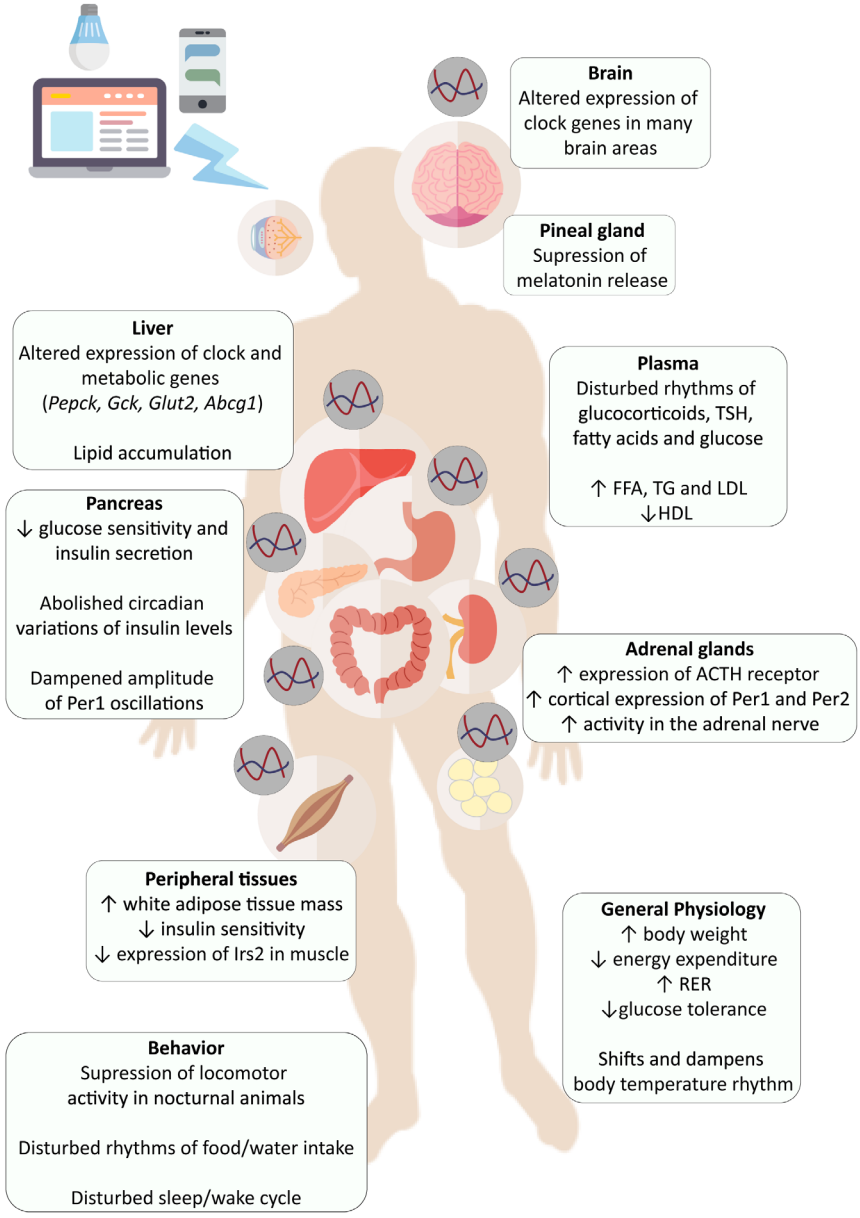

Figure 2. *Summary of the metabolic implications of exposure to light at night. TSH: thyroid-stimulating hormone, FFA: free fatty acids, TG: triglycerides, LDL: low-density lipoprotein, HDL: high-density lipoprotein, RER: respiratory exchange ratio.*

However, other, more acute experiments have shown that LAN also directly impacts peripheral tissues, independent of circadian rhythm disturbance, probably via the ventral region of the SCN or via non-SCN pathways. In the majority of cases, the metabolic consequences of acute LAN most likely will be limited, as homeostasis is very well able to cope with such challenges. On the other hand, it is not clear what the consequences are of prolonged and repeated exposures to acute LAN. However, in the real-life situation, most organisms (including humans) will be exposed to chronic LAN and experience the metabolic consequences of both the acute and chronic effects of light. The metabolic consequences of chronic LAN are mainly caused by its disruptive effects on the SCN. On the other hand, for the acute effects of LAN it is not clear whether they involve the SCN or not. In fact, also a combination of SCN-mediated and non-SCN mediated effects is possible. In addition, as for now, it is also not clear whether there are fundamental differences between the metabolic consequences of acute and chronic LAN, but it seems reasonable to assume that if anything the effects of acute LAN will enhance those of chronic LAN.

Moreover, the effects of LAN are wavelength dependent and differ within and amongst peripheral tissues, raising the question to what extent the effects of light are modulated by the SCN, and whether light also affects metabolism via non-SCN pathways (Figure 1). In this regard, several recent observations are interesting. First, using mice with a genetic ablation of all ipRGCs except those that project to the SCN, acute non-SCN dependent effects of light were shown on physiology and behavior (13,108). Secondly, Koronowsky et al. (45) showed that the autonomous oscillations of the liver clock are independent from all other clocks (including the SCN clock) yet depends on the presence of a light/dark cycle. Thirdly, subcutaneous WAT adipocytes in both mice and humans have been reported to be directly sensitive to light as these cells also express melanopsin (and encephalopsin, another photopigment) (109,110). Although, as for now, it is not clear whether these photopigments in WAT have any physiological relevance. Further research using LAN of different wavelengths will be necessary in answering these questions but will also help to determine what type of light has the most harmful consequences on health. As it has been shown that both acute and chronic LAN exposure impacts glucose metabolism and is associated with the development of overweight, obesity and cardiovascular disease in humans, it is of great importance that research on this topic continues.

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Table S1. Light conditions used in studies of acute effects of white light on metabolism.

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
50	Bedrosian et al., 2013	Hamsters	16/8 L/D after which 3 days of constant darkness	Diurnal light Light pulse	150lx 5lx, 150lx	30 min	1 hour before subjective lights off
72	Nagai et al., 2019	Mice	12/12 L/D	Diurnal light LED light pulse	100-300lx 10lx	30 min	2 hours after lights off
73	Masana et al., 1996	Mice	12/12 L/D after which constant darkness	Diurnal light Fluorescent light pulse	300lx 300lx	15 min	2, 6, 10, 14, 18 and 22 after activity onset
74	Aronin et al., 1990	Rats	12/12 L/D	Diurnal light	600lx		
75	Yan et al., 2002	Rats	12/12 L/D after which constant darkness	Diurnal light Fluorescent light pulse	300lx 600lx	30 min	4 hours after subjective lights off
76	Munch et al., 2002	Hamsters	12/12 L/D after which constant darkness	Diurnal light Light pulse	ND ND	1.5 hours	2 and 7 hours after subjective lights off
77	Thompson et al., 2008	Mice	12/12 L/D	Diurnal light Light pulse	380µW/cm2 380µW/cm2	20 min and 60 min	4 hours after lights off
78	Opperhuizen et al., 2017	Rats	12/12 L/dLAN	Mixed white diurnal light/ red dLAN Mixed white light pulse Mixed white light pulse	<150lx/5lx ~125lx 5, 20, 50 or 150lx	2 hours 2 hours	2 and 8 hours after lights off 2 hours after lights off
79	Cailotto et al., 2009	Rats	12/12 L/dLAN after which constant darkness	White diurnal light/red dLAN Light pulse	~200lx/<1lx ~200lx	1 hour	2 and 8 hours after lights off

Table S1. Light conditions used in studies of acute effects of white light on metabolism (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
80	Mohawk et al., 2007	Rats	12/12 L/D after which constant darkness	Diurnal light Light pulse or red dLAN for control sampling Light pulse or red dLAN for control sampling	~250lx ~250lx or ND ~250lx or ND	1 hour 1 hour	1, 5, 9, 13, 17 and 21 hours after subjective lights on 18 hours after subjective lights on
81	Kiessling et al., 2014	Mice	12/12 L/D after which constant darkness	Diurnal light Light pulse	100lx 35, 350 or 3500–4000lx	30 min	0, 2, 4, 6, 8, 10, 12, 15, 16, 18, 21/22, 23 hours after subjective lights on
82	Ishida et al., 2005	Mice	12/12 L/D after which constant darkness	Fluorescent diurnal light. Incandescent light pulse white light beam of glass fiber illumination apparatus	300lx, 400lx 300, 900 and 2000lx	30 min ND	2 hours after subjective lights off ND
85	Aras et al., 2019	Mice	12/12 L/D	Diurnal light Light pulse	ND 400lx	1 hour for 30 days	5 hours after lights off
86	Cho et al., 2018	Humans	ND	Diurnal light 5779.1K broad spectrum white light pulse (peak λ: 463.6 nm, center λ: 467.6 nm, centroid λ: 554.3 nm, dom λ: 501.4 nm)	ND 5 or 10lx	8 hours	1 whole night
87	Scheer et al., 1999	Humans	1 hour light or no light, 1 night complete darkness, 1 hour light or no light	Diurnal light White light pulse	ND 800lx	1 hour	1 hour before sleep onset or immediately after awakening
87	Scheer et al., 1999	Humans	16 hours of diurnal light after which 8 hours of darkness	Daylight mixed with artificial light for diurnal light	ND or 150lx	16 hours	1 whole day

Table S1. Light conditions used in studies of acute effects of white light on metabolism (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
88	Scheer et al., 1999	Humans	16 hours of diurnal light after which 8 hours of darkness	Diurnal light ND White light pulses	ND 100lx after which 800lx	10 min after which 10 min	15, 0, 8, 20, 24 hours after awakening
89	Versteeg et al., 2017	Humans	2 hours 200lx, 8 hours darkness, 6 hours light exposure	Diurnal light Morning light pulse	200lx 4000lx or 10lx	6 hours	Immediately after awakening
90	Scheer et al., 2004	Humans	16 hours of diurnal light after which 8 hours of darkness	Daylight mixed with artificial light for diurnal light	ND or 150lx	16 hours	1 whole day
90	Scheer et al., 2004	Humans	16 hours of diurnal light after which 8 hours of darkness	Diurnal light ND White light pulses	ND 100lx after which 800lx	10 min after which 10 min	15, 0, 8, 20, 24 hours after awakening
	Albreiki et al., 2017	Humans	ND	Diurnal light Fluorescent white light pulse	ND <5lx (0.001 w/m2 a 0.0008 w/m2) or 500lx (0.98 w/m2 and 0.73 w/m2)	12 hours	2 whole nights, start 06:00 PM, end 06:00 AM
89	Gil-Lozano et al., 2015	Humans	600lx for 5 hours, 10 hours dLAN or light exposure, 600lx for 6 hours	White diurnal light/red dLAN White light pulse	600lx/<2lx 600lx	10 hours	

All light used is white light unless specified otherwise.

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism.

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
36	Coomans et al, 2013	Mice	12/12 L/D after which constant darkness, then constant light	Diurnal light Constant light	ND >180lx	>7 days	After 3 days of darkness
37	Otah et al., 2005	Mice	12/12 L/D after which constant light	Diurnal light ND Constant light ND	ND 350lx	3-5 months	After 2 weeks of L/D
38	Fonken et al., 2010	Mice	16/8 L/D after which constant light or 16/8 L/dLAN	Diurnal light Constant light dLAN ND	~150lx ~150lx ~5lx	8 weeks	After 1 week of L/D
39	Madahi et al., 2018	Rats	12/12 L/D after which constant light, then 12/12 LD	Diurnal light ND Constant light ND	ND 250-350lx	23 days after which 67 in LD	As off birth
40	Depres-Brummer et al., 1995	Rats	12/12 L/D after which constant light, after which constant darkness, then constant light	Fluorescent diurnal light Constant fluorescent light	200-300lx 200-300lx	12 weeks after 4 weeks in constant darkness, after which 2 weeks in constant light	After 56 days of L/D and then after 4 weeks of constant darkness
42	Abílio et al., 1999	Rats	Constant light	Constant light ND	ND	36 days	ND
43	Fonken et al., 2009	Mice	16/8 L/D after which constant light	Fluorescent diurnal light Constant fluorescent light	ND ND	3 weeks	After 1 week of L/D

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism. (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
44	Qian et al., 2013	Rats	12/12 L/D after which constant light	Diurnal light ND Constant light ND	ND ND	10 weeks	After 2 weeks of L/D
45	Koronowski et al., 2019	Mice	12/12 L/D after which constant darkness	Diurnal light ND Constant darkness	ND	3-4 days	After 3-4 days of L/D
46	Fonken et al., 2013	Mice	14/10 L/D after which 14/10 L/dLAN	White LED diurnal light Cool white LED dLAN	~150lx 5lx	4 weeks	After 1 week of L/D
47	Ikeno et al., 2014	Hamsters	16/8 L/D after which 8/16 L/dLAN	Diurnal light ND dLAN ND	150lx 5lx	4, 8 or ~20 weeks	69-93 days after 16/8 L/D
48	Stenvers et al., 2016	Rats	12/12 L/D after which 12/12 L/dLAN	Fluorescent diurnal light LED dLAN	150-200lx 5lx	ND	ND
48	Stenvers et al., 2016	Rats	12/12 L/D after which specific dLAN or dark conditions, see duration	Fluorescent diurnal light Fluorescent dLAN Fluorescent dLAN Fluorescent dLAN	150-200lx 5lx 5 lx 5lx	2 weeks 30 days, 20 days D/D, 30 days L/D, 10 days D/D 16 days dLAN, after 22 days L/L 32 days dLAN, 14 days L/D, 16 days dLAN	7-10 days after L/D 10 days after L/D >10 days after L/D

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism. (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
49	Borniger et al., 2014	Mice	14/10 L/D after which 14/10 L/dLAN	Diurnal light ND Fluorescent dLAN LED dLAN	150lx ~5lx ~5lx	2 weeks 24 h, in metabolic chamber	After 4 weeks L/D and 24 h in metabolic chamber After 4 weeks L/D, after 2 weeks in fluorescent dLAN
50	Bedrosian et al., 2013	Hamsters	16/8 L/dLAN	Cool white fluorescent diurnal light Cool white fluorescent dLAN	150lx 5lx	ND	ND
51	Bedrosian et al., 2013	Hamsters	16/8 L/dLAN	Cool white fluorescent diurnal light Cool white fluorescent dLAN	150lx 5lx	4 weeks	ND
52	Panagiotou et al., 2019	Mice	12/12 L/D after which 12/12 L/dLAN	Fluorescent diurnal light Fluorescent dLAN	75lx 5lx	12 weeks	ND
53	Molcan et al., 2019	Rats	12/12 L/D after which 12/12 L/dLAN	Diurnal light ND dLAN ND	150lx 1-2lx	2 or 5 weeks	After 4 weeks of L/D
54	Rumanova et al., 2019	Rats	12/12 L/D after which 12/12 L/dLAN	Warm white diurnal light (3000K) dLAN ND	100-150lx ~2lx	2 or 5 weeks	After 1 month of L/D
55	Fonken et al., 2013	Mice	14/10 L/D after which 14/10 L/dLAN	Diurnal light ND dLAN ND	150lx 5lx	4 or 6 weeks	After 1 week of L/D

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism. (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
55	Fonken et al., 2013	Mice	14/10 L/D after which 14/10 L/dLAN	Diurnal light ND dLAN ND	150lx 5lx	5 nights	After 3 weeks of L/D
56	Aubrecht et al., 2015	Mice	16/8 L/D after which 16/8 L/dLAN	Diurnal light ND dLAN ND	~150lx ~5lx	6 weeks	After 1 week of L/D
57	Russart et al., 2019	Mice	L/dLAN 14/10 after which 14/10 L/D	Diurnal light ND dLAN ND	150lx 40lx	10 weeks, L/D for 4 weeks	ND
58	Rohling et al., 2011	Rats	12/12 L/D after which a 6 h delay or a 9 h advance of L/D regimen	Diurnal light ND	ND	24 h L/D after phase advance/ delay	'Lights on' was delayed 6 hours or advanced 9 hours after >3 weeks in L/D
60	Koo et al., 2016	Humans	Satellite data on outdoor ALAN exposure in South Korea, year 2015	High ALAN Low ALAN	>45 'digital numbers' <45 'digital numbers'	Time resolution of 1 year	Data collection from 8:30-10 PM
61	Xiao et al., 2020	Humans	Satellite data on outdoor ALAN exposure in the USA, year 1996	Q1 (lowest outdoor ALAN) Q2 Q3 Q4 Q5 (highest outdoor ALAN)	median 4.3nW/cm/sr median 12.6nW/ cm/sr median 25.1nW/ cm/sr median 3.0nW/cm/sr median 78.2nW/ cm/sr	ND	ND

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism. (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
62	Obayashi et al., 2013	Humans	Indoor LAN was measured every minute during the 'in-bed period' for two nights	LAN group Dim group	Avg >3lx, exposure to >10lx was 50.5min, exposure to >100lx was 42.3min on avg. Avg <3lx, exposure to >10lx was 2min, exposure to >100lx was 0min on avg	Mean sleep duration 423.6min Mean sleep duration 418.4min	Mean habitual bedtime 10:41 PM Mean habitual bedtime 10:30 PM
63	Obayashi et al., 2015	Humans	Indoor LAN was measured every minute during the 'in-bed period' for two nights	Q1 (lowest LAN) Q2 Q3 Q4 (highest LAN)	<0.1lx 0.1-0.7lx 0.7-3.3lx >3.3lx	Mean sleep duration 480.5min Mean sleep duration 484.4min Mean sleep duration 501.4min Mean sleep duration 513.1min	Mean habitual bedtime 10:28 PM Mean habitual bedtime 10:53 PM Mean habitual bedtime 10:36 PM Mean habitual bedtime 10:20 PM
64	Park et al., 2019	Humans	Inquiry of LAN conditions via questionnaire in the USA and Puerto Rico, year 2003-2009	LAN No LAN	Small nightlight, light outside room or light/ television in the room. No light reported or sleeping with mask	Mean sleep duration 7.2, 7.1 or 6.7 hours Mean sleep duration 7.1 hours	ND

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism. (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
65	McFadden et al., 2014	Humans	Inquiry of LAN conditions via questionnaire in the UK, year 2003-2012	Highest level Middle level Darkest level	Light enough to read or light enough to see across the room, but not read' 'Light enough to see your hand in front of you, but not to see across the room' 'Too dark to see your hand, or you wear a mask'	ND	ND
66	Rybnikova et al., 2016	Humans	Satellite data on global outdoor ALAN exposure, year 2010	Avg national ALAN was determined by matching ALAN satellite data with population density	Countries ranged from 5-1000 dimensionless ALAN units	ND	ND
67	Abay et al., 2018	Humans	Satellite data on outdoor ALAN exposure in Nigeria, year 2008 and 2013	Highest ALAN measured Lowest ALAN measured	63 'digital numbers' 0 'digital numbers'	ND	ND
68	Malhotra et al., 1998	Humans	Outdoor lighting of Antarctica, indoor lighting regimen ND	Outdoor light in June Outdoor light in December	ND	0 hours 24 hours	All day
69	Campbell et al., 1975	Humans	Outdoor lighting of Antarctica, indoor lighting regimen ND	ND	ND	ND	ND

Table S2. Light conditions used in studies on effects of chronic white light and dim light-at-night on metabolism. (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Intensity	Duration	Timing
70	Campbell et al., 1975	Humans	Outdoor lighting of Antarctica, indoor lighting regimen ND	ND	ND	ND	ND
71	Sawhney, et al., 1995	Humans	Outdoor lighting of Antarctica, indoor lighting regimen ND	Outdoor light May 31st - July 20th Outdoor light November 22nd -3rd week of January	ND	0 hours 24 hours	ND

All light used is white light unless specified otherwise.

Table S3. Light conditions used in studies of effects of light of different wavelengths on metabolism.

Ref. #	Reference	Species	LD regimen	Type of light exposure	Wavelength	Intensity/ Irradiance	Photon flux	Duration	Timing
50	Bedrosian et al., 2013	Hamster	16/8 L/dLAN	White diurnal light	broad spectrum	150lx	ND	8 hours for 4 weeks	whole night
				Fluorescent white LAN	broad spectrum	5 lx			
				Blue LAN	peak at ~480nm	5lx			
				Red LAN	>600nm	5lx			
51	Bedrosian et al., 2013	Hamster	16/8 L/D after which 3 days of constant darkness	White diurnal light	broad spectrum	150lx	ND	30 min	1 hour before subjective lights off
				White light pulse	broad spectrum	150lx			
				White light pulse	broad spectrum	5lx			
				Blue light pulse	peak at ~480nm	5lx			
				Red light pulse	>600nm	5lx			
72	Nagai et al., 2019	Mice	12/12 L/D	White diurnal light	ND	100–300lx	ND	30 min	2 hours after lights off
				White LED light pulse with blue light cut shield		10lx			
				Blue-filtered LED light pulse		10lx			
83	Masís-Vargas et al, 2020	Rats	12/12 L/D	Fluorescent white diurnal light/red dLAN	<150lx/5lx		$2.49x10^{13}$/ cm^2 /s	2 hours	2, 4, 6 or 8 hours after lights off
				Blue light pulse	peak at 455nm	10.76 µW/cm2	$2.75x10^{13}$/ cm^2/s		
				Green light pulse	peak at 595nm	9.54 µW/cm2	$2.4 x10^{13}$/ cm^2/s		
				White light pulse	peak at 520nm	9.48 µW/cm2			

Table S3. Light conditions used in studies of effects of light of different wavelengths on metabolism (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Wavelength	Intensity/ Irradiance	Photon flux	Duration	Timing
84	Fan et al, 2018	Mice	12/12 L/D	Diurnal light ND Blue LED light pulse Green LED light pulse	ND peak at 463 nm peak at 522nm	ND 4μW/cm^2 3μW/cm^2	 9.32 x10^{15}/ cm^2/s 7.88x10^{15}/ cm^2/s	1 hour for 10 days	1 hour after lights on
93	Pilorz et al., 2016	Mice	12/12 L/D	White diurnal light Violet light pulse Blue light pulse Green light pulse	ND 405nm 470nm 530nm	250lx	14.9 log quanta	1 hour	2 hours after lights off
78	Opperhuizen et al., 2017	Rats	12/12 L/D	Fluorescent white diurnal light/red dLAN Blue light pulse Green light pulse Red light pulse	<150lx/5lx peak at 457nm peak at 520nm peak at 633nm	52.95μW/cm^2 247.24μW/cm^2 23.23μW/cm^2	1.28×10^{14}/ cm^2/sec 1.28×10^{14}/ cm^2/s 0.74×10^{14}/ cm^2/s	2 hours	2 or 8 hours after lights off
94	Evans et al., 2007	Hamsters	14/10 L/D or 8/16 L/D, after which constant green light	Diurnal light ND Dim green light	ND peak at 560nm	100-300lx 1.3 × 10^{-9} W/ cm^2	3.7×10^9 photons/ cm^2/s	continuous light for 6 weeks	as from lights off
94	Evans et al., 2007	Hamsters	14/10 L/D	Diurnal light ND Green light pulse Green light pulse	ND peak at 560nm peak at 560nm	>100lx 1.3×10^{-9}W/cm^2 1.3×10^{-9} W/ cm^2	3.7×10^9 photons/ cm^2/s 3.7×10^9 photons/ cm^2/s	5 or 8 hours 2 hours	as from lights off 6 hours after lights off

Table S3. Light conditions used in studies of effects of light of different wavelengths on metabolism (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Wavelength	Intensity/ Irradiance	Photon flux	Duration	Timing
95	Zubidat et al., 2011	*Microtus Socialis*	8/16 L/D	Diurnal light ND Blue light pulse Yellow light pulse Red light pulse	ND 479nm 586nm 679nm	ND 293μW/cm^2 293μW/cm^2 293μW/cm^2	ND	30 min	8 hours after lights off
97	Masís-Vargas et al., 2019	*Arvicanthis Ansorgei*	12/12 L/ D	White diurnal lighting/red Blue light pulse	ND ~490nm	ND/5lx 4.26 μW/cm^2	1.06x10^{13}/ cm^2/s	1 hour	2 hours after lights off
96	Dauchy et al., 2015	Rats	12/12 L/ dLAN	Fluorescent white diurnal light red LAN	ND >620nm	123μW/cm^2 3.3μW/cm^2	ND	8 hours for 6 weeks	whole night
99	Brainard et al., 2008	Humans	darkness as from midnight	Blue light pulses Blue light pulse Blue light pulse	420nm 420nm 460nm	ND	10^{10} to 10^{14} photons/ cm^2 1.21×10^{13}/ cm^2/s 1.21×10^{13}/ cm^2/s	90 min	2 am
100	Thapan et al., 2001	Humans	12/12/ L/D	violet, blue and green light pulses	424, 456, 472, 496, 520 and 548 nm	5-8 different irradiances from 0.70-65 μW/cm^2	ND	30 min	4,5 hours after lights off
101	Cajochen et al., 2005	Humans	1,5h dLAN, 2h dark, 2h light exposure, 1,5h dLAN, 8h dark	dim polychromatic white light Blue light pulse Green light pulse	ND 460nm 550nm	2lx 12.1μW/cm^2 10.05μW/cm^2	2.8×10^{13}/ cm^2/s 2.8×10^{13}/ cm^2/s	2 hours	2 hours after lights off

Table S3. Light conditions used in studies of effects of light of different wavelengths on metabolism (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Wavelength	Intensity/ Irradiance	Photon flux	Duration	Timing
102	Figueiro et al., 2009	Humans	45 min of dark, 45 min of light, 45 min of dark, 45 min of light	Blue light pulse Red light pulse	peak 470nm peak 630nm	10μW/cm^2 and 40μW/cm^2 4.7μW/cm^2 and 19μW/cm^2	ND	45 min	45 min after lights off
103	Cajochen et al., 2011	Humans	30 min dim red light followed by 5 times 50 min screen exposure after which 10 min dim red light	red light LED screen non-LED screen	ND 400-480nm 400-480nm	<4lx 0.241 Watt/ (steradian×m^2) 0.099 Watt/ (steradian×m^2)	ND	5 hours	4.5 hours prior to usual bedtime
104	Figueiro et al., 2011	Humans	30 min dim red light, then 2 hours of computer use	red light computer monitor computer monitor with blue light enhancing goggles computer monitor with blue light filtering goggles	ND ND peak 470nm >525nm	<2lx 7lx 40lx (40μW/ cm^2) 7lx	ND	2 hours	2 hours after lights off
105	Heo et al., 2017	Humans	L/dLAN	dLAN conventional LED smartphone LED smartphone with blue light suppression	ND ND <450 and >470 nm	<3lx ND ND	ND	2.5 hours	30 min after lights off
106	Münch et al., 2006	Humans	1.5 dLAN, 2 h dark, 2 h light exposure, 105 min of dLAN	dim light blue light pulse green light pulse	ND 460nm 550nm	2lx	2.8×10^{13}/ cm^2/s	2 hours	2 hours after lights off

Table S3. Light conditions used in studies of effects of light of different wavelengths on metabolism (cont.)

Ref. #	Reference	Species	LD regimen	Type of light exposure	Wavelength	Intensity/ Irradiance	Photon flux	Duration	Timing
107	Kayaba et al., 2014	Humans	2 hours dark, 2 hours light exposure, 8 hours of dark, 4.5 hours of diurnal light	fluorescent diurnal light	ND	270lx			
				blue light pulse	465nm	7.02 μW/cm^2	ND	2 hours	2 hours after lights off
72	Nagai et al., 2019	Humans	ND	Blue light shield eyewear	filters 100% of 395-490nm light and 30% of all light	ND	ND	2-3 hours for 1 month	2-3 hours before bedtime

Part II

EFFECTS OF LIGHT ON GLUCOSE METABOLISM AND FOOD INTAKE IN A DIURNAL RODENT

Chapter 3

White light decreases glucose tolerance and reduces sugar and fat consumption in *Arvicanthis ansorgei.*

Anayanci Masís-Vargas

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Abstract

Our industrialized society has increased the exposure to light at night (LAN) enormously, due to the increasing technology and alternative working schedules or shift work. Evidence has shown that this may impact human health negatively by disrupting the function of the biological clock, modifying sleep-wake cycles and sleeping patterns, and causing metabolic dysregulation. Previously we reported white light at night acutely impairs glucose tolerance in Wistar rats, a nocturnal rodent. However, due to our diurnal nature, we wanted to see if the same effect was produced in the diurnal rodent Arvicanthis ansorgei (Sudanian grass rats). Hence, this study aimed to evaluate the acute effects of white artificial light at night (wALAN) at the beginning of the night on glucose metabolism and food intake in both male and female diurnal Sudanian grass rats fed either regular chow or a free choice high-fat high sucrose diet (HFHS). Chow fed female Arvicanthis exposed to a 1-hour of wALAN at ZT 14 showed higher areas under the curve during an oral glucose tolerance test (OGTT) without significant changes in plasma insulin. Conversely, in HFHS fed animals, wALAN did not significantly affect glucose tolerance. When food intake was analyzed, no significant changes in chow-fed animals were found, regardless of their sex. Surprisingly, in HFHS animals a decreased sucrose intake during the light phase and lower fat consumption during the dark phase was observed in the female Arvicanthis, while in males only an interaction effect was observed with sugar consumption during the light phase. These findings provide further proof for the deleterious effects of ALAN on glucose metabolism, open new questions regarding the effects of light on food intake, highlighting the importance of addressing sex differences in studies of the effects of light at night, metabolism and circadian biology.

Introduction

The World Health Organization estimates that in 2016 more than 1.9 billion adults were overweight, and of those 650 million were obese. In most countries, obesity accounts for more deaths than underweight (1). One of the common health consequences of overweight and obesity is type 2 diabetes (T2D). The prevalence of T2D rose from 108 million in 1980 to 422 million in 2014 (2). The causes of obesity and T2D are multiple and comprise several underlying physio-pathological mechanisms, however, lifestyle changes can not only prevent them but also are part of the main treatment.

Over the past century, several lifestyle changes have occurred in our society. These include greater availability of processed and highly palatable food that is rich in sugar and fat, less physically demanding jobs, and more industrialized means of transportation resulting in sedentary lifestyles. Additionally, access to new technologies has transformed our life into a 24-hour society, where shift work and exposure to artificial light at night (ALAN) is widely present across the globe. All these societal changes are thought to contribute to the increasing epidemy of overweight, obesity, and T2D.

Evidence from cross-sectional epidemiological and experimental studies in humans, as well as in animals, showing that sleep disruption and exposure to artificial light at night (ALAN) are risks factors for the development of obesity, T2D, and other metabolic disturbances, mainly due to circadian rhythms disruption, has been reviewed extensively elsewhere (3–5).

The circadian control of glucose homeostasis and metabolism is now well established (6–8). Even though the majority of our endogenous rhythms can oscillate without the presence of an external light/dark cycle, environmental light is the most important signal to entrain the intrinsic circadian rhythms to the 24-hour rhythm of the rotation of our planet. On the other hand, exposure to light at the wrong time of the day, i.e. during the dark phase, can be a potent circadian disruptor (5), changing the normal secretion of melatonin (9) and corticosterone (cortisol in humans) (10), altering the expression of circadian genes in the hypothalamus (11), pineal gland (12), adrenal (10) and liver (13), and strongly impacting glucose metabolism (14).

Mammals perceive light by rods, cones, and intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina, provoking both image- and non-

image-forming visual responses. The anatomical projections of the ipRGCs reach the suprachiasmatic nucleus (SCN) in the hypothalamus, where the main circadian clock is located, and other regions of the brain involved in the control of energy and glucose metabolism, reward and food intake (15–17). Together with the evidence previously mentioned that ALAN is a risk factor for metabolic diseases, we hypothesized that ALAN could alter food intake and glucose metabolism.

In a previous study from our group, it was shown that white light at night impairs glucose tolerance in Wistar rats by reducing beta-cell sensitivity and glucose uptake (14). However, the nocturnal nature of the Wistar rats is an important and complicating factor to consider when extrapolating these findings to the human situation. Hence, in the present study, we used athe diurnal rodent Arvicanthis ansorgei, to study the acute effects of white ALAN on glucose metabolism and on food intake of regular chow and palatable diets in both males and females.

Materials and Methods

Ethical approval

All the experiments were performed following the rules of the European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French Department of Agriculture (license no. 63-378 to JM).

Animals and housing

Thirty-five 12-weeks-old male (n=17) and female (n=18) Sudanian grass rats (Arvicanthis ansorgei) obtained from the platform Chronobiotron (UMS-3415, CNRS and the University of Strasbourg) with an average weight of 139.2 ± 15.9 g for males and 120.8 ± 13.6 g for females; were housed individually in Plexiglas cages with sawdust bedding, chow food, and tap water ad libitum. Animals were maintained under a controlled ambient temperature of 21-23 ºC, a relative humidity of 23-27% and a 12-h light/dark cycle (lights on at 07:00 hours, Zeitgeber time 0 (ZT0); lights off 19:00 hours, ZT12) with a dim red light at night (5 lux).

Daily food intake rhythm

Chow food (SAFE, 105, U8400G10R, Augy, France; 2.85 kcal/g, 23%

proteins, 65% carbohydrates, and 12% fat) and tap water was provided ad libitum to all animals.

Free choice high-fat high sucrose diet

Immediately after a week of acclimatization with chow food, animals were randomly assigned to two groups fed with different diets. The control group (8 males and 8 females) remained on the chow diet mentioned before and tap water. The free choice High-Fat High-Sugar (HFHS) group (9 males and 10 females) received in addition to the chow food and the tap water, a cube of saturated fat (beef lard, Vandemoortele, France; 9 kcal/g) and a bottle of 10% sugar water (commercial grade sucrose and tap water, 0,4 kcal/ml). Both groups were kept on their respective diets for 6 more weeks and animals were weighed weekly.

Table 1. *Spectral sensitivity of the white light condition used in the whole experiment.*

Retinal photopigment complement	White light
S-Cone (rNsc [λ])	7.19
Melanopsin (rNz [λ])	148.60
Rod (rNz [λ])	170.10
M-cone (rNmc [λ])	184.87
Irradiance (μW/cm^2)	80.50
Photon flux (1 cm^{-2} s^{-1})	2.23 x 10^{14}

Data based on calculations made with the Rodent Toolbox provided by Lucas et al (18) and the spectral power distributed by the manufacturer.
The first four rows represent the weighted contribution of rodent retinal photopigments (S-cone, ipRGC [melanopsin], rod, M-cone) in α-opic rodent lux. The last two rows (irradiance, photon flux) represent unweighted characteristics of the fluorescent lamp.

Light exposure protocol

To study the effects of the exposure to white light at night (wALAN) on glucose metabolism and food intake, at week 3 animals from both diet groups were randomly subdivided into two subgroups, dark controls (used also as controls for study the effects of blue light), and white light exposed. At ZT14 (two hours after lights off) individual cages of the animals from the light group were moved to a PVC cage (120cm x 60cm x 60cm), which had a fixed white light fluorescent tube lamp placed in the middle of the

ceiling and they were exposed to a 1-hour pulse of white light (see Table 1 for the irradiance spectrum (18)). Then the dark condition was restored and animals from the light group were moved back to their racks. Protocols to evaluate glucose metabolism, food intake, and light effects on hormones were applied immediately thereafter as described below. Between each light exposure, animals were left undisturbed for one week.

Oral glucose tolerance test (OGTT)

Immediately after animals were returned from the exposure to either the first pulse of white light or being kept in control dark conditions respectively, an OGTT was performed starting at ZT15. For this, all Arvicanthis were fasted for 6 hours before the test, i.e. from ZT9 onwards. Blood was taken from the tip of the tail at 0, 15, 30, 90, and 120 minutes for the measurement of whole blood glucose concentrations using an Accu-Chek Performa Nano glucometer (Roche Diabetes Care Limited). Immediately after the 0 minutes sample, D-glucose (2g of D-glucose per kg of body weight dissolved in 0.9% saline) was orally administered by gavage. Data of the OGTT were expressed as the delta between the basal (t=0) glucose and the measurement at each time point. Also, the increase of plasma glucose was analyzed by calculating the area under the curve (AUC) from the time of the baseline to the 120 min measurement, using the trapezoidal rule (19).

Food intake evaluation

One week after the OGTT experiment, animals received the same treatment as described above for the second time. This time all food components from both diet groups were weighed every 12 h during 2 LD cycles, starting at ZT12 of the dark phase before the light exposure until ZT12 of the light phase after, to measure possible changes in the daily food intake patterns due to the white light pulse at the beginning of the dark phase.

Blood sampling and serum analysis

During the last week of the experiment, a third light pulse was given and animals were deeply anesthetized immediately after (i.e. ZT15) with an overdose of isoflurane to recover the blood from the left ventricle with a cardiac puncture, using a 5 ml syringe with a 25G needle. Immediately after animals were perfused transcardially with PFA 4% wt/vol in 0.1 M phosphate buffer (pH=7.2) to recover brains for analysis in future studies.

Glucose levels in blood were determined as described before using a drop of the extracted blood. Then the blood was decanted into 15 ml Corning tubes containing 100 µl of 4% EDTA. Samples were centrifuged 5000 rpm at 4 ºC for 10 min, and plasma was stored at -80 ºC for determination of plasma insulin, corticosterone and leptin concentrations. Insulin was measured using an ELISA procedure with a Rat/Mouse Insulin ELISA Kit (EZRMI-13K, Merck-Millipore, Germany). The limit of sensitivity of the insulin kit was 0.1 ng/ml. Corticosterone was measured using an EIA procedure with a Rat/Mouse Corticosterone EIA Kit (AC-14F1, Immunodiagnostic Systems Ltd, UK). The limit of the sensitivity of the corticosterone kit was 0.55 ng/ml. The levels of leptin in plasma were measured using an ELISA procedure with a Rat Leptin Kit (EZRL-83K, Merck-Millipore, Germany). The limit of sensitivity of the leptin kit was 0.04 ng/ml.

Statistical analysis

Results were analyzed with and without considering the sex of the animals. All data are expressed as means ± standard deviation. The area under the curve (AUC) for the OGTT was calculated using the trapezoid rule. Unpaired two-tailed t tests were used to detect group differences in baseline concentration of fasting glucose before performing the OGTT, AUCs, non-fasted levels of glucose, and plasma hormones after the light exposure.

A two-way mixed model ANOVA was used to test the effects of the diets and light condition (Treatments), the effect of Time (samples during the OGTT or light and dark phases for the feeding measurements), and their Interaction on glucose tolerance and food intake pattern in response to the diet. Glucose and food intake responses to the white light exposure in either diet conditions were analyzed also with a mixed model ANOVA. A Sidak's multiple comparisons test was used as a post-hoc analysis. GraphPad Prism version 8.0 for Windows (GraphPad Software, La Jolla, CA, USA) was used for the whole analysis using a significance level of $p < 0.05$.

Results

Acute effects of white light in glucose metabolism

Baseline levels of glucose before starting the OGTT were similar for all diet and light groups (Figs.1A-1C and 2A-2C). In chow-fed animals, white light exposure caused a significant increase in plasma glucose 15 min after the oral administration of the glucose bolus (Fig.1D, $F_{(5,75)}=2.655$,

p<0.0290, post-hoc p=0.0289 for 15 min), as well as a significant increase in AUC (Fig.1G; $t_{(15)}$=3.229, p=0.005). When analyzed by sex there were no differences in the OGTT, however, the AUC was significantly higher in females (Fig.1I; $t_{(7)}$=3.982, p=0.005) exposed to white light. In HFHS-fed animals, no significant differences were found in plasma glucose or AUC (Figs.2D to 2I). These results indicated important sex and nutritional status dependent effect of white light on glucose metabolism.

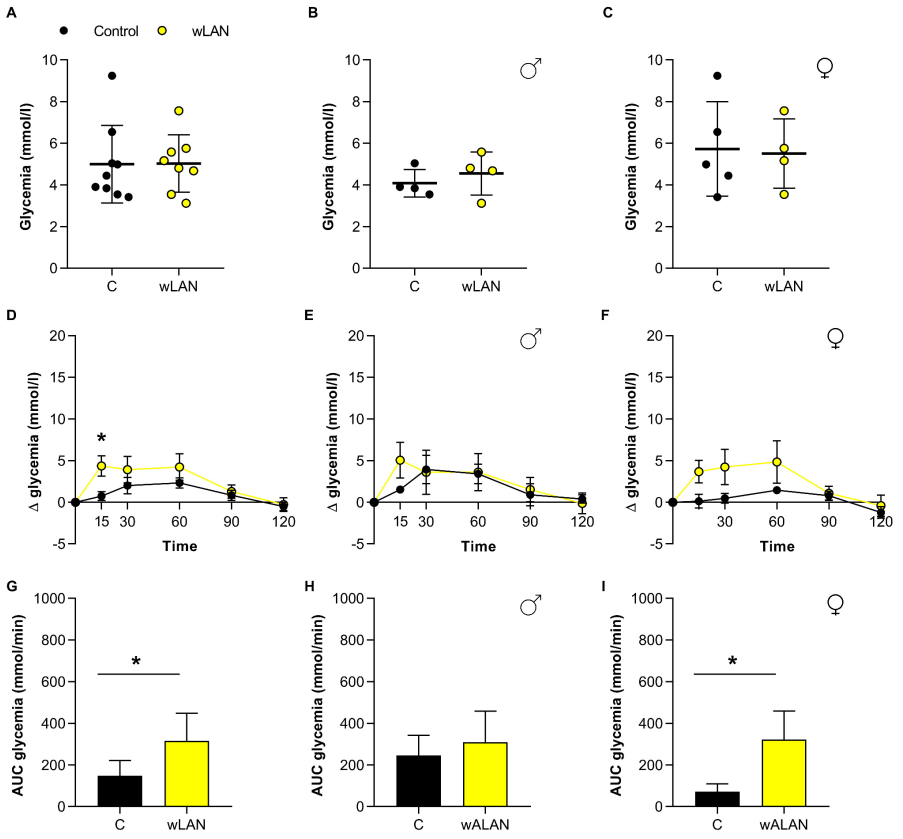

Figure 1. *Glycemia in chow-fed Arvicanthis. Baseline concentrations of glucose were not different between the dark control and wALAN in all animals (A), males (B) or females (C). wALAN from ZT14-15 in fasted grass rats affected glucose tolerance in all animals at t=15 min of the glucose bolus (D; *Post-hoc test, p=0.0289) and significantly increased the AUC (G; *t-test, p=0.005). The white light pulse did not affect the glucose response (E) nor the AUC (H) in male Arvicanthis. In females, a significantly higher AUC was observed (I; *t-test, p=0.005), even though no differences in the glucose response was detected (F).*

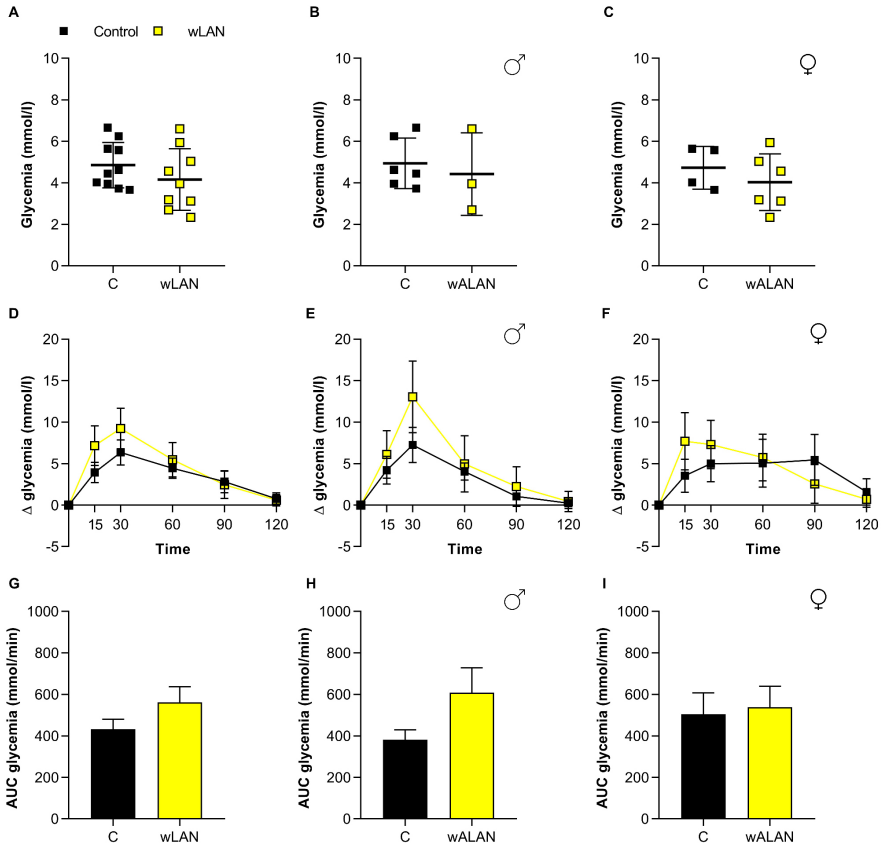

Figure 2. *Glycemia in HFHS-fed Arvicanthis. Baseline concentrations of glucose were not different between the dark control and wALAN sessions, in all animals (A), males (B) or females (C). wALAN from ZT14-15 in fasted grass rats did not affect glucose tolerance in all animals regardless of sex (D-F) nor significantly increased the AUC (G-I).*

Acute effects of white light on food intake

Next, we determined whether acute exposure to wALAN would change food intake. In Fig.3 food intake is shown as the difference in food consumption between the light and dark phases before, during, and after the wALAN exposure. When analyzing the total amount of kilocalories per gram of body weight, no significant effects of wALAN were found in the chow-fed group when analyzed both sexes together (Figs.3A), but when analyzed separately an Interaction effect was found in males (Fig. 3B: $F_{(1,12)}$=6.943, p=0.021, no significant post-hoc) but not in females (Fig. 3C). On the other

hand, in the HFHS-fed animals an Interaction effect was found in all of them (Fig.3D; $F_{(1,16)}$=24.34, p=0.0001, post-hoc p=0.007 for light phase). When it was analyzed by sex, we found an Interaction effect in males (Fig. 3E; $F_{(1,7)}$=6.236 p=0.041, no significant post-hoc) while in females we found both a significant effect of the wALAN and an interaction effect during both phases (Fig. 3F; Treatment $F_{(1,7)}$=10.94, p=0.013; Interaction $F_{(1,7)}$=18.72, p=0.003, post-hoc p=0.003 for light phase, p=0.040 for dark phase).

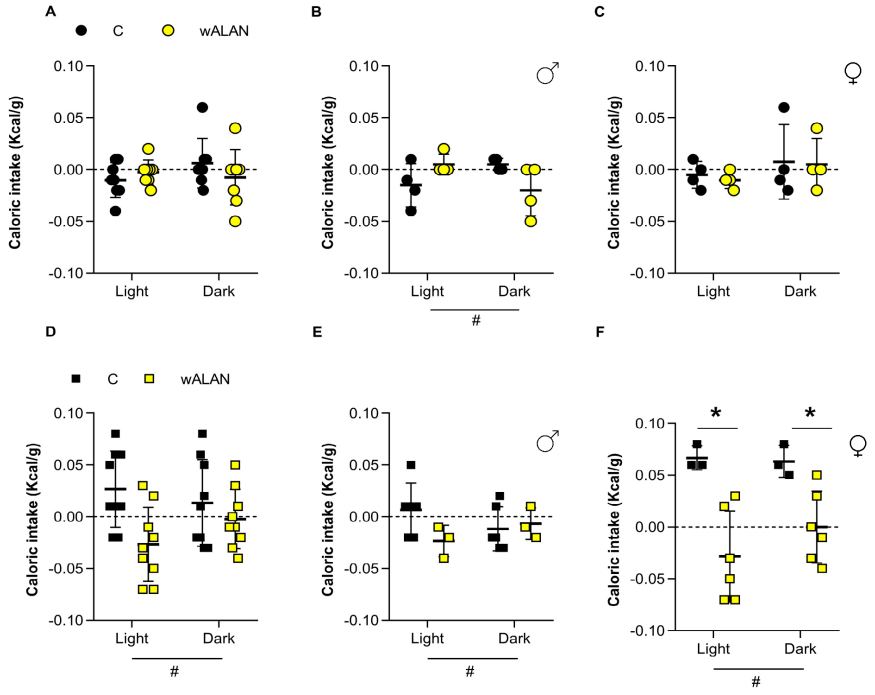

Figure 3. *Caloric intake in Arvicanthis during nocturnal light exposure. wALAN did not change the total calorie intake in all animals fed a chow diet regardless of sex and phase (light vs. dark phase) (A-C), but a significant Interaction effect (p=0.021) was found in males (B). The white light pulse did not affect the total caloric intake of all animals fed with a HFHS diet, but an interaction effect was found during the light phase (D; # Interaction, post-hoc test p=0.007),. When analyzed separately, males showed a significant Interaction effect (E; p=0.041), due to a lower intake in the light phase and higher intake during the dark phase. In HFHS-fed females we found a significant effect of the light Treatment in both phases and a significant Interaction effect, probably due to the stronger wALAN-induced decrease in the light phase (F; Treatment, p=0.013, Interaction, p=0.003). # indicates an Interaction effect, * indicates a Treatment effect.*

Additionally, when the different components of the HFHS diet were analyzed separately (Figs.4A-4I), we found an Interaction effect for sugar consumption in all animals (Fig. 4D; $F_{(1,16)}$=5.753, p=0.029). This effect was also found in males when analyzed by sex (Fig. 4E; $F_{(1,7)}$=18.05, p=0.003, post-hoc p=0.035 for light phase), indicating that sugar consumption was lower in the white light-exposed animals during the light phase. In females we observed a significantly lower sugar consumption during the light phase as an effect of the wALAN exposure (Fig.4F; $F_{(1,14)}$=14.08, p=0.021, post-hoc p=0.023 for light phase). In the same way, an effect of light exposure was observed during both the light and dark phase on fat consumption (Fig.4G, $F_{(1,16)}$=6.408, p=0.022, no significant post-hoc), but when analyzed by sex, a significantly lower consumption of fat after light exposure was only observed in female animals during the dark phase (Fig.4I, $F_{(1,7)}$=8.167, p=0.024, post-hoc for dark phase p=0.033).

Acute effects of white light on blood glucose and hormones

Plasma glucose levels were also measured, without fasting the animals, after the third exposure to one hour of white light (Figs.5A-5C and 6A-6C). No significant differences were found between dark controls and wALAN exposed animals fed with either diets at the same time point, plasma insulin, corticosterone, and leptin levels were also measured in all groups. In both diet groups, no significant differences were detected in insulin levels neither when both sexes were analyzed together or separately (Fig.5D-5F and Fig.6D-6F). Levels of plasma corticosterone were not different in chow-fed animals (Fig.5G), but on the HFHS diet higher levels of corticosterone were observed after exposure to wALAN (Fig.6G; $t_{(16)}$=3.070, p=0.007). The corticosterone analysis performed by sex showed that higher levels of corticosterone were significant only in HFHS fed females when exposed to the light pulse (Fig.6I; $t_{(7)}$=2.773, p=0.027). Lastly, no differences in plasma leptin levels were found due to the white light condition. HFHS-fed animals (Figs.6J-6L) had slightly higher leptin levels than the chow-fed Arvicanthis (Figs.5J-5L), but this difference did not reach statistical significance.

Discussion

The use of a diurnal rodent exposed to a high-fat high-sugar free-choice diet and white light resembles more the light-exposure and feeding conditions in humans, than those in nocturnal rodents with monochromatic light, and thus may provide a better model for translational studies. Here

we show for the first time the effects of acute wALAN exposure on glucose metabolism and food intake in the diurnal rodent Arvicanthis ansorgei, fed either a chow or a HFHS diet.

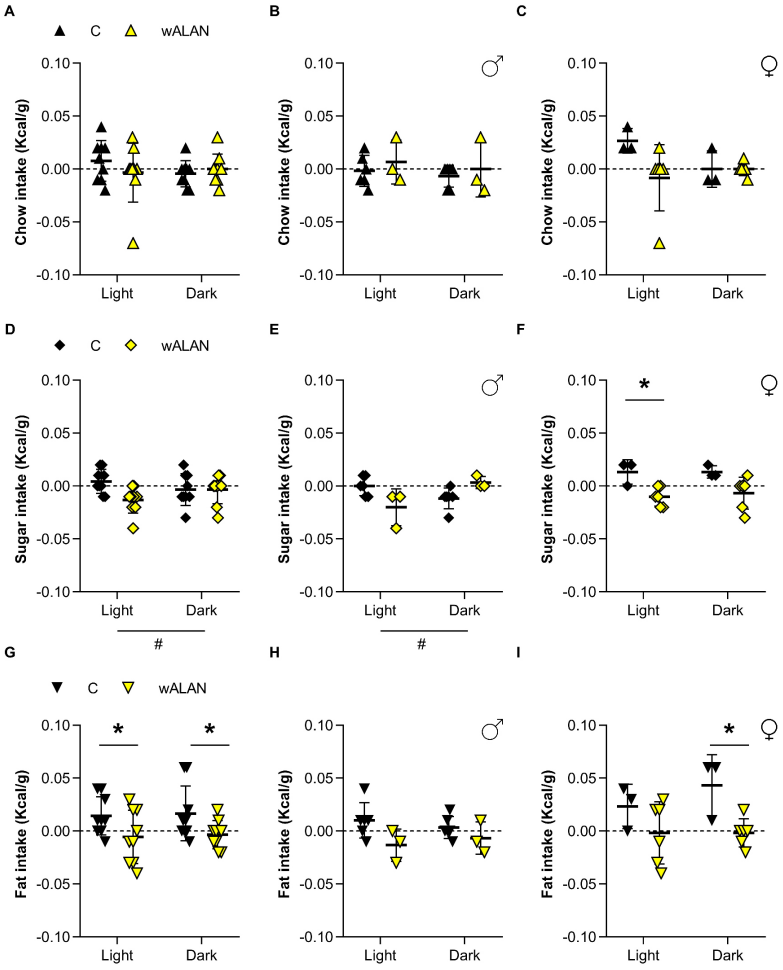

Figure 4. *Dietary intake in HFHS fed Arvicanthis. wALAN decreases the sugar intake of female grass rats during the light phase. wALAN from ZT14-15 did not affect the consumption of chow food in all HFHS fed animals, males or females (A-C). A significant Interaction effect was observed for sugar intake in all animals (D; p=0.013) and also for only males (E; p=0.035), while in females a significant effect of light Treatment was observed during the light phase (F; p=0.0021). wALAN also significantly decreased the amount of fat ingested by all animals during both the light and dark phase (G; p=0.022). When separated by sex, this effect only was significant in females during the dark phase (I; p=0.024).*

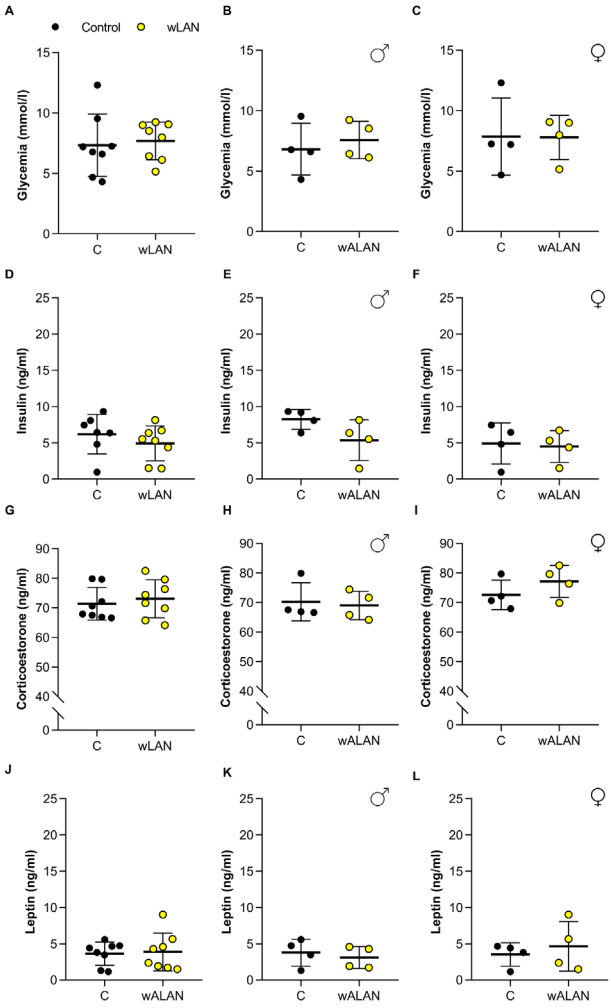

Figure 5. *Glucose and hormone levels after wALAN in chow fed Arvicanthis. Glycemia immediately after the light pulse was not affected in all chow-fed animals (A), nor in males (B) or females (C). wALAN also did not change plasma insulin, plasma corticosterone or plasma leptin concentrations in all animals (D, G, J), also not when analyzed separately for males (E, H, K) or females (F, I, L).*

Notably, the effects of wALAN on blood glucose, are only significant when animals are fed a chow diet, suggesting that the consumption of a high-fat high-sucrose diet may reduce the sensitivity of the circadian system components that are involved in the control of glucose metabolism or the sensitivity to light perception. The opposite effects of light on sugar intake in male and female animals indicate that also a sex-dependent mechanism is involved.

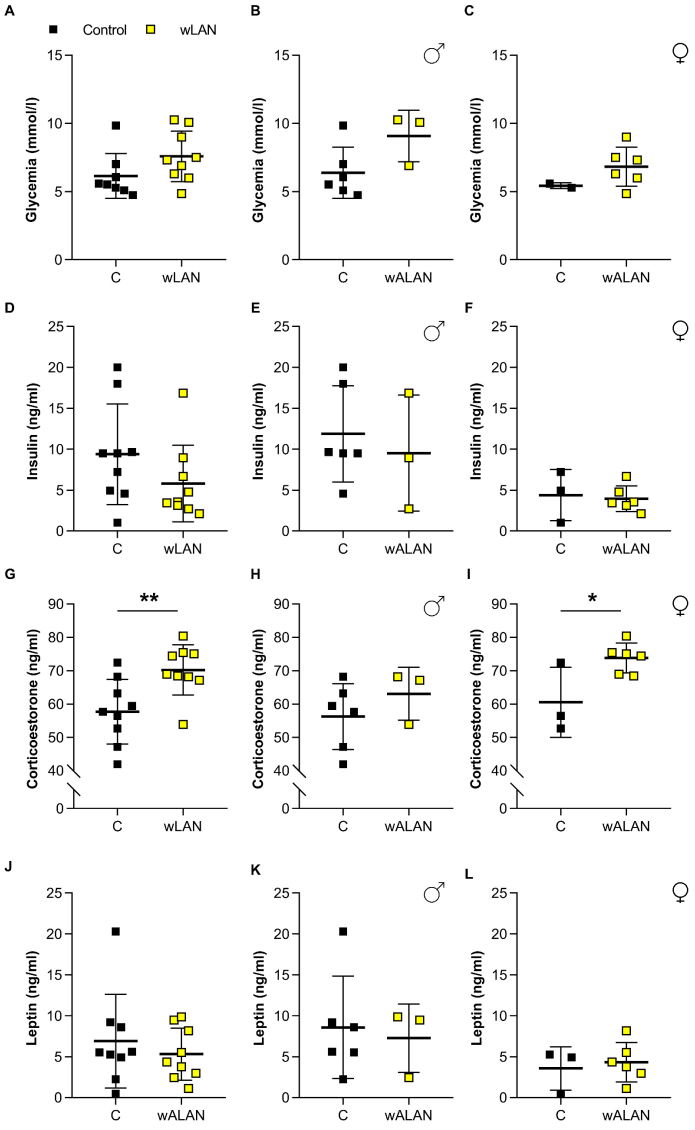

Figure 6. *Glucose and hormone levels after wALAN in HFHS fed Arvicanthis. wALAN from ZT14-15 in grass rats did not change blood sugar in all animals immediately after the light pulse regardless of sex (A-C). The white light also did not have any significant effect on the levels of plasma insulin in all animals (D), nor in only male (E), or only female (F) Arvicanthis. wALAN significantly increased plasma corticosterone concentrations in all animals (G; p=0.012). When separated by sex, this effect was not significant in males (H), but it was significant in females (I; p=0.039). The white light pulse did not change the plasma leptin levels in all animals (J), neither when analyzed separately in males (K) and females (L).*

Formerly this HFHS diet has been shown to induce snacking and obesity in Wistar rats (20) and in C57BL/6 mice (21), however, this is the first time that has been used in this diurnal rodent and also the first time that sex differences have been studied with this diet paradigm. Nonetheless, we did not observe a diet-induced increase in body weight in either sex (data are not shown). On the other hand, although adiposity was not measured, plasma leptin was slightly elevated in HFHS animals which may be a consequence of the HFHS regime.

In our current study, we observed marked glucose intolerance in chow-fed light-exposed females only. These changes were observed without any decrease in plasma insulin, indicating that exposure to wALAN may increase endogenous glucose production as also suggested elsewhere (13). Earlier in our group, we observed glucose intolerance after a 2-hour pulse of wALAN at ZT 15 in male Wistar rats, also without changes on plasma insulin (14). Hence, it was surprising that these effects were not observed on male diurnal rodents but in females. Whether female Wistar rats would present glucose intolerance after a white light pulse at the beginning of the dark phase remain to be investigated. Further studies are needed to evaluate more in-depth these effects light on glucose metabolism and whether sex-differences and effects on food intake are also present in rats. These contrasting results also emphasize the differences between species, which is an important factor to consider when trying to translate scientific result to human health.

Previously it has been described that via the SCN light during the dark phase can increase the sympathetic activity and decrease the parasympathetic activity of autonomic nerves that reach peripheral organs like the liver, the pancreas and the adrenal gland (10,22,23). These changes would result in increased gluconeogenesis and glycogenolysis, which could explain our current results in the glucose tolerance test. However, this mechanism seems to be somehow impaired in animals that have been feed a HFHS diet.

Animal and human studies providing evidence of the metabolic effects of environmental light have been reviewed extensively (3,4,24). In humans increases in obesity and diabetes match increased artificial light exposure (25) and it has been demonstrated that evening white light increases appetite (26). However, when we analyzed separately each component of the diet, we found an interaction effect in the sugar intake during the light phase in male animals after a 1 h white light pulse and in female animals we found a reduced sugar and fat intake in respectively the light and dark phase

after wALAN.

Our findings in this experiment show that sex is an important variable when studying effects of nocturnal light, not only regarding glucose metabolism but also for food intake. Unfortunately, possible mechanisms responsible for sex differences regarding light perception in rodents are virtually unknown. A study in humans demonstrated that compared to women, men show a stronger response to colder temperature light in the evening even at very low intensity (27) and this remains the only study in sex differences in light perception. Further research is needed to elucidate the physiology behind these differences, but according to our present results, it seems that somehow females are more sensitive to white light as it can even affect intake of palatable food. Whether this has potential as a possible therapy for treating excessive palatable food consumption in women is something that needs to be explored further.

A study in Arvicanthis niloticus (28), a diurnal rodent from the same genus as Arvichanthis ansorgei, pointed out that the main characteristics of the ipRGCs are fundamentally the same as those described in nocturnal rodents (16,29,30). Hence, among the areas that are innervated by the ipRGCs are the SCN, the intergeniculate leaflet (IGL), and the lateral hypothalamus (LH). Previous work from another group reported the acute effects of light on the brain of Arvicanthis (31), showing a significant number of cFos expressing cells in the IGL, the LH, and the lateral habenula (LHb).

The geniculohypothalamic tract (which includes the IGL), actively modulates SCN responses to retinal input (32) and might modulate metabolic signals to the SCN (33), and reward and metabolic signals of feeding to the LHb (34). Also, it is widely known that the LH and the LHb are deeply involved in the control of metabolism, food intake, and motivational processes (35,36), and that the projections of the LH to the LHb regulate feeding and reward (37), this together with the projections of the ipRGCs to the LHb (15,16) could explain our findings in this study, especially since the LHb directly and the SCN indirectly (via the medial preoptical nucleus) to the ventral tegmental area (VTA); which would explain the changes in palatable food consumption.

A possible explanation of why the light did not affect the glucose tolerance in HFHF-fed animals is that the diet disturbs the molecular clock in brain centers involved in metabolism and palatable food intake as it has

been described in our group previously (21). Together with this we can hypothesize that another plausible reason is changes in the retina due to the HFHS diet. It has been reported that Arvicanthis niloticus are an excellent model for diabetes and its complications, including diabetic retinopathy after only short time of been feed diets with high glycemic loads (38–40).

To conclude, we showed that in the diurnal rodent Arvicanthis ansorgei acute exposure to wALAN causes glucose intolerance in females, while it decreases the consumption of sugar in males and sugar and fat in females. Whether this effect is dependent on the activation of melanopsin in the ipRGCs and thus its projections to different parts of the brain, remains to be investigated. Nevertheless, our present results in this diurnal rodent might raise awareness about the deleterious effects of light at night on glucose metabolism, but also on the importance of sex differences in light perception and how light can influence appetite for palatable foods.

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Declarations of interest

None.

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Chapter 4

Blue light at night acutely impairs glucose tolerance and increases sugar intake in the diurnal rodent *Arvicanthis ansorgei* in a sex-dependent manner

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Abstract

In our modern society, exposure to light at night (LAN) has increased considerably, which may impact human health negatively. Especially exposure to light at night containing short wavelength emissions (~450–500 nm) can disrupt the normal function of the biological clock, altering sleep-wake cycles and inducing metabolic changes. Recently, we reported that light at night acutely impairs glucose tolerance in nocturnal rats. However, light at night in nocturnal rodents coincides with their activity period, in contrast to artificial light at night exposure in humans. The aim of this study was to evaluate the acute effects of blue (λ= 490 ± 20 nm) artificial light at night (bALAN) on glucose metabolism and food intake in both male and female diurnal Sudanian grass rats (*Arvicanthis ansorgei*) fed either regular chow or a free choice high-fat high sucrose diet (HFHS). In both chow and HFHS fed male *Arvicanthis*, 1-hour of bALAN exposure induced a higher glucose response in the oral glucose tolerance test (OGTT) accompanied by a significant decrease in plasma insulin. Furthermore, in HFHS fed animals, bALAN induced an increase in sucrose intake during the dark phase in males but not in females. Additionally, 1-h of bALAN increased the nonfasted glucose levels together with plasma corticosterone in female grass rats. These results provide new and further evidence for the deleterious effects of exposure to short-wavelength emission-containing artificial light at night on glucose metabolism in a diurnal rodent in a sex-dependent manner.

Introduction

Data from the World Health Organization show that the prevalence of obesity has increased almost three times since 1975. By 2016 more than 1.9 billion adults were overweight, of which 650 million were obese. Nowadays in most countries, obesity accounts for more deaths than underweight (1). It is widely known that obesity is a major risk factor for non-communicable diseases such as type 2 diabetes. The number of people suffering from type 2 diabetes has risen from 108 million in 1980 to 422 million in 2014 (2). The economic burden for healthcare systems associated with the diagnosis and health care of diabetic patients is increasing profoundly (3). Type 2 diabetes as much as obesity are multifactorial diseases with complex underlying physio-pathological mechanisms, nevertheless for both diseases many cases are preventable through lifestyle changes.

Epidemiological and experimental studies in humans have shown that sleep disruption and exposure to artificial light at night (ALAN) are risks factors for the development of obesity (4,5) and type 2 diabetes (6–8). Furthermore, animal studies have shown that exposure to ALAN increases body mass (9–13), exacerbates inflammatory responses (10,14), alters food intake (11), disrupts metabolism and circadian rhythms (9,15) and changes insulin sensitivity (16).

The strong relationship between circadian rhythms and metabolism is now well established, especially regarding the control of glucose homeostasis (17–19). Although most endogenous rhythms will continue to oscillate even without the presence of an environmental light/dark cycle, light is the most important environmental signal to entrain the intrinsic ~24h (i.e. circadian) rhythms to the 24-hour rhythm of the rotation of the earth. However, light can also be a potent circadian and endocrine disruptor when received at the wrong time of the day, i.e. during the dark phase (20), causing alterations in the secretion of melatonin (21) and corticosterone (cortisol in humans) (22), and changes in the expression of circadian genes in the hypothalamus (23), pineal gland (24), adrenal (22) and liver (25), all of them strongly involved in glucose metabolism.

In mammals, light is perceived by rod and cone photoreceptors in the retina eliciting both visual and non-image forming visual responses. Previous studies have revealed the existence of intrinsically photosensitive retinal

ganglion cells (ipRGCs) that express the photopigment melanopsin (with a peak spectrum of absorption at λ=480 nm, i.e. blue light), in addition to rods and cones. The anatomical projections of the ipRGCs directly reach the hypothalamic suprachiasmatic nucleus (SCN), the site of the main circadian clock, and other parts of the brain involved in energy metabolism, glucose homeostasis, reward and food intake (26–28). Combined with the evidence that ALAN is a risk factor for metabolic diseases, this leads to the hypothesis that ALAN can alter food intake and glucose metabolism.

In a previous study from our group, we showed that exposure to ALAN in male rats acutely induced glucose intolerance, most likely by reducing beta cell sensitivity and glucose uptake (29). However, the nocturnal nature of rats and the metabolic differences between sexes (30) is an important and complicating factor to consider when translating these findings to the human situation. Therefore, in the present study, we used a diurnal rodent to study the acute effects of ALAN on food intake and glucose metabolism in both males and females. In addition, we used a palatable diet and blue ALAN (bALAN), which both are widely present in human daily modern life.

Materials and Methods

Ethical approval

All the experiments were performed in accordance with the rules of the European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French Department of Agriculture (license no. 63-378 to JM). The investigators understand the ethical principles under which the journal operates, and all experiments comply with the policies and regulations set out in the editorial (31).

Animals and housing

Thirty-five 12-weeks-old male (n=18) and female (n=17) Sudanian grass rats (Arvicanthis ansorgei) obtained from the platform Chronobiotron (UMS-3415, CNRS and University of Strasbourg) weighing 131 ± 17 g were individually housed in Plexiglas cages with sawdust bedding, chow food and tap water ad libitum. Animals were maintained under a controlled ambient temperature of 21-23 ℃, a relative humidity of 23-27% and a 12-h light/dark cycle (lights on at 07:00 hours, Zeitgeber time 0 (ZT0); lights off 19:00

hours, ZT12) with dim red light at night (5 lux).

Daily food intake rhythm

Chow food (SAFE, 105, U8400G10R, Augy, France; 2.85 kcal/g, 23% proteins, 65% carbohydrates, and 12% fat) and tap water was provided ad libitum to all animals. After a week of acclimatization, food consumption was measured manually every 4 hours for 4 days, to assess the daily food intake pattern (Figure1).

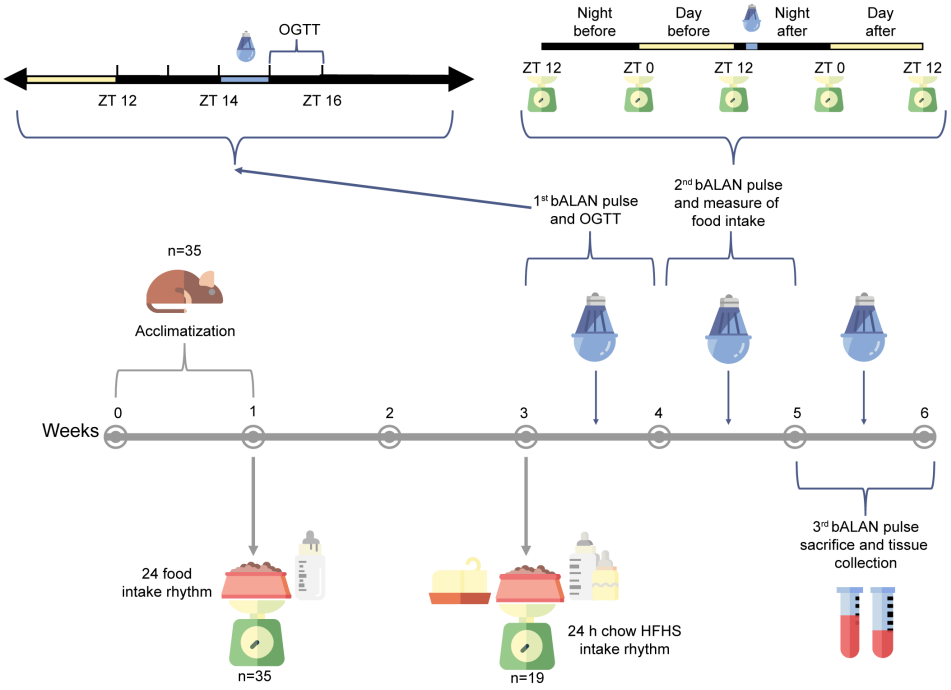

Figure 1. *Graphical representation of the experimental design.*

Free choice high-fat high sucrose diet

Immediately after measuring the daily food intake rhythm with chow food, animals were randomly assigned to two groups fed with different diets. The control group (n=8 per sex) remained on the chow diet mentioned before and tap water. The free choice High-Fat High-Sugar (HFHS) group (n=10 males and n=9 females) received in addition to the chow food and the tap water, a cube of saturated fat (beef lard, Vandemoortele, France; 9 kcal/g) and a bottle of 10% sugar water (commercial grade sucrose and tap water, 0,4 kcal/ml). In the HFHS model, animals are free to choose what

they prefer and are not forced to eat a high caloric diet as in other rodent models. This situation reflects human conditions in which people can choose from different diets (normocaloric vs. hypercaloric). After two weeks on the HFHS diet food consumption was again measured manually every 4 hours for 4 days, as described before, to assess the daily food intake rhythm when animals were fed with the HFHS diet. Both groups were kept on their respective diets for 4 more weeks and animals were weighed weekly.

Light exposure protocol

To study whether the exposure to blue light at night (bALAN) has an effect on glucose metabolism and food intake, starting in week 3 animals from both diet groups were randomly sub-divided in two subgroups, dark controls (n=9 chow-fed, 4 males and 5 females; n=10 HFHS-fed, 6 males and 4 females) and blue light exposed (n=7 chow-fed, 4 males and 3 females; n=9 HFHS-fed, 4 males and 5 females) (Figure1). At ZT14 (two hours after lights off) individual cages of the animals from the light group were moved to a PVC cage (120cm x 60cm x 60cm), which had a fixed blue light-emitting diode (LED) lamp placed in the middle of the ceiling and they were exposed to a 1 hour pulse of blue light (490±20 nm wavelength, see Table 1 for the irradiance spectrum (32)). Then the dark condition was restored and animals from the light group were moved back to their racks. Protocols to evaluate glucose metabolism, food intake and light effects on hormones were applied immediately thereafter as described below. Between each light exposure, animals were left undisturbed for one week.

Table 1. *Spectral sensitivity of the light condition used in the whole experiments.*

Retinal photopigment complement	Blue light
S-Cone (rNsc [λ])	0.00
Melanopsin (rNz [λ])	27.20
Rod (rNz [λ])	26.55
M-cone (rNmc [λ])	24.34
Irradiance (μW/cm^2)	4.26
Photon flux (1 cm^{-2} s^{-1})	1.06 x 10^{13}

Data based on the Rodent Toolbox provided by Lucas et al (2014). The first four rows represent weighted contribution of rodent reti- nal photopigments (S-cone, ipRGC [melanopsin], rod, M-cone) in a-opic rodent lux. The last two rows (irradiance, photon flux) rep- resent unweighted characteristics of the LEDs.

Oral glucose tolerance test (OGTT)

Immediately after animals were returned from the exposure to either the first pulse of blue light or being kept in control dark conditions respectively, an OGTT was performed starting at ZT15. For this, all *Arvicanthis* were fasted for 6 hours before the test, i.e. from ZT9 onwards. Blood was taken from the tip of the tail at 0, 15, 30, 90 and 120 minutes for the measurement of whole blood glucose concentrations using an Accu-Chek Performa Nano glucometer (Roche Diabetes Care Limited). Immediately after the 0 minutes sample, D-glucose (2g of D-glucose per kg of body weight dissolved in 0.9% saline) was orally administered by gavage.

Data of the OGTT were expressed as the delta between the basal (t=0) glucose and the measurement at each time point. In addition, the increase of plasma glucose was analyzed by calculating the area under the curve (AUC) from the time of the baseline to the 120 min measurement, using the trapezoidal rule (33).

Food intake evaluation

One week after the OGTT, animals received the same light treatment described above for the second time. All food components from both diet groups were weighed every 12 h during 2 LD cycles, starting at ZT12 or the dark phase before light exposure until ZT12 or the light phase after, in order to measure possible changes in the daily food intake patterns due to the blue light pulse at the beginning of the dark phase.

Locomotor activity recordings

To measure activity-rest cycles, general locomotor activity of all animals was monitored by using infrared detectors placed above the cages linked to an automated recording system (CAMS, Circadian activity monitoring system, Lyon, France). Data were recorded every 5 min. Clocklab software (Actimetrics, Wilmette, IL) was used to determine the activity profiles of each animal under different diet conditions (chow vs. HFHS).

Blood sampling and serum analysis

On the last week of the experiment, a third light pulse was given, and animals were deeply anesthetized immediately after (i.e. ZT15) with an overdose of isoflurane to recover the blood from the left ventricle with a

cardiac puncture, using a 5 ml syringe with a 25G needle. Immediately after animals were perfused transcardially with PFA 4% wt/vol in 0.1 M phosphate buffer (pH=7.2) to recover brains for analysis in future studies.

Glucose levels in blood were determined as described before using a drop of the extracted blood. Then the blood was decanted into 15 ml Corning tubes containing 100 µl of 4% EDTA. Samples were centrifuged 5000 rpm at 4ºC during 10 min, and plasma was stored at -80ºC for determination of plasma insulin, corticosterone and leptin concentrations.

Insulin was measured using an ELISA procedure with a Rat/Mouse Insulin ELISA Kit (EZRMI-13K, Merck-Millipore, Germany). The limit of sensitivity of the insulin kit was 0.1 ng/ml. Corticosterone was measured using an EIA procedure with a Rat/Mouse Corticosterone EIA Kit (AC-14F1, Immunodiagnostic Systems Ltd, UK). The limit of sensitivity corticosterone kit was 0.55 ng/ml. The levels of leptin in plasma were measured using an ELISA procedure with a Rat Leptin Kit (EZRL-83K, Merck-Millipore, Germany). The limit of sensitivity of the leptin kit was 0.04 ng/ml.

Statistical analysis

Results were analyzed with and without considering the sex of the animals. All data are expressed as means ± standard deviation. A two-way ANOVA was used to compare the intake of the three components of the HFHS diet between sexes per ZT, the activity levels and caloric intake. The area under the curve (AUC) for the OGTT was calculated using the trapezoid rule. Unpaired two-tailed t tests were used to detect group differences baseline concentration of glucose before performing the OGTT, AUCs, levels of glucose, and plasma hormones after the light exposure.

A two-way mixed model ANOVA was used to test the effects of the diets and light condition (treatments), the effect of time (during the OGTT or light and dark phases), and the interaction on activity and food intake pattern in response to the diet. Glucose and food intake responses in either diet conditions to the blue light exposure was analyzed also with a mixed model ANOVA. Additionally, a two-way ANOVA was used to analyze the consumption of the different components of the HFHS diet (chow vs. fat vs. sugar) after the light exposure. ANOVA's results were followed by a Sidak's multiple comparisons as a post-hoc test. Statistical power was calculated with a post hoc two-tail t-test (power= 85%, d=2.86, α=0.05) using G Power

3.1.9.2. GraphPad Prism version 7.01 for Windows (GraphPad Software, La Jolla, CA, USA) and IBM SPSS Statistics for Windows version 25 (Armonk, NY: IBM Corp.) using a significance level of $p<0.05$.

Results

Locomotor activity and daily food intake rhythms

Both male and female Arvicanthis fed with the chow diet showed stable and significant diurnal activity with a crepuscular pattern, with a higher locomotion before lights on and off, and continued stable during the light phase ($F(288, 4032)=33.1$, $p<0.001$; Fig. 2A–C). The daily food intake rhythm of how-fed male and female animals showed also a diurnal intake with a biphasic pattern ($F(23, 1150)=60.4$, $p<0.001$), which fits with the crepuscular pattern of locomotion (Fig. 2D–F). The crepuscular pattern of locomotor activity and food intake was also observed when animals from both sexes were fed with a HFHS diet (locomotion, $F(288, 4896)=33.4$, $p<0.001$; Feeding, $F(23, 1242)=16.9$, $p<0.001$; Fig. 3A–F). When we compare the daily rhythms of food intake for each component of the HFHS diet, we found significant differences in the consumption of the chow ($F(23,391)=1.8$, $p=0.01$) and fat ($F(23,391)=3.4$, $p<0.001$) by sex; being higher in males at ZT17 (Post-hoc, $p=0.006$) and ZT5 (Post-hoc, $p<0.001$) respectively (Fig. 3E and F). In the analysis of males and females Arvicanthis together, the total 24 h locomotor activity was not different between diet groups ($F(1,33)=3.9$, $p=0.05$; Fig. 4A).

However, a significant difference in locomotion for the factor phase (light vs. dark phase, $F_{(1,33)}=108.7$, $p<0.001$), and for the interaction between phase x diet factors was found ($F_{(1,33)}=5.2$, $p=0.029$), indicating that activity during the light phase was higher in chow-fed than HFHS-fed *Arvicanthis* (Post-hoc, $p=0.008$; Fig. 4A). When analyzed by sex, only a significant difference in the levels of locomotor activity for the factor phase (light vs. dark), but not for the factor diet or the interaction phase x diet, was found in both males ($F_{(1,15)}=38.6$, $p<0.0001$) and females ($F_{(1,16)}= 70.7$, $p<0.0001$), with a highest activity at the light phase (Fig. 4B–C). Caloric intake was higher at the light phase in both diet groups (Chow and HFHS) ($F_{(1,33)}=30.8$, $p<0.0001$; Fig. 4D). However, the 24 h total (light and dark phase) energy consumption was significantly higher in HFHS-fed animals ($F_{(1,33)}=7.5$, $p=0.009$; Fig. 4D). Separately, both males ($F_{(1,16)}=20.8$, $p<0.0001$) and females ($F_{(1,15)}=39.2$, $p<0.0001$) ate significantly more during the light phase (Fig. 4E and F).

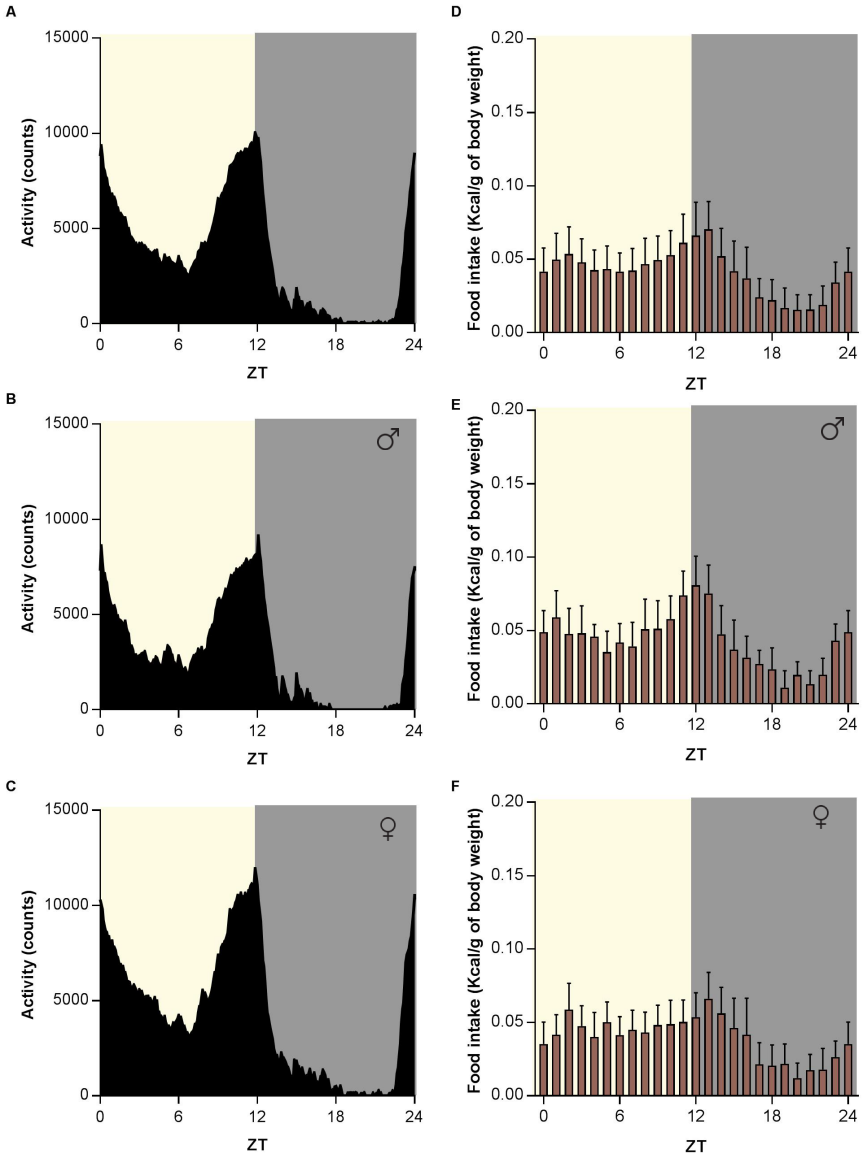

Figure 2. *Chow-fed Arvicanthis ansorgei show a biphasic crepuscular locomotor activity and food intake pattern. The plot of the mean 24-h locomotor activity of both sex shows a biphasic crepuscular shape (A), with similar behavior in male (B) and female (C) grass rats when fed a chow diet. The biphasic activity pattern correlates with the food intake rhythm in both sexes together (D). The two phases of the food intake, i.e., dusk and dawn, are observable in males (E) and females (F). Food intake is expressed as kilocalories consumed per gram of body weight. ZT, Zeitgeber Time (in hours).*

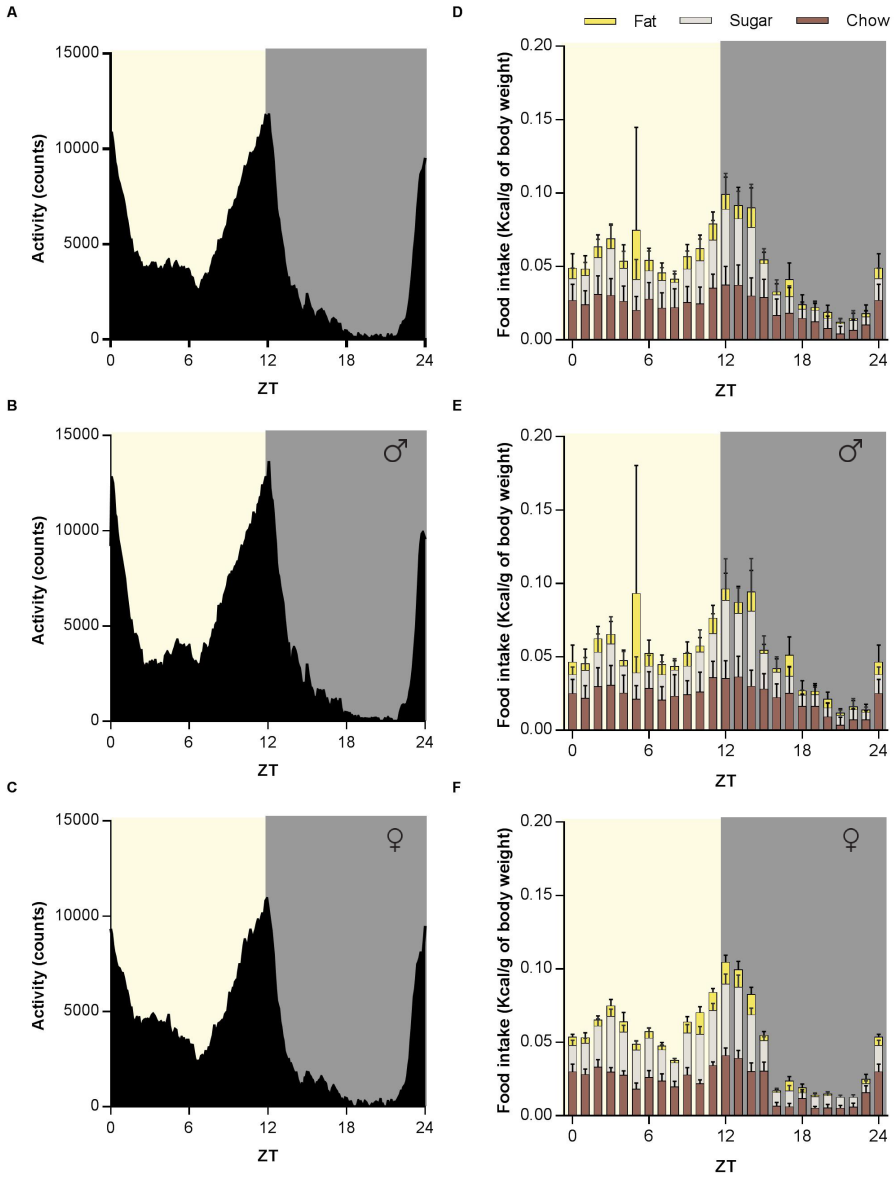

Figure 3. *HFHS-fed Arvicanthis ansorgei show a biphasic crepuscular locomotor activity and food intake pattern. The plot of the mean 24-hour locomotor activity of both sexes (A) shows a biphasic crepuscular shape. This activity pattern when separated is similar between male (B) and female grass rats (C) fed with a HFHS diet. The biphasic activity pattern correlates with the HFHS food intake rhythm in both sexes together (D). Food intake patterns of the different components of the HFHS diet (fat, sugar, chow) in both males (E) and females (F). Food intake is expressed as kilocalories consumed per gram of body weight. ZT, Zeitgeber Time (in hours).*

In HFHS-fed females, but not males, the 24 h total caloric intake was higher compared with chow-fed female *Arvicanthis*, although this effect was at the limit of significance ($F_{(1,15)}$=4.25, p=0.05; Fig. 4F). In HFHS-fed animals, when we analyzed calorie intake together in males and females *Arvicanthis* (Fig. 4G), the two-way ANOVA indicated a light versus dark phase difference ($F_{(2,102)}$=13.8, p=0.0003) and in the intake of the three components of the HFHS diet (chow vs. fat vs. sugar; $F_{(2,102)}$=32.5, p<0.001), but no for the factors interaction ($F_{(2,102)}$=1.7, p=0.18). However, when analyzed by sex, the intake of the three components of the diet (chow vs. fat vs. sugar) was significantly different between the light versus dark phase in females *Arvicanthis* (time x component interaction, $F_{(2,24)}$=4.1, p=0.02), showing a main sugar intake at the light phase (Fig. 4I). Although all animals increased their body weight during the study, with a difference between males and females, no significant differences were observed in body weight gain by light or diet exposure (Table 2).

Table 2. *Statistical analysis (three-way ANOVA) of percentage of body weight (BW) gain of the animals during the experiment per diet (Chow vs. HFHS), light treatment (dark vs. bALAN), and sex (male vs. female). Data represent the mean values ± the standard deviation.*

	Initial BW (g)	Final BW (g)	BW gain (%)	*p* value for diet	*p* value for light	*p* value for sex
Chow				0.25	0.78	0.02
♂ ♀	129.3 ±16.4	133.2 ±18.2	1.9 ± 7.5			
♂	136.2 ±17.7	146.5 ±5.9	4.7 ±3.3			
♀	122.4 ±12.4	120.0 ±5.3	-0.8 ± 9.7			
HFHS						
♂ ♀	131.1 ±16.9	139.2 ±19.8	4.2 ±7.0			
♂	138.6 ±15.9	150.7 ±16.3	4.6 ± 2.1			
♀	122.7 ±14.5	126.5 ±15.4	3.8 ±10.2			

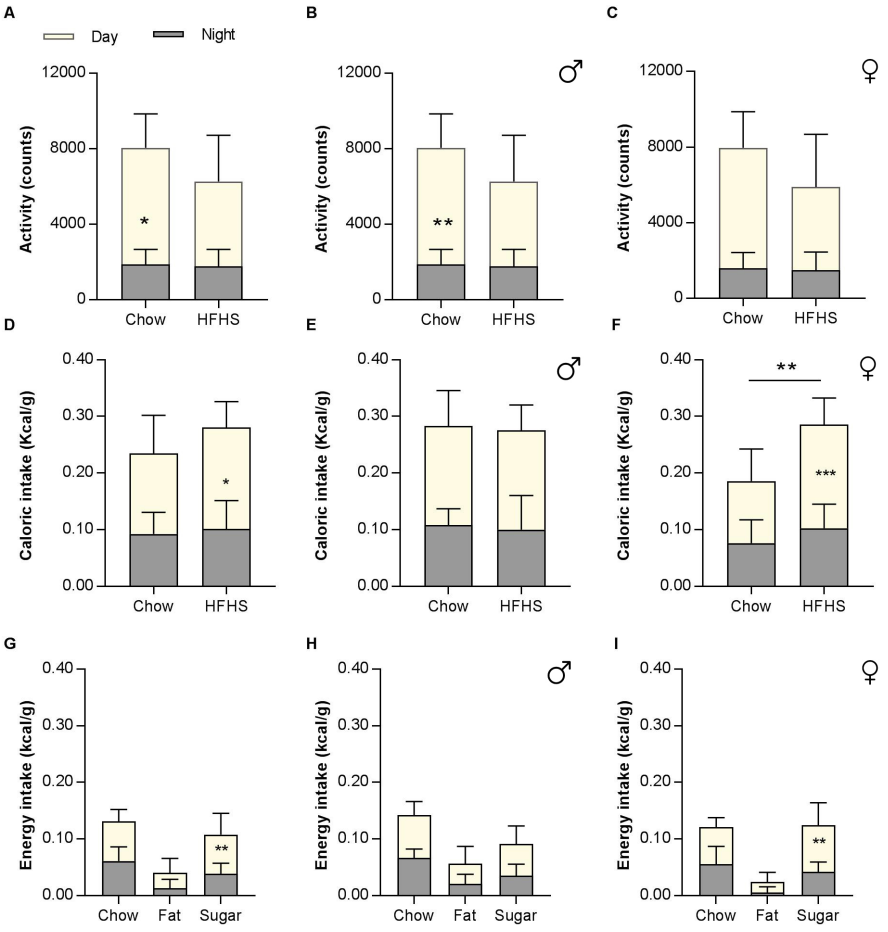

Figure 4. *HFHS diet induces changes in the total caloric intake and locomotor activity of Arvicanthis ansorgei. Light versus dark phase activity in males and females Arvicanthis together (A) or separated by sex (B and C) fed with a chow or a HFHS diet. Activity was significantly higher at the light phase in both males and females. Moreover activity at the light phase in both sexes together (A) was significant higher in chow-fed than HFHS-fed animals (*Post-hoc, P = 0.008). (D) Animals of both sexes from either diet group ate more total calories at the light phase than night. HFHS-fed animals ingest significantly more calories than the chow group (*Post-hoc, P = 0.009). Separately, in males (E) and females (F) grass rats caloric intake is higher at the light phase, but no differences in the total caloric intake between diet groups (chow vs. HFHS) were found. Intake at the light versus dark phase of the different components of the HFHS diet from both sexes together (G), or separately in males (H) and females (I). Female Arvicanthis drink significantly more sugar during the light phase than the dark phase (*Post-hoc, P = 0.0001).*

Acute effects of blue light in glucose metabolism

Baseline levels of glucose before starting the OGTT were similar between diet and light groups in both males and females (Figs. 5A–C and 6A–C). In chow-fed animals (males and females), blue light exposure caused a significant increase in plasma glucose 15 min after the glucose bolus (factor time, $F_{(5,70)}$=12.5, p<0.001; time x treatment interaction, $F_{(5,70)}$= 2.4, p=0.03; Post-hoc, p=0.04; Fig. 5D, Table 3), as well as a significant increase in the AUC values ($t_{(14)}$=3.7, p=0.002; Fig. 5G). When analyzed separately by sex, the AUC values were significantly higher in males exposed to bALAN ($t_{(6)}$=3.5, p=0.01; Fig. 5H), whereas no significant difference was found in females ($t_{(6)}$=1.2, p=0.2; Fig. 5I). In HFHS-fed animals, there were no differences between dark versus bALAN exposed-animals in plasma glucose in the OGTT ($F_{(5,85)}$=0.3, p= 0.8, Table 4) or in AUC glycemia values ($t_{(17)}$=1.5, p=0.1; Fig. 6D and G). However, when separated by sex, males showed a significant increase in AUC values after bALAN exposure ($t_{(8)}$=3.1, p=0.01; Fig. 6H), whereas in females no differences were observed between conditions ($t_{(7)}$=0.8, p=0.4; Fig. 6I). These results indicated a sex-dependent effect of blue light on glucose metabolism.

Table 3. *Statistical analysis (mixed-model ANOVA) of the raw glucose levels during the OGTT of the animals (males/females) fed with the chow diet and exposed to the light treatment (dark vs. bALAN).*

Groups		Time						p value treatment	p value time	p value interaction
		0	15	30	60	90	120			
♂ ♀	Dark	4.9 ±1.8	5.7 ±2.2	7.0 ±3.0	7.3 ±2.1	5.8 ±2.1	4.4 ±1.0	0.229	<0.001	0.018
	Blue	4.4 ±0.7	8.4 ±3.8	9.4 ±4.0	8.7 ±3.7	5.5 ±1.8	3.9 ±0.9			
♂	Dark	4.1 ±0.7	5.6 ±1.2	8.0 ±3.6	7.5 ±2.3	4.9 ±2.6	4.5 ±0.8	0.031	<0.001	0.036
	Blue	4.4 ±0.6	9.9 ±3.2	11.6 ±1.9	10.4 ±2.9	6.2 ±1.6	4.2 ±0.9			
♀	Dark	5.7 ±2.3	5.8 ±3.0	6.2 ±2.6	7.2 ±2.4	6.5 ±1.7	4.5 ±1.4	0.691	0.047	0.665
	Blue	4.4 ±0.9	6.5 ±4.2	6.5 ±4.7	6.5 ±3.9	4.5 ±1.8	3.7 ±1.1			

Data represent the mean values ± the standard deviation of the glucose measurement (mmol/L) during the test from 0 to 120 min after the bolus of glucose administration.

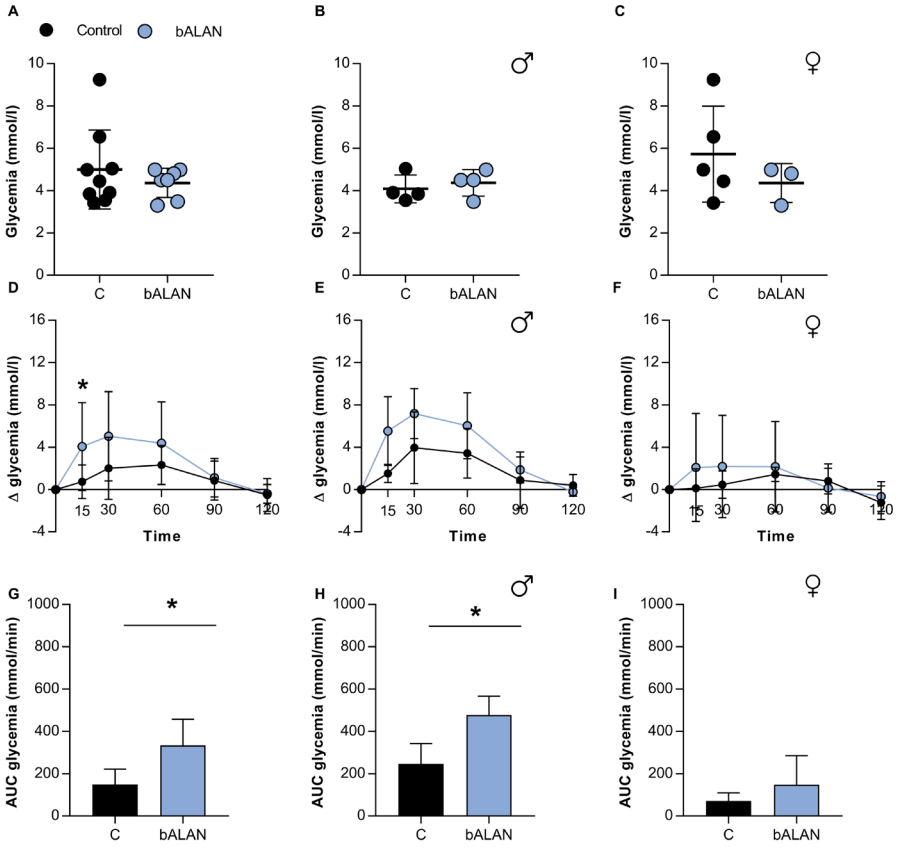

Figure 5. *In chow-fed Arvicanthis, baseline concentrations of glucose were not affected by bALAN in all animals (A), nor males (B) or females (C). bALAN exposure from ZT14-15 in fasted grass rats affects glucose tolerance in all animals after 15 min of the glucose bolus (D; *Post-hoc, P = 0.04) and increases the AUC values of glucose (G; *t-test,P = 0.002). The bALAN exposure altered the glucose response in male Arvicanthis (E) by increasing the AUC values of glucose (H; *t-test,P = 0.01). In females no significant change was observed in the glucose response (F) nor in the AUC (I) between control (dark-exposed) and bALAN-exposed animals.*

Acute effects of blue light on food intake

We next determined whether acute exposure to bALAN would change food intake. In Figure 7 food intake is depicted as the difference in food consumption between the light versus dark phases before and after the bALAN exposure. When analyzing the total amount of kilocalories per gram of body weight, no significant effects of bALAN exposure were found in the chow-fed ($F_{(1,14)}$=0.5, p=0.4) or HFHS-fed ($F_{(1,16)}$=0.2, p=0.6) groups in both

sexes together (Figs. 7A and D), neither when analyzed separately (chow-fed males, $F_{(1,6)}$=1.7, p=0.2, HFHS-fed males $F_{(1,7)}$=0.3, p=0.5; chow-fed females, $F_{(1,6)}$=0.9, p=0.3, HFHS-fed females $F_{(1,7)}$=4.8, p=0.06; Fig. 7B–C and E–F).

Table 4. *Statistical analysis (mixed model ANOVA) of the raw glucose levels during the OGTT of the animals (males/females) fed with the HFHS diet and exposed to the light treatment (dark vs. bALAN).*

Groups		Time						p value treatment	p value time	p value interaction
		0	15	30	60	90	120			
♂ ♀	Dark	4.9 ±1.1	8.8 ±3.5	11.2 ±4.9	9.3 ±4.3	7.7 ±4.7	5.6 ±2.2	0.050	<0.001	0.873
	Blue	4.2 ±0.9	10.3 ±2.9	11.4 ±3.8	10.3 ±6.1	7.2 ±4.5	5.6 ±2.7			
♂	Dark	4.9 ±1.2	9.1 ±3.4	12.2 ±5.1	9.0 ±2.8	6.0 ±0.8	5.2 ±1.2	0.506	<0.001	0.651
	Blue	3.8 ±0.7	9.4 ±1.4	12.6 ±4.3	11.9 ±8.3	8.4 ±6.7	6.9 ±3.8			
♀	Dark	4.7 ±1.0	8.3 ±4.1	9.7 ±4.9	9.8 ±6.4	10.2 ±7.2	6.3 ±3.4	0.118	0.858	0.100
	Blue	4.5 ±1.2	11.1 ±3.7	10.4 ±3.6	9.0 ±4.2	5.9 ±1.5	4.5 ±0.7			

Data represent the mean values ± the standard deviation of the glucose measurement (mmol/L) during the test from 0 to 120 min after the bolus of glucose administration.

Nevertheless, in the HFHS-fed group when the different components of the diet were analyzed separately (Fig. 8A–I), we found a significant difference for sugar consumption ($F_{(1,16)}$=11.6, p=0.004), suggesting that sugar intake was different between the dark- versus the bALAN-exposed animals. Moreover, when we analyze sugar intake with respect to sex, a significant interaction (treatment x phase) effect was observed in males ($F_{(1,7)}$= 11.8, p=0.011, post-hoc, p=0.008), indicating that bALAN-exposed male *Arvicanthis* drink more sugar at the dark phase than their respective controls (dark-exposed animals; Fig. 8E). In females, the ANOVA indicated a significant difference for the factor treatment (dark vs. bALAN) in sugar consumption ($F_{(1,7)}$=9.0, p=0.02; Fig. 8F); however, no differences were found for the factor phase (light vs. dark; $F_{(1,7)}$=1.1, p=0.3) or in the interaction between factors ($F_{(1,7)}$=1.1, p=0.3; Fig. 8F).

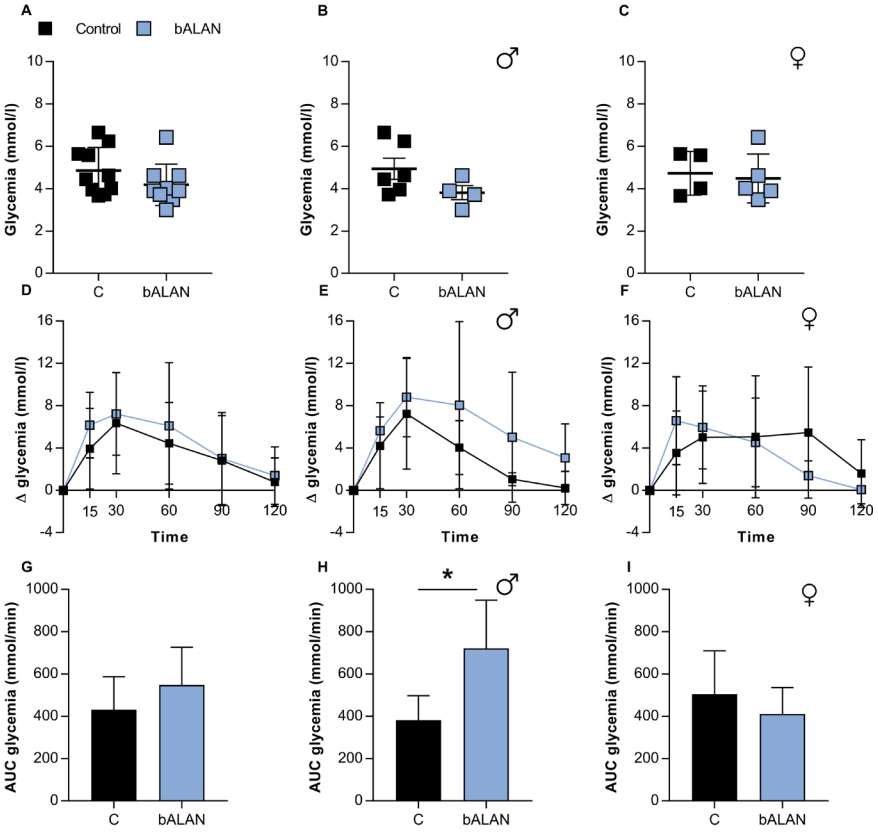

Figure 6. *In HFHS-fed Arvicanthis baseline concentrations of glucose were not affected by bALAN in all animals (A), nor males (B) or females (C). bALAN exposure from ZT14-15 in fasted grass rats did not affect glucose tolerance in all animals (D) nor significantly increased the AUC values (G). bALAN exposure changed the glucose response in male Arvicanthis (E) by increasing the AUC values of glucose (H; *t-test, P = 0.01). In females no significant change was observed in the glucose response (F) or in the AUC (I) between control (dark-exposed) and bALAN-exposed animals.*

Acute effects of blue light on blood glucose and hormones

Plasma glucose levels were also measured after the third 1h bALAN exposure (Figs. 9A–C and 10A–C). No significant differences were found between dark controls versus bALAN-exposed animals fed with a chow diet ($t_{(14)}$=0.7, p=0.4; Fig. 9A) neither in males ($t_{(6)}$=0.4, p=0.6; Fig. 9B) or females ($t_{(6)}$=1.1, p=0.2; Fig. 9C). On the other hand, in HFHS-fed animals, increased blood glucose was observed after animals had been exposed to bALAN

($t_{(15)}$=2.4, p=0.02; Fig. 10A). When analyzed by sex, the hyperglycemic effect of the bALAN exposure was significant only in females ($t_{(5)}$=2.5, p=0.04; Fig. 10C), although a nonsignificant tendency of hyper- glycemia was also observed in males ($t_{(8)}$=1.5, p=0.1; Fig. 10B). After the third exposure to 1 h of bALAN exposure, in the chow-fed group, no significant differences were detected in insulin levels when both sexes were analyzed together ($t_{(12)}$=1.7, p=0.1; Fig. 9D).

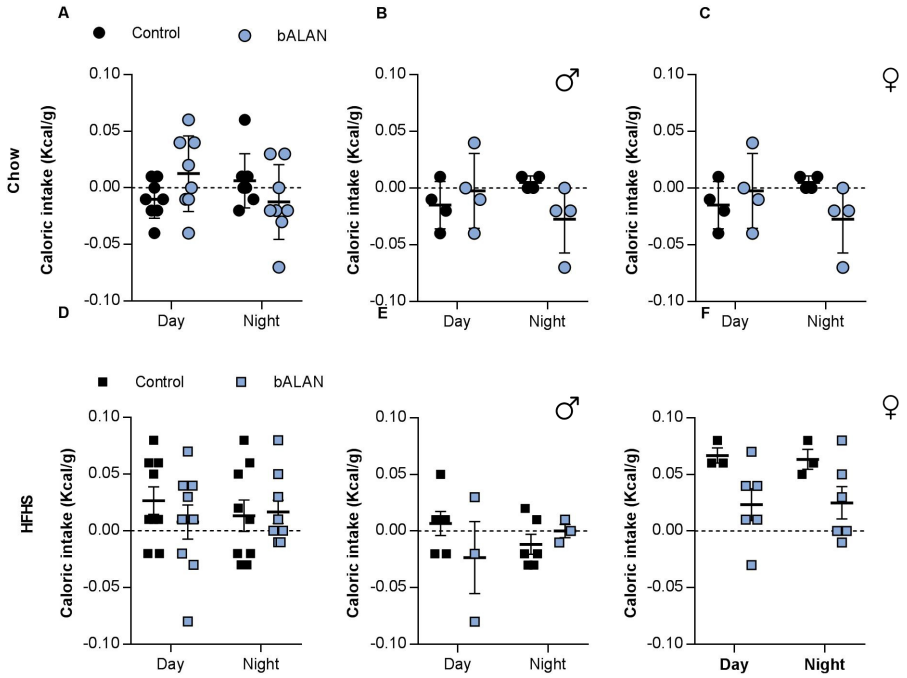

Figure 7. *bALAN exposure did not change the total calorie intake in either diet regardless of sex and phase (light vs. dark). bALAN exposure from ZT14-15 did not affect the calorie intake in all chow-fed animals (A) nor in males (B) neither in females (C). The bALAN exposure did not affect either the total caloric intake of all animals fed with a HFHS diet (D), neither separately in males (E) or females (F).*

However, in the group fed with the HFHS diet, animals exposed to bALAN exhibited significantly lower plasma insulin levels ($t_{(16)}$= 2.6, p=0.01; Fig. 10D). When analyzed by sex, a significant reduction in plasma insulin was observed in bALAN-exposed males, but not females animals, either fed chow ($t_{(6)}$=3.0, p=0.02; Fig. 9E) or the HFHS diet ($t_{(8)}$=2.4, p=0.03; Fig. 10E). Levels of plasma corticosterone were not different in chow-fed animals

($t_{(14)}$=1.1, p=0.12; Fig. 9G), but under HFHS diet significant higher levels of corticosterone were observed after the exposure to bALAN ($t_{(16)}$=2.8, p=0.01; Fig. 10G). The corticosterone analysis performed by sex showed higher levels of corticosterone only in HFHS-fed females when exposed to bALAN ($t_{(6)}$=2.6, p=0.03; Fig. 10I), but lower levels in males on chow-diet (Fig. 9H; $t_{(6)}$=2.7, p=0.03). Lastly, no differences in plasma leptin levels were found due to the bALAN exposure in animals fed with the chow ($t_{(14)}$=0.4, p=0.6; Fig. 9J) or the HFHS diet ($t_{(16)}$=0.1, p=0.8; Fig. 10J). HFHS-fed animals (Fig. 10J–L) had slightly higher leptin levels than the chow-fed *Arvicanthis* (Fig. 9J–L), but this difference did not reach statistical significance (Dark controls, $t_{(15)}$=1.5, p=0.1; bALAN-exposed, $t_{(15)}$=1.6, p= 0.1).

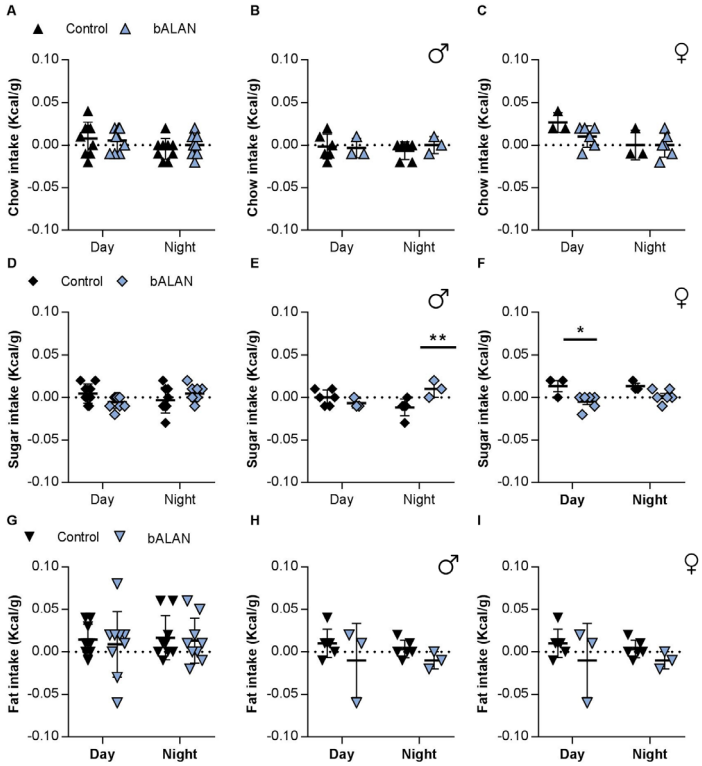

Figure 8. *In HFHS-fed Arvicanthis, bALAN exposure increases the sugar intake in male grass rats during the dark phase. bALAN exposure from ZT14-15 did not affect the consumption of chow food in all HFHS fed animals (A) nor in males (B) neither in females (C). The bALAN exposure did not affect either the sugar intake of all animals (D) but caused an increase in the sugar consumption during the dark phase in males (E; *Post-hoc, P = 0.008), but not in females (F). bALAN exposure did not changed the amount of fat ingested in all animals (G), nor when analyzed separately in males (H) or females (I).*

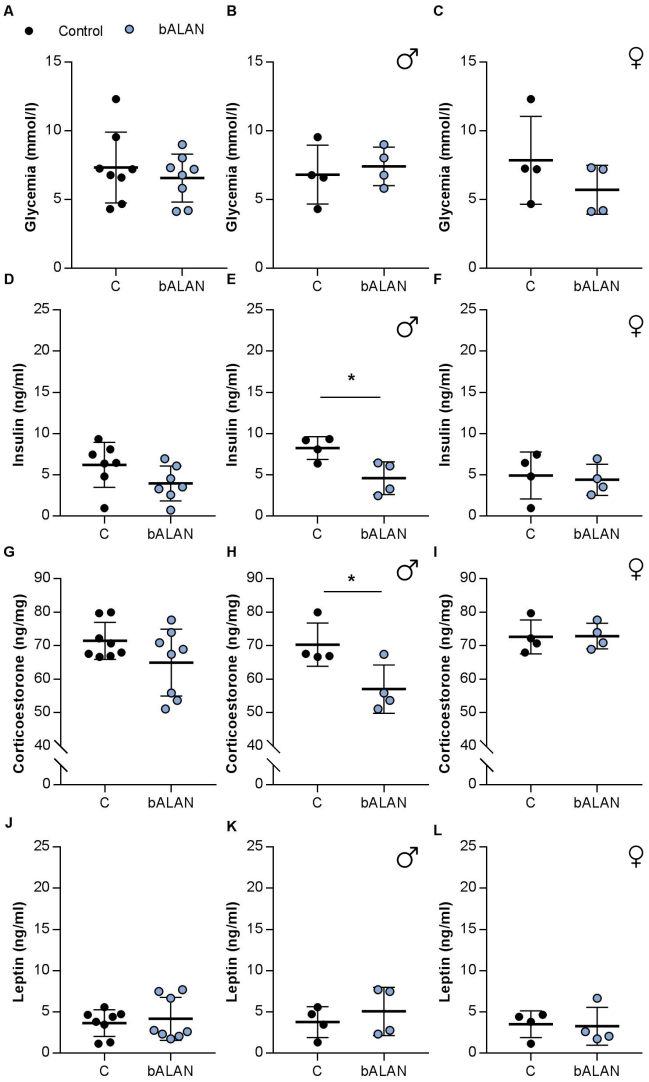

Figure 9. *bALAN exposure decreased plasma insulin and corticosterone in male Arvicanthis ansorgei on a regular chow diet. bALAN exposure from ZT14-15 in grass rats does not affect glycemia immediately after the light pulse in all animals (A), neither separately in males (B) or females (C). The bALAN exposure did not change the levels of plasma insulin in all animals (D), whereas it reduced the plasma insulin in male Arvicanthis (E; *t-test, P = 0.02), but not in females (F). bALAN exposure did not changed the plasma corticosterone concentrations in all animals (G); however, lower levels of corticosterone were observed in bALAN-exposed males (H; *t-test, P = 0.03), but not females (I), when compared to the levels of cortiscosterone in control dark-exposed Arvicanthis. The bALAN exposure pulse did not change plasmatic leptin levels in all animals (J), neither when analyzed separately for males (K) and females (L).*

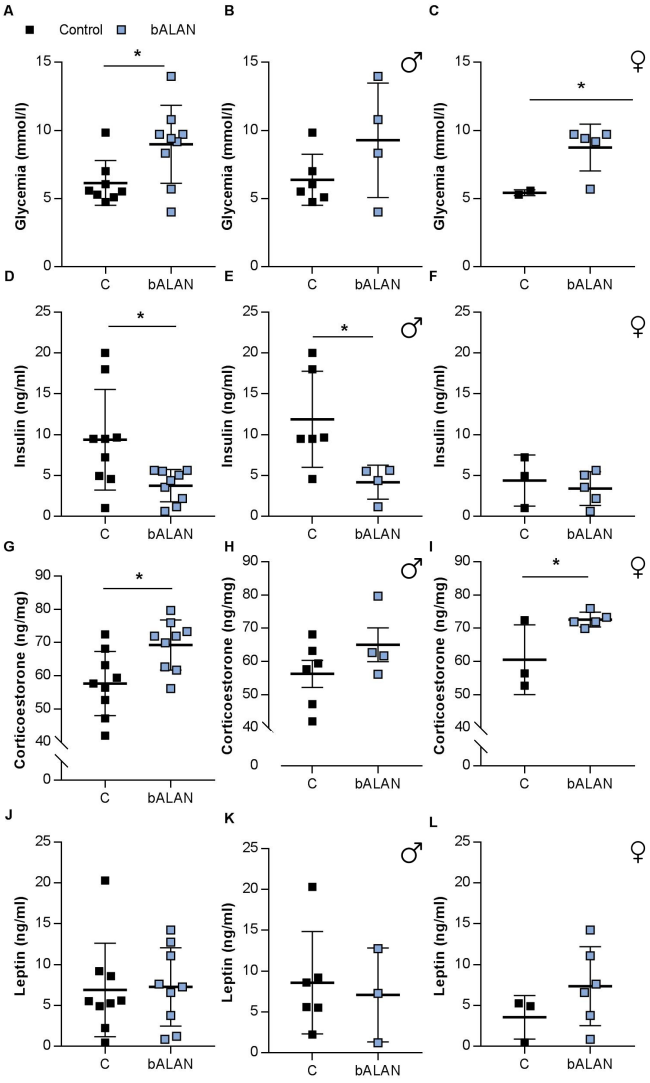

Figure 10. *bALAN exposure increased glycemia, decreased insulin, and increased corticosterone in Arvicanthis ansorgei on a HFHS diet. bALAN from ZT14-15 in grass rats increased blood sugar in all animals immediately after the light pulse (A; *t-test, P = 0.026). Nevertheless, it did not cause blood glucose changes in males (B), whereas a significant increase was observed in females (C; *t-test, P = 0.04). The bALAN exposure decreased the levels of plasma insulin in all animals (D; *t-test, P = 0.01), with a significant reduction also observed in the plasma insulin of males (E; *t-test, P = 0.03), but not in females Arvicanthis (F). bALAN exposure increased the amount of plasma corticosterone in all animals (G; P = 0.01). This effect was not significant separately in males (H), but it was significant in females (I; *t-test, P = 0.03). The bALAN exposure did not change the plasmatic leptin levels in all animals (J), neither when analyzed separately in males (K) and females (L).*

Discussion

To the best of our knowledge, this is the first study showing the effects of acute bALAN exposure on glucose metabolism and food intake in the diurnal rodent *Arvicanthis ansorgei*, fed either a chow or a HFHS diet. Importantly, the effects of bALAN on blood glucose and plasma insulin concentrations, as well as increased sugar intake were significant only in males, indicating a sex-dependent mechanism. The use of a diurnal rodent exposed to a high-fat high-sugar free choice diet more resembles light- exposure and feeding conditions in humans, than those in nocturnal rats or mice, and thus provides a better model for translational studies. Altogether, our results indicate that bALAN impairs glucose metabolism, especially in males, which in the long run could lead to the development of diabetes. Previously, the HFHS diet has been used successfully to induce obesity in rats (34,35) and mice (36), and here we used this dietary paradigm for the first time in the diurnal rodent *Arvicanthis ansorgei*. We did not observe a diet-induced increase in body weight in either sex, but although adiposity was not measured, plasma leptin was slightly elevated in HFHS animals. Moreover, we did observe marked glucose intolerance in both HFHS-fed males and females *Arvicanthis* like previously described in nocturnal rats and mice (34,36). After nocturnal exposure to blue light, increased glucose levels were observed in male *Arvicanthis* regardless of the type of diet, together with decreased plasma insulin levels. These results further stress the negative effects of nocturnal light on glucose tolerance, putatively by inhibiting insulin release, as previously also described in rats (29) and in healthy and T2D adults (37,38). It has been described before how light during the dark phase via the SCN can increase the sympathetic activity and decrease the parasympathetic activity of autonomic nerves that reach peripheral organs like the liver, the pancreas and the adrenal gland (22,39,40); this would lead to an increase in the hepatic gluconeogenesis, glycogenolysis, and a decrease in the release of insulin from the beta cells which can explain our current results in the glucose tolerance test.

Contrary to reports in nocturnal rats (35), a HFHS diet did not induce snacking behavior during the inactive phase in grass rats. However, exposure to the HFHS diet did to some extent reduce locomotor activity at the light phase in both sexes. Interestingly, although males did not change their food intake rhythm or increase their total caloric intake when exposed to a HFHS diet, an increased preference for fat consumption was observed at

the middle of the light phase (ZT5) in some animals. Females developed hyperphagia during their active phase when exposed to the palatable diet, with a ~50% increase in total caloric intake compared to chow-fed females. Previous studies in Wistar rats have shown that when rats are allowed to self-select the macronutrient composition of their meals, a high-protein high-fat composition is chosen by both sexes. Especially males have a higher fat intake after 6 weeks of age, mainly during their active phase (i.e., the dark period) (41), in agreement with the preference for fat at ZT5 in the male *Arvicanthis*. Earlier work from other groups has shown that female rats have a higher preference for diets with a high content of carbohydrates (42), which would be in agreement with the hyperphagia observed in female *Arvicanthis*, coming mainly from the higher intake of sugar.

Animal and human studies providing evidence of the metabolic effects of environmental light have been reviewed extensively (12,43). In humans, increases in obesity and diabetes match increased artificial light exposure (44) and it has been demonstrated that evening bright light increases appetite (45). Additionally, in a human study which aimed to assess the effect of bALAN exposure on the control of sleep/wakefulness and energy metabolism, it was reported that energy expenditure, oxygen consumption, carbon dioxide production and the thermic effect of breakfast were significantly lower in subjects who received bALAN compared to no light exposure (15). Opposed to studies using chronic exposure to dim light at night (11,46), we did not observe an increase in the total amount of calories ingested by animals as an acute effect of the light pulse. However, when we analyzed separately each component of the diet, we found a significant increased sugar intake during the dark phase in male animals after a 1 h of bALAN exposure, suggesting that blue light may affect specific brain sites regulating palatable food intake, in a sex-dependent manner.

Our observations in this experiment indicate that sex is an important variable when accounting for light effects not only on glucose metabolism but also on food intake. Although possible mechanisms responsible for sex differences regarding light perception in rodents are virtually unknown, a study in humans demonstrated that com- pared to women, men show a stronger response to blue- enriched light in the evening even at very low intensity (47). Clearly, further research is needed to elucidate the physiology behind these differences. Moreover, animal studies have reported how female animals are protected from circadian metabolic disruptions by high-

fat feeding due to the ovarian hormones (48), which may be a reason why we did not observe the same detrimental effects with the HFHS diet and the bALAN in females.

While the central projections of the ipRGCs have not been described in *Arvicanthis ansorgei*, previous work has been done in diurnal rodents from the same genus: *Arvicanthis niloticus* (49). The results of that study pointed out that the main characteristics of these cells are fundamentally the same as those described in nocturnal rodents (50–52). Thus, among the areas that are innervated by the ipRGCs are the SCN, the intergeniculate leaflet (IGL), and presumably the lateral hypothalamus (LH). In previous work from another group, the acute effects of light on the brain of *Arvicanthis* have been reported (53), showing a significant number of c-Fos activated cells in the IGL, the LH, and the lateral habenula (LHb), or the perihabenular nucleus (PHb) in mice (54). It has been previously shown that the geniculohypothalamic tract (which includes the IGL), actively modulates SCN responses to retinal input (55) and might modulate metabolic signals to the SCN (56), and reward and metabolic signals of feeding to the LHb (57). Also, it is widely known that the LH and the LHb are deeply involved in the control of metabolism, food intake and motivational processes (58,59), which could explain our findings in this study.

In previous studies from our group, we reported decreased glucose tolerance without an insulin response when Wistar rats were exposed to a two-hour bright or green light pulse from ZT14-16. However, this was not observed with blue light. Additionally, in that same study increased insulin responses with only small effects on glucose levels were observed when ALAN was administered close to the end of the dark phase, suggesting that not only wavelength but also time-of-day defines the effects of ALAN on glucose metabolism, probably through various mechanisms (29). However, there are several differences in the experimental set-up, such as the wavelength of the lights used, length of the light exposure, glucose administration route, blood sampling method, and the diurnality of the *Arvicanthis* versus the nocturnal nature of the rats that make it difficult to compare both experiments. Further studies with bALAN and diurnal species are needed to evaluate more in-depth the putative effects of blue light on glucose metabolism and food intake in humans. To conclude, we showed that in the diurnal rodent *Arvicanthis ansorgei* acute exposure to bALAN causes glucose intolerance, reduced insulin secretion and increased consumption of sugar, especially in

males. Whether this effect is dependent on the activation of melanopsin in the ipRGCs and thus its projections to different parts of the brain, remains to be investigated. Nevertheless, our present results in this diurnal rodent and previous results in rats (29) and humans (37,38) are sufficient to raise the awareness about the deleterious effects of light at night, especially short-wavelength coming from light-emitting diodes based products, on glucose metabolism and food intake.

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Conflict of Interest

None.

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Part III

EFFECTS OF LIGHT ON ENERGY METABOLISM OF RATS.

Chapter 5

Metabolic Effects of Light at Night are Time- and Wavelength-Dependent in Rats

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Abstract

Objective: Intrinsically photosensitive retinal ganglion cells are most sensitive to short wavelengths and reach brain regions that modulate biological rhythms and energy metabolism. The increased exposure nowadays to artificial light at night (ALAN), especially short wavelengths, perturbs our synchronization with the 24-hour solar cycle. Here, the time- and wavelength-dependence of the metabolic effects of ALAN are investigated.

Methods: Male Wistar rats were exposed to white, blue, or green light at different time points during the dark phase. Locomotor activity, energy expenditure, respiratory exchange ratio (RER), and food intake were recorded. Brains, livers, and blood were collected.

Results: All wavelengths decreased locomotor activity regardless of time of exposure, but changes in energy expenditure were dependent on the time of exposure. Blue and green light reduced RER at Zeitgeber time 16-18 without changing food intake. Blue light increased Period 1 (*Per1*) expression in the liver, while green and white light increased *Per2.* Blue light decreased plasma glucose and Phosphoenolpyruvate carboxykinase (*Pepck*) expression in the liver. All wavelengths increased c-Fos activity in the suprachiasmatic nucleus, but blue and green light decreased c-Fos activity in the paraventricular nucleus.

Conclusions: ALAN affects locomotor activity, energy expenditure, RER, hypothalamic c-Fos expression, and expression of clock and metabolic genes in the liver depending on the time of day and wavelength.

Study importance

• Acute exposure to bright morning light increases plasma triglyceride levels in healthy men and glucose levels in men with obesity with type 2 diabetes; blue enriched light in the morning and in the evening impacts glucose metabolism in healthy adults.

• Nocturnal light exposure in rats acutely induces glucose intolerance, depending on the time of day, intensity, and wavelength of the exposure.

• Here, we show that also the effects of light at night on energy metabolism are wavelength and time dependent.

Introduction

With our modern lifestyle, our daily pattern of light exposure is very different from that of the 24-hour solar cycle. Artificial light at night (ALAN) has been associated with multiple disturbances to the environment and to virtually every organism, being considered an endocrine disruptor (1). Studies in animals and in humans have shown how ALAN interferes with the normal secretion of hormones such as melatonin (2) and glucocorticoids (3); alters locomotor activity patterns, sleep, and alertness (4,5); and even alters body weight, food intake, and glucose metabolism (6,7,8,9,10). Our group reported that ALAN caused the upregulation of the enzyme phosphoenolpyruvate carboxykinase (PEPCK) in the liver of rats.

Because PEPCK is well known to play an important role in gluconeogenesis, this finding suggested that aberrant light exposure could affect glucose metabolism (11). More recently, our lab showed that 2 hours of ALAN in rats impaired glucose tolerance and that this effect was depending both on the time of the day and the wavelength (12). Most recently, we showed that 1 hour of ALAN induces changes in the hepatic transcriptome and that this effect was at least partially mediated via the autonomic nervous system (ANS). Additionally, it has been shown that light can acutely increase plasma triglycerides in healthy men and plasma glucose and triglycerides in men with obesity with type 2 diabetes, suggesting that exposure to artificial light can also alter lipid metabolism (13).

Light stimuli perceived by the retina reach the biological clock in the suprachiasmatic nucleus (SCN) via the retinohypothalamic tract. The entrained rhythmic information from the SCN subsequently is transmitted to the paraventricular nucleus of the hypothalamus (PVN), amongst others. The

preautonomic neurons in the PVN are responsible for balancing the activity of the sympathetic and parasympathetic branches of the ANS (14, 15), which is key for the control of daily carbohydrate and lipid metabolism in the liver. Hence, we hypothesized that (nocturnal) light may reach the PVN via the SCN and subsequently cause changes in the liver and energy metabolism. Therefore, the first and main objective of this work was to provide a detaileddescription of the time- and wavelength-dependent effects of light at night on energy metabolism in rats. The second aim was to investigate the time and wavelength dependency of light-induced changes in neural activity in the SCN and the PVN and, ultimately, the expression of hepatic clock and metabolic genes.

Methods

Animals and housing

Sixty-four male Wistar rats (Charles River Breeding Laboratories, Sulzfeld, Germany) weighing 240-280 g were individually housed under a controlled ambient temperature of 21°C to 23°C and 12-hour light/dark cycle (lights on at 07:00, Zeitgeber time 0 (ZT0); lights off 19:00, ZT12) with white fluorescent light (maximum, 150 lux) during the light phase and dim red light during the dark phase (< 5 lux). All animals had ad libitum access to chow food (Teklad Global Diet; Harlan, Horst, the Netherlands) and water. Body weight was measured weekly. All the experiments were performed in accordance with the Council Directive 2010/63EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures were also approved by the Animal Ethics Committee of the Royal Dutch Academy of Arts and Sciences (KNAW, Amsterdam, the Netherlands) and in accordance with the guidelines on animal experimentation of the Netherlands Institute for Neuroscience.

Experimental design

After an acclimatization week, animals were divided randomly into the following four groups: dark controls and blue, green, and white light (n = 16 each). Light-treated animals were divided further into two subgroups (n = 8 each), using blocked randomization by body weight; both subgroups received two different light pulses, one at the beginning and one toward the end of the dark phase. Subgroup 1 received a light pulse at ZT14-16

and ZT18-20, whereas subgroup 2 received light at ZT16-18 and ZT20-22 (Supporting Information Figure S1A).

Metabolic cages

To measure locomotor activity, food intake, energy expenditure, and respiratory exchange ratio (RER) before, during, and after the light exposure, animals were placed in Metabolic Pheno Cages (TSE systems, Bad Hombourg, Germany). Animals were individually housed and kept in these cages for 96 hours. The first 24 hours were taken as acclimatization and were not used for the analysis, the second day was used as a control day, the light treatment was given on the third day, and the fourth day was used as a control day to make sure the possible effects we would see were exclusively light dependent (Supporting Information Figure S1B, data of control days not shown). Data were acquired every 15 minutes for all the variables. For each parameter, the effects of light exposure were analyzed as the absolute change (Δ) between the eight measurements obtained from the metabolic cages during the light exposure and the last time point before lights went on.

Light exposure protocol

Metabolic cages were placed inside rectangular metal frames with LED strips around the perimeter. To determine which light intensity to use, all lights were measured with a spectrometer (Avaspec-3648-USB2; Avantes, Apeldoorn, the Netherlands). Using the rodent toolbox designed by Lucas et al. (16) we selected intensities such that roughly the same photon flux was obtained for each wavelength. Detailed characteristics of the light conditionsused can be found in Supporting Information Table S1. All animals in total received three 2-hour light pulses with ~2 wash-out weeks in between each light exposure (Supporting Information Figure S1).

Blood and tissue sampling

In the last week of the experiment, each group of eight animals was further divided into two subgroups of four animals each. Each subgroup received a third light pulse at one of the two time points they had been treated before (Supporting Information Figure S1). Immediately after this third 2-hour light pulse, animals were deeply anesthetized with an overdose of pentobarbital (intraperitoneal). A thoracotomy was performed, and blood was collected with a cardiac puncture from the left ventricle. A sample of

liver tissue was collected, frozen immediately in liquid nitrogen, and kept at −80°C until RNA isolation. All animals were perfused transcardially with paraformaldehyde (PFA) 4% weight/volume in 0.1 M phosphate buffer (pH= 7.2) and brains recovered for further analysis.

Plasma measurements

Blood glucose levels were determined by using blood glucose test strips with an accuracy of 0.1 mmol/l (FreeStyle; Abbot Diabetes Care, Alameda, California). Plasma insulin and corticosterone levels were measured by using a radioimmunoassay (Millipore, St. Charles, Missouri, and MP Biomedicals, Santa Ana, California, respectively).

RNA isolation and complementary DNA synthesis

Liver tissues were homogenized with tri-reagent using an Ultra-Turrax device (IKA Staufen, Germany) in order to prevent RNA degradation. RNA isolation and complementary DNA synthesis were performed as described elsewhere (17, 18) and in the Supporting Information.

Quantitative polymerase chain reaction

To measure the expression profiles of hepatic clock and metabolic genes, diluted complementary DNA (2:38) was used for all quantitative polymerase chain reaction (qPCR) in duplicate. The expression levels of the tested genes were standardized by dividing over the geometric mean of three reference genes Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) 40S ribosomal protein S18, (S18), Hypoxanthine-guanine phosphoribosyltransferase (HPRT). Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed by using a Light Cycler 480 (Roche). Gene expression levels were calculated with the software for linear regression of qPCR data LinRegPCR. Melting curves of the RT-qPCR were used as quality controls. Primer sequences are listed in Supporting Information Table S2.

Immunohistochemistry for c-Fos

Perfused brains were kept in paraformaldehyde (PFA) 4% for 24 hours and then changed to 30% sucrose with 0.05% sodium-azide until they were cut. Brains were cut at 30-μm thickness with the Cryostat NX50 (Thermo Fisher Scientific, Waltham, Massachusetts) and stored in cryoprotectant solution (30% v/v glycerol, 30% v/v ethylene glycol, 40% 0.1M Tris-buffered

saline (TBS) with 30% sucrose, and 0.05% sodium azide) at −20°C until the immunohistochemical analysis was performed with a protocol previously described (17), using a different primary antibody (c-Fos polyclonal rabbit SC-52, 1:1000; Santa Cruz Biotechnology, Dallas, Texas). A CCD camera (Sony Model 77CE; Tokyo, Japan) attached to a microscope (Zeiss Axios-kop with Plan-NEOFLUAR Zeiss objectives; Carl Zeiss GmbH, Oberkochen, Germany) was used to take micrographs with a 10 × 0.63 objective with identical lighting conditions for all animals. c-Fos-positive cells were counted using ImageJ 1.52a (NIH, Bethesda, Maryland).

Statistics

Data are presented as means (SEM). Mixed-effects models analysis and ANOVAs were used to analyze the changes in the parameters obtained from the metabolic cages during the 2-hour light exposure, and body weight gain, plasma metabolites, relative clock and metabolic gene expression, and number of c-Fos–positive cells in the brain areas were analyzed. Dunnet's multiple comparisons test was used for post hoc analysis when significant effects of light or interaction were found. Statistics were performed by using GraphPad Prism version 8.01 for Windows (GraphPad Software, La Jolla, California) with a significance level of $p<0.05$.

Results

Metabolic data

ALAN effects on locomotor activity showed significant effects of light ($p<0.001$ to $p<0.0001$) and interaction ($p<0.0001$) for all four time points, although the most pronounced effects were seen in the second half of the dark period. All three wavelengths contributed significantly to these effects of light on locomotor activity (Figure 1). Before each light exposure, locomotor activity showed a clear daily rhythm entrained to the light/dark cycle (Supporting Information Figure S3). A significant effect of light on energy expenditure was only found at ZT16-18 ($p=0.0453$), and significant interaction effects were found at ZT16-18 ($p<0.0001$), ZT18-20 ($p<0.0001$), and ZT20-22 ($p=0.0143$). This effect was mostly caused by blue ($p< 0.0001$) and white ($p=0.0159$) light at ZT16-18, blue ($p=0.0198$) and green ($p= 0.0245$) light at ZT 18-20, and the green light ($p=0.0205$) at ZT20-22 (Figure 2). For RER, a significant light effect was found at ZT16-18 ($P = 0.0289$) as well as significant interaction effects at ZT16-18 ($p<0.0001$) and ZT18-20

(p < 0.0001). The effects on RER were mostly caused by the effects of blue (p= 0.0026) and green (p=0.0427) light (Figure 3). Lastly, significant effects of light on food intake were found at ZT16-18 (p=0.0344) and ZT18-20 (p= 0.0335). A significant interaction effect was found at ZT16-18 (p=0.0045). The suppressive effects on food intake were mainly caused by the green (p=0.0224) and white (p=0.0360) light from ZT16-18 and the blue (p= 0.0481) and white (p=0.0196) light from ZT18-20 (Figure 4). The detailed statistical analysis of these metabolic data are displayed in Supporting Information Tables S5-S12.

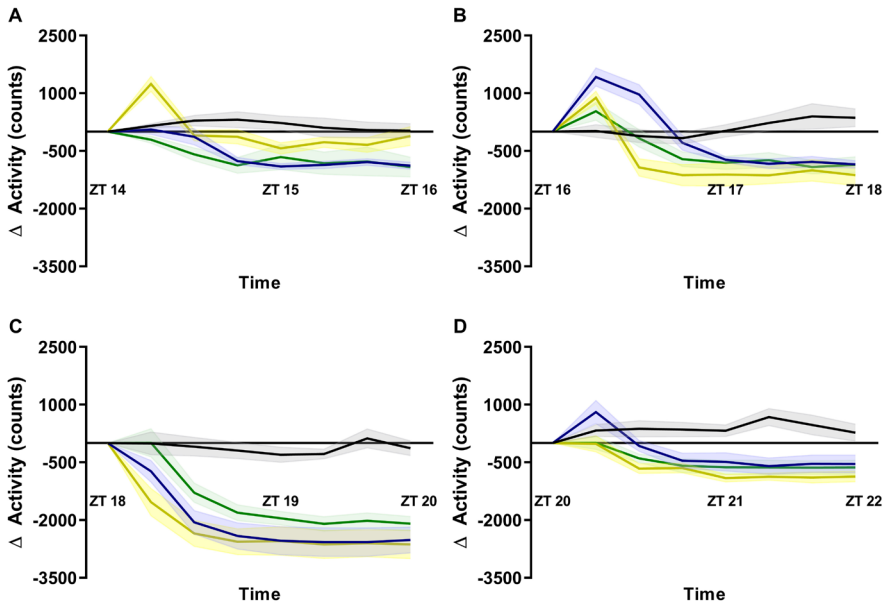

Figure 1. *ALAN effects on locomotor activity. ALAN significantly decreases locomotor activity regardless of the wavelength and time of the exposure. (A) ZT14-16 (p<0.0001), (B) ZT16-18 (p=0.0073), (C) ZT18-20 (P < 0.0001), (D) ZT20-22 (P = 0.0054). Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light-exposed animals.*

Surprisingly, with only three 2-hour nightly light pulses over a period of 6 weeks, we observed a significant difference in the percentage of body weight gain between light treatments, with the animals exposed to blue and green light showing a slightly lower weight gain over the course of the experiment (Supporting Information Figure S2, Supporting Information Tables S3-S4), which may be partially explained by the acute inhibitory effects of blue and green light described above.

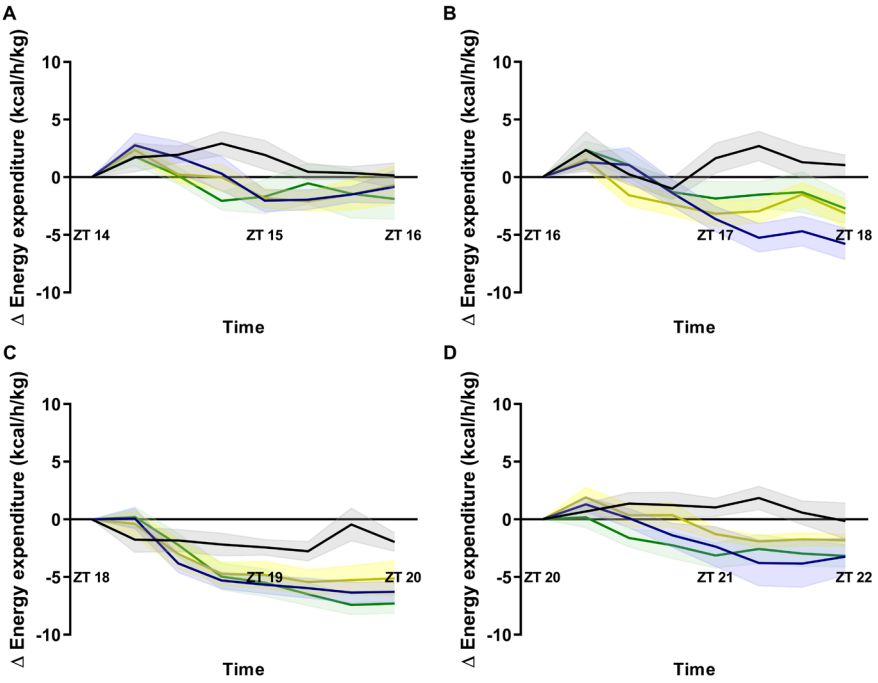

Figure 2. *ALAN effects on energy expenditure. Blue light significantly affects energy expenditure at ZT16-18 (p=0.0180) and ZT18-20 (p=0.0198), green light at ZT18-20 (p=0.0245) and ZT20-22 (p = 0.0205), and white light at ZT16-18 (p = 0.0159). Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light-exposed animals.*

Plasma measurements

Plasma glucose, insulin, and corticosterone concentrations were measured after the third 2-hour light exposure for each time point and wavelength. An overall significant effect of ALAN was observed in plasma glucose (p=0.0019, Figure 5A), with a significant effect especially from blue light (p=0.0312). No significant differences were found at any time point in the plasma insulin or corticosterone levels of light-exposed animals compared with dark controls, regardless of the wavelength used (Figures 5B-5C). A detailed statistical analysis of the changes in plasma values is shown in Supporting Information Tables S13-S15.

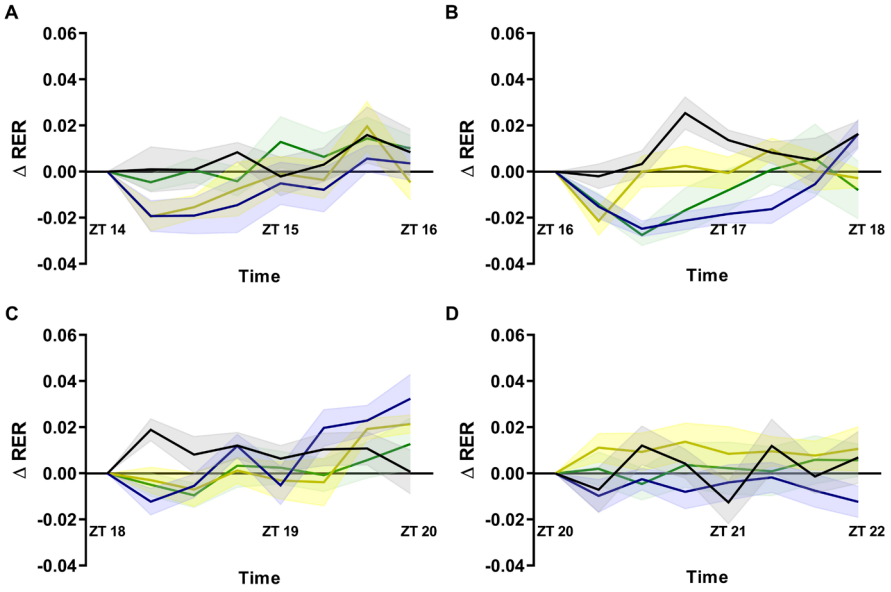

Figure 3. *ALAN effects on the respiratory exchange ratio (RER). Blue light (P = 0.0026) and green light (p=0.0427) caused a significant reduction in the RER at ZT16-18. Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light–exposed animals.*

Clock and metabolic genes in the liver

No significant ALAN-induced changes in the relative expression of the clock genes cryptochrome (*Cry*), brain and muscle Arnt-like protein-1 (Bmal1), Circadian Locomotor Output Cycles Kaput (*Clock*), or *Rev-erb* (Figure 6) were found, regardless of timing and wavelength of the light exposure. Moreover, a significant increase in the expression of *Per1* was found in the blue light–exposed group (p=0.0155), whereas increases in *Per2* were observed with green (p=0.0004) and white light (p=0.0004). The detailed statistical analysis of the changes in hepatic clock gene expression is shown in Supporting Information Tables S16-S18.

For the genes involved in carbohydrate metabolism, ALAN caused significant changes in the expression of Phosphoenolpyruvate carboxykinase (*Pepck*) (p=0.0072), Glucokinase (*Gck*) (p=0.0112), Acetyl-CoA carboxylase 1 (*Acc1*) (p=0.0005), Fatty acid synthase (*Fas*) (p=0.0002), and Peroxisome proliferator-activated receptor alpha (*Pparα*) (p=0.0003). Exposure to blue light caused a significant reduction in the expression of *Pepck* (p=0.0404)

(Figure 7A). Additionally, *Gck* expression was significantly increased with blue (p=0.0107), green (p=0.0002), and white (p=0.0386) light (Figure 7B), while *Pdk4* expression decreased with blue light exposure (p=0.0306) (Figure 7D).

Among the genes involved in lipids metabolism, *Acc1* significantly increased with all three wavelengths (p=0.0128 for blue, p=0.0076 for green, and p=0.0195 for white) (Figure 7E), and such an effect was also observed for the expression of *Fas* (p=0.0344 for blue, p<0.0001 for green, and p=0.0071 for white) (Figure 7F). Lastly, *Pparα* only showed a significant decrease with green light exposure (p=0.0032) (Figure 7G). A detailed statistical analysis of the changes in metabolic gene expression is shown in Supporting Information Tables S19-S21.

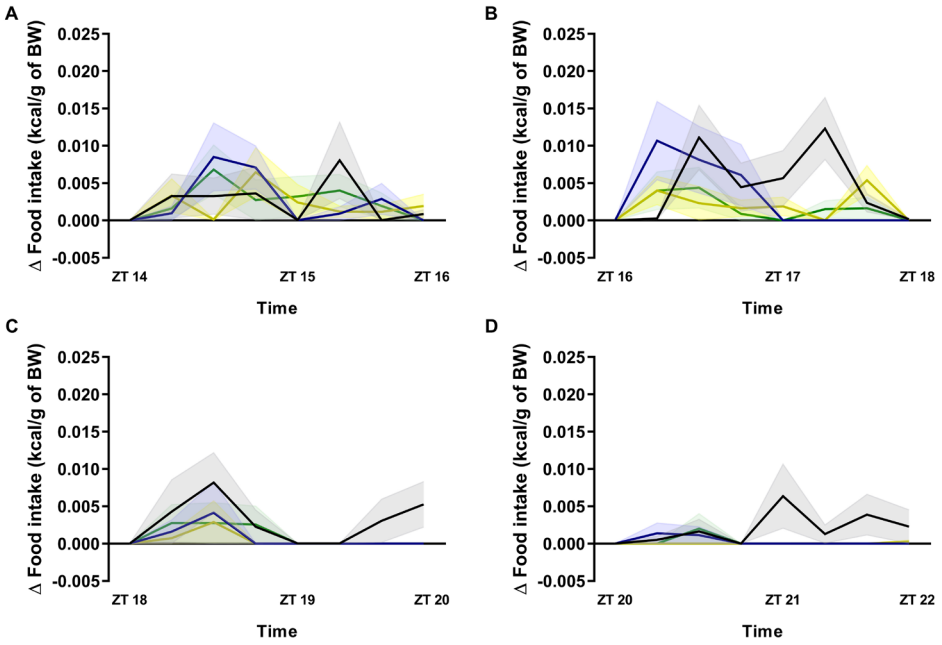

Figure 4. *ALAN effects on food intake. Green light (p = 0.0224) and white light (p = 0.0180) changed food intake during ZT16-18, while only white light affected it from ZT18-20 (p= 0.0183). Blue (p=0.0325) and green (p=0.0283) light decreased food intake at the end of the dark phase (i.e., ZT20-22). Black: dark controls; yellow: bright light-exposed animals; blue: blue light–exposed animals; green: green light–exposed animals.*

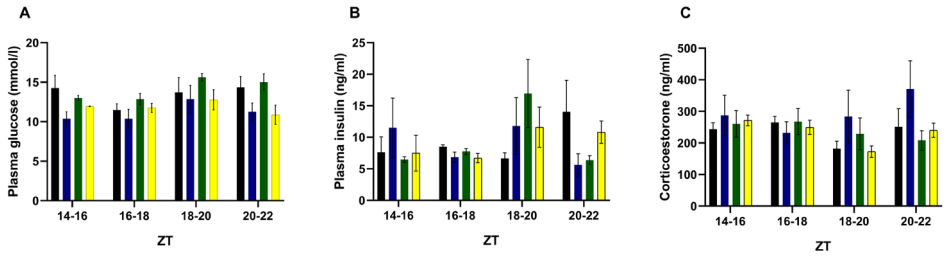

Figure 5. *ALAN effects on plasma glucose, insulin, and corticosterone concentrations. Blue light reduced plasma glucose concentrations (p= 0.0312), while no other changes in plasma levels of (A) glucose, (B) insulin, or (C) corticosterone were observed regardless of wavelength and time of the exposure. Black: dark controls; yellow: bright light-exposed animals; blue: blue light–exposed animals; green: green light- exposed animals.*

cFos expression SCN and PVN

ALAN showed significant effects of light ($p<0.0001$) and interaction ($p=0.0003$) on c-Fos expression in the SCN as well as a significant effect of light ($p=0.0010$) for c-Fos expression in the PVN. The number of c-Fos–positive cells in the SCN was significantly increased by all wavelengths compared with dark controls ($p=0.0132$ for blue, $p=0.0004$ for green, and $p<0.0001$ for white), with the highest numbers observed after exposure to white light (Figure 8A).

The effects of blue light were only significant at ZT20-22, whereas the effects of green and white light were apparent at all four time points. Moreover, in the PVN, we observed that the number of c-Fos–positive cells was significantly decreased in the blue ($p=0.0099$) and green light ($p= 0.0019$) exposed animals. This inhibitory effect of ALAN was only observed at the beginning of the dark period. No differences were found for c-Fos expression in the PVN between dark controls and animals exposed to white light. A more detailed statistical analysis of the c-Fos results can be found in Supporting Information Tables S22-S24.

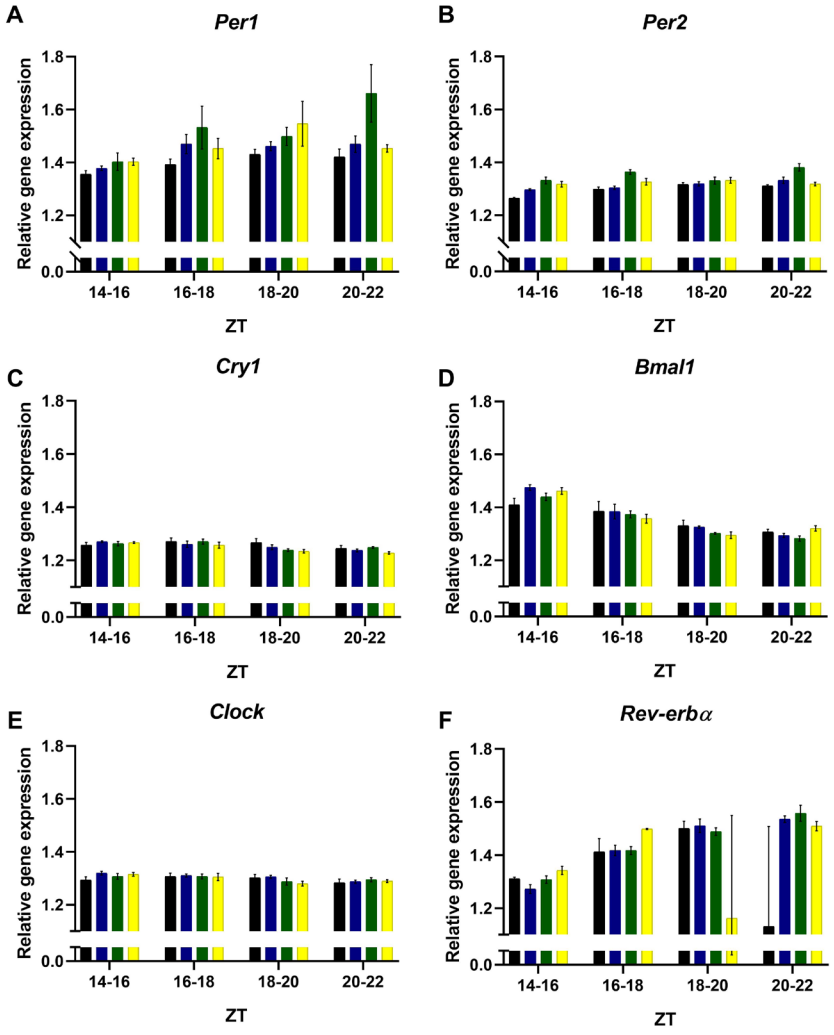

Figure 6. *Effects of ALAN on liver clock genes. (A) Per1 significantly changed with blue light (p=0.0155). (B) Green (p =0.0004) and white (p=0.0004) light increased the expression of Per2. ALAN did not change the expression of (C) Cry, (D) Bmal1, (E) Clock, or (F) Reverbα. Black: dark controls; yellow: bright light–exposed animals; blue: blue light-exposed animals; green: green light–exposed animals.*

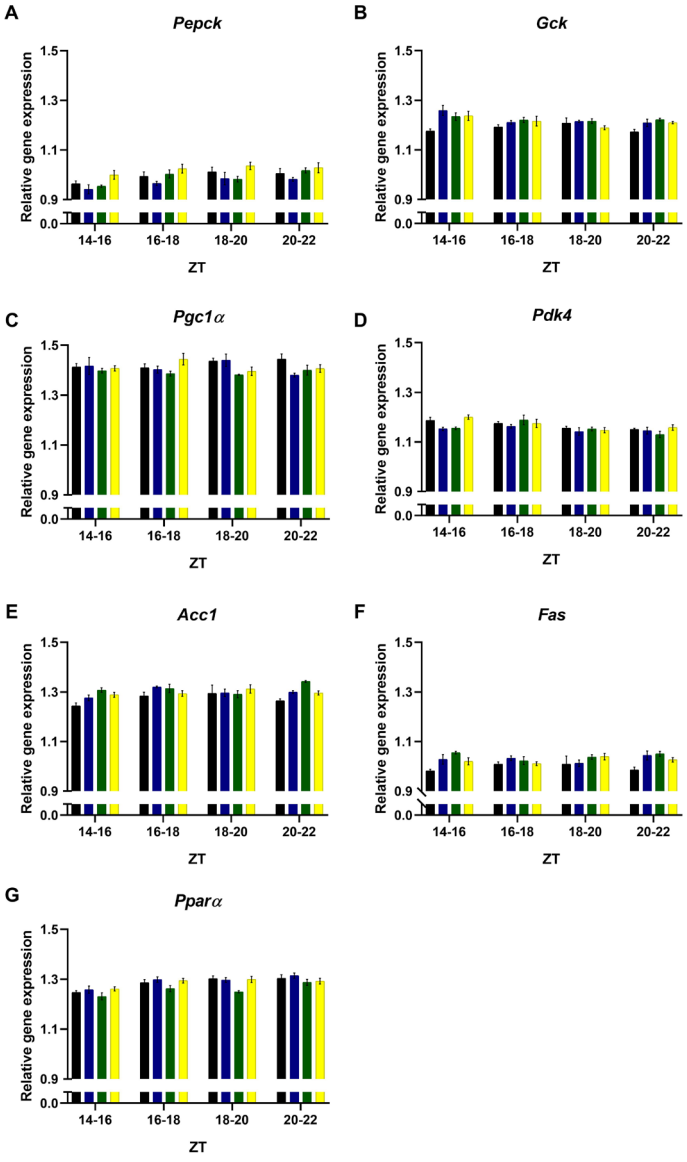

Figure 7. *Effects of ALAN on metabolic genes in the liver. (A) Pepck decreased significantly with blue light (p=0.0404). (B) Gck increased with blue (p=0.0107), green (p=0.0002), and white (p=0.0386) light. (C) Pgc1α expression decreased with green light (p=0.0025).(D) Pdk4 was downregulated by blue light (p=0.0306). (E) Acc1 increased significantly with all wavelengths (p=0.0128 for blue, p=0.0076 for green, and p=0195 for white).(F) Fas expression increased by blue (p=0.0344), green (p< 0.0001), and white (p=0.0071) light exposure. (G) Pparα expression decreased with green light exposure (p=0.0032). Black: dark controls; yellow: bright light–exposed animals; blue: blue light–exposed animals; green: green light–exposed animals.*

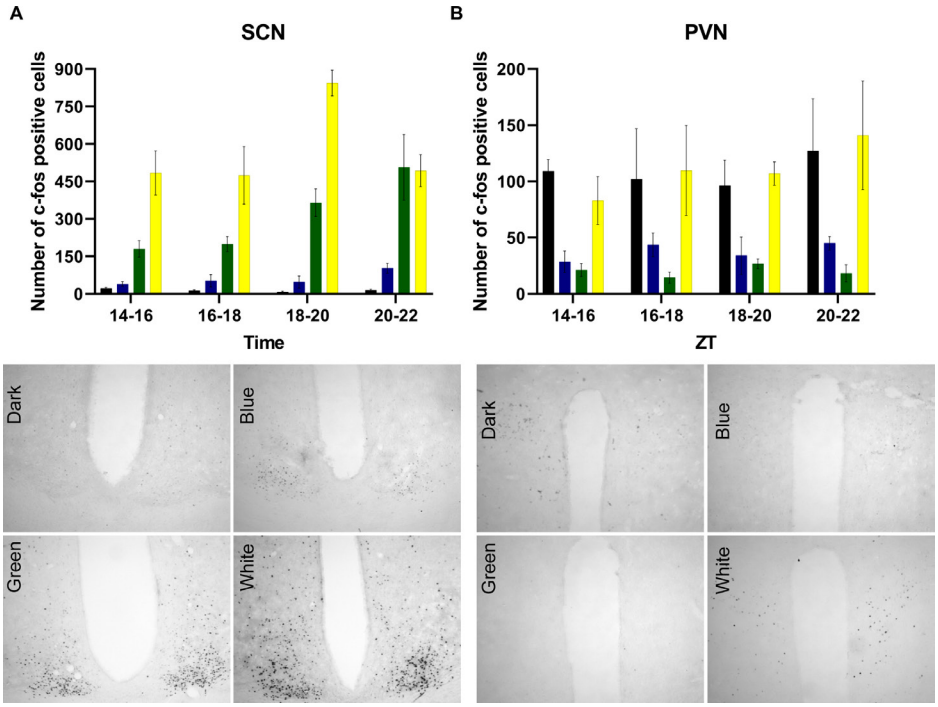

Figure 8. *ALAN effects on the number of c-Fos–positive cells in the SCN and the PVN. (A) Blue (p= 0.0132), green (p = 0.0004), and white (p < 0.0001) light significantly increased the expression of c-Fos in the SCN. (B) Blue (P = 0.0099) and green (p = 0.0019) light decreased the number of c-Fos–positive cells in the PVN. Pictures show representative sections of each area with every light exposure. Black: dark controls; yellow: brightlight–exposed animals; blue: blue light–exposed animals; green: green light–exposed animals.*

Discussion

Here, we show that 2 hours of ALAN acutely causes metabolic alterations and changes in the expression of clock and metabolic genes in the liver, and that these effects are dependent on both the timing and wavelength of the light exposure. In addition, we show that next to irradiance and photon flux, light-induced activation of c-Fos expression in the SCN also depends on wavelength. Remarkably, the effect of nocturnal light exposure on c-Fos expression in the PVN was completely opposite to that in the SCN. Contrary to the stimulation observed in the SCN, ALAN decreased c-Fos expression in the PVN. Moreover, inhibition of c-Fos expression in the PVN was only observed after blue and green light exposure, whereas white light was most effective in the SCN.

Our results show that ALAN in rats reduces locomotor activity regardless of the wavelength and time of exposure, which proves that in rats, negative masking (19) occurs independently of the wavelength and time of the dark phase when the light pulse is given. The robustness of this effect is clearly indicated by the fact that a similar analysis on the day before or after the light exposure did not reveal such a reduction. Remarkably, though, on some occasions, increased activity was also observed, especially during the first 15-minute period (that is, contrary to decades of negative masking data). The effect was observed mostly during the first half of the dark phase and especially with white and blue light. We do not have a straightforward explanation, although changes in the relative contribution of photoreceptors shortly after the onset of light exposure and possible daily rhythms therein could be involved (20, 21); often, a short bout of increased activity was also observed after regular lights-on with polychromatic light at ZT0 (Supporting Information Figure S3). Therefore, we think it more resembles a rarely studied phenomenon in the context of chronobiology called light-induced locomotor activity/activation (22, 23, 24).

Interestingly, although the reduction of locomotor activity by ALAN was significant for all time points and wavelengths, a significant concomitant reduction in energy expenditure was observed only at ZT16-18 with blue and white light. This apparent discrepancy could be merely a technical issue, as a decreasing trend was observed after each light exposure, with changes in the locomotor activity being just easier to detect than changes in energy expenditure. Moreover, whereas a significant decrease in energy expenditure was only observed at ZT16-18, the largest decrease in locomotor activity was observed at ZT18-20, indicating light might also have an activity independent effect on energy expenditure.

At ZT16-18, also a significant reduction in the RER was observed, both during blue and green light exposure. The decrease in RER (i.e., indicating a higher lipid metabolism) was accompanied by a reduction in food intake during green light exposure but not during blue light exposure. The discrepancy between feeding behavior and peripheral metabolism indicates that light may also have independent effects on these parameters.

Surprisingly, white and green light–exposed animals showed no significant changes in plasma glucose values. Previously, our laboratory showed that ALAN, especially white and green light, acutely impaired glucose tolerance in rats (12) and we observed a similar effect with blue light in the

diurnal rodent *Arvicanthis ansorgei* (25). However, in both these experiments, a glucose tolerance test was used to investigate glucose metabolism, which prevents a direct comparison with the current results. Moreover, animals were fasted for a few hours before the glucose tolerance tests, and the fasting state not only changes the glucose response but also changes SCN activity (26, 27). Moreover, when the Arvicanthis were not fasted (25) we did not observe any significant effects of ALAN on basal plasma glucose concentrations, whereas in the current experiment, we found that blue light reduced blood glucose. The difference between the effects of blue light on nonfasted blood glucose in these two experiments could be because *Arvicanthis* are diurnal rodents, whereas here we used nocturnal rats. Additionally, the setup and characteristics of the light exposure equipment differed slightly between the two experiments.

Although previous animal studies have shown that ALAN acutely increases the sympathetic and decreases the parasympathetic tone of the autonomic nerves that innervate peripheral organs such as the pancreas, adrenals, and liver (3, 28, 29), we did not find any significant changes in plasma insulin or corticosterone levels. In the existing literature, conflicting results have been reported regarding changes in corticosterone levels in response to acute LAN. Direct changes in absolute levels have not always been reported, and even opposite changes can be found (3, 11, 12, 25, 30, 31). These inconsistent findings may be related to differences among studies including the intensity, duration, wavelength, and circadian phase of the light exposure as well as sex and species involved. Moreover, our basal corticosterone levels are rather high (150-250 ng/mL); therefore, we cannot exclude these values are affected by the stress of the pentobarbital anesthesia, thoracotomy, and cardiac puncture, and it should be considered a limitation of our study. Nonetheless, even if our experimental conditions affected the baseline concentrations of corticosterone in all groups (dark controls included), we did not find any differences among the groups because of the different light conditions. We also did not observe a significant increase in the liver expression of *Pepck* as was previously reported with white light (11). Moreover, we did observe an unexpected decrease in the expression of this gene with blue light, which matches the observed reduction in plasma glucose but goes against previous evidence that exposure to blue-enriched white light at the beginning of the resting phase (i.e., ZT14) has a detrimental effect on plasma glucose in mice (32). However, we measured plasma glucose immediately after a 2-hour light pulse, whereas Nagai *et al.* (32) reported

significant changes in plasma glucose 48 hours after the light pulse. Second, the Nagai *et al*. (32) study was performed using C57BL/6J mice, indicating again that the effects of ALAN may be species dependent.

The results of the current study did confirm the light-induced increased expression of *Per2* in the liver observed before (11, 32). In addition, we found an increased expression of *Gck* with all the wavelengths and a decreased expression of *Pgc1α* and *Pdk4* with green and blue light exposure, respectively, providing further evidence for light-induced changes in carbohydrate metabolism. Likewise, the increased expression of Acc1 and Fas caused by ALAN indicates that light at the wrong time of the day can also acutely affect hepatic lipid metabolism regardless of the wavelength. In fact, the increased lipid metabolism indicated by the changes in gene expression nicely fits with the decrease in RER observed. Unfortunately, plasma fatty acids and triglycerides were not measured, but the current findings support the changes in liver lipids observed in zebra finches (33) and lipid metabolism in rats (34) and men (13) reported previously.

Although we did find lower energy expenditure and changes in oxygen consumption with blue light as was reported in humans before (35), contrary to what was expected, green and not blue light was better mimicking the detrimental effects of white light overall. Thus, in accordance with our previous study on glucose tolerance (12), we show that green light causes more metabolic changes than blue light. Our results with green light once more indicate a putative role for middle-wavelength cones in the effects induced by ALAN. Because both blue and green light are able to suppress melatonin (36), it is not likely that the marked difference between the metabolic effects of green and blue light are melatonin dependent.

The intrinsically photosensitive retinal ganglion cells (ipRGCs) have been described extensively before in humans (37), mice (38, 39, 40), rats (41) and diurnal rodents (42). These ipRGCs are known to contain melanopsin, a photosensitive pigment with a peak of action at short wavelength light (465-485 nm, i.e., the blue-appearing portion of the visible spectrum). In addition to their intrinsic photosensitivity, ipRGCs also receive light information via the rods and cones (16). Our data suggest that M-cones, which are sensitive to wavelengths of ~520 nm (green light), might be playing an important role in the metabolic effects of ALAN. Although only few studies have reported on the possible physiological effects of green light, a study in mice (4) did report effects on sleep/arousal and corticosterone

release. In that study, it was proposed that blue light mainly stimulates the SCN via the M1 subtype of ipRGCs, whereas green light stimulates another type of ipRGCs that mainly project to the ventrolateral preoptic area (4, 43, 44). However, according to our immunohistochemical results, in rats, green light activates the SCN more effectively than blue light. We did not study the ventrolateral preoptic area, but in the PVN, we found an inhibition of c-Fos expression with blue and green light but not with white light. Thus, although these rat and mice results are not identical, both studies clearly indicate that different wavelengths may affect the SCN and other brain areas differently.

It is highly unlikely that S-cones play a major role in our observations because they were barely stimulated with any of the light conditions we used (Supporting Information Table S1). Moreover, in our setup, the green and white light stimulated the rods much more effectively than the blue light, which in turn may change the information that ipRGCs send to the SCN. However, it is important to mention that although we equated the photon flux between the three light conditions, this does not mean that the α-opic irradiance and illuminance is the same for all wavelengths used, which makes comparisons among the different light conditions difficult. For example, the blue light used activated melanopsin to a greater extend (Supporting Information Table S1), but it also activated rods and cones, which in turn influences the firing rate of the ipRGCs (16). However, the green and white light activated the same photoreceptors, albeit to a different degree. Nevertheless, because one of the properties of photoreceptors is the principle of univariance (i.e., it cannot distinguish between changes in intensity and in wavelength), in principle, all lights could be equally effective in activating them, which can be considered a limitation of our study. A better approach for the future will be to study the role of ipRGCs in the effects of ALAN on metabolism by using the method of silent substitution (45).

The inhibitory effects of nocturnal light on c-fos expression in the PVN were to be expected in view of the abundant presence of GABA-containing neurons in the SCN and the strong stimulation of c-fos activity in the SCN by nocturnal light. In fact, previously, we have shown that SCN-mediated gamma-Aminobutyric acid (GABA) release in the PVN is essential for the inhibitory effect of nocturnal light on melatonin release (2, 46). However, the differential effects of wavelength on c-Fos expression in the SCN and PVN indicate that the inhibitory effects of light on the PVN may also be mediated via direct projections of the ganglion cells to the PVN and not indirectly via

the SCN. Additionally, ipRGCs also project to the lateral hypothalamic area where the major population of orexin neurons is located; because these orexin neurons, either glutamatergic or GABAergic, among others, project to spinally projecting PVN neurons (47, 48), this may be another pathway for light to reach preautonomic neurons in the PVN and hence affect peripheral metabolism. Indeed, recent publications clearly indicated alternative pathways not involving the SCN for light to affect peripheral clocks and metabolism (49, 50, 51), nicely fitting with the existence of separate populations of ipRGCs projecting to the SCN and to non-SCN target areas (52, 53).

The increased gene expression in the liver (*Per2, Gck, Acc1, Fas*) nicely correlates with the effects of light on c-Fos expression in the SCN, whereas the decreased gene expression in the liver (*Pgc1α, Pdk4*) correlates with the wavelength-dependent effects of light on c-Fos expression in the PVN. The selective inhibition of neural activity in the PVN with blue and green light makes it unlikely that the metabolic effects of ALAN, particularly those of white light, are only due to changes in ANS activity, which supports a recent observation from our group on ALAN-induced changes in the liver transcriptome (54).

Even though the particular effects of each wavelength may differ from one study to the other, all the evidence points toward the fact that exposure to ALAN acutely disturbs energy metabolism, which with repeated exposure, in the end, may lead to metabolic diseases such as obesity and diabetes, even in low intensities (7, 34, 55, 56). We are aware that the sample sizes for our immunohistochemical analysis and gene measurements were small (that is, n=4 per time point). Also, the interpretation and translation of our current results in nocturnal rats to human health must be done cautiously; nonetheless, they go hand in hand with the detrimental effects of light on energy metabolism in humans reported before (13, 57). Together with all the evidence of ALAN affecting energy metabolism in both an acute and chronic way (6, 11, 12, 25, 32, 54, 55), this results in several recommendations being made regarding the spectrum of light we should use (58). The current study provides further evidence to aid our understanding of ALAN, shiftwork, and metabolic disorders. However, additional studies involving other species, genetically modified animals, and humans are needed to further elucidate the metabolic effects of different wavelengths and the neural pathways involved.

Conclusion

We demonstrated in rats that exposure to ALAN causes changes in energy expenditure, the RER, and clock and metabolic gene expression in the liver. All these effects were dependent on the time of day, which suggests an interaction with the circadian timing system. Moreover, the effects observed were also dependent on wavelength, suggesting involvement of ipRGCs, M-cones, and rods. Our work warrants further precaution in the use of electronic devices that emit low- and middle-length wavelengths of light at night in order to prevent negative metabolic consequences among users.

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Disclosure

The authors declare no conflict of interest.

Author contributions

AMV designed research, executed all experiments, analyzed samples, performed statistical analysis, created the figures, and wrote the manuscript. WIGRR assisted during experiments and revised the manuscript. JM advised on experimental design and revised the manuscript. AK designed research and wrote the manuscript. AK is the guarantor of this work and, as such, has full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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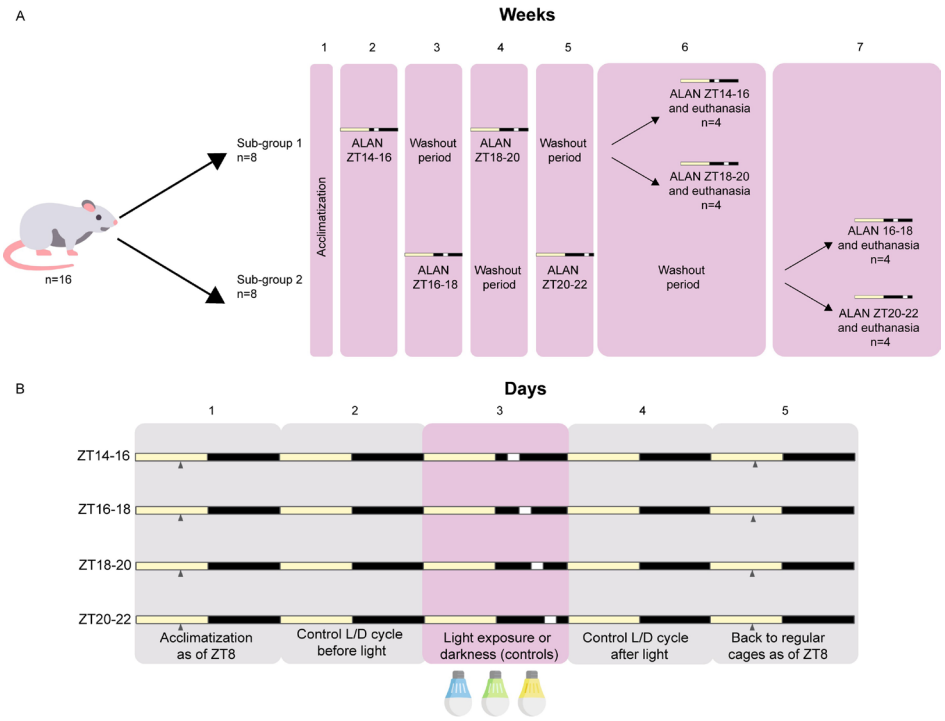
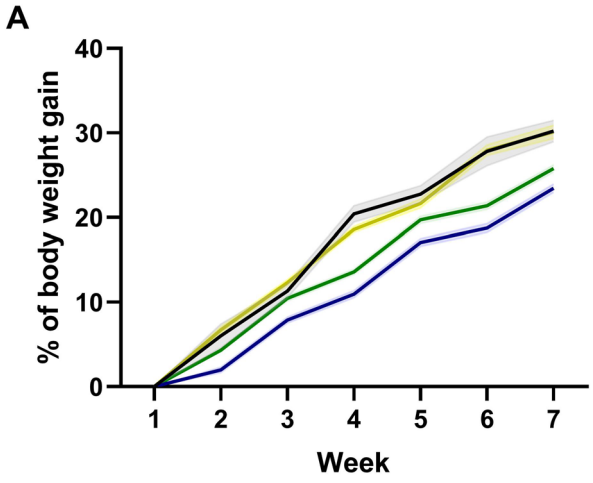
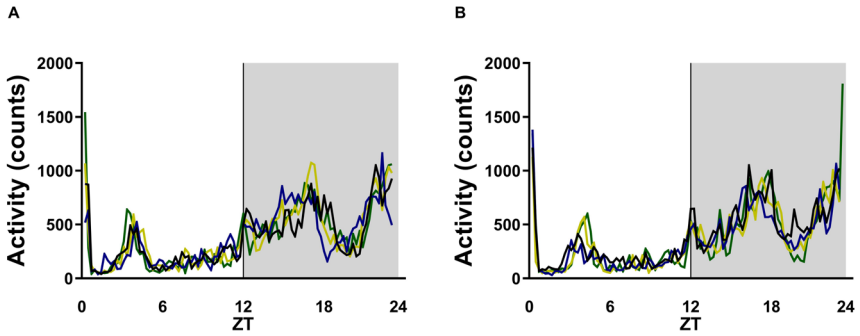


Supp. Figure 1. *Graphical representation of the experimental design.*



Supp. Figure 2. *ALAN effects on body weight. Percentage of body weight gain for each experimental group during the experiment.*



Supp. Figure 3. *Locomotor activity pattern on the days before light exposure. (A) 24-h locomotor activity before the first light pulse. (B) 24-h locomotor activity before the second light pulse.*

Supp. Table 1. *Spectral sensitivity of light conditions used in the experiments.*

Retinal photopigment complement	White	Blue	Green
S-Cone (rNsc [λ])	0.05	0.07	0.00
Melanopsin (rNz [λ])	13.69	58.77	38.67
Rod (rNz [λ])	16.39	43.13	50.71
M-cone (rNmc [λ])	18.31	35.04	53.60
Irradiance (μW/cm^2)	9.54	10.76	9.48
Photon flux (1 cm^{-2} s^{-1})	2.75x10^{13}	2.49x10^{13}	2.40x10^{13}
Peak spectral irradiance (nm)	595	455	520

Supp. Table 2. *Primer sequences used for qPCR on liver tissue.*

Gene name	Gene	Sequence F primer 5'-3'	Sequence R primer 5'-3'
		Clock genes	
Period 1	*Per1*	CGCACTTCGGGAGCTCAAACTTC	GTCCATGGCACAGGGCTCACC
Period 2	*Per2*	CACCCTGAAAAGAAAGTGCGA	CAACGCCAAGGAGCTCAAGT
Cryptochrome 1	*Cry1*	AAGTCATCGTGCGCATTTCA	TCATCATGGTCGTCGGACAGA
Brain and muscle aryl hy-drocarbon receptor nuclear translocator (ARNT)-like PGC-alpha	*Bmal1*	CCGATGACGAACTGAAACACCT	TGCAGTGTCCGAGGAAGATAGC
Circadian Locomotor Out-put Cycles Kaput	*Clock*	CGATCACAGCCCAACTCCTT	TTGCAGCTTGAGACATCGCT
Reverb alpha (gene: nr1d1)	*Rev-erbα*	ACAGCTGACACCACCCAGATC	CATGGGCATAGGTGAAGATTTCT
		Metabolic genes	
Phosphoenolpyruvate carboxykinase	*Pepck*	GTGTCCCCCTTGTCTACGAA	GGTCGTGCATGATGACCTT
Glucokinase	*Gck*	CAAGCTGCACCCGAGCTT	TGATTCGATGAAGGTGATTTCG
Peroxisome proliferator-activated receptor gamma coactivator 1-alpha	*Pgc1α*	TGCCATTGTTAAGACCGAG	GGTCATTTGGTGACTCTGG
Pyruvate Dehydrogenase Kinase 4	*Pdk4*	TGGTTTTGGTTACGGCTTGC	TGCCAGTTTCTCCTTCGACA
Acetyl-coenzyme A car-boxylase alpha	*Acc1*	GATGATCAAGGCCAGCTTGT	CAGGCTACCATGCCAATCTC
Fatty acid synthase	*Fas*	CTTGGGTGCCGATTACAACC	GCCCTCCCGTACACTCACTC
Peroxisome-Proliferator Activated Receptor alpha	*Pparα*	TCACACAATGCAATCCGTTT	GGCCTTGACCTTGTTCATGT
		Reference genes	
Glyceraldehyde 3-phosphate dehydrogenase	*GAPDH*	TGAACGGGAAGCTCACTGG	TCCACCACCCTGTTG CTGTA
Ribosomal protein S18	*S18*	CTCTTCCACAGGAGGCCTACACG	TGGCCAGAACCTGGCTATACTTCC
Hypoxanthine-guanine phosphoribosyltransferase	*HPRT*	GCAGTACAGCCCCAAAATGG	AACAAAGTCTGGCCTGTATCCAA

Supplementary Methods

RNA isolation

Liver tissues were homogenized with tri-reagent using an Ultra-Turrax device (IKA) in order to prevent RNA degradation. RNA isolation was performed with the ISOLATE II RNA Mini Kit (Bioline) and eluted from the spin colum with 40 µl of ultra-pure water. RNA purity and concentration were measured using a DS-11 (DeNovix) spectrophotometer. For purity a 260/280 ratio within the range of 1.8-2.0 was considered acceptable, whereas the 260/230 ratio was considered acceptable between 2.0-2.2. Quality of the RNA was further analyzed with the Agilent 2100 Bioanalyzer (Agilent Technologies). All samples had an RNA integrity number (RIN value) above 8.

cDNA synthesis

An input of 500 ng of RNA was used with the Transcriptor First Strand cDNA synthesis kit (Roche) and oligo-dT primers. Two additional samples without reverse transcriptase (RT-) were used as negative controls to check for DNA contamination. RT-PCRs were run with an UNO-Thermoblock (Biometra) 30 min at 55 °C, 5 min at 85 °C. Samples were stored at 4 °C for one day until qPCRs were run.

Supp. Table 3. *Statistical analysis of the effects of light on percentage body weight.*

Light	Time	Interaction
$F_{(3,60)}$=24.72	$F_{(6, 360)}$= 1926	$F_{(18, 360)}$=13.28
p<0.0001*	*p*<0.0001*	*p*<0.0001*

Supp. Table 4. *Post-hoc statistical analysis of percentage body weight gain.*

Time (weeks)	*p* for blue	*p* for green	*p* for white
1	>0.9999	>0.9999	>0.9999
2	0.0001*	0.1865	0.8087
3	0.0012*	0.6807	0.6043
4	<0.0001*	<0.0001*	0.1461
5	<0.0001*	0.0049*	0.5371
6	<0.0001*	<0.0001*	0.9968
7	<0.0001*	<0.0001*	0.9996

p values compared to dark controls

Supp. Table 5. *Statistical analysis of the effects of light on locomotor activity, energy expenditure, RER, and food intake*

Variable	Time point	*Light*	*Time*	*Interaction*
Locomotor activity	ZT14-16	$F_{(3, 28)}$=8.774	$F_{(7, 196)}$=17.46	$F_{(21, 196)}$=4.455
		p=0.0003*	*p*<0.0001*	*p*<0.0001*
	ZT16-18	$F_{(3, 28)}$=4.907	$F_{(7, 196)}$=55.57	$F_{(21, 196)}$=13.74
		p=0.0073*	*p*<0.0001*	*p*<0.0001*
	ZT18-20	$F_{(3, 28)}$=14.26	$F_{(7, 196)}$=95.18	$F_{(21, 196)}$=10.59
		p<0.0001*	*p*<0.0001*	*p*<0.0001*
	ZT20-22	$F_{(3, 28)}$=12.13	$F_{(7, 196)}$=13.75	$F_{(21, 196)}$=4.119
		p<0.0001*	*p*<0.0001*	*p*<0.0001*
Energy expenditure	ZT14-16	$F_{(3, 28)}$=1.293	$F_{(7, 196)}$=6.995	$F_{(21, 196)}$=1.174
		p=0.2964	*p*<0.0001*	*p*=0.2777
	ZT16-18	$F_{(3, 28)}$=3.041	$F_{(7, 196)}$=14.21	$F_{(21, 196)}$=3.818
		p=0.0453*	*p*<0.0001*	*p*<0.0001*
	ZT18-20	$F_{(3, 28)}$=2.345	$F_{(7, 196)}$=51.28	$F_{(21, 196)}$=4.952
		p=0.0944	*p*<0.0001*	*p*<0.0001*
	ZT20-22	$F_{(3, 28)}$=2.448	$F_{(7, 196)}$=9.562	$F_{(21, 196)}$=1.878
		p=0.0845	*p*<0.0001*	*p*=0.0143*
RER	ZT14-16	$F_{(3, 28)}$=1.230	$F_{(7, 196)}$=6.103	$F_{(21, 196)}$=0.8044
		p=0.3173	*p*<0.0001*	*p*=0.7126
	ZT16-18	$F_{(3, 28)}$=3.483	$F_{(7, 196)}$=6.777	$F_{(21, 196)}$=3.815
		p=0.0289*	*p*<0.0001*	*p*<0.0001*
	ZT18-20	$F_{(3, 28)}$=0.4957	$F_{(7, 196)}$=9.838	$F_{(21, 196)}$=3.540
		p=0.6882	*p*<0.0001*	*p*<0.0001*
	ZT20-22	$F_{(3, 28)}$=1.466	$F_{(7, 196)}$=1.139	$F_{(21, 196)}$=1.061
		p=0.2451	*p*=0.3409	*p*=0.3933
Food intake	ZT14-16	$F_{(3, 224)}$=0.08098	$F_{(7, 196)}$=2.872	$F_{(21, 224)}$=1.082
		p=0.9703	*p*=0.0069*	*p*=0.3691
	ZT16-18	$F_{(3, 224)}$=2.931	$F_{(7, 196)}$=3.938	$F_{(21, 224)}$=2.091
		p=0.0344*	*p*=0.0085*	*p*=0.0045*
	ZT18-20	$F_{(3, 224)}$=2.952	$F_{(7, 196)}$=2.866	$F_{(21, 224)}$=0.4435
		p=0.0335*	*p*=0.0495*	*p*=0.9846
	ZT20-22	$F_{(3, 224)}$=0.9692	$F_{(7, 196)}$=1.007	$F_{(21, 224)}$=0.9974
		p=0.4080	*p*=0.3232	*p*=0.4676

Light (all wavelengths), Time (data collection from the metabolic cages, every 15 min), and Interaction effects were determined using repeated measures two-way ANOVA comparing all 4 treatment groups.

Supp. Table 6. *Statistical analysis of the effects of blue light on locomotor activity, energy expenditure, RER, and food intake.*

Variable	Time point	*p for Light*	*p for Time*	*p for Interaction*
Locomotor activity	ZT14-16	<0.0001*	0.0006*	<0.0001*
	ZT16-18	0.2301	<0.0001*	<0.0001*
	ZT18-20	<0.0001*	<0.0001*	<0.0001*
	ZT20-22	0.0054*	0.0018*	<0.0001*
Energy expenditure	ZT14-16	0.1531	0.0004*	0.0647
	ZT16-18	0.0180*	<0.0001*	<0.0001*
	ZT18-20	0.0198*	<0.0001*	<0.0001*
	ZT20-22	0.0730	0.0157*	0.0067*
RER	ZT14-16	0.1847	0.0176*	0.4221
	ZT16-18	0.0026*	<0.0001*	<0.0001*
	ZT18-20	0.9541	<0.0001*	<0.0001*
	ZT20-22	0.5941	0.0147*	0.1923
Food intake	ZT14-16	0.8991	0.0473*	0.2393
	ZT16-18	0.3110	0.0074*	0.0138*
	ZT18-20	0.0503	0.0845	0.8902
	ZT20-22	0.0325*	0.4955	0.3028

p values were determined using repeated measures two-way ANOVA comparing blue light exposed animals against dark controls.

Supp. Table 7. *Statistical analysis of the effects of green light on locomotor activity, energy expenditure, RER, and food intake.*

Variable	Time point	*p for Light*	*p for Time*	*p for Interaction*
Locomotor activity	ZT14-16	0.0045*	0.0847	0.0042*
	ZT16-18	0.0208*	0.0003*	<0.0001*
	ZT18-20	<0.0001*	<0.0001*	<0.0001*
	ZT20-22	0.0003*	0.2848	0.0002*
Energy expenditure	ZT14-16	0.1129	0.1112	0.0951
	ZT16-18	0.1756	0.0043*	0.0093*
	ZT18-20	0.0245*	<0.0001*	<0.0001*
	ZT20-22	0.0205*	0.0602	0.0105*
RER	ZT14-16	0.9966	0.1694	0.6208
	ZT16-18	0.0427*	0.0119*	0.0016*
	ZT18-20	0.3582	0.3851	0.0107*
	ZT20-22	0.1822	0.0438*	0.5516
Food intake	ZT14-16	0.9059	0.1174	0.7565
	ZT16-18	0.0224*	0.0033	0.0426*
	ZT18-20	0.0896	0.1434	0.7527
	ZT20-22	0.0283*	0.4148	0.3574

p values were determined using repeated measures two-way ANOVA comparing green light exposed animals against dark controls.

Supp. Table 8. *Statistical analysis of the effects of white light on locomotor activity, energy expenditure, RER, and food intake.*

Variable	Time point	*p for Light*	*p for Time*	*p for Interaction*
Locomotor activity	ZT14-16	0.2639	0.0003*	<0.0001*
	ZT16-18	0.0079*	<0.0001*	<0.0001*
	ZT18-20	<0.0001*	<0.0001*	<0.0001*
	ZT20-22	<0.0001*	0.0459*	<0.0001*
Energy expenditure	ZT14-16	0.0426*	0.0097*	0.1797
	ZT16-18	0.0159*	0.0036*	0.0075*
	ZT18-20	0.1258	<0.0001*	0.0004*
	ZT20-22	0.0778	0.0489*	0.0493*
RER	ZT14-16	0.2815	0.0053*	0.4239
	ZT16-18	0.1061	<0.0001*	0.0478*
	ZT18-20	0.4411	0.0169*	0.0002*
	ZT20-22	0.2295	0.6659	0.3360
Food intake	ZT14-16	0.7767	0.2121	0.4077
	ZT16-18	0.0180*	0.0179*	0.0037*
	ZT18-20	0.0183*	0.1074	0.7449
	ZT20-22	0.3383	0.4280	0.4427

p values were determined using repeated measures two-way ANOVA comparing white light exposed animals against dark controls.

Supp. Table 9. *Post-hoc statistical analysis of the effects of light on locomotor activity, energy expenditure, RER, and food intake at ZT 14-16.*

Variable	Time point (min)	*p for blue*	*p for green*	*p for white*
Locomotor activity	15	>0.9999	>0.9999	>0.9999
	30	0.8494	0.0162*	0.0016*
	45	0.2588	0.0030*	0.1595
	60	0.0022*	0.0033*	0.2920
	75	0.0007*	0.0883	0.0360*
	90	0.0160*	0.0797	0.4557
	105	0.0108*	0.1843	0.4080
	120	0.0030*	0.0278*	0.9388
Energy expenditure	15			>0.9999
	30			0.9531
	45			0.5510
	60			0.1544
	75			0.0453*
	90			0.2359
	105			0.4922
	120			0.9084

Supp. Table 10. *Post-hoc statistical analysis of the effects of light on locomotor activity, energy expenditure, RER, and food intake at ZT 16-18.*

Variable	Time point (min)	*p for blue*	*p for green*	*p for white*
Locomotor activity	15	>0.9999	>0.9999	>0.9999
	30	0.0009*	0.2286	0.0079*
	45	0.0110*	0.9927	0.0339*
	60	0.9241	0.1035	0.0284*
	75	0.0048*	0.0085*	0.0185*
	90	0.0028*	0.0125*	0.0015*
	105	0.0229*	0.0137*	0.0171*
	120	0.0022*	0.0042*	0.0026*
Energy expenditure	15	>0.9999	>0.9999	>0.9999
	30	0.8425	>0.9999	0.9121
	45	0.9150	0.9166	0.5090
	60	0.9906	0.9957	0.7111
	75	0.0025*	0.0690	0.0067*
	90	<0.0001*	0.0206*	0.0011*
	105	0.0005*	0.2297	0.1828
	120	<0.0001*	0.0440*	0.0218*
RER	15	>0.9999	>0.9999	>0.9999
	30	0.2259	0.2547	0.0820
	45	0.0040*	0.0029*	0.9674
	60	0.0004*	0.0104*	0.1439
	75	0.0003*	0.1400	0.2425
	90	0.0210*	0.6867	0.9940
	105	0.6735	>0.9999	0.9656
	120	>0.9999	0.2353	0.0406*
Food Intake	15	>0.9999	>0.9999	>0.9999
	30	0.1942	0.3811	0.2047
	45	0.9323	0.4431	0.2259
	60	0.9794	0.5980	0.7463
	75	0.3579	0.3593	0.6655
	90	0.0504	0.0860	0.0508
	105	0.2153	0.9645	0.4624
	120	0.1407	0.2447	0.2267

Supp. Table 11. *Post-hoc statistical analysis of the effects of light on locomotor activity, energy expenditure, RER, and food intake at ZT 18-20.*

Variable	Time point (min)	*p for blue*	*p for green*	*p for white*
Locomotor activity	15	>0.9999	>0.9999	>0.9999
	30	0.2178	>0.9999	0.0141*
	45	0.0006*	0.0102*	0.0004*
	60	0.0004*	0.0002*	0.0002*
	75	0.0007*	<0.0001*	0.0007*
	90	0.0007*	<0.0001*	0.0006*
	105	0.0002	<0.0001*	<0.0001*
	120	0.0002*	<0.0001*	0.0003
Energy expenditure	15	>0.9999	>0.9999	>0.9999
	30	0.4390	0.3870	0.7155
	45	0.2942	0.9839	0.8162
	60	0.0607	0.2089	0.2139
	75	0.0191*	0.1191	0.2419
	90	0.0489*	0.0315*	0.1666
	105	0.0115*	0.0036*	0.0573
	120	0.0074*	0.0012*	0.2178
RER	15	>0.9999	>0.9999	>0.9999
	30	0.0028*	0.0105*	0.0265*
	45	0.3690	0.2018	0.3887
	60	>0.9999	0.7674	0.4284
	75	0.5411	0.9504	0.6609
	90	0.7122	0.5632	0.5444
	105	0.4834	0.9352	0.6413
	120	0.1032	0.7493	0.1699
Food Intake	15			>0.9999
	30			0.7612
	45			0.5902
	60			0.6462
	75			0.6462
	90			0.6462
	105			0.6107
	120			0.2751

Supp. Table 12. *Post-hoc statistical analysis of the effects of light on locomotor activity, energy expenditure, RER, and food intake at ZT 20-22.*

Variable	Time point (min)	*p for blue*	*p for green*	*p for white*
Locomotor activity	15	>0.9999	>0.9999	>0.9999
	30	0.3889	0.4112	0.4489
	45	0.2367	0.0220*	0.0036*
	60	0.0246*	0.0071*	0.0019*
	75	0.0345*	0.0044*	<0.0001*
	90	0.0026*	0.0015*	0.0003*
	105	0.0276*	0.0097*	0.0019
	120	0.0567	0.0197*	0.0024*
Energy expenditure	15	>0.9999	>0.9999	>0.9999
	30	0.8561	0.9518	0.5797
	45	0.6274	0.0775	0.7983
	60	0.2500	0.1193	0.9000
	75	0.2332	0.0231*	0.0726
	90	0.0724	0.0427*	0.0202*
	105	0.1883	0.0849	0.1706
	120	0.4048	0.2826	0.6404
Food Intake	15	>0.9999	>0.9999	
	30	0.8797	0.6445	
	45	0.9884	0.9977	
	60	>0.9999	>0.9999	
	75	0.3781	0.3781	
	90	0.6190	0.6190	
	105	0.4112	0.4112	
	120	0.6345	0.6345	

Supp. Table 13. *Statistical analysis of the effects of light on plasma metabolites.*

Metabolite	*p for Light*	*p for Time*	*p for Interaction*
Glucose	0.0019*	0.0878	0.7302
Insulin	0.9969	0.1900	0.1428
Corticosterone	0.3358	0.3265	0.5584

Supp. Table 14. *Statistical analysis of the effects of each wavelength of light on plasma metabolites.*

Metabolite	*p for blue*	*p for green*	*p for white*
Glucose	0.0312*	0.4108	0.4366
Insulin	0.9062	0.9242	0.9760
Corticosterone	0.1541	0.8754	0.9299

Supp. Table 15. *Post-hoc statistical analysis of the effects of blue light on plasma glucose.*

Metabolite	Time point (ZT)	*p for blue*
Glucose	14-16	0.2078
	16-18	0.7900
	18-20	0.9739
	20-22	0.2803

Supp. Table 16. *Statistical analysis of the effects of light on liver clock genes expression.*

Gene	*p for Light*	*p for Time*	*p for Interaction*
Per1	0.1219	0.0032*	0.1142
Per2	<0.0001*	0.0016*	0.0313*
Cry1	0.2243	0.0016*	0.4734
Bmal1	0.4096	<0.0001*	0.1877
Clock	0.6545	0.0171*	0.5758
Rev-erbα	0.6942	0.4637	0.4094

Supp. Table 17. *Statistical analysis of the effects of each wavelength of light on liver clock genes expression.*

Gene	*p for blue*	*p for green*	*p for white*
Per1	0.0155*	0.1011	0.0552
Per2	0.0577	0.0004*	0.0004*
Cry1	0.4658	0.504	0.0740
Bmal1	0.4420	0.5306	0.9525
Clock	0.4046	0.8767	0.9735
Rev-erbα	0.3501	0.3079	0.7847

Supp. Table 18. *Post-hoc statistical analysis of the effects of light on liver clock genes expression.*

Gene	Time point (ZT)	*p for blue*	*p for green*	*p for white*
Per1	14-16	0.4331		
	16-18	0.2649		
	18-20	0.5046		
	20-22	0.5830		
Per2	14-16		0.0247*	0.0281*
	16-18		0.0043*	0.2492
	18-20		0.6661	0.5482
	20-22		0.0249*	0.7183

Supp. Table 19. *Statistical analysis of the effects of light on liver metabolic genes expression.*

Gene	*p for Light*	*p for Time*	*p for Interaction*
Pepck	0.0072*	0.0023	0.8930
Gck	0.0112*	0.0512	0.0898
Pgc1α	0.0632	0.9677	0.1471
Pdk4	0.0640	0.0010*	0.2287
Acc1	0.0005*	0.0643	0.1008
Fas	0.0002*	0.7995	0.2562
Pparα	0.0003*	<0.0001*	0.7332

Supp. Table 20. *Statistical analysis of the effects of each wavelength of light on liver metabolic genes expression.*

Gene	*p for blue*	*p for green*	*p for white*
Pepck	0.0404*	0.6603	0.0784
Gck	0.0107*	0.0002*	0.0386*
Pgc1α	0.2692	0.0025*	0.3062
Pdk4	0.0306*	0.1726	0.7191
Acc1	0.0128*	0.0076*	0.0195*
Fas	0.0344*	<0.0001*	0.0071*
Pparα	0.3978	0.0032*	0.8112

Supp. Table 21. *Post-hoc statistical analysis of the effects of light on liver metabolic genes expression.*

Gene	Time point (ZT)	*p for blue*	*p for green*	*p for white*
Pepck	14-16	0.6449		
	16-18	0.4238		
	18-20	0.6947		
	20-22	0.5605		
Gck	14-16	0.0413*	0.0526	0.0768
	16-18	0.3183	0.1787	0.5959
	18-20	0.9653	0.9594	0.7447
	20-22	0.2205	0.0194*	0.0539
Pgc1α	14-16		0.7229	
	16-18		0.5592	
	18-20		0.0705	
	20-22		0.3335	
Pdk4	14-16	0.1231		
	16-18	0.5531		
	18-20	0.7487		
	20-22	0.9572		
Acc1	14-16	0.2194	0.0142*	0.0687
	16-18	0.1957	0.4749	0.9243
	18-20	0.9997	0.9994	0.9199
	20-22	0.0277*	0.0013*	0.0685
Fas	14-16	0.1944	0.0004*	0.1483
	16-18	0.2583	0.8022	0.9968
	18-20	0.9992	0.7465	0.7276
	20-22	0.0905	0.0156*	0.0850
Pparα	14-16		0.6537	
	16-18		0.4292	
	18-20		0.0568	
			0.7450	

Supp. Table 22. *Statistical analysis of the effects of light on c-fos expression in the SCN and the PVN.*

Area	*p for Light*	*p for Time*	*p for Interaction*
SCN	<0.0001*	0.0030*	0.0003*
PVN	0.0010*	0.4832	0.9490

Supp. Table 23. *Statistical analysis of the effects of each wavelength of light on c-fos expression in the SCN and the PVN*

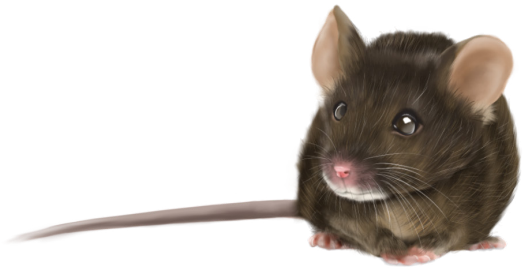
Area	*p for blue*	*p for green*	*p for white*
SCN	0.0132*	0.0004*	<0.0001*
PVN	0.0099*	0.0019*	0.9449

Supp. Table 24. *Post-hoc statistical analysis of the effects of light on c-fos expression in the SCN and the PVN.*

Area	Time point (ZT)	*p for blue*	*p for green*	*p for white*
SCN	14-16	0.3189	0.0369*	0.0284*
	16-18	0.4016	0.0177*	0.0587
	18-20	0.3753	0.0162*	0.0011*
	20-22	0.0385*	0.0698	0.0106*
PVN	14-16	0.0190*	0.0020*	
	16-18	0.5454	0.3345	
	18-20	0.1715	0.1105	
	20-22	0.3823	0.2551	

Part IV

EFFECTS OF LIGHT ON GLUCOSE, ENERGY METABOLISM, AND FOOD INTAKE IN MICE.



Chapter 6

Blue light at night modulates glucose metabolism and increases fat intake in female mice fed a free choice high-fat high-sucrose diet

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Abstract

Several brain structures implicated in the regulation of food intake and glucose metabolism receive projections from melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs). Thus, light might have a direct impact on these physiological variables. In the current study, we investigated the acute effects of blue and white light at night on food intake and glucose metabolism of C57BL/6J female mice exposed to a regular chow diet or a free choice high-fat high sucrose diet (HFHS). Mice, under a 12h/12h light-dark cycle, were exposed to a 1h light pulse (either blue or white) starting at ZT14 (two hours after lights-off) and immediately thereafter intraperitoneal glucose tolerance tests were performed. After one week a second light pulse was given and food intake was measured during the 24 hours preceding and following the light pulse. Finally, to evaluate the role of melanopsin in the effects of blue and white light on food intake, we exposed melanopsin mutant mice (*Opn4 -/-*) to the same light-exposure conditions. Both white and blue light exposure reduced locomotor activity in mice regardless of the diet. No significant differences in glycemic response were found between light-exposed (either spectrum), and dark control animals on a chow diet. Animals on the HFHS diet, however, showed an improved glucose tolerance, when exposed to blue or white light for the 1st time. Food intake in the chow animals showed no significant changes due to light exposure. In HFHS animals, however, we found a significant increase in caloric intake during the following dark period only in blue light-exposed animals. Interestingly, the extra calories consumed mainly came from fat, suggesting that blue light stimulates the drive for hedonic feeding in mice. Blue light and white light at night also increased food intake in melanopsin mutant mice, but now by increasing the amount of chow eaten. Our results indicate that the effects of light at night on hedonic food intake are melanopsin dependent.

Introduction

In nature, the endogenously generated circadian (circa "about" and dies "day") rhythms of organisms are synchronized with the 24-hour solar cycle. Therefore, light is the most important environmental cue to entrain these circadian rhythms to the solar cycle, although they are able to maintain rhythmicity without the presence of environmental light/dark cycles (1). In mammals, environmental light is not only perceived by the rods and cones, but also by the intrinsically photosensitive retinal ganglion cells (ipRGCs), expressing the photopigment melanopsin (2,3). In addition to the visual system, this light input is also sent to the bilateral suprachiasmatic nucleus (SCN) in the hypothalamus, containing the central circadian pacemaker of the brain (4,5). Light is the main environmental cue or Zeitgeber (German for time giver) for the SCN to be entrained (i.e. phase-adjusted) with our environment. However, over the last century, the introduction of electrical light has caused an aberrant exposure to artificial light at night for at least 80% of the world population (6), which is much more than the maximum amount of natural light that can be received at night during a full moon, which does not go beyond 1 lux (7). Hence, it is expected that this aberrant light exposure pattern could affect behavior and physiology.

Nowadays, the artificial light we receive comes not only from incandescent or fluorescent electrical bulbs but also from electronic devices including television, computer screens, tablets, and smartphones. This does not only increase the total amount of light we receive in the evening but due to new technologies, such as the light-emitting diodes (LEDs), also the wavelength of the light we are exposed to has changed (8). These LEDs produce more short-wavelength (i.e. blue) light than the incandescent or fluorescent electrical bulbs. It is also known that melanopsin, the photopigment expressed in ipRGCs, is the most sensitive photopigment to short-wavelength blue light (9–11). Moreover, it has previously been shown that short-wavelength light is most potent in suppressing melatonin in humans (12). Melatonin is involved in the control of several physiological processes, such as energy metabolism, hormone synthesis, and body mass regulation (13). Consequently, previous studies have shown that exposure to short-wavelength light during the dark period leads to various physiological (14–16) and behavioral consequences (17), amongst others these alterations in food intake and metabolism (18–20). Due to the fact that ipRGCs not only project to the SCN, but also to other hypothalamic, limbic and mood-

regulation areas (21,22), feeding behavior and appetite regulation may be altered acutely by aberrant light exposures. Here we aimed to explore the effects of white and blue light exposure at night on glucose metabolism and food intake of wild type mice exposed on a regular chow diet or a free choice high-fat high sucrose diet (HFHS). Furthermore, to study the possible role of melanopsin in the effects of light at night on food intake we determined this behavioral response in melanopsin deficient mice (Opn4 -/-) and their wild-type littermates.

Materials and Methods

Animals and housing

For the first experiment, we used sixty C57Bl6/J adult (10 weeks-old) female mice weighting 21.2 ± 4.1 g. Furthermore, nineteen adult (10 weeks old) male and female *Opn4 -/-* mice (on a C57Bl6/J background (23) and their respective littermates (Opn4 +/+, n=11) were used. Animals were obtained from the platform Chronobiotron, UMS n° 3415, CNRS and University of Strasbourg. Mice were individually housed in Plexiglas cages with sawdust bedding, chow food, and tap water ad libitum. Mice were maintained under a controlled ambient temperature of 21-23 ºC, relative humidity of 23-27% and a 12-h light/dark cycle (lights on at 07:00 hours, Zeitgeber time 0 (ZT0); lights off 19:00 hours, ZT12) with dim red light at night (<5 lux). All animals were given a wooden chow stick measuring 50 mm x 5 mm x 5mm and a cotton square as environmental enrichment.

All experiments were performed in accordance with the rules of the European Committee Council Directive of November 24, 1986 (86/609/EEC) and the French Department of Agriculture (license no. 63-378 to JM).

Food intake rhythm

To determine the time of light exposure at night time we measured the daily rhythm of Chow food intake (SAFE, 105, U8400G10R. Augy, France. 2.85 kcal/g, where: 23% proteins, 65% carbohydrates and 12% fat). Thus, after a week of acclimatization food consumption of C57Bl6/J mice was measured manually every 4 hours for 4 days. The time of 1h light exposure was selected at the time at which food intake was the highest (ZT-15; Figure 1).

Free choice high-fat high sucrose diet

After measuring the daily food intake rhythm with chow food, animals were randomly assigned to two groups fed with a different diet. The chow group (n=28) remained on the chow diet mentioned before and tap water. The free choice High-Fat High-Sugar (HFHS) group (n=26) received a cube of saturated fat (beef lard, Vandemoortele, France; 9 kcal/g) and a bottle with 10% sugar water (commercial grade sucrose and tap water, 0,4 kcal/ml) in addition to the chow food and tap water (15,24). All groups were kept on their respective diets for 4 more weeks and animals were weighed weekly.

Light exposure protocol

Animals from both diet groups were randomly assigned to three subgroups, dark controls (n=6-7, per diet group), blue (n=6-7, per diet group), and white light-exposed (n=6-7, per diet group). At ZT14 (i.e. two hours after lights -off) the regular white light use during the day was turn on for the white light-exposed group or a blue LED lamp was placed in front of the cabinets where the mice cages were placed for the blue light exposed ones. Animals were exposed to a 1-hour pulse of white or blue light (490±20 nm wavelength, see Table 1 for the irradiance spectrum (2)), then the dark condition was restored. Protocols to evaluate glucose metabolism, food intake, and lights effects on hormones were applied immediately hereafter as described below. Between each light exposure, animals were able to rest for one week. For food intake experiments in *Opn4 +/+* and *Opn4 -/-* the same light exposure protocol was used.

Table 1. *Spectral sensitivity of the light condition used in the whole experiments.*

Retinal photopigment complement	Blue light	White light
S-Cone (rNsc [λ])	0.00	7.19
Melanopsin (rNz [λ])	27.20	148.60
Rod (rNz [λ])	26.55	170.10
M-cone (rNmc [λ])	24.34	184.87
Irradiance (μW/cm^2)	4.26	80.50
Photon flux (1 cm^{-2} s^{-1})	1.06 x 10^{13}	2.23 x 10^{14}

Data based on the Rodent Toolbox provided by Lucas et al (2014). T

Intraperitoneal glucose tolerance test (IPGTT)

Immediately after animals had been exposed to the first pulse of light (either white or blue) or kept in control dark conditions, an IPGTT was performed starting at ZT15 as previously reported (15). All mice were fasted for 6 hours before the test, i.e. from ZT9 onwards. Blood was taken from the tip of the tail at 0, 15, 30, 90, and 120 minutes for the measurement of whole blood glucose concentrations using an Accu-Chek Performa Nano glucometer (Roche Diabetes Care Limited). Immediately after the 0 minutes sample, D-glucose (2g of D-glucose per kg of body weight dissolved in 0.9% saline, with a concentration of 100 mg/ml) was administered i.p. Data of the IPGTT were expressed as the change compared to the basal (t=0) glucose. In addition, the increase of plasma glucose was analyzed by calculating the area under the curve (AUC) from the time of the baseline to the 120 min measurement, using the trapezoidal rule (25).

Effects of light on food intake

One week after, animals received the same light treatment as described above and all food components from both diet groups were weighted every 12 h starting at ZT12 on the day before the light exposure and until ZT12 on the day after, in order to record the possible changes in the day-night intake pattern due to the blue or white light pulse at the beginning of the night. Food intake changes by light exposure were also determined in Opn4+/+ and Opn4-/- mice. For a representative timeline of the experiment see Fig.1.

Locomotor activity recordings

To measure light effects on locomotor activity, animals were monitored by using infrared detectors placed above the cages. Data were recorded every 10 min. Clocklab software (Actimetrics, Wilmette, IL) was used to determine the activity of each animal during the whole experiment. Locomotor activity data is expressed as the difference between the activity at ZT14 of the day before the light exposure and ZT14 of the day with the first light pulse.

Blood sampling and serum analysis

At least one week after the second light pulse a third light pulse was given to the animals and they were deeply anesthetized immediately after (i.e. ZT15) with an overdose of isoflurane. Blood was taken from the left ventricle with cardiac puncture using a 2 ml syringe with a 27G needle. Immediately

after animals were perfused transcardially with PFA 4% in 0.1 M phosphate buffer and brains were collected for future analysis. Glucose levels in blood were determined as described before using a drop of the extracted blood. Then it was decanted into 15 ml Corning tubes containing 100 µl of 4% EDTA. Afterward, samples were centrifuged at 4 ºC with a speed of 5000 rpm during 10 min, and plasma was stored at -80 ºC for determination of plasma insulin corticosterone, and ghrelin concentrations. Insulin was measured using an ELISA procedure with a Rat/Mouse Insulin ELISA Kit (EZRMI-13K, Merck-Millipore, Germany). The limit of sensitivity of the insulin kit was 0.1 ng/ml. Corticosterone was measured using an EIA procedure with a Rat/Mouse Corticosterone EIA Kit (AC-14F1, Immunodiagnostic Systems Ltd, UK). The limit of sensitivity of the corticosterone kit was 0.55 ng/ml. Ghrelin was measured using a Rat/mouse Ghrelin (active) ELISA kit (EZRGRA-90K, Merck-Millipore, Germany) with a sensitivity limit of 8 pg/ml.

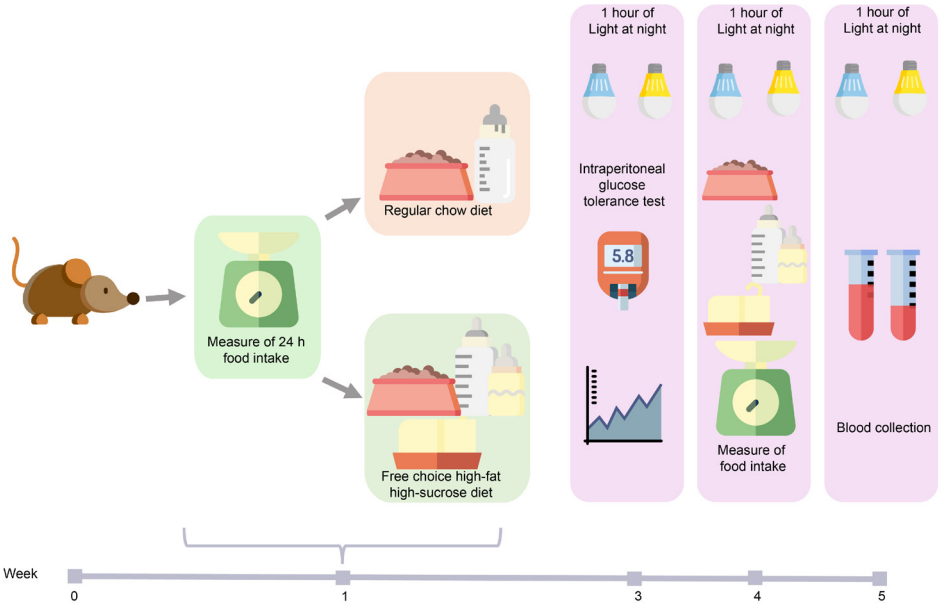

Figure 1. *Graphical representation of the experimental design.*

Statistical analysis

Data are expressed as means ± SEM. The area under the curve (AUC) for the IPGTT was calculated using the trapezoid rule. One-way ANOVAs were used to detect group differences in locomotor activity, baseline concentration for glucose before performing the IPGTT, differences in the AUC obtained from the test results, to compare the levels of glucose, plasma insulin, corticosterone, and ghrelin after the third light pulse, and the immunohistochemistry data. A mixed-effects model was used to identify the changes in body weight and to test the effects of Treatment (dark control vs. light conditions), Time (t=0 – t=90, day vs. night respectively), and Interaction on the glucose and feeding responses in both diet conditions. If an effect of Treatment, Time or Interaction was found a Dunnett's multiple comparison tests were performed to determine the post-hoc differences. All statistical analyses were performed with GraphPad Prism version 8.01 for Windows (GraphPad Software, La Jolla, CA, USA) using a significance level of $p < 0.05$.

Results

C57Bl6/J mice

Daily food intake rhythm

As expected, we observed a predominantly nocturnal pattern in the daily food intake rhythm (Fig.2), with the highest intake at ZT15. Since our main aim was to study the effects of blue light on food intake and glucose metabolism, we decide to give the 1-hour blue light pulse immediately before the moment where mice ate the most (i.e. ZT14).

Food intake per diet and body weight

In accordance with their nocturnal physiology, mice ate most of their calories at dark than during the light phase, regardless of the diet. Nonetheless, animals on a HFHS diet ate significantly more calories on each phase and in total during the day compared to chow-fed animals (Fig. 3a, *Diet* $F(1, 52)=34.79$, $p<0.0001$, *Time* $F(1, 52)=195.4$, $p<0.0001$, *Diet x Time* $F(1, 52)=11.49$, $p=0.001$). Significant differences in body weight were observed between chow and HFHS fed animals (Fig.3b and 3c, *Diet* $F(1, 38)=4.851$, $p=0.033$, *Diet x Time* $F(5, 190)=6.261$, $p<0.0001$; post-hoc $p=0.010$ for week 5). No significant differences were observed among groups fed with the same diet regardless of the light treatment.

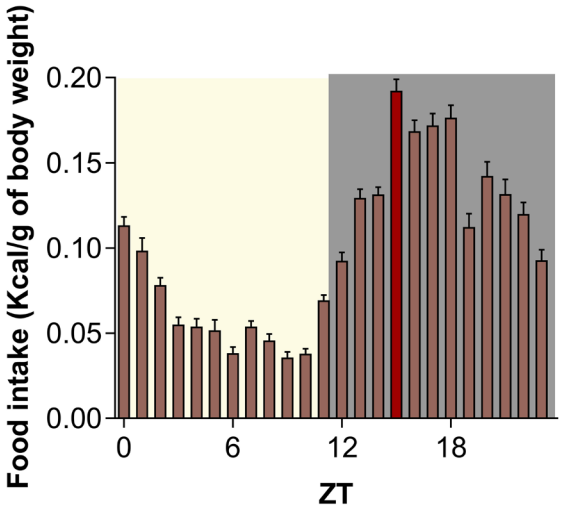

Figure 2. *Food intake rhythm of chow food in C57Bl6/J female mice. Bars with light yellow background indicate food consumption during the light phase while the grey background indicates food intake dark phase. The red bar indicates ZT15 with the highest food intake. (n=40)*

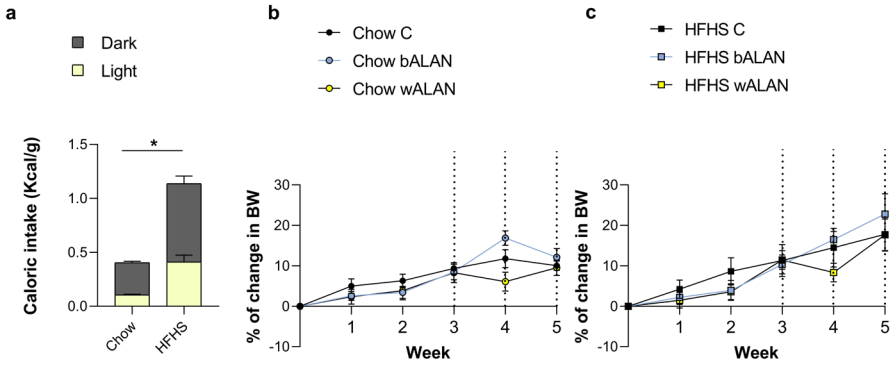

Figure 3. *Food intake during dark and light phases, and animal's body weight gain throughout the experiment. (a) Caloric intake is higher during the dark phase regardless of the diet (p<0.0001), however, HFHS animals have a significant main total caloric intake (p<0.0001); (b) there were no differences in body weight between chow-fed animals nor (c) between HFHS-fed mice, however animals fed a free-choice high-fat high-sucrose diet were significantly heavier than those fed a regular chow diet (p=0.033). Grey portion of the bar indicates the dark phase, light yellow indicates the light phase. (n=6-7 per group), (*) p<0.05 for Treatment. Dotted lines mean the week of light exposure.*

Acute effects artificial light at night in locomotor activity and glucose metabolism

When animals received the first light pulse, we observed a statistically significant decrease in the locomotor activity of both chow-fed (Fig. 4a, $F(2, 9)=6.789$, $p=0.015$; post hoc $p=0.043$ for blue, $p=0.011$ for white) and HFHS-fed mice (Fig. 4b, $F(2, 9)=22.71$, $p=0.0003$, post hoc $p=0.014$ for blue, $p=0.0002$ for white) compared to their respective dark controls. After the light pulse, we did not detect any differences in the blood glucose levels regardless of the diet and the light condition (Fig. 4c and 24d). In chow-fed animals, there were no differences between blue light or white light exposed, and dark control animals in the plasma glucose at any point of the IPGTT (Fig. 4e,), neither in the AUC (Fig. 4g), although a significant effect of Time was observed ($F(5, 85)= 61.84$ $p<0.0001$) as expected.

In contrast, animals fed a HFHS diet that were exposed to either light condition showed significantly lower glucose levels during glucose tolerance test, as compared to the dark controls (Fig. 4f, *Treatment* $F(2, 17)=7.405$, $p=0.004$; *Time* $F(5, 85)=113.1$, $p<0.001$; *Interaction* $F(10, 85)=7.738$, $p<0.001$). The white light-exposed group showed a significantly lower glucose level after 15 min (post hoc $p=0.037$), white the group that received blue light has significantly lower glucose levels after 15 (post hoc $p<0.0001$), 30 (post hoc $p<0.0001$) and 60 minutes (post hoc $p=0.003$). Also significantly lower AUC (Fig. 4h, $F(2, 17)=30.94$, $p<0.0001$, post hoc $p<0.0001$ for blue, $p=0.033$ for white). This indicates an improved glucose tolerance in response to the blue light in the animals on a HFHS diet. It is worth mentioning that when dark controls of both diets were compared among each other, significant effects of the diet were observed, since animals after only 2 weeks of being fed a HFHS diet developed glucose intolerance (*Diet* $F(1, 10)=8.004$, $p=0.017$; *Time* $F(5, 50)=67.39$, $p<0.0001$; *Time x Diet* $F(5, 50)=7.647$, $p<0.001$).

Acute effects of artificial light at night food intake

After the second light pulse, we measured food intake during the light and the dark phases before, during, and after the light exposure. In animals on the chow diet, we did not observe any significant differences in food intake during the light or dark period regardless of the wavelength used (Fig. 5a). However, in the mice on the HFHS diet we observed an increase in the number of calories consumed during the night when the blue light

pulse was given (Fig. 5b, *Treatment* F(2, 23)=7.694, p=0.002, *Time* F(1, 23)=4.852, p=0.037, *Interaction* F(2, 23)=4.363, p=0.024, post hoc p=0.003 for blue during the dark phase). Interestingly, when the three components of the HFHS diet were analyzed separately, we did not see any increase in the consumption of chow food (Fig. 6a). However, we did observe an Interaction effect on fat intake (Fig. 6b, *Interaction* F(2, 46)=4.090, p=0.023) indicating a higher consumption of fat during the dark phase on blue-light exposed animals (post hoc p=0.019). Moreover, a significant Time effect for sugar intake was observed (Fig. 6c, F(1, 46)=7.911, p=0.007; post-hoc p=0.014 for white light) showing a decrease in the sugar intake during the dark phase in white light-exposed animals.

Acute effects artificial light at on blood glucose and hormones

After the third light pulse blood, glucose, and plasma levels of insulin and corticosterone were measured. We did not observe any significant difference in the blood glucose in either diet group regardless of the light condition (Fig. 7a and 7b). We also did not find any significant effects of blue or white light on plasma insulin levels of chow-fed animals (Fig. 7c). Although in HFHS-fed mice plasma insulin levels were higher in blue light-exposed mice this difference was not statistically significant (Fig. 7d). Levels of plasma corticosterone did not differ regardless of the diet or light condition (Fig. 7e and 7f). Nor did we detect any significant differences in plasma ghrelin concentrations (Fig. 7g and 7h).

Opn4 -/- mice

Food intake per diet

We hypothesize that the effect of light on feeding and metabolism are melanopsin dependent, we analyzed the food intake per type of diet in both *Opn4-/-* and *Opn4+/+* mice. There were no differences in the total amount of calories consumed between chow-fed and HFHS-fed *Opn4+/+* animals. However, we observed that in HFHS-fed animals, most of the caloric intake happened during the light phase, contrary to chow-fed animals who ate most of their calories during the dark phase (Fig. 8a, *Time* F(1, 22)=26.65, p<0.0001; post hoc p=0.004 for chow and p=0.001 for HFHS).

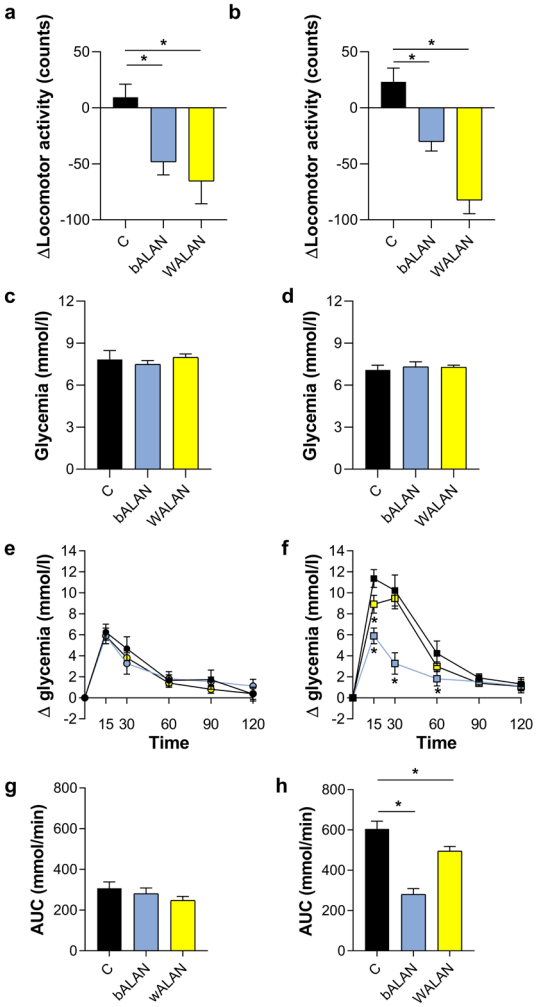

Figure 4. *Effects of light on locomotor activity and glucose tolerance. (a)Blue (p=0.043) and white light (p=0.011) caused a significant reduction of the locomotor activity in chow-fed animals, likewise in (b) HFHS-fed animals both light spectra caused a significant reduction in the locomotor activity (p=0.014 for blue, p=0.0002 for white). No significant differences were observed in the basal glucose levels of chow-fed animals (c) neither in HFHS-fed mice (d) regardless of the light treatment. (e) Neither blue nor white light affected the glucose tolerance in animals fed only with chow food. (f) In HFHS-fed animals, white light improved glucose tolerance after 15 min of starting the IPGTT (p=0.037) while blue light improved glucose tolerance at 15 (p<0.0001), 30 (p<0.0001), and 60 (p=0.003) min of the tolerance test. (g) No significant differences were observed in the AUC of chow-fed animals. (h) Both blue (p<0.0001) and white light (p=0.033) exposed HFHS-fed animals showed a lower AUC compared to dark controls. (n=6-7 per group). (*) p<0.05 for Treatment.*

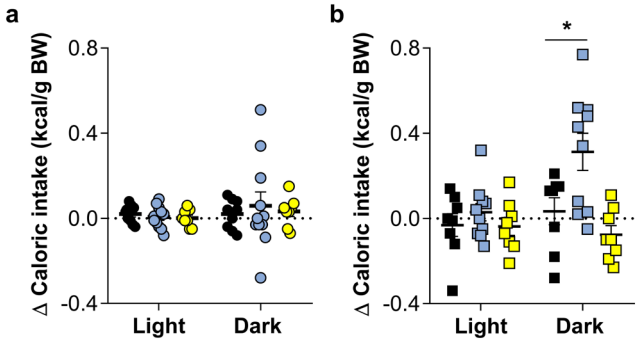

Figure 5. *Effects of light on food intake. (a) Food intake of chow-fed animals did not change during either phase or light exposure condition. (b) Animals fed a HFHS diet ate significantly more calories during the dark phase when a 1-hour blue light pulse was given (p=0.003). (n=8-10 per group). (*) p<0.05 for Treatment.*

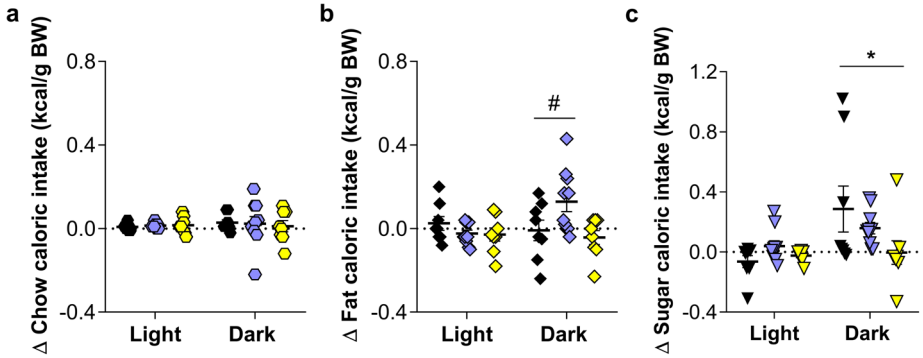

Figure 6. *Intake of the different components of the HFHS diet. (a) Light did not cause any changes in the amount of chow food eaten by the mice regardless of the wavelength or the phase, (b) blue, but not white light, increased the amount of fat eaten during the dark phase (p=0.023), (c) animals exposed to white light ate significantly less sugar (p=0.014) than the dark controls and blue light exposed mice. (n=8-10 per group). (*) p<0.05 for Treatment, (#) p<0.05 for Interaction.*

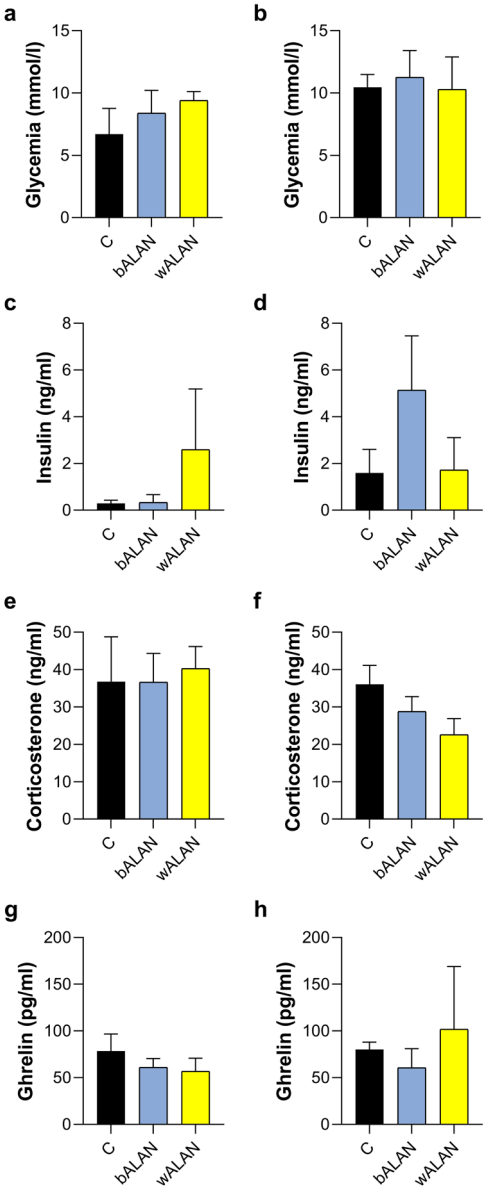

Figure 7. *Light effects in plasma metabolites. No significant effects of either lights were observed in the plasma glucose of (a) chow-fed nor (b) HFHS fed animals. Although plasma insulin appeared to be higher in chow-fed white light-exposed (c) and in HFHS-fed blue light-exposed mice, this was not statistically significant. Corticosterone levels did not change regardless of the light treatment neither in chow (e) nor in HFHS groups (f). Light did not cause any significant changes in active plasma ghrelin in chow-fed animals (g) nor in HFHS-fed (h). (n=8-10 per group)*

In the case of the *Opn4-/-* mice, there was an Interaction effect of the diet, showing that animals in a HFHS diet not only eat fewer calories during the whole day but also, similar to their wildtype littermates, ate more during the light phase than during the dark phase (Fig. 8b, *Time* F(1, 17)=78.89, p<0.0001; *Interaction* F(1, 22)=4.742, p=0.043). When comparing animals of different genotypes fed the same diet, we observed a significant difference between wild type and *Opn4 -/-* only when they were chow-fed. *Opn4-/-* chow-fed animals ate a higher amount of calories compared to their wild type littermates (Fig. 8, *Genotype* F(1, 12)=76.14, p<0.0001).

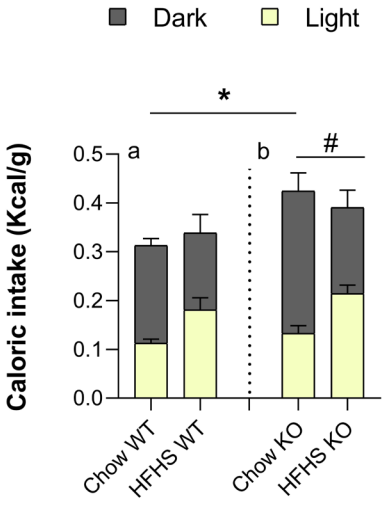

Figure 8. *Food intake during dark and light phases in wildtype littermates and Opn4 -/- mice. No differences were observed in the total amount of calories consumed between chow and HFHS-fed wild type animals, however (a) chow-fed animals ate most of their food during the dark phase (p=0.004) while HFHF animals ate more during the light phase (p=0.001). In melanopsin deficient mice (b) there was no difference in the total caloric intake among diet groups. Chow-fed knockouts ate most part of their calories during the dark phase, while HFHS-fed mice ate more during the light phase. (p=0.043). When animals from different genotypes but same diet treatment were compared, we observed that chow-fed knockout mice ate more than their wildtype litter mates (p<0.0001). Grey portion of the bar indicates the dark phase, light yellow indicates the light phase. (*) p<0.05 for Genotype, (#) p<0.05 for Interaction (Time x Diet).*

Acute effects of artificial light at night food intake

Similar to what we observed in C57Bl6J mice, neither blue nor white light changed the intake of chow food in mice fed a regular diet regardless

of their genotype (Fig. 9a). On the other hand, in HFHS-fed animals, we observed higher intake of food at night in both blue and white light exposed *Opn4+/+* littermate animals (Fig. 9b, left panel; *Treatment* F(2, 10)=7.378, p=0.010; *Interaction* F(2, 10)=7.690, p=0.029) while no significant effects in the total caloric intake were observed in *Opn4-/-* mice (Fig. 9b, right panel).

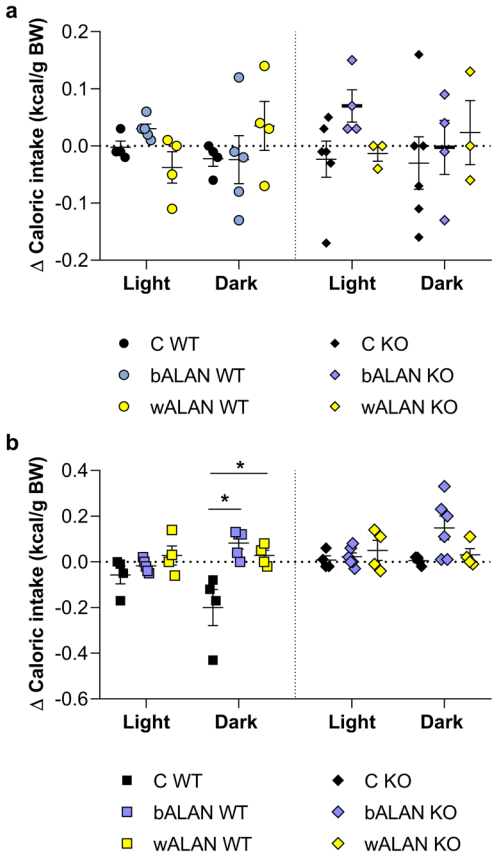

Figure 9. *Effects of light on food intake of Opn4 -/- mice. (a) Food intake of chow-fed animals did not change during either phase regardless of their genotype or light exposure condition. (b) Wild type animals fed a HFHS diet ate significantly more calories during the dark phase when a 1-hour of white or blue light pulse was given (p=0.010). (n=4-8 for wildtypes, n=7-10 for Opn4 -/-). (*) p<0.05 for Treatment. Left panels: wild-type litter mates. Right panels: melanopsin deficient mice.*

Breaking down the calories into the three different diet components, we noticed that both Opn4+/+ (Fig. 10a, left panel; *Treatment* F(2, 10)=8.548, p=0.006; post hoc p=0.001 for blue and p=0.003 for white) and *Opn4 -/-* (Fig.

10a, right panel; *Time* F(1, 9)=6.062, p=0.036, *Interaction* F(2, 9)=10.08, p=0.005; post hoc p=0.0001 for blue and p=0.015 for white) ate significantly more chow food when they were exposed to both light spectra.

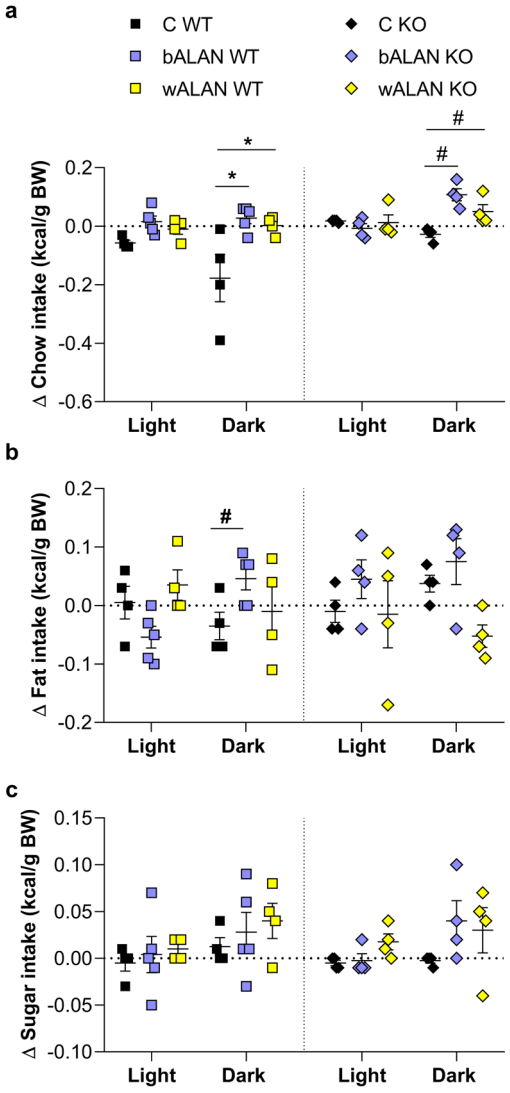

Figure 10. *Intake of the different components of the HFHS diet in Opn4 -/- mice. (a) Both blue and white light increased the amount of chow food eaten by the mice regardless of the genotype (p=0.006 for wild types, p=0.005 for knockouts) (b) blue but not white light increased the amount of fat eaten during the dark phase only in wild type animals (p=0.015), (c) No significant changes were observed in the sugar intake. (n=8-10 per group). (*) p<0.05 for Treatment, (#) p<0.05 for Interaction. Left panels: wildtype littermates. Right panels: melanopsin deficient mice.*

Additionally, *Opn4+/+* animals ate considerably more fat when exposed to blue light (Fig. 10b left panel, *Interaction* $F(2, 20)=5.164$, $p=0.015$), but no light effects were observed in the consumption of sucrose (Fig 10c left panel). Contrary, in *Opn4-/-* deficient mice exposed to light (blue or white), no changes were observed in the consumption of the highly palatable components of the HFHS diet in comparison to the dark controls (Fig 10a-10c right panels).

Discussion

In the present study, we showed that a 1-hour pulse of white and, to a larger extend, of blue light can improve glucose tolerance in animals that were fed a free-choice high-fat high sucrose diet. Also, we demonstrated that blue light at the beginning of the dark phase (i.e. ZT14) triggers the intake of fat in female mice. This effect in fat intake was not observed in melanopsin deficient mice, suggesting that the effects of artificial light at night on palatable food intake might be melanopsin dependent.

In one of our previous studies (15) using the same type of diets, we showed that in the diurnal rodent Arvicanthis ansorgei a 1-hour pulse of blue light at ZT14 causes no effects in the glucose tolerance of chow-fed animals, but that when animals were fed the HFHS diet, blue light acutely induced glucose intolerance in males (but not females) animals, even when obesity was not induced by the diet. Contrary, here we showed that hyperphagia, obesity, and glucose intolerance can be induced by the HFHS diet in female mice just as it was reported in rats and in male mice before (24,26), and that white and blue light can (acutely) improve glucose tolerance in these female mice. Initially, one can think that these diametrically opposite effects of light at night are because Arvicanthis are diurnal while mice are nocturnal animals, hence the observed effects may be the opposite. However, in previous work from one of our labs, it was shown that light (white and green) at the beginning of the dark phase induces glucose intolerance in male Wistar rats without changes in plasma insulin, suggesting that light either increases endogenous glucose production or inhibits glucose uptake (27). On the other side, in Arvicanthis we did observe a concomitant reduction of plasma insulin, which goes in accord to previous work that has shown that light during the light phase can increase the sympathetic and decrease the parasympathetic activity of autonomic nerves via the SCN, decreasing, in turn, insulin release and increasing hepatic gluconeogenesis (28,29). However, it is important to note that first these previous studies have been done in rats,

so one must account for species differences and that previously in one of our labs it was demonstrated that the hepatic effects of light are only partially mediated by autonomic innervation (30). Here, although we noticed higher levels of plasma insulin in blue light-exposed animals, due to high variations the differences where not statistically significant, nonetheless it can partially explain the apparently improved glucose tolerance in mice that presented diet-induced obesity and glucose intolerance. It is worth mention that these effects are only present when female mice are exposed for a short period to the HFHS diet (i.e. 2 weeks, like we did here) but not after animals have been fed that type of diet for more than eight weeks. In unpublished experiments from our lab (data not shown) when C57Bl6/j female mice were exposed to either white or blue light before an IPGTT no significant effects of light were observed. This indicates that the possible mechanism that causes changes in glucose metabolism due to the exposure to light may be corrupted in obese animals.

Another study reported the effects of white and different monochromatic lights in glucose tolerance of C57Bl6/j male mice, fed either a normal chow diet or a high-fat diet, observed that green light was the wavelength that caused significant metabolic disturbances, glucose intolerance included (31). Nonetheless, even when this support further our hypothesis that light spectra has an impact on metabolic health, this study is not comparable to ours, since they substituted the white environmental light for the monochromatic ones for 2 months and also because the study was performed in male mice. While the effects of a single light pulse in the glucose tolerance of male mice are still not reported and although it is virtually unknown how light perception and physiological effects can be sex-dependent and because of our previous results in Arvicanthis (15) and previous result in humans (32), we hypothesized that likewise in mice, sex plays an important role in the metabolic and behavioral effects of light. Also, it has been previously reported that female mice are more resistant to the western diets that induce obesity and type 2 diabetes (33), even when here we observed that HFHS fed mice developed obesity, glucose intolerance, and hyperphagia, it is possible that other environmental factors that cause metabolic disruption, such as exposure to light during the dark phase, can be less harmful in female animals due to sex hormones or other less studied physiological differences.

Similar to what we observed in male diurnal rodents (15), blue light

increased the consumption of palatable components of the HFHS diet in C57Bl6/j female mice, but in this case, what we observed was a higher intake of fat. Although in Wistar rats, a study reported that when animals are allowed to self-select the macronutrients of their diet, females always tend to consume more fat than males regardless of the age (34), this may explain the preferences observed in our current study. Also, even when a decrease in the consumption of sugar was observed among the white light-exposed mice, we did not observe any differences in the total caloric intake. Unfortunately, since the measures in the food consumption where done manually every 12 hours, we do not know the specific time when the animals stopped consuming sugar, but one can speculate that this reduction in the intake of sugar during the dark phase may not be a direct effect of the light in the food intake per se but more a result of the strong negative masking induced by white light. The reduction in locomotor activity was also observed with blue light, although less prominent which may be related to the fact that, as it has been shown before, sleep induction is delayed in response to blue light exposure (35).

Additionally, when we executed this experiment with Opn4 -/- (and their wildtype littermates) we observed that both blue and to a lesser extend white light, triggered a higher intake in chow food. Even when this effect was also observed in their littermates (but no in C57Bl6/j mice), melanopsin deficient mice did not eat more fat when they received the blue light pulse, just like C57Bl6/j and their wildtype littermates did, suggesting that the preference for the consumption of highly palatable food triggered by blue light exposure at the beginning of the dark phase appears to be melanopsin dependent. Nonetheless, a limitation of our study is that for this second experiment using Opn4 +/+ and Opn4 -/- mice, we used animals of both sexes due to the lower availability that we have to that genotype.

The effects of light in the consumption of palatable food in wild type animals could be explained because the ipRGCs project directly to areas of the brain knowingly involved in controlling metabolic homeostasis such as the lateral hypothalamus, and to areas involved in reward processes like the lateral habenula (LHb), the amygdala and the ventral tegmental area (indirectly via the LHb or from the SCN via the medial preoptical nucleus) (36). However, even when further studies are needed to elucidate more about the role of melanopsin in non-imaging forming visual functions, our results add feeding behavior to the list of light-induced physiological changes that might be regulated by melanopsin (35).

To conclude, even though the direction of the effects of light in glucose metabolism are contradictory among rodent species, all the evidence points out that artificial light at night during the dark phase can alter the metabolic homeostasis, especially in those individuals that consume a diet high in fat and sugar. To translate this study into human health, our results give further proof that avoiding aberrant light exposure (in time and spectra) is as important as a healthy diet to maintain metabolic health.

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Declarations of interest

None.

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Chapter 7

Acute effects of light of different wavelengths and at different times in the night on energy metabolism of C57Bl6/J and BMAL1 -/- mice.

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In preparation

Abstract

Light is the main cue for the entrainment of our daily biological rhythms, but besides the circadian clock in the suprachiasmatic nuclei (SCN) it also reaches non-image-forming brain areas that are responsible for the control of energy and glucose metabolism, food intake and motivated behavior. Hence exposure to light could modulate these physiological processes independent from its circadian effects. Previous evidence from studies in humans, as well as earlier results from our group in rodents, pointed out that the effects of artificial light at night (ALAN) on physiology and behavior are wavelength and time-dependent. Here we aimed to study the effects of different wavelengths of light at different time points during the dark phase on energy metabolism of C57Bl6/J and BMAL1 deficient mice. The current results demonstrate that ALAN produces changes in energy expenditure, RER, and plasma glucose in mice and that these effects are dependent on the time of the exposure. Also, we provide evidence that the integrity of the molecular clock is necessary to observe these metabolic disturbances caused by light at night. Moreover, we again observed that these effects are dependent on the wavelength of light used, indicating that ipRGCs, cones, and rods are all part of the pathways involved.

Introduction

Numerous studies in animal models together with interventional and epidemiological studies in humans have shown that exposure to artificial light at night (ALAN) is associated with higher body weight (1–5), inflammation (1), endocrine and metabolic disruption (6,7), cardiovascular diseases (8), cancer (9–11), and increased food intake (3,12) and appetite (13). It is well established that exposure to light at the wrong time of the day disrupts the temporal organization of our biological clocks, which is most likely the cause of all the negative health consequences behind ALAN, although further research is needed to clarify the exact mechanisms.

Light perceived by retinal photoreceptors is the major environmental cue for the entrainment of the mammalian biological clock. Cones and rods are hyperpolarized by the photons and are mainly involved in the visual processes linked to light perception. The rod and cone signals are relayed to the brain via the retinal ganglion cells. A small proportion of these ganglion cells express the pigment melanopsin, which makes them intrinsically photosensitive (ipRGCs) (14,15). Both direct and indirect signals are integrated and transmitted as outputs to different brain areas responsible for non-image-forming visual functions, such as circadian photoentrainment. Major site of these projections is the suprachiasmatic nucleus in the hypothalamus (SCN), where the master biological clock is located, but the ipRGCs also project to other areas of the brain involved in the control of energy and glucose metabolism, food intake, and motivated behaviors (14,16). Hence exposure to light could also modulate these physiological processes directly, i.e. without passing by the SCN.

It is known that melanopsin is primarily depolarized by short-wavelength light, with its highest peak of depolarization around 480 nm (blue light), but its appearance varies from slightly violet, to blue and turquoise green (i.e. 445-520 nm) (17–19). This together with previous human evidence (20–24) and recent results from our group in rodents points out that the effects of ALAN are wavelength and time-dependent with white, green (25), and blue light (12) disrupting significantly glucose metabolism, food intake, locomotion and energy expenditure (26).

However, the cellular and molecular brain mechanisms implicated in the light effects on metabolism and behavior are not fully known yet. The molecular basis of circadian clocks consists of a number of transcriptional-

translational feedback loops that involve a group of 10 -15 clock genes, such as *Clock, Bmal1, Per* and *Cry* (27). Some of these genes are inducible by light (28,29). Furthermore, previous studies indicate that clock genes may also be involved in the regulation of energy balance and glucose metabolism (30–32). Hence, we hypothesized that animals lacking one of the core clock genes will not show time dependent metabolic disruptions when exposed to ALAN. Therefore, to assess the role of clock genes in the physiological and behavioral effects of ALAN, here we determined whether light exposure to different wavelengths at different time points during the night (dark phase) affects energy metabolism of C57Bl6/J and *BMAL1* deficient mice.

Materials and Methods

Animals and housing

Sixty-four eight weeks old male C57Bl6/J mice (Charles River Breeding Laboratories, Sulzfeld, Germany) and eight (8-10 weeks old) global male *BMAL1 -/-* mice (33) on a C57Bl6/J background (from our breeding facility) were individually housed under a controlled ambient temperature of 21-23°C and a 12-hour light/dark cycle (lights on at 07:00, Zeitgeber time 0 (ZT0); lights off 19:00, ZT12) with white fluorescent light (maximum, 150 lux) during the light phase and dim red light during the dark phase (< 5 lux). Mice had ad libitum access to chow food (Teklad Global Diet; Harlan, Horst, the Netherlands) and water. Body weight was measured weekly.

All the experiments were performed following the Council Directive 2010/63EU of the European Parliament and the Council of 22 September 2010 on the protection of animals used for scientific purposes. All procedures were also approved by the Animal Ethics Committee of the Royal Dutch Academy of Arts and Sciences (KNAW, Amsterdam, the Netherlands) and under the guidelines on animal experimentation of the Netherlands Institute for Neuroscience.

Experimental design

C57Bl6/J animals were given at least one week to get adjusted to the conditions of our animal facility and then were divided randomly into the following four groups: dark controls and blue, green, or white light (n=16 per group) exposed animals. All light-treated animals were subdivided into two subgroups (n=8 each), using blocked randomization by body weight. Each subgroup received two different light pulses, one at the beginning and one

towards the end of the dark phase, with at least one week in between each light exposure. Subgroup 1 received a light pulse at ZT14-16 and ZT18-20, while subgroup 2 received light at ZT16-18 and ZT20-22.

Due to the small number of *BMAL1 -/-* mice available to us (n=8), the time points and wavelengths of the light exposure were decided after the analysis of the metabolic data for the wild-type animals was done, to test the two conditions with the most significant impact on metabolic variables also in the knock-out mice. Therefore, *BMAL1* mutant mice were exposed only to blue (n=4) and green (n=4) light at ZT14-16 and ZT20-22, respectively.

Metabolic cages

Locomotor activity, food intake, energy expenditure, and respiratory exchange ratio (RER) were measured before, during and after the light exposure by placing animals inside Metabolic Pheno Cages (TSE systems, Bad Hombourg, Germany). Mice were individually housed and kept in these cages for 96 hours. The first 24 hours were taken as acclimatization and were not used for the analysis, the second day was used as a control day, the light treatment was given on day number three, and the fourth day was used as a control day to make sure the possible effects we would see on day three were exclusively dependent on the light exposure (data not shown). Data were acquired every 15 minutes for all the variables, and analyzed as the absolute change (Δ) between the eight measurements obtained from the metabolic cages during the 2-hour light exposure and the last time point before lights went on. For the *BMAL1 -/-*, due to the limited availability of these animals, no dark control group was available and the day before the light exposure was used as the dark control condition.

Protocol used for light exposure

We used rectangular metal frames with LED strips around the perimeter of the metabolic cages to administer the light pulses to the animals. A detailed characterization of the light conditions used can be found below (Table 1). Wild types animals received three 2-hour light pulses in total, while KO animals received only two 2-hour light pulses. For both genotypes light exposures were given with at least one wash-out week in between.

Table 1. *Spectral sensitivity of light conditions used in the experiments.*

Retinal photopigment complement	White	Blue	Green
S-Cone (rNsc [λ])	0.05	0.07	0.00
Melanopsin (rNz [λ])	13.69	58.77	38.67
Rod (rNz [λ])	16.39	43.13	50.71
M-cone (rNmc [λ])	18.31	35.04	53.60
Irradiance ($\mu W/cm^2$)	9.54	10.76	9.48
Photon flux ($1\ cm^{-2}\ s^{-1}$)	$2.75x10^{13}$	$2.49x10^{13}$	$2.40x10^{13}$
Peak spectral irradiance (nm)	595	455	520

Blood and tissue collection

During the last week of the experiment, each group of eight WT animals was further divided into two subgroups of four animals each and received a third light pulse at one of the two-time points they had received light before. As soon as this third light pulse finished, animals were deeply anesthetized with an intraperitoneal overdose of pentobarbital. Blood was collected via a cardiac puncture from the left ventricle. Samples of liver, adipose tissue and skeletal muscle were gathered and after animals were perfused transcardially with PFA 4% in 0.1 M phosphate buffer and brains were collected for future analysis. Tissues and brains were stored for future studies.

Plasma measurements

Blood glucose levels were determined by using blood glucose test strips with an accuracy of 0.1 mmol/l (FreeStyle; Abbot Diabetes Care, Alameda, California). Plasma insulin was measured using an ELISA procedure with a Rat/Mouse Insulin ELISA Kit (EZRMI-13K, Merck-Millipore, Germany). Corticosterone levels were measured by using a radioimmunoassay (MP Biomedicals, Santa Ana, California).

Statistics

Data are presented as means (±SEM). Mixed-effects model analysis were used to analyze the changes in the parameters obtained from the

metabolic cages during the 2-hour light exposure and plasma measurements of C57Bl6/J animals. One-way ANOVAs were used for the plasma glucose levels in *BMAL1* mutant mice. Dunnet's multiple comparisons test was used for post-hoc analysis when significant effects of light (=Treatment) or the Time x Treatment interaction (=Interaction) were found in the mixed model analysis, while Sidak's multiple comparisons test was used as a post-hoc test for the ANOVAs. Statistics were performed by using GraphPad Prism version 8.01 for Windows (GraphPad Software, La Jolla, California) with a significance level of $p<0.05$.

Results

Metabolic effects of light in C57Bl6/J mice

Locomotor activity

When mice were exposed to different wavelengths of light at ZT14-16, we observed a significant effect of *Time* and *Interaction* (Fig. 1A, Time $F(7, 196)=9.161$, $p<0.0001$) and *Interaction* ($F(21, 196)=2.002$, $p=0.007$) in the overall comparison, but no significant *Treatment* effect was found (Supp. Table 1). When comparing each wavelength separately with the dark controls an *Interaction* effect was observed only for blue ($p=0.0082$) and white ($p=0.005$) light (Supp. Table 2–4), with a decrease in the locomotor activity after the first 15 minutes of light exposure at ZT14. When the light pulse was given at ZT16-18 or ZT18-20 (Fig. 1B-1D) no significant effects of *Treatment* or *Interaction* were found (Supp. Table 1), but at ZT20-22 green light increased locomotor activity, resulting in a significant *Treatment* effect ($p=0.030$) (Table 3).

These results suggest that the extent to which light affects locomotor activity in mice strongly depends on the time of the dark phase when the light is given. ZT14-16 at the beginning of the dark phase being the time point where most negative masking was observed, and blue and white light the wavelengths that reduced locomotor activity the most. On the other hand, at the end of the night green light had a stimulatory effect on locomotor activity.

Energy expenditure

Light exposure from ZT14-16 caused a significant effect of *Time* ($F(7, 196)=6.212$, $p=0.0001$) and *Interaction* ($F(21, 196)=6.212$, $p=0.048$) on the energy expenditure of mice (Supp. Table 1). The *Interaction* effect at ZT20-

22 just missed significance (p=0.0560). Post-hoc analysis showed the effect at ZT14-16 to be due to a significant decrease in energy expenditure by blue light 30 min after starting the light pulse (Fig. 2A, post-hoc t30min p=0.001, t60min p<0.0001, t75min p=0.001), indicating a decrease in the energy expenditure. Additionally, a *Time* effect was found at ZT16-18 (Fig. 2B, F(7, 196)=3.042, p=0.004) and ZT20-22 (Fig. 2D, F(7, 196)=2.340, p=0.025). No other significant effects were observed with other wavelengths or at other time points (Fig.2C; Tables 1-4). Overall, it is apparent from Fig.2 that ALAN tended to reduce energy expenditure, with blue light being most effective. Remarkably, at the end of the night, green (and white) light had a stimulatory effect again, although non-significant (p=0.093 for white light).

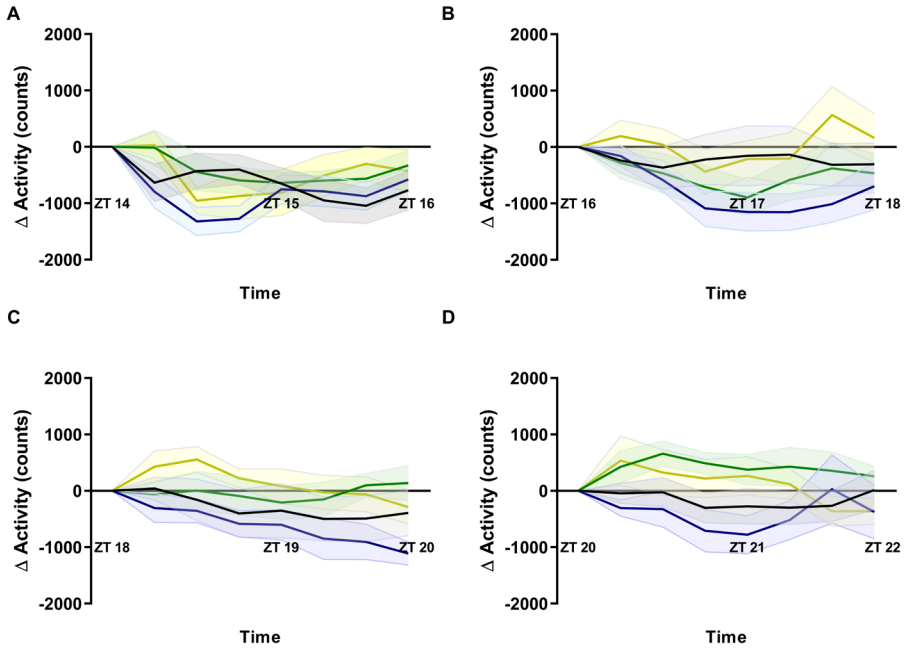

Figure 1. *ALAN effects on locomotor activity. An Interaction (p =0.007) effect of ALAN was observed in the locomotor activity at ZT14-16 (A). No significant Treatment or Interaction effects were observed at ZT16-18 (B), ZT18-20 (p=0.019) (C) or ZT20-22 (D). Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light-exposed animals. The black and colored straight bold lines indicate the mean levels of the dark control and 3 wavelength groups, whereas the shaded gray and colored areas delimited by the thin lines show the SEM. n=8.*

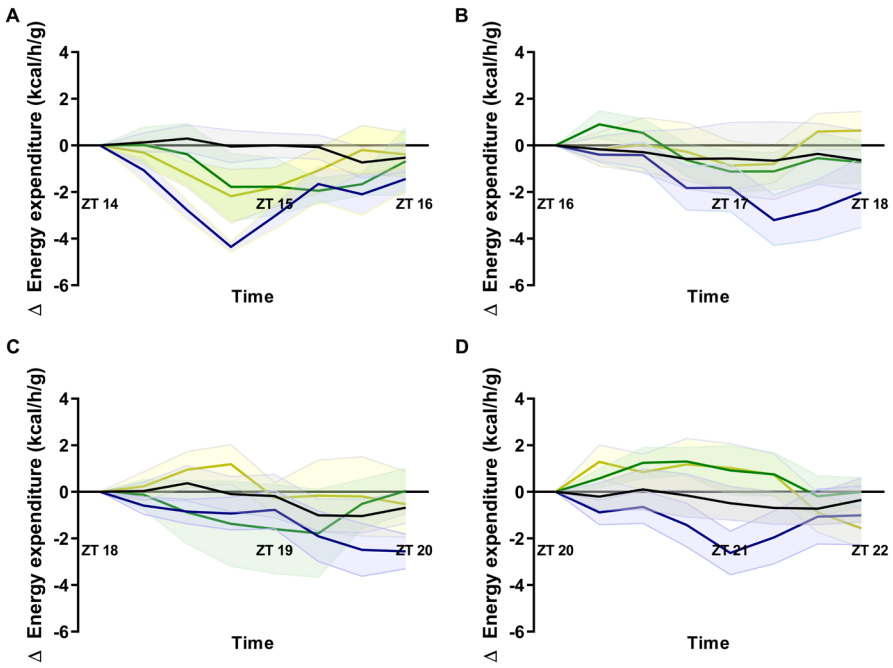

Figure 2. *ALAN effects on energy expenditure. Blue light caused a significant Interaction (p=0.048, post-hoc t30min p=0.002, t60min p=0.001, t75min p=0.004) effect on energy expenditure at ZT14-16 (A). No significant effects on energy expenditure were observed at ZT16-18 (B), ZT18-20 (C) or ZT20-22 (D). Black: dark controls; yellow: bright light–exposed animals; blue: blue light–exposed animals; green: green light–exposed animals. The black and colored straight bold lines indicate the mean levels of the dark control and 3 wavelength groups, whereas the shaded gray and colored areas delimited by the thin lines show the SEM. n=8.*

Respiratory exchange ratio (RER)

During the light pulse from ZT14-16 (Fig. 3A) we observed significant effects of *Time* (F(7, 196)=15.69, p<0.0001), *Interaction* (F(21, 196)=2.546, p=0.001) and almost *Treatment* (p=0.070)(Supp. Table 1). Post-hoc analysis revealed a significant reduction of RER with all wavelengths (post-hoc p=0.032 for blue, p=0.013 for green, and p=0.033 for white). Furthermore, when the light pulse was given from ZT16-18, also a significant *Time* (Fig. 3B, F(7, 196)=2.460 , p=0.023) and *Interaction* effect were observed, this time due to the white light exposure (Fig. 3B, F(21, 196)=3.078 , p<0.0001; post-hoc p=0.038 for blue and p<0.0001 for white light). The light pulse given from ZT18-20 evoked only a significant effect of *Time* (F(7, 196)=3.313, p=0.013) (Fig. 3C), but not *Treatment* or *Interaction.* In contrast, when light was given

at the end of the night (i.e. ZT20-22), a significant *Treatment* effect was observed again (Supp. Table 1). Post-hoc analysis revealed this was mostly due to the white light exposed animals (Fig. 3D, p=0.003 for Interaction) and a reduction in the RER especially after ZT21. Remarkably, green light again showed an opposite effect.

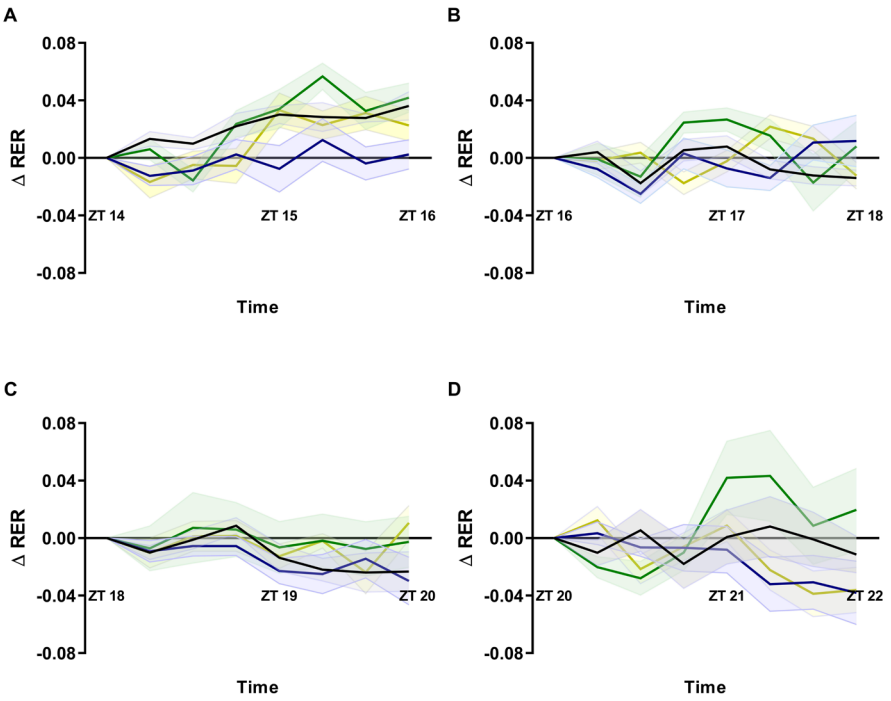

Figure 3. *ALAN effects on the respiratory exchange ratio (RER). Blue caused a significant Treatment effect (p=0.032) and green light a significant Interaction effect (p=0.001) in the RER at ZT14-16 (A). A significant Interaction effect was also observed with blue (p=0.038) and white light (p<0.0001) at ZT16-18 (B). No significant Treatment or Interaction effects were observed at ZT18-20 (C). An Interaction effect (p=0.003) was again observed with white light at ZT20-22 (D). Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light-exposed animals. The black and straight colored bold lines indicate the mean levels of the dark control and 3 wavelength groups, whereas the shaded gray and colored areas delimited by the thin lines show the SEM. n=8.*

Food Intake

We also wanted to assess whether light exposure with different wavelengths and at different times of the dark phase could affect food intake in mice. When the light pulses were given at ZT14-16 (Fig.4A) we detected a significant effect of *Time* ($F(7, 224)=2.918$ $p=0.001$), but not *Treatment* or *Interaction.* Also at ZT16-18 only a *Time* effect was observed (Fig.4B, $F(7, 196)=2.208$ $p=0.003$). However, for light given from ZT18-20 we observed an *Interaction* effect on food intake (Fig. 4C, $F(21, 196)=2.018$, $p=0.007$), with white light being most effective (Table 4, $p=0.038$), due to an increase in food intake after ZT19 (Fig. 4C). At ZT20-22 (Fig.4D) no significant effects of *Time, Treatment* or *Interaction* were found, irrespective of the wavelength used. Overall, the effects of ALAN on food intake were very limited, with only blue and white light having (a tendency) for a stimulatory effect.

Plasma measurements in C57Bl6/J mice

Two-hour light pulses of different wavelength and at different times of the dark phase did not have a direct effect on the blood glucose concentration of the mice, nonetheless we observed a significant *Time* (Fig. 5A, $F(3, 26)=11.26$, $p<0.0001$) and *Interaction* ($F(9, 36)= 3.435$, $p=0.003$) effect at ZT14-16(post hoc $p=0.036$ for white light) and ZT16-18 (post hoc $p=0.046$ for green light and $p=0.005$ for white light), due to an increase in the plasma glucose levels of white and green light exposed animals. No significant effects were observed regardless of the time point and the type of light for plasma insulin (Fig.5B) and plasma corticosterone (Fig.5C).

Metabolic effects of light in BMAL1 -/- mice

Since blue light at ZT14-16 and green light at ZT20-22 had the most significant effects on the metabolic variables of wild type mice (Figs.1A, 2A, 3A and 1D, 2D, 3D, respectively), we decided to use this combination of time points and wavelengths in the *BMAL1 -/-* animals. It has been previously described that this genetic model has endocrine and metabolic alterations due to the global deficiency of the *BMAL1* clock gene (34), which makes this animal model more vulnerable for circadian disruptions, like ALAN. When KO animals were exposed to blue light from ZT14-16 we did not observe any significant differences (Supp. Table 5) in the locomotor activity (Fig. 6A), the energy expenditure (Fig.6B) or the RER (Fig.6C) compared to the dark condition at the same time of the night the day before.

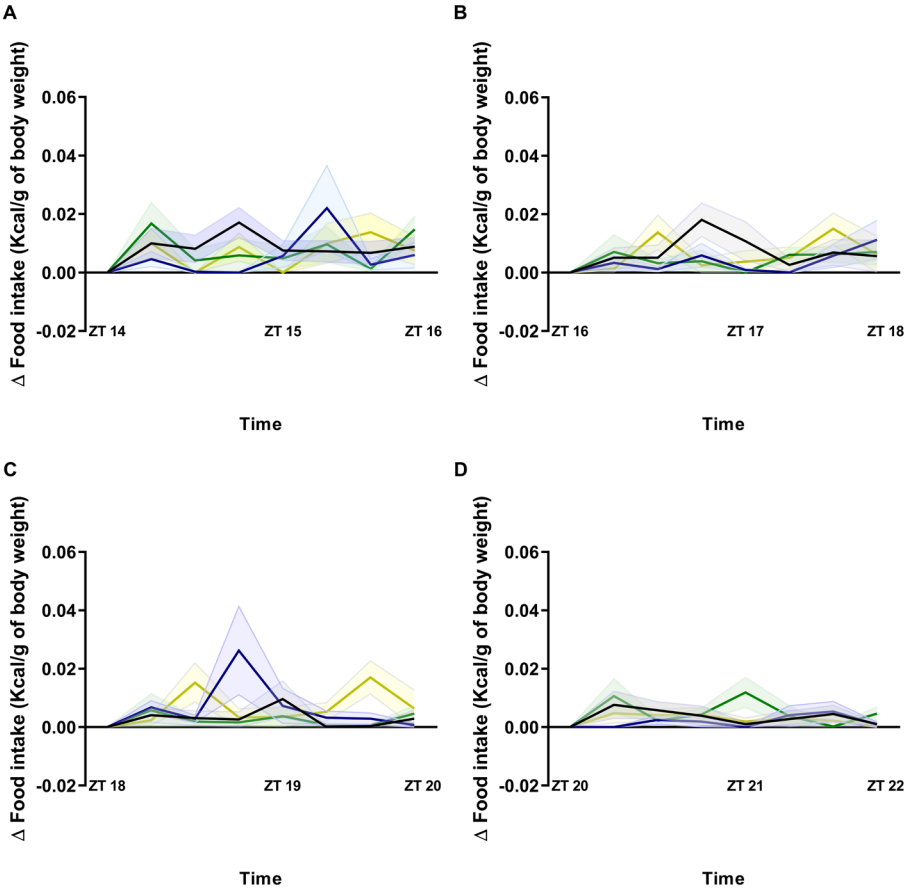

Figure 4. *ALAN did not cause any significant changes in food intake at ZT14-16 (A) or ZT16-18 (B). At ZT18-20 (C) an Interaction effect was observed in the food intake (p=0.038) for white light. No effects were observed at ZT20-22 (D). Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light-exposed animals. The black and colored straight bold lines indicate the mean levels of the dark control and 3 wavelength groups, whereas the shaded gray and colored areas delimited by the thin lines show the SEM. n=8.*

Also when *BMAL1* -/- mice were exposed to green light from ZT20-22 no significant effects of Treatment or Interaction were found, and we only observed an effect of Time on locomotor activity ($F(7, 42)= 4.439$, $p=0.001$; Fig.6D), energy expenditure ($F(7, 42)= 4.350$, $p=0.001$; Fig.6E) and RER ($F(7, 42)= 2.298$, $p=0.004$; Fig.6F)(Supp. Table 6). Because these animals had difficulties eating from the food baskets of the metabolic cages, we had

to place food on the bottom of the cages to prevent inanition and weight loss. The food on the bottom of the cage prevented us to track eating behavior in the same way as we did in the wild type animals.

Plasma measurements in *BMAL1 -/-* mice

When *BMAL1 -/-* were exposed to blue light from ZT14-16 significantly higher plasma glucose levels were observed compared to wild type animals in dark conditions or exposed to blue light at the same time point (Fig.7A). Although, plasma insulin levels were exceptionally low in the knockout animals, no statistically significant differences were observed (Fig.7C). Neither did we observe any significant differences in plasma corticosterone levels (Fig.7E). In addition, green light from ZT20-22 induced no statistically significant changes in plasma glucose, insulin or corticosterone, when comparing knock out animals with wild type animals in the same light condition or with C57Bl6/J dark controls at the same time point (Fig.7B, 7D, 7F).

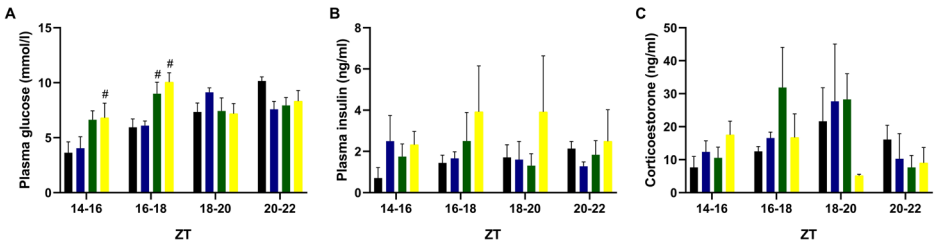

Figure 5. *ALAN effects on blood glucose and plasma insulin and plasma corticosterone concentrations. Nocturnal light exposure also affected plasma glucose concentrations. Post-hoc analysis revealed an increase in plasma glucose levels of animals exposed to white light at ZT14-16 (p=0.036), and to white (p=0.005) and green light (p=0.046) at ZT16-18 (A). No other changes in (B) plasma insulin, or (C) plasma corticosterone were observed, regardless of wavelength and time of the exposure. Black: dark controls; yellow: bright light-exposed animals; blue: blue light-exposed animals; green: green light-exposed animals. n=4 for all groups. #: p<0.05 Post-hoc white light vs. dark control.*

Discussion

This is, to our knowledge, the first time that the acute effects of nocturnal light on the energy metabolism of mice have been investigated in such detail, i.e. taking into account three different wavelengths and four different exposure times during the dark phase. Moreover, these effects were

studied in both wild type and *BMAL1* deficient mice. We showed that in wild type mice the masking effects of light during the dark period on locomotor activity depend on both the wavelength of the light used as well as on the moment of the dark phase during which the animals receive the light pulse. Similarly, also the metabolic effects of light on energy expenditure and the respiratory exchange ratio (RER) depended on the wavelength used as well as the time of exposure. Surprisingly, we found no significant effects of ALAN on these parameters in *BMAL1* deficient mice, although in these animals only two wavelengths and two time points could be investigated.

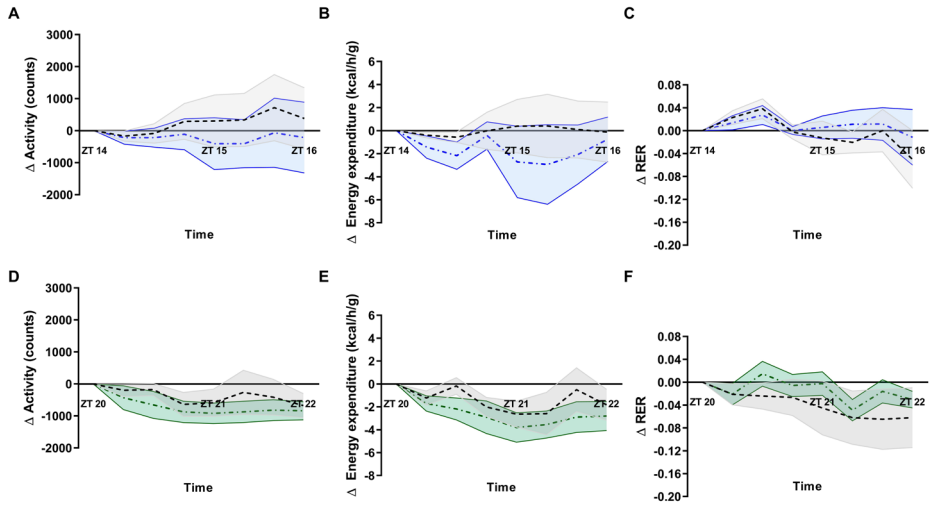

Figure 6. *Effects of nocturnal blue and green light on the locomotor activity, energy expenditure and respiratory exchange ratio (RER) of BMAL1 -/- mice. No significant changes were induced by the blue light exposure (ZT14-16) in the locomotor activity (A), energy expenditure (B) or RER (C) of BMAL1-/- mice. Also the nocturnal exposure to green light (ZT20-22) caused no significant changes in the locomotor activity (D), energy expenditure (E) or RER (F) of BMAL1-/- mice The dotted blue line indicates mean levels during exposure to blue light. The blue background delimited by thin lines indicates the SEM (A-C). The dotted green line indicates mean levels during exposure to green light. The green background delimited by thin lines indicates the SEM (D-F). The dotted black line in A-F indicates mean levels of the dark control day before light exposure. The gray background delimited by thin lines indicates the SEM. n=4 per timepoint.*

Blue light at the beginning of the dark phase (i.e. ZT14-16) had the most pronounced (decreasing) effect on locomotor activity and probably consequently also on energy expenditure. This is in contrast with our previous findings in Wistar rats where we observed a significant reduction

in locomotor activity with all three wavelengths used and at all four time points tested. However, in the same study significant reductions in energy expenditure were only observed at ZT16-18 (26), which might indicate that the reduction in energy expenditure is not only dependent on the change in locomotor activity.

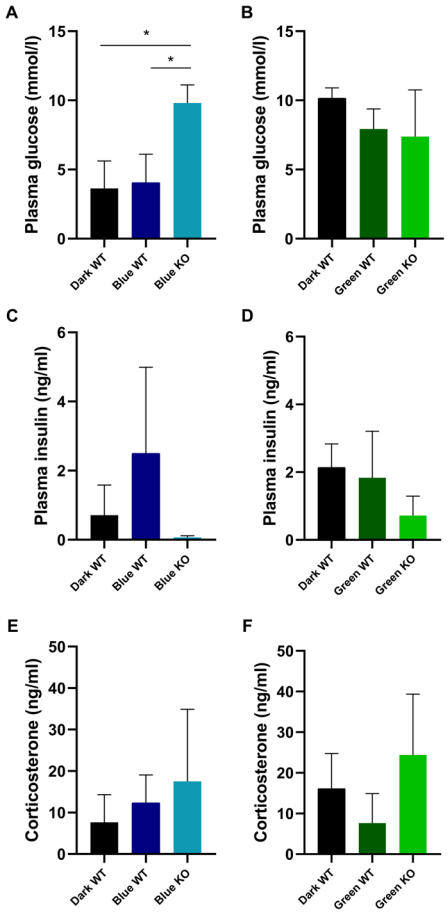

Figure 7. *Effects of blue and green light on blood glucose, plasma insulin and plasma corticosterone concentrations of BMAL1 -/- mice. Blue light increased blood glucose concentrations in BMAL1-/- mice exposed to blue light at ZT14-16 compared to wild type mice exposed to blue-light or darkness during the same hours of the dark period (A). No significant changes in blood glucose levels were induced by green light (B). Both blue and green light exposed animals showed no significant changes in plasma insulin (C-D) or plasma corticosterone (E-F). n=4 for all groups. *= p<0.05*

Interestingly, at ZT14-16 blue light also induced a significant decrease in RER but without any significant changes in food intake. In our previous rat study blue and green light evoked a decrease in RER, but from ZT16-18 (26). These data indicate that both in rats and mice ALAN induces changes in locomotor activity, energy expenditure and RER. Both rats and mice seem to be most sensitive to the effects of blue light, but the exact timing of the light-induced changes differs between both species. Overall, more light-induced changes were found in the rat study, but this might be merely due to the larger variation in the mice study.

The RER is calculated by dividing the amount of carbon dioxide produced by the energy metabolism by the amount of oxygen consumed. Due to the different ratios for glucose and lipid metabolism this ratio also gives an indication for the main fuel that is used as a source of energy for the body. A lower RER (0.7-0.8) indicates that mainly lipids are used as the main source of energy (like in fasting states or when consuming a low carbohydrate diet), while the opposite is the case when carbohydrates are used as the main fuel (RER = 0.9-1.0) (35). Therefore, usually the RER decreases when a decrease in the food intake is observed. Our current results at ZT14-16 indicate that blue light somehow shifts metabolism to a more fat-oxidative state, which is the normal state when sleeping, albeit the fact that no reduction in food intake was observed.

Previous studies in WT and *Opn4 -/-* mice have shown that blue light induces higher c-Fos expression in the SCN, while green light produces a greater response in the ventrolateral pre-optic area (VLPO). Indicating that ALAN can have both an arousing and a sleep-promoting effect and that these effects are mediated via pathways with different spectral sensitivities and partly melanopsin dependent (36). Different from our experiment, in that study light pulses, were given only for one hour starting at ZT14. Nevertheless, at none of the four time points studied we observed an arousing effect with blue light, in fact, we observed a higher reduction in locomotor activity with blue than with green light. In addition, we also noticed that green and to a lesser extent white light promoted arousal at the end of the dark phase (ZT20-22), suggesting that there is an important circadian component in the pathways stimulated by the different spectra and that the exact effects of ALAN vary according to the time of the exposure (37–39).

Another important metabolic effect that we observed to be wavelength and time-dependent was the effect of light on blood glucose concentrations.

White light from ZT14-16 caused a significant increase in blood glucose, and a similar increase was notable at ZT16-14 with white and green light. The increased plasma glucose levels occurred without any significant increases in food intake or changes in plasma insulin; suggesting that in mice white light during the first half of the night may increase endogenous glucose production. These results are in line with previous observations in rats from our group. We observed a hyperglycemic effect in rats exposed to white and also to green light at ZT14-16 (25) after a glucose tolerance test and when we observed an increased expression of Pepck in the liver of rats exposed to white light (40), suggesting again that light activates gluconeogenesis. Moreover, in the study of Opperhuizen et al. the hyperglycemic effect was observed at the beginning of the night, but not at the end of the night. In addition, green but not blue or red light mimicked the effect of white light. Similar trends are notable in the current study, with hyperglycemic effects of white and green, but not blue light during the first but not second half of the dark period.

Because we observed that the metabolic effects of blue and green light showed an important time-dependent component, we decided to test the effect of those two light spectra either at the beginning or at the end of the dark phase in an animal model without a functional clock, i.e. *BMAL1 -/-* mice. To our surprise we did not observe any significant changes in locomotor activity, energy expenditure or RER in the *BMAL1 -/-* mice during blue or green light exposure compared to dark conditions. These observations provide further support for the hypothesis that metabolic disturbances caused by ALAN are due to alterations in the normal functioning of the circadian timing system. However, the number of knockout animals utilized was low (n=4 per group) compared to the number of wildtype animals we used (n=8), which is a limitation of our study, and further research is needed.

It has been reported that global disruption of the *BMAL1* clock component leads to hypo-insulinemia and diabetes, whereas liver-specific *BMAL1-/-* mutations result in hypoglycemia (34). Although the differences were not significant, also in the current study we detected lower levels of plasma insulin in these mice, as well as higher levels of blood glucose in the blue exposed *BMAL1* deficient animals in comparison to blue light exposed C57Bl6/J mice. Unfortunately, we were not able to measure blood glucose and plasma insulin in KO mice under control dark conditions, which is another limitation of our study.

An additional constraint of this part of our study is that we could not track properly the food intake of the mutant animals. *BMAL1-/-* present premature aging (41), muscle (42), mandibular (43), and bone problems (44), which made it difficult for them to eat from the food baskets used in the metabolic cages.

In conclusion, we showed that ALAN produces changes in locomotor activity, energy expenditure, RER and blood glucose in mice. All these effects are dependent on their timing within the dark phase as well as on an intact molecular clock, which further stresses the important role of the circadian timing system, although further research is needed. The fact that these effects are also dependent on the light spectra used suggests that ipRGCs, rods and cones are all involved in the responsible pathways. Further studies are needed to elucidate the ideal light spectral composition to use during the dark phase in order to prevent metabolic disturbances due to ALAN.

Overall, even though the existing evidence on the effects of light on metabolism differs considerably due to different setups, species, light characteristics or experimental designs, the different studies do point out that ALAN disturbs energy metabolism. It is to be expected that when these exposures occur repeatedly or even become chronic, this will severely disrupt the circadian timing system and increase the risk for metabolic diseases such as obesity and diabetes.

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Supp. Table 1. *Statistical analysis of the overall effects of light on locomotor activity, energy expenditure, RER, and food intake of wild type mice.*

Variable	Time point	*Treatment*	*Time*	*Interaction*
Locomotor activity	ZT14-16	0.599	<0.0001*	0.007*
	ZT16-18	0.234	0.002*	0.388
	ZT18-20	0.163	0.001*	0.302
	ZT20-22	0.126	0.419	0.132
Energy expenditure	ZT14-16	0.154	<0.0001*	0.048*
	ZT16-18	0.547	0.004*	0.598
	ZT18-20	0.734	0.494	0.826
	ZT20-22	0.206	0.025*	0.056
RER	ZT14-16	0.070	<0.0001*	0.0004*
	ZT16-18	0.690	0.023*	<0.0001*
	ZT18-20	0.782	0.013*	0.635
	ZT20-22	0.541	0.019*	0.0004*
Food intake	ZT14-16	0.619	0.006*	0.322
	ZT16-18	0.425	0.035*	0.204
	ZT18-20	0.243	0.129	0.007*
	ZT20-22	0.139	0.104	0.301

Treatment (light condition), Time (time of the measurement) and Interaction effects were determined for each 2-hour experiment using mixed model analysis.

Supp. Table 2. *Statistical analysis of the effects of blue light as compared to dark controls on locomotor activity, energy expenditure, RER, and food intake of wild type mice.*

Variable	Time point	*Treatment*	*Time*	*Interaction*
Locomotor activity	ZT14-16	0.529	<0.0001*	0.008*
	ZT16-18	0.184	0.048*	0.124
	ZT18-20	0.411	0.0005*	0.764
	ZT20-22	0.546	0.106	0.610
Energy expenditure	ZT14-16	0.002*	<0.0001*	<0.0001*
	ZT16-18	0.356	0.085	0.468
	ZT18-20	0.247	0.0007*	0.687
	ZT20-22	0.356	0.110	0.656
RER	ZT14-16	0.032*	0.003*	0.114
	ZT16-18	0.925	0.018*	0.038*
	ZT18-20	0.730	0.0003*	0.852
	ZT20-22	0.495	0.215	0.099
Food intake	ZT14-16	0.216	0.186	0.124
	ZT16-18	0.140	0.069	0.413
	ZT18-20	0.251	0.033*	0.103
	ZT20-22	0.211	0.279	0.610

Treatment (blue light condition), Time (time of the measurement) and Interaction effects were determined for each 2-hour experiment using mixed model analysis.

Supp. Table 3. *Statistical analysis of the effects of green light as compared to dark controls on locomotor activity, energy expenditure, RER, and food intake of wild type mice.*

Variable	Time point	*Treatment*	*Time*	*Interaction*
Locomotor activity	ZT14-16	0.480	0.001*	0.296
	ZT16-18	0.545	0.490	0.713
	ZT18-20	0.516	0.628	0.642
	ZT20-22	0.030*	0.887	0.624
Energy expenditure	ZT14-16	0.334	0.129	0.433
	ZT16-18	0.951	0.346	0.883
	ZT18-20	0.717	0.441	0.618
	ZT20-22	0.271	0.410	0.765
RER	ZT14-16	0.864	<0.0001*	0.013*
	ZT16-18	0.344	<0.0001*	0.257
	ZT18-20	0.583	0.084	0.801
	ZT20-22	0.601	0.015*	0.107
Food intake	ZT14-16	0.637	0.040*	0.456
	ZT16-18	0.199	0.304	0.249
	ZT18-20	0.740	0.200	0.915
	ZT20-22	0.368	0.130	0.299

Treatment (green light condition), Time (time of the measurement) and Interaction effects were determined for each 2-hour experiment using mixed model analysis.

Supp. Table 4. *Statistical analysis of the effects of white light as compared to dark controls on locomotor activity, energy expenditure, RER, and food intake of wild type mice.*

Variable	Time point	*Treatment*	*Time*	*Interaction*
Locomotor activity	ZT14-16	0.721	0.002*	0.005*
	ZT16-18	0.543	0.862	0.572
	ZT18-20	0.258	0.080	0.768
	ZT20-22	0.442	0.194	0.183
Energy expenditure	ZT14-16	0.307	0.393	0.084
	ZT16-18	0.807	0.824	0.922
	ZT18-20	0.611	0.327	0.966
	ZT20-22	0.478	0.023*	0.093
RER	ZT14-16	0.272	<0.0001*	0.033*
	ZT16-18	0.485	0.011*	<0.0001*
	ZT18-20	0.417	0.022*	0.243
	ZT20-22	0.542	0.016*	0.003*
Food intake	ZT14-16	0.359	0.058	0.525
	ZT16-18	0.760	0.117	0.088
	ZT18-20	0.127	0.137	0.038*
	ZT20-22	0.535	0.144	0.991

Treatment (white light condition), Time (time of the measurement) and Interaction effects were determined for each 2-hour experiment using mixed model analysis.

Supp. Table 5. *Statistical analysis of the effects of blue light at ZT 14-16 on locomotor activity, energy expenditure, RER, and food intake of BMAL -/- mice.*

Variable	*Treatment*	*Time*	*Interaction*
Locomotor activity	0.484	0.879	0.984
Energy expenditure	0.542	0.955	0.879
RER	0.625	0.275	0.941

Treatment (blue light or dark control), Time (time of the measurement) and Interaction effects were determined for the ZT14-15 light exposure experiment using mixed model analysis.

Supp. Table 6. *Statistical analysis of the effects of green light at ZT 20-22 on locomotor activity, energy expenditure, RER, and food intake of BMAL -/- mice.*

Variable	*Treatment*	*Time*	*Interaction*
Locomotor activity	0.150	0.001*	0.097
Energy expenditure	0.439	0.001*	0.775
RER	0.506	0.044*	0.808

Treatment (green light or dark control), Time (time of the measurement) and Interaction effects were determined for the ZT20-22 light exposure experiment using mixed model analysis.

Chapter 8

General discussion and future directions

Discussion

The 24-hour duration of the light-dark cycles produced by the rotation of the Earth around its axis made organisms adapt their physiology to the illumination characteristics of the environment during evolution. Virtually all organisms developed a circadian timing system that allowed their biology and their behavior to be synchronized with the external environment. Circadian rhythms, as described by their name, last about but not exactly 24 hours. In order for these rhythms to stay synchronized with the exact 24-hour rhythms of the environment, they are reset every day by the exposure to environmental cues or Zeitgebers, light (or light-dark cycles) being the most important of them (1).

Over the past century, the use of artificial light at night (ALAN) has spread widely, bringing urbanization and a highly industrialized lifestyle. This allowed humans to expand their day activities far beyond sunset time and, amongst others, lead to the introduction of shift work. Additionally, technological advances starting towards the end of the 20th century have given most of the western population access to television screens, computers, tablets, e-readers and, cellphones; increasing, even more, the amount of light that we receive at night. It is estimated that 80% of the European and American populations experience night pollution (2). All these changes induced by our modern lifestyle have led to an aberrant daily light exposure, i.e. ALAN, which in the long run may cause circadian disruption.

Numerous epidemiological studies have reported a higher incidence of cardiometabolic diseases among shift workers (3–12) due to circadian disruption but also related to changes in food intake behavior (13). These findings have also been supported by evidence from animal studies (14–18), which provided further evidence for the metabolic and behavioral effects of nocturnal light exposure (19). Moreover, scientific proof for the deleterious effects on metabolism and behavior of the light-emitting diodes (LED) in bulbs and screens has raised further concern about the use of those devices (20–28). Consequently, in our studies we took into account the role of the wavelength of the nocturnal light as well. Although scientific evidence is abundant, the mechanisms responsible for the metabolic and behavioral disruption caused by ALAN are still being investigated. The general aim of this thesis was to study further the metabolic and physiologic effects of acute exposure to ALAN. This objective was approached from several angles, first by studying the effects of light in two different feeding conditions (a balanced

diet and a free choice high-fat, high-sucrose diet) hence expanding our understanding of the interactive effects of ALAN and diet on feeding behavior and food preference. Second, investigating more in-depth the important role of the timing and the light spectra for the behavioral and metabolic outcomes. Third, to explore the role of a functional circadian rhythm and the protein melanopsin in the acute effects of ALAN by using genetically engineered mice models. Fourth, the utilization of different animal species, including a diurnal rodent, as well as animals of both sexes gave us further insight regarding future directions and possible translation to humans.

In **Part I, Chapter 2** we reviewed the current evidence on the metabolic implications of exposure to ALAN, gathering not only the latest evidence from animal studies but also all the data from human interventional and epidemiological studies. We focused on the impact of both chronic and acute light exposures, also taking into account the effects of different wavelengths. Indeed, rodent studies with chronic exposure to ALAN have shown disruptions in locomotor activity, food intake, body temperature, altered rhythms of melatonin, glucocorticoids, plasma glucose, and lipid metabolism; mainly caused by its disruptive effects on the master clock in the hypothalamic suprachiasmatic nucleus (SCN) (29–32). Interestingly, studies showing short-term or acute effects of light exposure also demonstrated negative effects on metabolically active tissues, these may be driven not only by the SCN (since they are time-dependent) but also by SCN-independent pathways (33–35). Evidence of experiments using different wavelengths of light highlights the importance of melanopsin and also shows that each wavelength of light may have a different effect on metabolism. Furthermore, exposure to ALAN has shown to alter glucose homeostasis and to increase the risk of developing overweight, obesity, and atherosclerosis (18,19,36–39). Clearly, the evidence from animal models and human studies confirmed the deleterious effects of ALAN, but further studies exploring the putative mechanisms are needed.

Part II (Chapters 3 and 4) of this thesis focused on the effects of ALAN on glucose metabolism and food intake in the diurnal rodent *Arvicanthis ansorgei,* and we briefly explored also the role of sex in the metabolic and physiologic outcomes of acute light exposure at night. We think exposing a diurnal rodent to a high-fat high-sucrose (HFHS) diet better resembles the conditions to which humans are exposed than the usual studies in chow-fed nocturnal rodents. In **Chapter 3** the effects of a 1-hour pulse of white light

at the beginning of the night (i.e. Z14) were explored, while in **Chapter 4** we used a similar experimental set-up to study the effects of blue light at the same time of the dark phase (rest period for diurnal rodents). Remarkably, the effects on glucose metabolism, food intake, and plasma metabolites of both light spectra depended on the sex of the animals. Additionally, light did not impact energy metabolism the same way when animals were fed with regular chow diet than when they had the option to eat from the different components of an HFHS diet. While white light caused glucose intolerance in female, but not in male *Arvicanthis* fed a regular chow diet, blue light altered glucose tolerance only in male animals fed the same diet. The wavelength-dependent effects of light on glucose metabolism depend were in line with what was reported in Wistar rats before (19), but the current experiments indicated that these effects are also sex-dependent. In HFHS fed animals we only found a detrimental effect in male animals exposed to blue light, which points out two important points. First, we confirmed that female animals may be protected from the negative metabolic effects of an HFHS diet probably by sexual hormones (40–42). Secondly, in male animals, the nutritional status determines the extent to which ALAN affects their metabolism, this might be either because once they gain weight the retinal-brain mechanism involved in feeding behavior control that is disrupted by ALAN has already been corrupted by the diet or because the diet induces retinal damage. Indeed, *Arvicanthis* can show a diabetic phenotype (retinopathy included) even after short periods of exposure to high glycemic loads (43–45).

In males, the reduction in plasma insulin after the blue light pulse may explain in part the decreased glucose tolerance, however in female mice exposed to white light no differences in plasma insulin were observed. The latter suggests that in females the mechanism by which glucose tolerance was impaired, may be either because of higher endogenous glucose production or because of lower glucose uptake. However, how sex and wavelength can cause these different outcomes for glucose metabolism remains to be understood. Likewise, the opposite effects and interaction of wavelength and sex on the intake behavior of the palatable components of the HFHS diet need to be investigated further.

Future directions

The diurnal nature of *Arvicanthis* makes it a really good model for studying circadian disruption due to ALAN. In humans, aberrant exposure to ALAN usually occurs by receiving light after dusk or before dawn, due

to discrepancies between social (school, work, leisure) and biological time (46). Previous evidence obtained in Wistar rats has shown that the effects of light on glucose metabolism are time-dependent and also that green light has a more detrimental effect than blue light (19). Here we tested only the effects of white and blue light at ZT14 (the beginning of the dark phase), but to get better insights into the effects of light in this diurnal rodent, light effects at the end of the dark phase (ZT22 for example) and additional experiments with green light should be added in the future. Chronic effects of green light on obesity and metabolic disorders have been reported in high-fat-fed C57B6/J mice (39), but the acute effects of green light pulses on food preference have not been investigated yet. Furthermore, due to the striking differences between males and females and since only a couple of studies have reported different anatomical differences, such as L to M cone ratio (47), and light perception discrepancies between men and women (48), studying the effects of sex hormones, as protective molecules, should also be one of the next steps. A better understanding of the sex differences and the possible brain areas involved in the metabolic effects that light at night has in *Arvicanthis* can be obtained with histological techniques, such as immunohistochemistry or in situ hybridization or with more state of the art technology such as RNAscope. Using those techniques, special attention should be given to those areas that are involved in the control of metabolism and food intake and that receive direct or indirect inputs from the intrinsically photosensitive retinal ganglion cells (ipRGCs), like the lateral hypothalamus (LH), the perihabenular complex (PHb), the paraventricular nucleus of the hypothalamus (PVH), the intergeniculate leaflet (IGL), the ventral tegmental area (VTA) and the amygdala (49).

Previously it has been shown that light at night impairs glucose tolerance in Wistar rats in a time-, intensity- and wavelength-dependent manner (19), in **Part III, Chapter 5** we explored the effects of different wavelengths of light at four different time points during the dark phase on energy metabolism, neural activity in the SCN and PVH, and the expression of hepatic clock and metabolic genes. Remarkably, we observed discrepancies between locomotor activity and energy expenditure, and also between respiratory exchange ratio (RER) and food intake, indicating that despite the physiological correlation between these parameters, ALAN may affect them independently.

Regarding the expression of hepatic clock genes, we did not find a

significant effect of white light on *Per1* as it has been reported before (34), but blue light did increase its expression significantly. In addition, white and green light increased the expression of *Per2* while no effects were observed on *Cry, Bmal1, Clock, or Rev-erbα*. The effect of white light on Per2 expression is in line with previous evidence (34). A possible mechanism responsible for the light-induced changes in *Per1* has been reported in mice, and involves epigenetic changes such as histone acetylation and deacetylation (50). With regard to the metabolic genes in the liver, we observed that all wavelengths of light affected the expression of genes involved in carbohydrate metabolism. Earlier it has been reported that white light at ZT20 reduced the expression of glucokinase (*Gck*), whereas at ZT14 and ZT20 it increased the expression of phosphoenolpyruvate carboxykinase (*Pepck*), indicating that exposure to white light at night promotes gluconeogenesis (34). Surprisingly, we did not observe the same effects with blue light, if anything we observed a reduction of *Pepck* with blue light, meaning that blue light in Wistar rats could reduce gluconeogenesis. Green light may have a similar effect on this pathway by reducing the expression of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (*Pgc1α*)(Figure 1). in addition, with all three wavelengths tested, we saw an increment in acetyl-CoA carboxylase 1 (*Acc1*) and fatty acid synthase (*Fas*), indicating that light can increase lipogenesis, which matches the observed decrease in RER despite no changes in food intake. Moreover, these data nicely support previous results in human studies (51). While it has been already reported that the effects of light on hepatic metabolism are mediated both by the SCN and by autonomic innervation (34,35), how different wavelengths of light can affect the hepatic expression of metabolic genes differently remains to be understood. The same applies to the c-Fos activation observed in the SCN and the PVH, which makes studying how different wavelengths activate different parts of the brain an approach that should be explored further.

Future directions

Based on our findings in mice and the Arvicanthis, it is evident that sex plays an important role in the metabolic and physiologic effects of light. However, no studies have explored this in female Wistar rats, thus this should be addressed in future investigations. Findings in humans have shown that the perception of light, wavelength, intensity, brightness, and its impact on cognitive functions varies in men and women (48), hence we could expect that similar processes can apply to the metabolic effects of light. Animal

studies using animals of both sexes, with different wavelengths of light and with different intensities (in a dose-response manner) will be helpful to increase our knowledge in this regard.

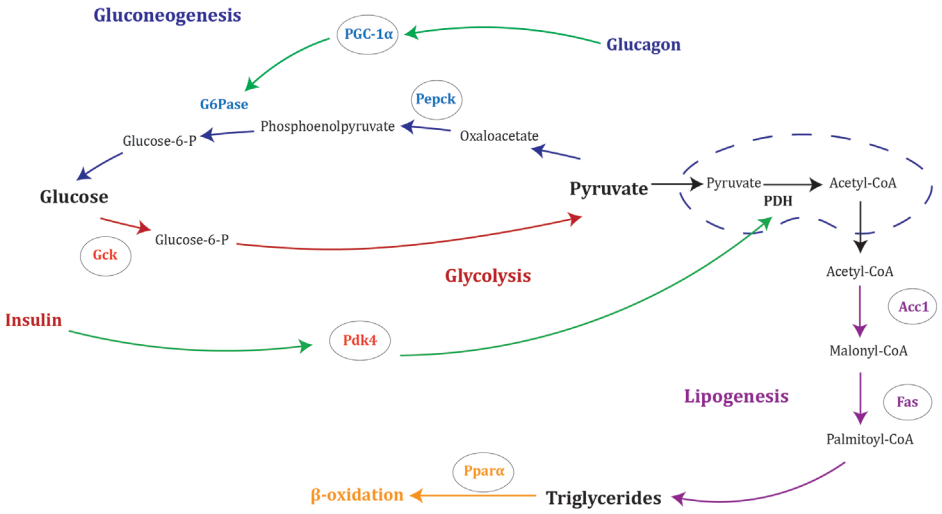

Figure 1. *Simplified schematic representation of biochemical pathways involved in the metabolism of carbohydrates and lipids, and the role of the genes measured in rat hepatocytes.*

Since the current work and previous work from our group (19,34,35) has demonstrated that indeed ALAN affects glucose and energy metabolism via both the SCN and the autonomic nervous system, more studies using liver denervations and different light spectra should be performed to understand at which extent each wavelength changes hepatic metabolism and how much these wavelength-dependent effects are SCN dependent too. Likewise, since the timing of the light exposure in rats changes the outcome for energy and glucose metabolism, experiments combining different light colors and time of the exposures with glucose kinetic studies, such as endogenous glucose production, hyperinsulinemic-euglycemic clamps, and new techniques like bioluminescent-based imaging for in vivo quantification of glucose uptake could be insightful (52).

Lastly in **Part IV,** we studied the metabolic effects of ALAN in C57Bl6/j mice. Our results in **Chapters 6 and 7** showed the striking difference in physiology among different rodent species. In mice, blue and white light improved glucose tolerance by decreasing the glycemic response during the glucose tolerance test, but only in HFHS fed animals. Moreover, although

the differences in plasma insulin levels did not reach significance, blue light-exposed animals showed higher levels of plasma insulin, indicating a putative mechanism for the decreased glycemic response during the tolerance test. Additionally, blue light increased the consumption of fat during the dark phase when animals received the light pulse, suggesting that blue light triggers appetite for palatable food items. It is important to mention that these experiments were performed in female mice and because of the previously discussed sex differences in *Arvicanthis*, it is highly likely that comparable sex differences are also present in mice. Nevertheless, this remains to be studied. We also investigated the effects of light on food intake in melanopsin deficient mice (*Opn4 -/-*). It was remarkable to see that in the *Opn4 -/-* mice blue light increased the consumption of chow food, but not the palatable food (i.e. sugar of fat), pointing out that the retina-brain pathway involved in feeding behavior is melanopsin dependent.

Furthermore, we studied the effects of three different wavelengths of nocturnal light (blue, green, and white) on the metabolism of C57Bl6/j mice and *Bmal1 -/-* mice. We showed that ALAN changed locomotor activity, energy expenditure, RER, and blood glucose and that these changes are dependent not only on the wavelength but also on the time of the exposure in wild type mice. These observations provide further support for the idea that the effects of nocturnal light on metabolism involve the circadian timing system. Although further research is needed, the first observations in animals lacking the clock gene *Bmal1* support this idea, since such time-dependent effects of light were not in these animals. Finally, the different wavelength effects of light indicate that besides the central circadian pacemaker also ipRGCs, rods, and cones might be involved in the underlying mechanism and this should be addressed in future research.

Future directions

To complement further our previous experiments, testing the effects of blue light on glucose tolerance in *Opn4 -/-* mice is necessary. Also, because our results and those of other research groups have shown the deleterious effect of short-wavelength light (SWL) on the circadian timing system and energy metabolism in rodents (19,39,53,54), it is necessary to study further whether the negative effects of SWL are fully melanopsin dependent or if cones and rods are involved too. The use of a genetically engineered mouse like *Nrl -/-* (55) could be extremely helpful here. In the mouse retina this mutation results in a lack of rods and an increased number of shortwave

cones, which could give more insights regarding the retinal circuits involved behind the effects of SWL on metabolism. Also mice models that completely lack the cone and rod functions can be used (56), as this will show whether melanopsin alone is responsible for the non-image forming effects of light, particularly in this case on energy metabolism. Thereafter, to further explore the brain areas implicated in the mechanisms behind the effects of ALAN it would be really interesting to use a transgenic mice model that has no ipRGCs projections to the SCN, PHb and LH (57,58). Lastly, using a mice model that only has ipRGCs projections to the SCN can prove if the observed metabolic effects of light are SCN-dependent or if other areas play an important role in it (59). And as a general conclusion, as mentioned before, due to cumulating evidence on physiological differences between sexes, the use of animals (or subjects in the case of human studies) of both sexes in any future experiment is not only highly encouraged but extremely necessary.

Suggestions for the use, reporting, and handling of light in future studies

One of the major limitations encountered while performing our studies was the great difficulty to make comparisons between the different studies. As we summarized in the supplementary tables of Chapter 2, the unstandardized way in which authors report their light treatments makes experiments virtually unreproducible, which is a major pitfall in science. Although lately, most authors including ourselves have reported the light conditions used using the toolbox designed by Lucas et al. (60), recently these guidelines have been superseded by the International Commission on Illumination (61). Details on how to report light conditions properly have been described extensively now by Spitschan et al. (62), hence using these new guidelines is urged. Likewise, future scientific research focusing on non-visual effects of light should refrain from describing light only by its apparent hue, since there are multiple ways of making light to appear as a specific color (63).

When studying the non-image forming effects of light what needs to be accounted for is its melanopic effect, since these effects are mostly driven by ipRGCs. Also, intensity should be reported especially in studies using monochromatic light, because by the principle of univariance different combinations of wavelengths can elicit the same response on the photoreceptors; meaning that photopigments cannot distinguish between

changes in intensity or wavelength (64). Hence, the principle of silent substitution is another interesting approach to study the contributions of melanopsin to the metabolic and physiologic effects of light (65).

Clinical relevance

The current worldwide epidemic of obesity and type 2 diabetes along with our failure to address it with lifestyle changes based solely on diet and physical activity, or medication, makes it imperative to look at other environmental causes contributing to these diseases. Previous evidence has shown how these pathologies go hand in hand with increasing light pollution (66). Increasing our knowledge on how light and its characteristics, such as wavelength and intensity, impact energy metabolism can help us with optimizing the artificial light we use in our daily life activities. Clearly, our highly industrialized and 24-hour active lifestyle will not go back to what it was more than a hundred years ago, i.e. before electrical light was introduced in human life. But combining scientific discoveries in this area of research with state of the art technology, such as artificial intelligence and smart materials, will make it possible to develop adaptive lighting products and light-emitting devices that are able to adjust their spectra to the time of the day. Such an approach could contribute to the prevention and better treatment of metabolic disorders in the near future.

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Appendices

Summary

The 24-hour Earth's rotation determines the alternation between day and night. Exposure to variations in illuminance (light intensity, spectra, and contrasts) together with daily temperature changes have played an important role in the evolution of virtually all organisms. These environmental conditions lead to the development of body clocks that synchronize many physiological and behavioral processes, including the daily cycle of wakefulness and sleep, to the rhythmic changes in the environment. However, this master clock, located in the hypothalamic suprachiasmatic nucleus (SCN), oscillates with a period of approximately 24 hours, which is why it is called circadian (circa "about" and dies "day"). This rhythmic oscillation originates at a cellular level and is being fine-tuned by a molecular clock consisting of several transcriptional-translational feedback loops. In most vertebrates light is the most effective environmental cue (Zeitgeber) to synchronize the internal circadian rhythm to the exact external 24-hour rhythm of the Earth's rotation. The previously mentioned molecular clock has also been found to be present in peripheral tissues such as but not limited to liver, muscle, and adipose tissue. For peripheral clocks, it is thought that food, i.e. the cycles of feeding and fasting, is the most important *Zeitgeber*.

Our industrialized and highly technological lifestyle is confronting us with major changes in the patterns of light exposure, as well as changes in our activity and food intake patterns. In the long term, this can impair physiological processes by disrupting their circadian rhythms. Mounting evidence from the last thirty years has shown that disruption of the circadian rhythm can result in metabolic diseases such as obesity and type 2 diabetes. In this Ph.D. project, we aimed to study the metabolic and physiological effects of acute exposure to light at night in both nocturnal and diurnal rodents.

This thesis is divided into several sections. First **Part I, Chapter 1** includes a brief introduction to circadian rhythms and light, providing the necessary physical principles for understanding our research. Additionally, at the end of this chapter, the scope of this thesis is described. In **Chapter 2** we included an exhaustive review of animal and human studies describing the metabolic implications of artificial light at night (ALAN). To analyze better the existing evidence, we divided the reviewed studies between chronic and acute exposures to ALAN. A separate section analyzing the effects of different wavelengths was also included. Our overview of the existing literature showed that most studies that employed chronic ALAN

caused metabolic inefficiency by disturbing daily behavioral rhythms via its impact on the SCN. On the other side, in experiments using acute exposure to ALAN, disturbances in peripheral tissues were reported independent of disturbances in SCN circadian rhythms, possibly via ALAN effects on the ventral region of the SCN, via non-SCN brain areas or a combination of both pathways. Furthermore, several publications indicated that the effects of ALAN are wavelength dependent and these effects differ among peripheral tissues, providing another indication that these effects might be independent of SCN disturbances. This literature review clearly stressed the need for further research in this field, especially to determine which light spectrum is most harmful to human health, and in particular to human metabolism.

In **Part II,** we explored the effects of ALAN on food intake and glucose metabolism in the diurnal rodent *Arvicanthis ansorgei*, a rodent model with circadian physiology that resembles more that of humans. To mimic the human conditions even further, animals were also offered the possibility to choose between a palatable or a more healthy diet. For this we used for the very first time a free-choice high-fat high-sucrose diet (fcHFHS) in these animals, aiming to study the effects of the light exposure at night on the intake of the different dietary components (regular chow food, sugar, and animal lard). **Chapter 3** was dedicated to exploring the effects of white light, whereas in **Chapter 4** the effects of blue light on glucose metabolism and food intake were addressed. We observed that in *Arvicanthis* exposure to white light at ZT14 caused glucose intolerance in females but not in males. No changes in plasma insulin levels were observed, indicating that the effects of ALAN were possibly induced by increasing endogenous glucose production or decreasing glucose uptake. In addition, white light decreased sugar intake in males and fat intake in females. On the other hand, we found that acute exposure to blue light at that same time of the night caused glucose intolerance by reducing plasma insulin. It also increased sugar consumption. Both effects were observed in males only. Hence, our findings in these two chapters indicate that the effects of light on glucose metabolism and food intake vary depending on the wavelength used and that each light spectrum affects the two sexes differently. The different effects observed with each type of light indicate the involvement of the intrinsically photosensitive retinal ganglion cells (ipRGCs). In addition, sex hormones seem to play an important too, however, this remains to be studied further.

In **Part III, Chapter 5** we used male Wistar rats, a nocturnal rodent, with

the main aim to further explore the time-and wavelength-dependent effects of light at night on energy metabolism. As a second objective, we studied the time- and wavelength-dependent effects of light on neural activity in the SCN and the paraventricular nucleus of the hypothalamus (PVH), as well as on the expression of hepatic clock genes and metabolic genes. We showed that ALAN of any wavelength and at any time point during the dark phase reduced the locomotor activity in Wistar rats. On some occasions during the first 15 minutes of light exposure, especially blue light, some increases in locomotor activity were observed, probably due to a phenomenon not so well known, called light-induced locomotor activity. Remarkably, when locomotor activity was reduced, the expected associated decrease in energy expenditure was observed only with blue and white light, which indicates that light may also have activity independent effects on energy expenditure.

A similar discrepancy was observed between the respiratory exchange ratio (RER) and food intake, as we detected a decrease in the RER (indicating a higher lipid metabolism) when animals were exposed to blue light at ZT16-18, without any reduction in food intake. Showing again that light may have an independent effect on these two variables. Moreover, we observed an increased expression of the clock gene *Per 2,* and the metabolic genes *Gck*, *Acc1*, and *Fas*, which correlated with the effects of light on c-fos expression in the SCN. Similarly, the observed decreased expression of *Pgc1α* and *Pdk4* in the liver matched the wavelength-dependent effects of light on c-fos expression in the PVH, especially with blue and green light. All in all these effects were dependent on the time of the dark phase when the light pulse was given, which indicates a strong interaction with the circadian timing system. Because the effects observed were also wavelength-dependent we speculate that the ipRGCs, together with the cones and rods, are involved in the mechanisms responsible for the effects of ALAN observed.

Lastly, in **Part IV** we focused on the effects of ALAN on glucose and energy metabolism of wild type mice and two knock-out mice models. The development of genetically engineered mice has increased our ability to study the role of specific genes and proteins on animal physiology enormously over the last decade. Intrinsically photosensitive retinal ganglion cells are known to be required for the non-image-forming (NIF) physiological responses to light. Their intrinsic light sensitivity is obtained from the presence of a protein called melanopsin, which makes this protein an important target for studying NIF effects of light.

In **Chapter 6**, the effects of light (white and blue) on glucose metabolism and food intake were investigated using a combination of C57Bl/6J wild type mice with genetically engineered mice that were melanopsin deficient. Both light spectra decreased locomotor activity regardless of the diet. Contrary, in chow-fed animals no significant differences in glycemic response were found between light-exposed (either wavelength) and dark control animals. However, animals fed the HFHS diet, showed an improved glucose tolerance, when exposed to blue or white light. Additionally, food intake in the chow-fed groups showed no significant changes due to light exposure. In HFHS animals, however, a significant increase in caloric intake during the following dark period was observed, but only in blue light-exposed animals. Remarkably, the extra energy intake came mainly from fat, suggesting that blue light stimulates hedonic feeding in mice. Both wavelengths also increased food intake in the melanopsin mutant mice, but this time by increasing the amount of chow food eaten. Our findings indicate that the effects of light at night on hedonic food intake may be melanopsin dependent.

Finally, in **Chapter 7** our objective was to study the effects of different wavelengths of light at different time points during the dark phase on energy metabolism of C57Bl6/J and *BMAL1* deficient mice. *BMAL1* is one of the most important clock genes and it has been shown that besides disturbed circadian rhythms *BMAL1* deficient mice also have endocrine and metabolic alterations. We hypothesized that these endocrine alterations would make this animal more vulnerable to further circadian disruptions due to ALAN, or, on the contrary, that due to its already existing disrupted circadian physiology the effects of light on metabolism would not be observed. The results in this chapter show that ALAN alters energy expenditure, RER, and plasma glucose levels in mice in a time-dependent manner. We also obtained some first evidence that the integrity of the molecular clock is necessary to observe these metabolic disturbances caused by ALAN, although this needs to be further investigated. Additionally, we observed that also in mice these effects are dependent on the wavelength of light used, indicating again that ipRGCs, cones, and rods may be part of the pathways involved.

To conclude, although the exact results may vary between species, this thesis provides further evidence on the need for caution when being exposed to artificial light at night, to prevent detrimental metabolic consequences. This also includes light-emitting electronic devices, especially those that emit short- and middle-wavelength light. However, still more research is

needed in this area to further elucidate the exact mechanisms behind it and to optimize the light spectra we use in our daily life activities. Nevertheless, we expect that by combining these scientific discoveries with state of the art technology will make it possible in the future to develop adaptive lighting products and light-emitting devices that will be able to adjust their spectra to the time of the day, to prevent metabolic diseases.

Samenvatting

De 24-uurs rotatie van de aarde bepaalt de afwisseling tussen dag en nacht. Blootstelling aan deze dagelijkse variaties in de hoeveelheid licht (lichtintensiteit, spectra en contrasten) hebben samen met temperatuursschommelingen een belangrijke rol gespeeld in de evolutie van vrijwel alle organismen. Deze omgevingscondities resulteerden in de ontwikkeling van lichaamsklokken die een veelheid aan fysiologische en gedragsmatige processen, inclusief de slaap en waak cyclus, synchroniseren met de omgeving. Echter, deze hoofdklok, gelegen in de hypothalame suprachiasmatische kern (SCN), oscilleert met een periode van ongeveer 24 uur, wat de reden is waarom deze ritmes ook wel circadiane ritmes worden genoemd, (*circa* = ongeveer, *dies* = een dag). Deze circadiane ritmes ontstaan op cellulair niveau en worden gegenereerd door een moleculaire klok door middel van een aantal transcriptie-translatie feedback loops. In de meeste gewervelde dieren is licht de meest effectieve stimulus in de omgeving (*Zeitgeber*) om het interne circadiane ritme te synchroniseren met het externe exacte 24-uursritme van de rotatie van de aarde. De eerder genoemde moleculaire klok is ook aanwezig in perifere weefsels zoals, maar niet beperkt tot, lever, spier en vetweefsel. De algemene gedachte is dat voor deze zogenaamde perifere klokken, voedsel, of eigenlijk de cyclus van het voeden en vasten, de belangrijke Zeitgeber is.

Onze geïndustrialiseerde en hoogtechnologische levensstijl heeft gezorgd voor grote veranderingen in de blootstellingspatronen aan licht, als ook grote veranderingen in onze activiteits- en voedselinnamepatronen. Op de lange termijn kunnen deze veranderingen ook fysiologische processen verstoren door het verstoren van hun circadiane ritme. De afgelopen dertig jaar is gebleken dat verstoringen in de circadiane ritmiek kan resulteren in stofwisselingsziekten zoals obesitas en type 2 diabetes. In dit proefschrift hebben we ons gericht op het bestuderen van de metabole en fysiologische effecten van acute blootstelling aan nachtelijk licht in zowel nacht-actieve als dag-actieve knaagdieren.

Dit proefschrift is onderverdeeld in verschillende secties. **Deel I, Hoofdstuk 1**, bevat een korte inleiding tot de circadiane ritmes en effecten van licht, en biedt de nodige fysische principes voor het begrijpen van ons onderzoek. Daarnaast wordt aan het eind van dit hoofdstuk het doel van het onderzoek in dit proefschrift beschreven. **Hoofdstuk 2** bevat een uitgebreid overzicht van eerdere dier- en mensstudies die de metabole implicaties van

kunstmatig licht ‘s nachts hebben onderzocht. Om het bestaande bewijs beter te analyseren, hebben we de beschreven studies verdeeld in studies met een chronische dan wel een acute blootstelling aan nachtelijk (kunst) licht (ALAN). Ook bevat dit hoofdstuk een aparte sectie waarin we de effecten van verschillende golflengtes analyseren. Dit literatuuroverzicht laat zien dat de meeste studies die gebruik maken van chronische ALAN metabole inefficiëntie veroorzaakten door het verstoren van de dagelijkse gedragsritmes via de impact van licht op de SCN. Aan de andere kant, in de experimenten met acute blootstelling aan ALAN, zijn verstoringen in perifere weefsels gerapporteerd onafhankelijk van de SCN klok, mogelijk via het ventrale deel van de SCN, via niet-SCN hersengebieden of een combinatie van beide paden. Bovendien laten verschillende publicaties zien dat de effecten van ALAN afhankelijk zijn van de golflengte en dat deze effecten verschillen tussen de perifere weefsels, wat de vraag doet rijzen of deze waarnemingen al dan niet SCN-afhankelijk zijn. Dit literatuuroverzicht toonde duidelijk de noodzaak van verder onderzoek op dit gebied aan, met name om te bepalen welk lichtspectrum het meest schadelijk is voor de gezondheid van de mens, en in het bijzonder voor het energie metabolisme van de mens.

In **Deel II** hebben we de effecten van kunstmatig licht ‘s nachts op de voedselinname en het glucosemetabolisme onderzocht in de *Arvicanthis ansorgei* onderzocht, een dag-actief knaagdier met een circadiane fysiologie die meer lijkt op die van de mens. Om de menselijke condities nog beter na te bootsen, zoals de mogelijkheid om te kiezen tussen een smakelijker of een gezonder dieet, gebruikten we voor het eerst een vrij te kiezen suiker- en vetrijk dieet (HFHS) bij deze dieren, met als doel de effecten van de blootstelling aan nachtelijk licht op de inname van de verschillende dieetbestanddelen (regulier voedsel, suiker en dierlijke reuzel) te bestuderen. **Hoofdstuk 3** is gewijd aan het onderzoeken van de effecten van wit licht, terwijl in **Hoofdstuk 4** de effecten van blauw licht op het glucosemetabolisme en de voedselinname werden onderzocht. We merkten op dat in de *Arvicanthis* de blootstelling aan wit licht op ZT14 wel glucose-intolerantie bij de vrouwelijke dieren veroorzaakt, maar niet bij mannen. Deze veranderingen gingen niet gepaard met veranderingen in de plasma insuline concentraties, mogelijk door dat nachtelijk licht de endogene glucoseproductie verhoogt of de glucose opname verlaagt. Nachtelijke blootstelling aan wit licht verminderde ook de suikerinname bij de manlijke dieren en de vetinname bij de vrouwelijke dieren. Aan de andere kant lieten de resultaten na een acute blootstelling aan blauw licht op hetzelfde moment

van de nacht een verminderde glucose-tolerantie zien die gepaard ging met een verlaging van de plasma insuline concentratie. Blauw licht verhoogde de suikerconsumptie, in dit geval alleen in de manlijke dieren. Gezamenlijk laten de bevindingen in deze twee hoofdstukken zien dat de effecten van nachtelijk licht op de glucosestofwisseling en voedselinname variëren afhankelijk van de gebruikte golflengte en dat elk lichtspectrum verschillende effecten kan hebben in de beide geslachten. Deze effecten van licht worden mogelijk gemedieerd door de intrinsiek foto-sensitieve ganglion cellen in de retina (ipRGC's). Ook lijken geslachtshormonen een belangrijke rol te spelen, maar dit moet nog verder worden onderzocht.

In **Deel III, Hoofdstuk 5,** gebruikten we manlijke Wistar ratten, een nacht-actief knaagdier. Het belangrijkste doel van deze studie was om de tijd- en golflengte-afhankelijke effecten van nachtelijke licht op het energiemetabolisme verder te onderzoeken. De tweede doelstelling was het bestuderen van de tijd- en golflengte-afhankelijke effecten van licht op de neurale activiteit in de SCN en de paraventriculaire kern van de hypothalamus (PVH) en op de expressie van klok- en metabole genen in de lever. We toonden aan dat ALAN van elke golflengte en op elk moment tijdens de donker fase de bewegingsactiviteit van de Wistar ratten verminderde. Bij sommige gelegenheden werd tijdens de eerste 15 minuten van blootstelling aan (vooral blauw) licht, ook een verhoging van de bewegingsactiviteit waargenomen, waarschijnlijk als gevolg van een niet zo goed bekend fenomeen genaamd licht-geïnduceerde bewegingsactiviteit. Opmerkelijk is dat, wanneer een afname van de locomotorische activiteit werd waargenomen, de verwachte bijbehorende afname in energieverbruik alleen werden waargenomen met blauw en wit licht. Deze discrepantie geeft aan dat licht een activiteit-onafhankelijk effect op de energie-uitgaven kan hebben. Een vergelijkbare discrepantie werd waargenomen tussen de respiratie quotiënt (RER) en de voedselinname, we vonden bijvoorbeeld een afname van de RER (dus een hoger vetmetabolisme) toen de dieren werden blootgesteld aan blauw licht op ZT16-18 zonder enige vermindering van de voedselinname. Dus een effect van nachtelijk licht op de RER , onafhankelijk van een effect op de voedselinname. In deze experimenten vonden we ook een verhoogde expressie van het klokgen *Per 2*, en de metabole genen *Gck*, *Acc1*, en *Fas* in de lever, die correleren met de effecten van licht op de c-fos expressie in het SCN. De waargenomen verminderde expressie van *Pgc1α* en *Pdk4* in de lever, met name met blauw en groen licht, kwam overeen met de golflengte-afhankelijke effecten van licht op c-fos expressie in het

PVH. Al deze effecten waren afhankelijk van de tijd van de donker fase waarop de lichtpuls werd gegeven, wat duidt op een sterke interactie met het circadiane systeem. Omdat de waargenomen effecten ook golflengte-afhankelijk waren is het aannemelijk dat de ipRGC's, samen met de staafjes en kegeltjes, betrokken zijn bij het mechanisme dat verantwoordelijk is voor de waargenomen effecten van ALAN.

Tot slot hebben we ons in **Deel IV** gericht op de effecten van ALAN op het glucose- en energiemetabolisme van wild-type muizen en twee knock-out muizenmodellen. De ontwikkeling van genetisch gemanipuleerde muizen in de afgelopen tien jaar heeft de mogelijkheden voor het bestuderen van de rol van specifieke genen en eiwitten op de dierfysiologie enorm uitgebreid. De ipRGC's staan erom bekend dat ze nodig zijn voor de niet-beeldvormende (NIF) fysiologische reacties op licht. De aanwezigheid van een eiwit genaamd melanopsine maakt deze cellen lichtgevoelig, met als gevolg dat dit eiwit een bekend doelwit is voor het bestuderen van NIF-effecten van licht. In **Hoofdstuk 6** worden de effecten beschreven van nachtelijk licht (wit en blauw) op het glucosemetabolisme en de voedselinname in C57Bl/6J wildtype muizen en in muizen met een melanopsine deficiëntie. Beide lichtspectra verminderden de locomotorische activiteit ongeacht het dieet. In chow-gevoede dieren werden geen significante verschillen in de glycemische respons gevonden tussen licht blootgestelde (beide golflengtes) en de donkere controle dieren. Daarentegen vonden we in dieren op een HFHS-dieet, een verbeterde glucosetolerantie wanneer ze werden blootgesteld aan blauw of wit licht. De chow-gevoede dieren lieten ook geen significante veranderingen zien in de voedselopname als gevolg van de nachtelijke blootstelling aan licht. Bij HFHS-dieren werd echter een significante toename van de calorie-inname waargenomen tijdens de volgende donkere periode, maar alleen bij dieren die aan blauw licht werden blootgesteld. Opmerkelijk is dat de extra energie-inname voornamelijk afkomstig was van vet, wat suggereert dat blauw licht vooral de hedonische honger of het hedonisch eten van de muizen stimuleert. Beide golflengten verhoogden ook de voedselopname bij de mutante muizen zonder melanopsine, maar dit keer door de hoeveelheid gegeten chow te verhogen. Deze bevindingen geven aan dat de effecten van nachtelijk licht op de hedonische voedselinname mogelijke afhankelijk zijn van melanopsine.

In **Hoofdstuk 7** was ons doel tenslotte om de effecten van verschillende golflengten van licht op verschillende tijdstippen tijdens de donkere fase op het energiemetabolisme van C57Bl6/J en *BMAL1* deficiënte muizen te

bestuderen. *BMAL1* is een belangrijk klokgen en *BMAL1* deficiënte muizen vertonen dan ook geen circadiane ritmes meer. Daarnaast is het aangetoond dat *BMAL1* deficiënte muizen ook endocriene en metabolische veranderingen laten zien. We veronderstelden dat deze endocriene veranderingen dit dier kwetsbaarder zouden maken voor verdere circadiane verstoringen als gevolg van ALAN, of integendeel, dat door de verstoorde circadiane fysiologie de effecten van licht op het metabolisme niet zouden worden waargenomen. De resultaten in dit hoofdstuk laten zien dat ALAN het energiegebruik, de RER en de plasma glucose concentratie in muizen op een tijdsafhankelijke manier verandert. De experimenten met de *BMAL1*-KO muizen leverden het eerste bewijs dat de integriteit van de moleculaire klok noodzakelijk is om deze metabole verstoringen veroorzaakt door ALAN waar te kunnen nemen, hoewel dit verder moet worden onderzocht. Daarnaast hebben we nu ook bij muizen waargenomen dat de effecten nachtelijk licht afhankelijk zijn van de golflengte, wat aangeeft dat waarschijnlijk ipRGC's, staafjes en kegeltjes deel uitmaken van het betrokken neurale mechanisme.

Concluderend kan worden gesteld dat, hoewel de resultaten verschillen per diersoort, deze dissertatie verder bewijs levert voor de noodzaak van voorzichtigheid bij blootstelling aan kunstmatig licht 's nachts om schadelijke metabole gevolgen te voorkomen. Dit geldt zeker ook voor het gebruik van elektronische apparaten, met name apparaten die licht met korte en middellang golflengtes uitstralen. Er is meer wetenschappelijk onderzoek nodig op dit gebied om de achterliggende mechanismen verder op te helderen en om de lichtspectra die we in ons dagelijks leven gebruikten te optimaliseren. Het is waarschijnlijk dat in de toekomst de combinatie van nieuwe wetenschappelijke ontdekkingen op dit terrein tezamen met de nieuwste technologie zal resulteren in de ontwikkeling van adaptieve lichtproducten en lichtgevende apparaten die in staat zijn om hun spectra aan te passen aan het tijdstip van de dag, wat zal helpen om metabole ziekten te voorkomen en beter te behandelen.

Résumé de la thèse

La rotation de la Terre sur 24 heures détermine l'alternance entre le jour et la nuit. L'exposition aux variations d'éclairement (intensité lumineuse, spectres et contrastes) ainsi que les changements quotidiens de température ont joué un rôle important dans l'évolution de pratiquement tous les organismes. Ces conditions environnementales conduisent au développement d'horloges corporelles qui synchronisent de nombreux processus physiologiques et comportementaux, y compris le cycle quotidien de veille et de sommeil, aux changements rythmiques de l'environnement. Cependant, cette horloge maîtresse, située dans le noyau hypothalamique suprachiasmatique (SCN ang.), oscille avec une période d'environ 24 heures, c'est pourquoi elle est appelée circadienne (*circa* «environ» et *dies* «jour»). Cette oscillation rythmique prend naissance au niveau cellulaire et est affinée par une horloge moléculaire constituée de plusieurs boucles de rétroaction transcriptionnelles-traductionnelles. Chez la plupart des vertébrés, la lumière est le signal environnemental le plus efficace (*Zeitgeber*) pour synchroniser le rythme circadien interne avec le rythme externe exact de 24 heures de la rotation de la Terre. L'horloge moléculaire mentionnée précédemment s'est également avérée être présente dans les tissus périphériques tels que, mais sans s'y limiter, le foie, les muscles et le tissu adipeux. Pour les horloges périphériques, on pense que la nourriture, c'est-à-dire les cycles d'alimentation et de jeûne, est le *Zeitgeber* le plus important.

Notre mode de vie industrialisé et hautement technologique nous confronte à des changements majeurs dans les modèles d'exposition à la lumière, ainsi qu'à des changements dans nos habitudes d'activité et de consommation alimentaire. À long terme, cela peut altérer les processus physiologiques en perturbant leurs rythmes circadiens. De plus en plus de preuves des trente dernières années ont montré que la perturbation du rythme circadien peut entraîner des maladies métaboliques telles que l'obésité et le diabète de type 2. Dans ce projet de doctorat nous avons visé à étudier les effets métaboliques et physiologiques de l'exposition aiguë à la lumière la nuit chez les rongeurs nocturnes et diurnes.

Cette thèse est divisée en plusieurs sections. Premièrement, la **partie I**, **chapitre 1** comprend une brève introduction aux rythmes circadiens et à la lumière, fournissant les principes physiques nécessaires pour comprendre notre recherche. De plus, à la fin de ce chapitre, la portée de cette thèse est décrite. Dans le **chapitre 2**, nous avons inclus une revue exhaustive des

études animales et humaines décrivant les implications métaboliques de la lumière artificielle pendant la nuit (trad. ang. artificial light at night ALAN). Pour mieux analyser les preuves existantes, nous avons divisé les études examinées entre les expositions chroniques et aiguës à l'ALAN. Une section distincte analysant les effets de différentes longueurs d'onde a également été incluse. Notre aperçu de la littérature existante a montré que la plupart des études utilisant l'ALAN chronique provoquaient une inefficacité métabolique en perturbant les rythmes comportementaux quotidiens via son impact sur le SCN. D'un autre côté, dans des expériences utilisant une exposition aiguë à l'ALAN, des perturbations dans les tissus périphériques ont été rapportées indépendamment des perturbations des rythmes circadiens du SCN, peut-être via les effets de l'ALAN sur la région ventrale du SCN, via des zones cérébrales non SCN ou une combinaison des deux voies. En outre, plusieurs publications ont indiqué que les effets de l'ALAN dépendent de la longueur d'onde et que ces effets diffèrent selon les tissus périphériques, fournissant une autre indication que ces effets pourraient être indépendants des perturbations du SCN. Cette revue de la littérature a clairement souligné la nécessité de poursuivre les recherches dans ce domaine, notamment pour déterminer quel spectre lumineux est le plus nocif pour la santé humaine, et en particulier pour le métabolisme humain.

Dans la **deuxième partie**, nous avons exploré les effets de l'ALAN sur la prise alimentaire et le métabolisme du glucose chez le rongeur diurne *Arvicanthis ansorgei*, un modèle de rongeur dont la physiologie circadienne ressemble davantage à celle des humains. Pour imiter davantage les conditions humaines, les animaux se sont également vu offrir la possibilité de choisir entre une alimentation savoureuse et une alimentation plus saine. Pour cela, nous avons utilisé pour la toute première fois un régime au choix, riche en matières grasses et riche en saccharose (trad. ang. free-choice high-fat high-sugar fcHFHS) chez ces animaux, dans le but d'étudier les effets de l'exposition à l'ALAN sur la consommation des différents composants diététiques , sucre et saindoux animal). Le **chapitre 3** était consacré à l'exploration des effets de la lumière blanche, tandis que dans le **chapitre 4**, les effets de la lumière bleue sur le métabolisme du glucose et la prise alimentaire ont été abordés. Nous avons observé que chez *Arvicanthis*, l'exposition à la lumière blanche à ZT14 provoquait une intolérance au glucose chez les femelles mais pas chez les mâles. Aucun changement dans les taux d'insuline plasmatique n'a été observé, ce qui indique que les effets de l'ALAN ont peut-être été induits par une augmentation de la production endogène de glucose ou une

diminution de l'absorption du glucose. De plus, la lumière blanche a réduit la consommation de sucre chez les mâles et la consommation de graisses chez les femelles. D'autre part, nous avons constaté qu'une exposition aiguë à la lumière bleue à la même heure de la nuit provoquait une intolérance au glucose en réduisant l'insuline plasmatique. Celle-ci a également augmenté la consommation de sucre. Les deux effets n'ont été observés seulement chez les mâles. Par conséquent, nos résultats dans ces deux chapitres indiquent que les effets de la lumière sur le métabolisme du glucose et la prise alimentaire varient en fonction de la longueur d'onde utilisée et que chaque spectre lumineux affecte différemment les deux sexes. Les différents effets observés avec chaque type de lumière indiquent l'implication des cellules ganglionnaires rétiniennes intrinsèquement photosensibles (abrev. Ang. ipRGCs). De plus, les hormones sexuelles semblent également jouer un rôle important, mais cela reste une piste à étudier plus loin.

Dans la **partie III, chapitre 5**, nous avons utilisé des rats mâles Wistar, un rongeur nocturne, dans le but principal d'explorer davantage les effets dépendant du temps et de la longueur d'onde de la lumière la nuit sur le métabolisme énergétique. Comme deuxième objectif, nous avons étudié les effets dépendant du temps et de la longueur d'onde de la lumière sur l'activité neuronale dans le SCN et le noyau paraventriculaire de l'hypothalamus (PVH), ainsi que sur l'expression des gènes de l'horloge hépatique et des gènes métaboliques. Nous avons montré que l'ALAN de n'importe quelle longueur d'onde et à tout moment pendant la phase sombre réduisait l'activité locomotrice chez les rats Wistar. À certaines occasions pendant les 15 premières minutes d'exposition à la lumière, en particulier à la lumière bleue, des augmentations de l'activité locomotrice ont été observées, probablement en raison d'un phénomène moins connu, appelé activité locomotrice induite par la lumière. Remarquablement, lorsque l'activité locomotrice était réduite, la diminution associée attendue de la dépense énergétique a été observée uniquement avec la lumière bleue et blanche, ce qui indique que la lumière peut également avoir des effets indépendants de l'activité sur la dépense énergétique. Un écart similaire a été observé entre le ratio d'échange respiratoire (RER) et la prise alimentaire, car nous avons détecté une diminution du RER (indiquant un métabolisme lipidique plus élevé) lorsque les animaux étaient exposés à la lumière bleue à ZT16-18, sans aucune réduction de la prise alimentaire. . Montrant à nouveau que la lumière peut avoir un effet indépendant sur ces deux variables. De plus, nous avons observé une expression accrue du gène d'horloge *Per 2* et des gènes

métaboliques *Gck, Acc1* et *Fas*, qui étaient en corrélation avec les effets de la lumière sur l'expression de c-fos dans le SCN. De même, la diminution de l'expression observée de *Pgc1α* et *Pdk4* dans le foie correspondait aux effets dépendant de la longueur d'onde de la lumière sur l'expression de c-fos dans le PVH, en particulier avec la lumière bleue et verte. Dans l'ensemble, ces effets dépendaient de l'heure de la phase sombre lorsque l'impulsion lumineuse a été donnée, ce qui indique une forte interaction avec le système de chronométrage circadien. Les effets observés étant également dépendants de la longueur d'onde, nous supposons que les ipRGCs, avec les cônes et les bâtonnets, sont impliqués dans les mécanismes responsables des effets de l'ALAN observés.

Enfin, dans la **partie IV**, nous nous sommes concentrés sur les effets de l'ALAN sur le métabolisme du glucose et de l'énergie des souris de type sauvage et de deux modèles de souris knock-out. Le développement de souris génétiquement modifiées a considérablement augmenté notre capacité à étudier le rôle de gènes et de protéines spécifiques sur la physiologie animale au cours de la dernière décennie. On sait que les cellules ganglionnaires rétiniennes intrinsèquement photosensibles sont nécessaires pour les réponses physiologiques non formatrices d'image (trad. Ang. non-image-forming NIF) à la lumière. Leur sensibilité à la lumière intrinsèque est obtenue à partir de la présence d'une protéine appelée mélanopsine, ce qui fait de cette protéine une cible importante pour l'étude des effets NIF de la lumière. Au **chapitre 6**, les effets de la lumière (blanche et bleue) sur le métabolisme du glucose et la prise alimentaire ont été étudiés en utilisant une combinaison de souris de type sauvage C57Bl6/J avec des souris génétiquement modifiées déficientes en mélanopsine. Les deux spectres lumineux ont diminué l'activité locomotrice quel que soit le régime alimentaire. Au contraire, chez les animaux nourris à la nourriture (ang. chow-food), aucune différence significative de réponse glycémique n'a été trouvée entre les animaux exposés à la lumière (l'une ou l'autre longueur d'onde) et ceux de contrôle dans l'obscurité. Cependant, les animaux nourris avec le régime HFHS ont montré une meilleure tolérance au glucose lorsqu'ils sont exposés à la lumière bleue ou blanche. De plus, la prise de nourriture dans les groupes nourris à la nourriture n'a montré aucun changement significatif en raison de l'exposition à la lumière. Chez les animaux HFHS, cependant, une augmentation significative de l'apport calorique au cours de la période d'obscurité suivante a été observée, mais uniquement chez les animaux exposés à la lumière bleue. Fait remarquable, l'apport énergétique

supplémentaire provenait principalement des graisses, ce qui suggère que la lumière bleue stimule l'alimentation hédonique chez les souris. Les deux longueurs d'onde ont également augmenté l'apport alimentaire chez les souris mutantes de la mélanopsine, mais cette fois en augmentant la quantité de nourriture consommée. Nos résultats indiquent que les effets de la lumière la nuit sur l'apport alimentaire hédonique peuvent être dépendants de la mélanopsine.

Finalement, au **chapitre 7**, notre objectif était d'étudier les effets de différentes longueurs d'onde de lumière à différents moments de la phase sombre sur le métabolisme énergétique des souris C57Bl6 / J et déficientes en *BMAL1*. *BMAL1* est l'un des gènes d'horloge les plus importants et il a été démontré qu'en plus des rythmes circadiens perturbés, les souris déficientes en *BMAL1* présentent également des altérations endocriniennes et métaboliques. Nous avons émis l'hypothèse que ces altérations endocriniennes rendraient cet animal plus vulnérable à de nouvelles perturbations circadiennes dues à l'ALAN ou, au contraire, qu'en raison de sa physiologie circadienne déjà perturbée, les effets de la lumière sur le métabolisme ne seraient pas observés. Les résultats de ce chapitre montrent que l'ALAN modifie la dépense énergétique, le RER et les niveaux de glucose plasmatique chez la souris en fonction du temps. Nous avons également obtenu une première preuve que l'intégrité de l'horloge moléculaire est nécessaire pour observer ces perturbations métaboliques causées par l'ALAN, bien que cela doive être approfondi. De plus, nous avons observé que chez la souris également, ces effets dépendent de la longueur d'onde de la lumière utilisée, indiquant à nouveau que les ipRGCs, les cônes et les bâtonnets peuvent faire partie des voies impliquées.

Pour conclure, bien que les résultats exacts puissent varier selon les espèces, cette thèse apporte des preuves supplémentaires sur la nécessité d'être prudent lors d'une exposition à la lumière artificielle la nuit, pour éviter des conséquences métaboliques néfastes. Cela comprend également les appareils électroniques électroluminescents, en particulier ceux qui émettent une lumière de longueur d'onde courte et moyenne. Cependant, des recherches supplémentaires sont nécessaires dans ce domaine pour élucider davantage les mécanismes exacts qui le sous-tendent et pour optimiser les spectres lumineux que nous utilisons dans nos activités de la vie quotidienne. Néanmoins, nous prévoyons qu'en combinant ces découvertes scientifiques avec une technologie de pointe, il sera possible à l'avenir de développer des

produits d'éclairage adaptatif et des dispositifs électroluminescents qui pourront ajuster leurs spectres à l'heure de la journée, afin de prévenir les maladies métaboliques.

Université de Strasbourg

Anayanci Masís Vargas

Effets métaboliques et physiologiques de l'exposition aiguë a la lumière artificielle pendant la nuit

École Doctorale des Sciences de la Vie et de la Santé STRASBOURG

Résumé

Cette thèse a étudié les effets métaboliques et physiologiques de l'exposition aiguë à la lumière artificielle la nuit (ALAN) chez les rongeurs nocturnes et diurnes. La lumière blanche et bleue a provoqué une intolérance au glucose et a affecté la prise alimentaire d'une manière dépendante du sexe et de la longueur d'onde chez Arvicanthis. Chez le rat, nous avons observé les effets de la lumière sur l'activité locomotrice (LA), la dépense énergétique (EE), le rapport d'échange respiratoire (RER), l'apport alimentaire et l'expression hépatique des gènes de l'horloge et du métabolisme en fonction du temps et de la longueur d'onde. Chez la souris, la lumière blanche et bleue a diminué la LA, avec un régime riche en graisses et en saccharose, une amélioration de la tolérance au glucose a été observée après une exposition à la lumière. Les animaux nourris au HFHS exposés à la lumière bleue ont augmenté leur apport en graisses. Les deux longueurs d'onde ont augmenté la consommation de nourriture lorsque nous avons utilisé des souris mutantes de mélanopsine. Les souris exposées à l'ALAN ont montré une altération des taux d'EE, de RER et de glucose plasmatique en fonction du temps. Nous avons montré qu'une horloge moléculaire intacte est nécessaire pour observer les perturbations métaboliques causées par ALAN à l'aide de souris BMAL1 - / -. Ces effets dépendaient de la longueur d'onde, ce qui indique que les ipRGC, les cônes et les bâtonnets peuvent faire partie des voies impliquées. Nous avons fourni des preuves supplémentaires sur la nécessité de faire preuve de prudence lors d'une exposition à l'ALAN, afin d'éviter des conséquences métaboliques néfastes.

Mots clés : lumière artificielle pendant la nuit, circadien, obésité, diabète de type 2.

Résumé en anglais

This thesis investigated the metabolic and physiologic effects of acute exposure to artificial light at night (ALAN) in nocturnal and diurnal rodents. White and blue light caused glucose intolerance and affected food intake in a sex- and wavelength-dependent manner in Arvicanthis. In rats, we observed effects of light in locomotor activity (LA), energy expenditure (EE), respiratory exchange ratio (RER), food intake, and hepatic expression of clock and metabolic genes in a time- and wavelength-dependent fashion. In mice, white and blue light decreased LA; when fed a free-choice high fat high-sucrose diet an improved glucose tolerance was observed after light exposure. HFHS-fed animals exposed to blue light increased fat intake. Both wavelengths increased chow food intake when we used melanopsin mutant mice. Mice exposed to ALAN showed an altered EE, RER, and plasma glucose levels in a time-dependent manner. We showed that an intact molecular clock is necessary to observe metabolic disturbances caused by ALAN using BMAL1 -/- mice. These effects were dependent on the wavelength, indicating that ipRGCs, cones, and rods may be part of the pathways involved. We provided further evidence on the need for caution when being exposed to ALAN, to prevent detrimental metabolic consequences.

Key words: artificial light at night, circadian, obesity, type 2 diabetes.

PhD Portfolio
Name PhD candidate: Anayanci Masís Vargas
PhD period: October 2015 – July 2020
Name PhD supervisors: J. Mendoza and A. Kalsbeek

1. PhD Training:

a. Socio-professional

• Guided visit to the European Parliament.	2015	Strasbourg, France.
• Guided visit to the European Council.	2015	Strasbourg, France.
• Brexit from the point of view of the United Kingdom and its consequences.	2016	Strasbourg, France.
• Sensitisation to entrepreneurship or the thesis work viewed from the entrepreneurship point of view.	2016	Strasbourg, France.
• Industrial and intellectual property protected by patents.	2016	Strasbourg, France.

b. Scientific

• Introduction to R.	2016	Strasbourg, France.
• Effecting scientific writing in English.	2016	Strasbourg, France.
• Practical Bioinformatics.	2016	Strasbourg, France.

c. Workshops, meetings and masterclasses

• The attentive brain, the deluded brain, what is reality?	2015	Mittelwihr, France.
• From maps to circuits: models and mechanisms for generating neural connections.	2015	Strasbourg, France.
• Guided visit to Roche headquarters.	2016	Basel, Switzerland.
• Actelion pharmaceutical clinical trials in drug development.		Basel, Switzerland.
• "Circadian rhythms: The biology of measuring time."	2016	Sèvres, France.
• Does optogenetics still shine after 11 years?	2016	Strasbourg, France.
• Circadian clocks and metabolic health: from basic science to clinical implications.	2016	Strasbourg, France.
• 10th anniversary of the joint Master in Neuroscience.	2016	Strasbourg, France.
• Memory lab tour Neurocampus.	2016	Strasbourg, France. Freiburg, Germany. Basel, Switzerland.
• Retinal circadian clocks.	2017	Strasbourg, France.
• The arcuate nucleus: sensor of time and metabolism.	2018	Strasbourg, France.
• Weekly research meeting Endocrinology and Metabolism, Amsterdam UMC.	2017-2019	Amsterdam, the Netherlands.

2. Conferences

a. Oral presentations

• NeuroTime Anual meeting: "Effects of acute and chronic light exposure on hedonic eating behavior: molecular and neurobiological mechanisms."	2016	Strasbourg, France.
• NeuroTime Anual meeting: "Effects of acute light exposure on hedonic eating behavior and glucose metabolism."	2017	Amsterdam, the Netherlands.
• XV European Biological Rhythms Society Congress: "Acute effects of light on eating behavior and glucose metabolism of rodents."	2017	Amsterdam, the Netherlands.
• Society for Light Treatment and Biological Rhythms: "Acute Exposure to Blue Light at Night Impairs Glucose Tolerance, Alters Insulin Secretion and Increase Sugar Intake in Diurnal Rodents."	2018	Groningen, the Netherlands.
• Muscle clocks & Diabetes meeting: "Acute exposure to blue light at night impairs glucose tolerance, alters insulin secretion and increases sugar intake in diurnal rodents."	2019	Amsterdam, the Netherlands.
• XVI European Biological Rhythms Society Congress: "Light at night affects energy metabolism in rodents depending on the species, wavelength and time of the exposure."	2019	Lyon, France.

b. Poster presentations

• 45ième Congrès de la Société Francophone de Chronobiologie: "Acute effects of light on eating behavior and glucose metabolism of mice."	2016	Strasbourg, France.
• Neurex meeting Addiction: A neurobiological and cognitive brain disorder: "Acute effects of light on eating behavior and glucose metabolism of mice."	2017	Strasbourg, France.
• Society for Reseach on Biological Rhythms: "Acute effects of light on eating behavior and glucose metabolism of mice."	2018	Florida, United States of America.
• Society for the Study of Ingestive Behavior: "Acute effects of light on feeding behavior, glucose and energy metabolism in rodents."	2019	Utecht, the Netherlands.

3. Teaching

• Brain Awareness week class "Brain and feeding" Lycée André Maurois.	2016	Bischwiller, France.
• Brain Awareness week class "Brain and feeding" Lyée Stanislas.	2016	Wissenbourg, France.
• Supervision of Eva van Sambeek, Bachelor student Utrecht University.	2018	Amsterdam, the Netherlands.
• Supervision of Lizette Hogenberg, High School student during her vocational internship.	2019.	Amsterdam, the Netherlands.
• Supervision of Esther Bürhman, Bachelor student UvA.	2019	Amsterdam, the Netherlands.

4. Parameters of esteem

• Best Poster prize during the 45ième Congrès de la Société Francophone de Chronobiologie.	2016	Strasbourg, France.
• Travel Grant from the Société Francophone de Chronobiologie to attend the European Biological Rhythm Society meeting.	2017	Amsterdam, the Netherlands.
• Diversity Award from Society for Reseach in Biological Rhythms during their meeting.	2018	Florida, United States of America.
• Society for Light Therapy and Biological Rhythms Travel Grant to attend their meeting.	2018	Groningen, the Netherlands.

5. Publications

a. Peer reviewed

• **Masís-Vargas A,** Hicks D, Kalsbeek A, Mendoza J. Blue light at night acutely impairs glucose tolerance and increases sugar intake in the diurnal rodent Arvicanthis ansorgei in a sex-dependent manner. Physiol Rep. 2019;7(20):1–19.

• Fleury G, **Masís-Vargas A**, Kalsbeek A. Metabolic implications of exposure to light at night: a compilation of animal and human evidence. Obesity. (Silver Spring). 2020 Jul;28 Suppl 1:S18-S28.

• **Masís-Vargas A**, Ritsema WIGR, Mendoza J, Kalsbeek A. Metabolic effects of light at night are time- and wavelength-dependent in rats. Obesity. (Silver Spring). 2020 Jul;28 Suppl 1:S114-S125.

b. Press releases

• **EurekAlert!:** Blue light at night increases the consumption of sweets in rats. Available at: https://www.eurekalert.org/pub_releases/2019-07/sfts-bla070519.php.

• **Science daily:** Blue light at night increases the consumption of sweets in rats. Available at: https://www.sciencedaily.com/releases/2019/07/190709091120.htm.

• **Daily Mail, UK:** Could using your phone or tablet at night give you a sweet tooth? Study shows exposing rats to just one hour of blue light in the evening makes them crave sugar. Available at: https://www.dailymail.co.uk/health/article-7228619/Light-emitted-phones-tablets-night-causes-sugar-cravings-study-rats-finds.html

• **DW, Germany**: Studie: Smartphone-Licht macht Appetit auf Süßes. Avalible at https://www.dw.com/de/studie-smartphone-licht-macht-appetit-auf-s%C3%BC%C3%9Fes/a-49534566.

• **Men's Health, Australia:** This Night Time Activity Could Be Responsible For Your Sugar Cravings. Available at https://www.menshealth.com.au/blue-light-from-screens-may-cause-sugar-cravings?fbclid=IwAR3d7xaUwWSAfNpqCWfGeQlNZjmdL_01_Uv-SY974148iP-7wJvwOOn6cK8.

• **Futura sciences, France:** La lumière bleue altère la sécrétion d'insuline chez les souris. Available at https://www.futura-sciences.com/sante/actualites/biologie-lumiere-bleue-altere-secretion-insuline-chez-souris-76840/.

• **Women's Health, the Netherlands:** Dit zou zomaar eens de reden kunnen zijn dat je continu snakt naar zoete dingen. Available at https://www.womenshealthmag.com/nl/gezondheid/a28409895/schermen-elektronica-trek-zoete-dingen/

6. Others

• Organization Trainee Day at XVI European Biological Rhythms Society biannual international meeting. 2019 Lyon, France.

• Trainee Board delegate at the European Biological Rhythms Society board. 2019-2020.

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About the author

Anayanci Masís Vargas was born 5th of May 1985 in San José, Costa Rica, and lived the first twenty-five years of her life in Vázquez de Coronado, a town in the eastern mountains of that capital city. She obtained her general baccalaureate with a specialization in Mathematics and Computer Science from the Madre del Divino Pastor High School. After this, she studied Human Nutrition at the University of Costa Rica and received her BSc degree in 2009. Throughout her bachelor's she worked with both pediatric and adult type 2 diabetic patients as part of her clinical internships, which strengthen her conviction to actively contribute to research in this field. After that, during the first half of 2010, she worked as a teacher assistant in the Physiology Department of the School of Medicine of her alma mater. In August of the same year, she received a one-year Erasmus scholarship from the European Union and moved to Stockholm, Sweden to study Public Health Epidemiology at Karolinska Institute. Following this experience, she returned to Costa Rica and decided to start a Master in Biomedical Sciences and to follow the Physiology track. Simultaneously, she resumed teaching at the Physiology Department of the School of Medicine to further her experience. Towards the end of her master's, she was presented with the opportunity of joining the Center for Research in Neuroscience (CIN) where she did her master thesis and collaborated with projects studying Parkinson's disease, both in animal models and in humans under the supervision of Prof. Dr. Jaime Fornaguera Trías. Soon before her master's graduation in 2015, she had successfully applied for a fellowship to pursue a joint Ph.D. within the framework of the NeuroTime Programme between the University of Strasbourg and the University of Amsterdam. In September of the same year, she moved to Strasbourg, France to start her doctorate project. During the first two years of her Ph.D., she worked at the Institute of Cellular and Integrative Neuroscience (INCI) in Strasbourg, under the supervision of Dr. Jorge Mendoza. The following two years she continued her project under the supervision of Prof. Dr. Andries Kalsbeek working at the Netherlands Institute for Neuroscience (NIN) and the Department of Endocrinology and Metabolism of Amsterdam UMC. Anayanci received several prizes and travel grants for presentations of her Ph.D. results at international meetings and the main product of her doctorate project is this thesis. Owing to the relevance of her scientific results, part of her work has been published in press releases and magazines across the world in multiple languages. She also participated in the organization of the Trainee Day at the biannual meeting of the European Biological Rhythms Society (EBRS), where she advocated for the creation of a Trainee Board, a formal body representing the interests of Ph.D. students and post-docs with the EBRS board. Consequently, she became the first Trainee Board delegate to work alongside the EBRS board on expanding the involvement of trainees in the activities and projects of the organization. Currently, she is working as a postdoctoral researcher at the Department of Endocrinology and Metabolism of Amsterdam UMC in the group of Prof. Dr. Susanne E. la Fleur and lives in Amsterdam with her husband Călin.

Acerca de la autora

Anayanci Masís Vargas nació el 5 de mayo de 1985 en San José, Costa Rica, y vivió los primeros veinticinco años de su vida en Vázquez de Coronado. Obtuvo su bachillerato en educación diversificada en el Colegio Madre del Divino Pastor. Estudió Nutrición Humana en la Universidad de Costa Rica y recibió su bachillerato universitario en el 2009. Durante su bachillerato trabajó con pacientes pediátricos y adultos con diabetes tipo 2 como parte de sus pasantías clínicas, lo que fortaleció su convicción de contribuir activamente a la investigación en este campo. Durante el primer semestre del 2010, trabajó como profesora asistente en el Departamento de Fisiología de la Facultad de Medicina de su alma mater. En agosto del mismo año, recibió una beca Erasmus de la Unión Europea y se mudó a Estocolmo, Suecia, para estudiar Epidemiología de la Salud Pública en el Instituto Karolinska. Luego regresó a Costa Rica e inició una Maestría en Ciencias Biomédicas con énfasis en Fisiología. Simultáneamente, reanudó la enseñanza en el Departamento de Fisiología de la Facultad de Medicina ampliando experiencia docente. Al final de su maestría, se le presentó la oportunidad de unirse al Centro de Investigación en Neurociencia (CIN) donde realizó su tesis de maestría y colaboró con proyectos que estudiaban la enfermedad de Parkinson, en modelos animales y pacientes bajo la supervisión del Prof. Dr. Jaime Fornaguera Trías. En el 2015, fue admitida a un doctorado con doble titulación entre la Universidad de Estrasburgo y la Universidad de Ámsterdam, financiado por el Programa NeuroTime de la Unión Europea. En septiembre del mismo año, se mudó a Estrasburgo, Francia, para inciar su doctorado. Durante los primeros dos años, trabajó en el Instituto de Neurociencias Celulares e Integrativas (INCI) en Estrasburgo, bajo la supervisión del Dr. Jorge Mendoza. Los dos años siguientes continuó su proyecto bajo la supervisión del Profesor Dr. Andries Kalsbeek trabajando en el Instituto de Neurociencias de los Países Bajos (NIN) y el Departamento de Endocrinología y Metabolismo del Centro Médico de la Universidad de Ámsterdam (Amsterdam UMC). Ella recibió varios premios y becas de viaje por las presentaciones de los resultados en congresos internacionales y el producto de su e doctorado es esta tesis. Debido a la relevancia de sus resultados científicos, parte de su trabajo ha sido publicado en comunicados de prensa y revistas de todo el mundo en varios idiomas. También participó en la organización del precongreso dedicado a estudiantes e investigadores jóvenes en la reunión bianual de la Sociedad Europea de Ritmos Biológicos (EBRS), donde abogó por la creación de una Junta de Estudiantes, un organismo formal que representa los intereses de los estudiantes de doctorado y los postdoctorandos ante la junta de EBRS. Como consecuencia, se convirtió en la primera delegada de la Junta de Estudiantes en trabajar junto con la Junta de EBRS para ampliar la participación de los investigadores jóvenes en las actividades y proyectos de esa organización. Actualmente, trabaja como investigadora postdoctoral en el Departamento de Endocrinología y Metabolismo del Centro Médico de la Universidad de Ámsterdam (Amsterdam UMC) en el grupo de la Prof. Dra. Susanne E. la Fleur y vive en Ámsterdam con su esposo Călin.

Anayanci Masís Vargas

Effets métaboliques et physiologiques de l'exposition aiguë a la lumière artificielle pendant la nuit

Résumé

Cette thèse a étudié les effets métaboliques et physiologiques de l'exposition aiguë à la lumière artificielle la nuit (ALAN) chez les rongeurs nocturnes et diurnes. La lumière blanche et bleue a provoqué une intolérance au glucose et a affecté la prise alimentaire d'une manière dépendante du sexe et de la longueur d'onde chez Arvicanthis. Chez le rat, nous avons observé les effets de la lumière sur l'activité locomotrice (LA), la dépense énergétique (EE), le rapport d'échange respiratoire (RER), l'apport alimentaire et l'expression hépatique des gènes de l'horloge et du métabolisme en fonction du temps et de la longueur d'onde. Chez la souris, la lumière blanche et bleue a diminué la LA, avec un régime riche en graisses et en saccharose, une amélioration de la tolérance au glucose a été observée après une exposition à la lumière. Les animaux nourris au HFHS exposés à la lumière bleue ont augmenté leur apport en graisses. Les deux longueurs d'onde ont augmenté la consommation de nourriture lorsque nous avons utilisé des souris mutantes de mélanopsine. Les souris exposées à l'ALAN ont montré une altération des taux d'EE, de RER et de glucose plasmatique en fonction du temps. Nous avons montré qu'une horloge moléculaire intacte est nécessaire pour observer les perturbations métaboliques causées par ALAN à l'aide de souris BMAL1 - / -. Ces effets dépendaient de la longueur d'onde, ce qui indique que les ipRGC, les cônes et les bâtonnets peuvent faire partie des voies impliquées. Nous avons fourni des preuves supplémentaires sur la nécessité de faire preuve de prudence lors d'une exposition à l'ALAN, afin d'éviter des conséquences métaboliques néfastes.

Mots clés : lumière artificielle pendant la nuit, circadien, obésité, diabète de type 2.

Résumé en anglais

This thesis investigated the metabolic and physiologic effects of acute exposure to artificial light at night (ALAN) in nocturnal and diurnal rodents. White and blue light caused glucose intolerance and affected food intake in a sex- and wavelength-dependent manner in Arvicanthis. In rats, we observed effects of light in locomotor activity (LA), energy expenditure (EE), respiratory exchange ratio (RER), food intake, and hepatic expression of clock and metabolic genes in a time- and wavelength-dependent fashion. In mice, white and blue light decreased LA; when fed a free-choice high fat high-sucrose diet an improved glucose tolerance was observed after light exposure. HFHS-fed animals exposed to blue light increased fat intake. Both wavelengths increased chow food intake when we used melanopsin mutant mice. Mice exposed to ALAN showed an altered EE, RER, and plasma glucose levels in a time-dependent manner. We showed that an intact molecular clock is necessary to observe metabolic disturbances caused by ALAN using BMAL1 -/- mice. These effects were dependent on the wavelength, indicating that ipRGCs, cones, and rods may be part of the pathways involved. We provided further evidence on the need for caution when being exposed to ALAN, to prevent detrimental metabolic consequences.

Key words: artificial light at night, circadian, obesity, type 2 diabetes.