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LIGHT FROM DAWN TO DUSK

Human Entrainment in a Changing Environment

M. C. Giménez

ISBN: 978-90-367-6156-7 (printed version)

ISBN: 978-90-367-6217-5 (digital version)

Cover design and lay-out by M.F. Masman.

Cover illustration: "Hanging on lightening time" by M.F. Masman.

This image was inspired not only on the scientific content of this thesis but also on one of the most famous silent movies ever; Safety last! (1923). Sir Harold Lloyd hanging from the hands of a broken clock high above the street is one of the most enduring cinematographic images of all times. This design contains elements from the DesignBum website.

Printed in the Netherlands by Print Partners Ipskamp, Enschede.

An electronic version in Adobe® portable document format (PDF) is available on the internet at:

http://dissertations.ub.rug.nl/faculties/science/2013/m.c.gimenez/

The research reported in this thesis was carried out at the Department of Chronobiology at the University of Groningen (RuG), The Netherlands. All studies were approved by the Medical Ethics Committee of the University Medical Center of Groningen (UMCG).

This research was supported by the Universtiy of Groningen. Further support was given by the 6th Framework Project EUCLOCK (No. 018741), by Philips Lighting, Philips Consumer Lifestyle, and by Bühlmann laboratories.

The printing of this thesis was financially supported by the following organizations:

Department of chronobiology

The university library

Graduate School of Behavioral and Cognitive Neuroscience (BCN)

Nederlandse Vereniging voor Slaap-Waak Onderzoek (NSWO)

Stichting Onderzoek Licht en Gezonheid (SOLG)

RIJKSUNIVERSITEIT GRONINGEN

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Human Entrainment in a Changing Environment

Proefschrift

ter verkrijging van het doctoraat in de
Wiskunde en Natuurwetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. E. Sterken,
in het openbaar te verdedigen op
maandag 10 juni 2013
om 11.00 uur

door

Marina Cecilia Giménez
geboren op 16 maart 1978
te Buenos Aires, Argentinië

Promotores:	Prof. dr. D.G.M. Beersma
	Prof. dr. S. Daan
Copromotor:	Dr. M.C.M. Gordijn
Beoordelingscommissie:	Prof. dr. E. J. W. van Someren
	Prof. dr. C. Midden
	Prof. dr. D. Skene

"Esa hora que puede llegar alguna vez fuera de toda hora, agujero en la red del tiempo, esa manera de estar entre, no por encima o detrás sino entre, esa hora orificio a la que se accede al socaire de las otras horas, de la incontable vida con sus horas de frente y de lado, su tiempo para cada cosa, sus cosas en el preciso tiempo…" (Prosa del observatorio, 1972, J. Cortázar)

"…toda distracción profunda entreabre ciertas puertas, y cómo hay que distraerse si no se es capaz de concentrarse" (La vuelta al día en ochenta mundos, 1967, J. Cortázar)

A mis viejos queridos,
A mi hermano del alma
Y a la memoria de tatate
Donde quiera que vaya los llevo conmigo

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Chapter 1

General introduction:

Marina C. Giménez

The Biological Clock: General Aspects

Due to the rotation of the Earth around the sun and the tilt of its axis relative to its orbital plane, virtually all places on Earth are exposed to a never-ending sequence of days and nights. Specifically, the alternation between light and darkness occurs on average every 24 hours. In response to the predictable changes that accompany the 24-hour day, such as daily variations in light, temperature, food availability, etc., biological clocks have evolved in practically all organisms, including humans. This generalization across organisms indicates the fundamental relevance of the biological clock. The biological clock has a strong adaptive significance by allowing organisms to functionally anticipate (in contrast to merely react to) the daily changes in the environment. This anticipation occurs thanks to changes in physiology and behavior known as biological rhythms. The field that studies these rhythms is known as chronobiology (from *chronos* = time, *bios* = life, and *logos* = study).

Biological rhythms are defined as regular oscillations of biological processes and they represent an internal notion of time. Biological rhythms are found in many frequency ranges but some are associated with cosmic cycles. These are known as the circarhythms: circadian rhythms (from latin *circa* = around, *dies* = day) with a period close to 24 hours, circannual rhythms with a period close to 365 days, circalunar rhythms with a period close to 29.5 days, and circatidal rhythms with a period close to 12.4 hours. Biological rhythms can be further described based on their period (the duration of one complete cycle), their amplitude, and their phase (the time of an event within the cycle relative to a specific reference point).

This thesis focuses on circadian rhythms in humans. The endogenous nature of these rhythms is demonstrated by the fact that under constant conditions (i.e., constant light, temperature, humidity, etc.) circadian rhythms do persist, however, they do not do so with an exact 24-hour period but rather with a close-to-24-hour period (hence they are referred to as circadian). Under constant conditions, these rhythms are referred to as free running. The period of this free running rhythm is called tau (τ) and its duration depends on the light conditions at which it is measured (Aschoff, 1965; Pittendrigh and Daan, 1976a). Circadian rhythms are usually self-sustained, which means that they continue cycling in constant conditions without a dampening of their amplitude. Furthermore, temperature has little influence on τ, allowing the clock machinery to function properly under a wide range of temperatures. This phenomenon is known as temperature compensation (Pittendrigh, 1954).

To match the organisms' internal timing with the timing of the external world, the circadian clock needs to be entrained. Light is the main environmental cue that serves as a Zeitgeber ("time giver" in German Aschoff, 1958) for circadian entrainment (Pittendrigh, 1960). Entrainment occurs when in the presence of a Zeitgeber,

which is characterized by a period T (T = 24 hours in our daily light/dark cycle), the internal circadian period (τ) is adjusted in such a way that $\tau = T$ (Pittendrigh, 1981). As a result a stable phase relationship between the internal and the external oscillations is established. This is known as phase of entrainment. Hence, the physiological and behavioral functions associated with activity are in phase with the external daytime in diurnal organisms. Whereas, the physiology and behavior associated with rest are in phase with the nighttime. Apart from entraining the internal rhythms, environmental signals can also influence those rhythms (e.g., sleep) by masking. Masking is defined as the direct effect of environmental stimuli, such as light, on the expression of an overt rhythm without affecting the underlying generating mechanisms of the rhythm. Masking can be positive or negative and it generally reflects a superficial change. Light, for instance, not only adjusts the phase of the rhythm of alertness, but it has also direct alerting effects (Cajochen, 2007; Campbell and Dawson, 1990; Rüger et al., 2006). All these effects of light are known as non-image-forming effects.

In mammals, to achieve the above mentioned non-image-forming effects light has to enter the system via the eyes. Light is absorbed by photopigments, which are light sensitive molecules located within the photoreceptor cells in the retina. Rods and cones are the classical photoreceptor cells. They contain rhodopsin as a photopigment. Once light is absorbed, the rhodopsin molecules switch to a high-energy-state that leads to physical-chemical changes affecting the conformation of the photoreceptor. This process of phototransduction ultimately evokes physiological responses within the organism. The rhodopsin molecules fall apart during this process and new rhodopsin molecules have to be constructed to replace those that have been used up.

The spectral sensitivity of phototransduction depends on the absorption characteristics of the photopigments. In humans, rods show a peak of sensitivity at around 498 nm, while cones peak at 437 nm (blue cones), 533 nm (green cones), and 564 nm (red cones). In the 1990s behavioral studies on circadian entrainment in animal models challenged the idea of rods and cones being the only photoreceptors present in the retina (Freedman et al., 1999; Lucas et al., 2001; 1999). This led to the discovery of a third type of photoreceptor, the intrinsically photosensitive retinal ganglion cells (ipRGCs). ipRGCs belong to a subset of retinal ganglion cells that contains the photopigment melanopsin, with a peak of sensitivity in the short wavelengths around 480 nm (Gooley et al., 2001; Hattar et al., 2002; Provencio et al., 2000; Lucas et al., 2001). Light signals originating in rods and cones can also activate ipRGCs (Dacey et al., 2005; Perez-Leon et al., 2006) . Melanopsin, unlike the photopigments found in rods and cones that have to be regenerated, shows two stable states: the 11-cis-retinal state (melanopsin, R state) and the all-trans-retinal state (metamelanopsin, M state) (Melyan et al., 2005; Mure et al., 2007). These two states exist in equilibrium under broadband light conditions. When melanopsin is exposed to monochromatic light, short wavelengths (480 nm) ini-

tiate the phototransduction cascade from R to M state, while long wavelengths (620 nm) restore responsiveness by regeneration of the M to the R state (Mure et al., 2007). This latter transition does not activate the phototransduction cascade. Via the retinohypothalamic tract (RHT) that originates at the retinal ganglion cells layer, light information is transferred to the suprachiasmatic nucleus (SCN) of the anterior hypothalamus, just above the crossing of the optic nerves, where the master biological clock is situated (Moore and Eichler, 1972; Stephan and Zucker, 1972). The ipRGCs also project to other brain areas of relevance for non-image-forming processes beyond entrainment. Namely, the olivary pretectal nucleus (OPN) cirtical in pupillary light reflexes, and the ventrolateral preoptic area (VLPO), which plays a role in sleep regulation. (Berson, 2003; Hattar et al., 2006).

The Biological Clock in Humans

More than 200 years ago Christoph Wilhelm Hufeland, a general practitioner in Germany, noticed the presence of rhythms in the physiology and behavior of human subjects (Aschoff, 1998; García Jordá, 2007; Lemmer, 2009). Several statements in "Makrobiotik – oder die Kunst das Leben zu verlaengern" (1823) made him a pioneer in the field of human chronobiology; a field that only began to be developed a century later. Here I include two citations from Hufeland's work: "*The 24-hour period which is imparted to all inhabitants of the terrestrial body by its uniform rotation is especially distinct in the physical economy of man. In all diseases this regular period makes its appearance, and all other so marvelously punctual terms in our physical history are, after all, determined by this single period.*" Regarding sleep he said: "*It is certainly not one and the same to sleep for 7 hours during daytime or for 7 hours at night; to sleep through 2 hours before midnight is of greater value for the body than 4 hours of sleep during daytime.*" (see Aschoff, 1998 for an overview of Hufeland's life). Later in 1866, William Ogle was the first one to measure his own temperature not only for several months but also, and more importantly, at different time points during the 24-h day. He observed that body temperature followed a 24-h rhythm with an increase in the early morning and a decrease in the early evening, even when he was not sleeping. Because he could not find a correlation with the sleep-wake cycle, nor with the environmental conditions he concluded that the variations in body temperature had to be due to variations in body functions (in Refinetti, 2006). Although the rhythmicity in the above aspects of human physiology was clear, the endogenous nature of these rhythms was still to be proven. In the 1930's Nathaniel Kleitman and colleagues conducted the first studies under isolation. They secluded themselves in the Kentucky's Mammoth Cave shielded from external influences during 4 weeks, although artificial light was on during the waking hours. In the course of this time they lived under a 28-h day and thus became the pioneers of a protocol that is now known as forced desynchrony. Under this 28-h day they measured body temperature every 2 hours during the waking period and 4-hourly during the resting period. They hypothesized that if rhythms would change to a 28-h day rhythm, the 24-h observed rhythms under natural conditions could be just due to a reaction to the exter-

nal world. Unfortunately their results were inconclusive. While one subject changed to the 28-h day, the other one retained the 24-h rhythm (Kleitman, 1987). Later in the 1960's, Jürgen Aschoff and Rütger Wever were the first to systematically study human subjects in isolation from time cues. In a special underground isolation facility they studied subjects for up to 3-4 weeks, and measured many variables, including body temperature, activity, and urine production. Under constant conditions (in the absence of external cues) they were able to prove that humans show biological rhythms with a period of *circa* 24 hours (Aschoff and Wever, 1962). Aschoff and Wever demonstrated that the observed rhythms were not merely a response to changes in the environment but intrinsic to the subject.

Later on, special laboratory facilities free of time cues (isolation facilities) as well as specific techniques such us constant routines and forced desynchrony have been developed to distinguish between circadian variations due to behavior (the sleep-wake cycle) and variations independent of behavior.

In the following sections I will describe the output parameters most commonly measured in human studies that are relevant for the present thesis. I will also present an overview of some aspects of the non-image-forming effects of light in humans in order to provide additional context for the studies presented in this thesis.

The Hands of the Human Clock

The "hands of the clock" is a term use to refer to the observed overt rhythms. These rhythms are driven by underlying mechanisms and can be used to characterize the biological clock. The rhythm of melatonin has been described as the most robust signal available for studying circadian rhythms in humans (Klerman et al., 2002). This is due to the fact that basically only light can mask melatonin rhythms while other rhythms such as core body temperature (Benloucif et al., 2005) and cortisol (Wehr et al., 2001a) can be influenced to a much larger extent by activity and posture. Although large differences in melatonin profiles can be found between individuals, within individuals, daily melatonin profiles are remarkably similar from day to day, which allows for the assessment of even small differences in phase (Klerman et al., 2002; Revell et al., 2005b). Melatonin is a hormone that is synthesized from tryptophan. In mammals this synthesis occurs mainly in the pineal gland and almost exclusively at night. Serotonin n-acetyltransferase (NAT), the most important enzyme in this pathway, shows high levels at night and low levels during the day, leading to rhythmicity in the secretion of melatonin. This rhythmic behavior is under the control of the biological clock. Melatonin rhythms can be shifted by light (Lewy et al., 1985), and light can also acutely inhibit melatonin production (Lewy et al., 1980). Melatonin signals back to the SCN where melatonin receptors are found (Reppert and Weaver, 1995) The Mel1a receptor is associated with acute suppression of SCN activity. By doing this melatonin could be involved in determining the sensitivity

of the biological clock. The Mel1b receptor is mainly involved in phase shifting (Liu et al., 1997). Different parameters have been used to characterize the phase of melatonin rhythm in humans. Dim Light Melatonin Onset (DLMO) is the most frequently used and serves as a phase marker (Lewy, 2007). For the assessment of DLMO, saliva or blood samples are collected under dim light conditions (to avoid melatonin suppression by light) and 'the onset' of the melatonin rhythm is estimated. This refers to the time in the evening at which a certain criterion value of melatonin concentration is reached. The choice for this criterion value varies between studies. Typical values used in the literature are 25 %, 50 %, and 3 pg/ml (Benloucif et al., 2008). The phase relationship between DLMO and sleep is rather invariant between subjects, with sleep onset occurring on average around 2 hours after DLMO (Burgess et al., 2003). The timing of the melatonin peak, the offset, the amplitude, the duration, and the percentage of melatonin suppression by light are also use for characterization of melatonin rhythms. This last measure is generally use to estimate the sensitivity of the non-image-forming system in humans (Brainard et al., 1997).

What makes studies in human subjects unique is the possibility of direct assessment of the effects of a certain intervention by asking questions about emotions and other psychological aspects (e.g., alertness/sleepiness, depression scores, appreciation, etc.), which then allows for correlations with physiological data. By means of a simple tool such as a questionnaire, insight into human entrainment can be gained. A clear example of the advantage of using questionnaires for circadian rhythm research is demonstrated through the use of the Munich ChronoType Questionnaire (MCTQ), developed by Roenneberg and colleagues (2003), to assess the distribution of the phase of entrainment. They defined Chronotype as the time of mid sleep on free days (i.e., days with no social obligations, MSF). MSF shows a slightly skewed unimodal distribution with slightly larger numbers of late chronotypes than early chronotypes. Early chronotypes wake up spontaneously at an early hour and have difficulties in staying up late at night, while the opposite occurs with the late types (Roenneberg et al., 2003; Zavada et al., 2005). Not only is the timing of sleep different between chronotypes, but also their performance, alertness and physiological responses are shifted (Baehr et al., 2000; Duffy et al., 1999; Kerkhof, 1998; Kerkhof and Van Dongen, 1996). Chronotype therefore has a major impact on everyday life when individuals have to cope with waking up early to go to work/school during working days, and with staying late for social interaction during weekends. In particular, the work/school schedule has a negative impact on late chronotypes who have to perform at a suboptimal phase and suffer from sleep deprivation during workdays. The leisure schedule presents, on the other hand, a challenge for the early chronotypes. This discrepancy between internal and external timing has been conceptualized in the expression 'social jetlag' (Wittmann et al., 2006). Up to today around 100,000 individuals mainly from central Europe have completed the MCTQ. These data allows for epidemiological studies on how chronotype relates to factors such

as age, gender, daily light exposure, time of sunrise, consumption of stimulants, mental distress, and obesity (Roenneberg et al., 2007a; Wittmann et al., 2006; Roenneberg et al., 2012).

The Non-Image-Forming Effects of Light

Light has a major impact on our everyday life. Light is the signal that sets the phase of our biological clock, which in turn synchronizes our physiological and psychological rhythms to the 24h rhythm of the environment. These are known as circadian effects of light. When entrainment is impaired it can lead to discomfort and to higher risks for certain diseases (Rajaratnam and Arendt, 2001; Rüger and Scheer, 2009). Light also has non-circadian effects. For example, light can have acute alerting and activating effects (Cajochen, 2007) and when applied at night it can acutely suppress melatonin production (Lewy et al., 1980). Non-circadian effects of light take place within a short interval after the beginning of the light exposure (Cajochen, 2007; Rüger et al., 2003) whereas the circadian effects are integrative and slow by nature (Revell and Eastman, 2005).

Timing, intensity, and spectral composition of the light stimulus are key features in determining the non-image-forming effects of light. How the timing of light exposure affects the phase of the clock is empirically assessed in a phase response curve (PRC) (Pittendrigh and Daan, 1976b). Honma and Honma (1988) conducted the first PRC in human subjects (Honma, 1988). Since then several other human PRC's have been reported (Beersma and Daan, 1993 ; Czeisler et al., 1989; Jewett et al., 1997; Khalsa et al., 2003; Minors et al., 1991; Revell and Eastman, 2005; Revell et al., 2012; Rüger et al., 2013; St.Hilaire et. al., 2012). Humans' PRCs (as in any other species studied so far) are characterized by phase delays in the early subjective night and phase advances in the late subjective night with slightly larger delays than advances. Light during the subjective day causes smaller phase shifts but there is no complete "dead zone" where no shifts are achieved. Another mechanism by which light can entrain the circadian clock is by accelerating or decelerating the system, that is to say, by changing τ (Aschoff, 1963). Until now the effects of timing of light exposure on τ have not been studied experimentally in human subjects. However, via a modeling approach it was shown that changes in τ could increase the accuracy of the biological clock by about 25% (Beersma et al., 1999).

The first studies on how light affects alertness/activation were mainly conducted during the biological night, when the drive towards sleep is at its maximum. These studies showed that, in comparison to dim light, exposure to bright light increases alertness (Badia et al., 1991; Cajochen et al., 2000; Campbell and Dawson, 1990; Daurat et al., 2000; Rüger et al., 2003). More recent studies have shown that the alerting/activating effects of light are not restricted to the nighttime. Exposure to light during the day can also increase alertness (Phipps-Nelson et al., 2009; Rüger et al., 2006; Smolders and de Kort, 2012).

With regard to light intensity, the first studies conducted in humans led to the conclusion that only high light intensities

(> 1000 lux), as compared to indoor intensities, could have an effect (Lewy et al., 1980; Wever, 1989). Recent studies show that low light intensities can also elicit a response. Responses to light intensities follow a dose-response curve (Cajochen et al., 2000; Zeitzer et al., 2000; 2005). While a specific level of intensity is generally needed to elicit a response, this intensity is far lower than the intensities suggested in those previous studies. The phase shifting effects of light as well as light's suppressing effects on melatonin secretion have in fact turned out to be possible at light intensities as low as 100 lux (Zeitzer et al., 2000; Boivin et al., 1996; Boivin and Czeisler, 1998). Similar to the circadian effects of light, the alerting/activating effects of light as measured by means of subjective ratings of sleepiness, slow-eye-movements, and EEG, also show a dose response relationship with light intensities; in fact 100 lux at night was sufficient to reach half of the maximum alerting response (Cajochen et al., 2000; Cajochen, 2007).

The discovery of intrinsically photosensitive retinal ganglion cells and their key role in non-image-forming responses have added the spectral composition of light as a quality feature to be considered for biological processes. The first studies in humans showing evidence for the presence of a new photoreceptor came from action spectrum studies (Brainard et al., 2001; Hankins and Lucas, 2002; Thapan et al., 2001). In these studies it was obvious that the sensitivity of the non-image-forming system differed from the spectral sensitivities of the rods and cones. After showing that ipRGCs were also present in the human retina (Provencio et al., 2000) many studies have focused on comparing the effects of different monochromatic lights. For instance, it was shown that short wavelengths produce both phase advances and delays more efficiently than longer wavelengths (Lockley et al., 2003; Revell et al., 2005a; Wright et al., 2004). Non-circadian effects of light are also enlarged by short wavelength light (Cajochen et al., 2005; Lockley and Gooley, 2006; Revell et al., 2006). In a recent study Gooley et al. (2010) studied the dose response effect of blue and green light on melatonin suppression and phase shifting effects. The authors found that at low light intensities and at the beginning of the light pulse, (green) cones contribute significantly, changing to a more melanopsin-dominant-response when exposed to higher light intensities and during extended exposure (Gooley et al., 2010). Other studies have focused on studying the effects of more realistic light sources. For example, exposure to blue-enriched light at work place for 4 consecutive weeks in comparison to white light was found to lead to improvement of subjective ratings of alertness, sleep quality, and performance (Viola et al., 2008). In a study comparing the effects of polychromatic and monochromatic light matched for melanopsin-stimulating photons, showed that melatonin suppression was more effective under the polychromatic light source, suggesting that the melatonin suppression response is not driven merely by melanopsin (Revell and Skene, 2007). Also, when the phase shifting effects of blue-enriched polychromatic light sources were assessed they did not differ from those achieved by white light (Smith et al., 2009; Smith and Eastman, 2009). Considering the dose response curve described above for the phase

shifting effects of light, the intensities used in these studies were on the saturating part of the curve. Therefore, it is likely that the light intensities used by Smith and coworkers (2009) were too high to see an effect.

Ultimately, the non-image-forming effects of light will depend on subject's sensitivity. Prior exposure to light (the "light history") is one factor by which sensitivity can vary. In particular, a prolonged exposure to dim light conditions as opposed to bright light (Hébert et al., 2002; Owen and Arendt, 1992) or darkness (Smith et al., 2004), can increase light sensitivity.

The Effects of Seasons

Variations in the characteristics of light come naturally with the changes in seasons. An extensive review of the literature available until 1989 was performed by Lacoste & Wirz-Justice (1989) on seasonal changes in human subjects. The authors reported several behavioral and physiological variables that were shown to be different at different times of the year (Lacoste & Wirz-Justice, 1989). Another extensive analysis of (admittedly incomplete) worldwide databases on human reproduction, with the earliest record being available from 1669 and the latest from 1981, was performed by Roenneberg and Aschoff (1990). They observed seasonal patterns of conception with two peaks, one in spring and one in winter (Roenneberg and Aschoff, 1990a; 1990b) that mainly correlated with photoperiod and temperature (Roenneberg and Aschoff, 1990b). Industrialization had a great impact on the amplitude and regularity of these seasonal observations, followed by a further decline that correlates with the introduction of cooling/heating systems.

In the 1980's the first systematic studies describing natural exposure to light during different seasons started. Since then few studies have been published on exposure to seasonal changes in natural light exposure; two studies were conducted in the context of seasonal affective disorder (SAD) (Eastman, 1990a, Guillemette et al., 1998), another investigated light exposure during onw week across four seasons at two different latitudes (Cole et al., 1995), and the last two studies investigated 1-week light exposure in summer and winter (Hébert et al., 1998; Thorne et al., 2009). Thorne et al. took the analysis a step further by studying the spectral composition of light in summer and winter measuring the red, green and blue components of the light to which subjects exposed themselves. Except from Thorne et al. who also looked into sleep rhythms, all the other studies only investigated light exposure and no physiological data was collected.

Melatonin is thought to be a key physiological response that signals the duration of the night. In this way melatonin could transfer information about seasons; for instance, that there are long days (short nights) in summer and short days (long nights) in winter (Menaker, 1997; Lincoln et al., 2006). Several studies in humans have been conducted on melatonin responses to photoperiodic changes, both artificial and natural. Under laboratory conditions melatonin secretion measured in dim light was longer after one week of short artificial days (long nights) than after one week of long artificial days (short nights) (Wehr et al., 1993).

Under extreme photoperiodic changes in Antarctica, melatonin rhythms were phase delayed in winter (Yoneyama et al., 1999). Also at moderate latitudes a phase delay has been reported in winter as compared to summer (Bojkowski and Arendt, 1988; Honma et al., 1992; Illnerová et al., 1985; Vondrasová et al., 1997). Unfortunately, light profiles were not systematically assessed throughout these studies. An integrative approach on light exposure and melatonin rhythms is needed to gain insight on the effects of seasonal changes in light.

THE EFFECTS OF AGEING: YOUNG VERSUS OLD EYES

The natural aging of the ocular lens changes the intensity and spectral composition of the light reaching the retina. With aging the lens becomes denser and transmits less light, in particular of short wavelengths (Giménez et al., 2010a; Van Norren and Vos, 1974). The discovery of the ipRGCs has triggered new functional questions on the effects of these changes. Specifically, are the disruptions of the circadian system that are observed in the elderly population perhaps caused by the ageing of the lens? These disruptions are particularly related to weaker rhythmicity. Common disturbances are: nocturnal awakening, difficulties in falling asleep, and early awakening. A decrease in melatonin production has also been observed. At the level of the SCN the amount of synthesized vasopressin, a neuropeptide that shows circadian oscillation is also reduced (see review, (Van Someren, 2000a and 2006). Due to the increasing number of elderly subjects in the population, improving healthy ageing, which is seriously jeopardized by sleep disturbances, is of increasing importance. In 2000 the Journal of Biological Rhythms dedicated a complete issue to aging pointing out the relevance of this topic.

It is therefore important to have a better understanding of the relationship between changes in lens transmission due to aging and circadian rhythmicity. By means of questionnaires it has been investigated how sleep quality (i.e., poor sleep, frequent awakenings, and difficulty in falling asleep after nocturnal awakening) developed 1 and 9 months after cataract surgery in Norway (Asplund and Lindblad, 2004; 2002). The authors however did not quantify sleep quality but instead gave the proportion of subjects who reported an increase in sleep quality.

The relationship between lens transmittance and circadian system has also been assessed by indirect measurements. Two studies were conducted at Surrey University in which the responses of elderly and young subjects to two different monochromatic light sources (456 nm versus 548 nm) were compared. Sletten et al. (2009) found no significant differences between age groups on the phase advancing effects of light. In contrast, a reduction in the acute responses to short wavelengths was observed in the elderly group in comparison to the younger subjects (Herljevic et al., 2005). The reduction in the acute responses denotes a decline in the amount/quality of light entering the eye, which in part can be explained by the aged lens. Sletten et al. (2009) attributed the lack of age differences in phase shifting effects to the integration over time of light information that characterized circadian responses. Clearly, further studies investigat-

ing the causal relationship between the aged lens and non-image-forming responses are required.

Entrainment Under Natural Conditions

Laboratory conditions in which human studies are conducted differ from the natural environment in which humans live. Laboratory studies are designed to control the environmental conditions and to reduce the influences of confounding variables. This means that not only can the timing and intensity of light exposure be manipulated, but also its spectral composition can be altered, by exposing subjects to monochromatic light sources. Subject's behavior can be restricted in order to optimize the effects of treatments and subjects' can also be manipulated in order to stabilize light exposure. This information is certainly needed for understanding the circadian system. However, the insights that arise from the lab studies have to be brought to the field.

Thesis Overview

As outlined above, we still know very little about entrainment in the natural environment. In the present thesis I aimed to bring the research on non-image-forming light effects a step closer to real life conditions.

The studies presented in this thesis were conducted within the EUCLOCK framework, a European network working on entrainment of the circadian clock (www.euclock.org). In particular, the present work was part of the EUCLOCK work-package "Entrainment in humans". All of the studies presented in this thesis were designed to assess the non-image-forming effects of light under conditions that were as natural as possible.

The thesis can be divided into 2 sections. The first section including chapters 2 and 3 deals with a more general picture on how natural alterations that occur due to changes in seasons might affect not only the timing of our behavior and physiology but also our mood and performance and how, by simple manipulations, we can influence some of these aspects. The second section includes chapters 4, 5 and 6. These chapters focus on the natural phenomenon of aging by which exposure to light is reduced in its intensity and in the amount of short wavelengths, and how this affects the circadian organization of human subjects under natural entrainment.

In CHAPTER 2 we assess the effects of seasons (winter and summer) and the accompanying changes in ambulatory light exposure on the rhythms of melatonin production and sleep-wakefulness alternation in healthy young subjects. In the context of natural entrainment, melatonin is assessed in two different ways: under dim light conditions as it is usually measured to assess the phase of the clock, but also under natural light conditions to assess the real melatonin signal that represents the signal of darkness to a wide variety of body functions, including the SCN itself.

In CHAPTER 3 we investigate, in a home study, during the dark winter months the potential benefits of a device that elicits an artificial dawn signal 30 minutes before the alarm goes off (Wake-UP Light; Philips Consumer Lifestyle, The Netherlands) in

lessening the period of reduced alertness that follows awakening, known as sleep inertia. We further investigated whether a reduction in sleep inertia complaints is accompanied by a shift in the rhythm of melatonin.

In CHAPTER 4 we describe a method to quantify *in vivo*, in an easy and quick way, the spectral composition of light and the light levels that reach the retina. This technique could have a great impact on our understanding of the optimal range of light levels/spectral composition for eliciting a response within the non-image-forming light perception pathways. To illustrate its impact we assessed the difference in lens transmittance before and after cataract surgery. This is of interest in the field due to the changes in particular of short-wavelengths transmission that occur in the lens of the eye with aging and that are modified after surgery where the old lens is replaced by a new transparent one.

In CHAPTER 5 we further investigate the causal relationship between the age related changes in non-image-forming responses to light and the aging of the ocular lens. We took advantage of the natural phenomenon of cataract development, and assessed melatonin and sleep-wake rhythms in elderly subjects before and after cataract surgery.

In CHAPTER 6 we followed up on the study in chapter 5 by assessing the effects of reduced (short wavelength) light exposure on melatonin and sleep wake rhythms as well as in the acute suppressing effects of light on melatonin in healthy young subjects. This study addresses the short and intermediate effects of changes in light exposure. It also contributes to understanding the aging effects of the lens (simulated by wearing continuously soft orange contact lenses) without the confounding effects of other aging processes.

In CHAPTER 7 the main findings of this thesis are integrated and discussed. §

Chapter 2

An Integrative Study on Humans' Sleep, Melatonin Rhythms, and Light Exposure During Summer and Winter

Marina C. Gimenéz, Maan van de Werken, Domien G. M. Beersma, Marijke C. M. Gordijn

Department of Chronobiology, Center for Life Sciences, University of Groningen, The Netherlands

Abstract

We conducted an integrative study to assess the effects of winter (short days) and summer (long days) on sleep and melatonin rhythms and on light exposure in human subejcts. Fifteen subjects participated for two consecutive weeks both in winter and summer. Sleep timing and quality were assessed on a daily basis and light exposure was recorded continuously. Melatonin rhythms were evaluated under laboratory dim light conditions as well as at the participant's home environment without restrictions on light exposure (real life). We observed a delay of sleep and melatonin rhythms in winter as compared to summer. The delay we observed on days off in winter was larger than the 1-hour expected by daylight saving time, whereas the delay on workdays was smaller. The average amplitude of the melatonin rhythms assessed under dim light conditions was larger than the amplitude observed under real life light conditions. The timing of the melatonin rhythm was not affected by the light conditions. Day to day variations in light exposure were shown to correlate to the daily changes in sleep timing and quality, and to the assessed timing and amplitude of the melatonin rhythm.

Introduction

Seasonal changes are the consequence of the rotation of the earth around the sun and the tilt of the Earth's axis relative to its orbital plane. The long days (and short nights) in summer and short days (and long nights) in winter lead to changes in physiology and behavior in many mammalian species, known as photoperiodic responses. The biological clock mediates many of these responses. In particular, the nocturnal melatonin hormone plays a key role in signaling seasonal changes (Menaker, 1997; Arendt, 1998; Goldman, 2001; Hut and Beersma, 2011). In humans, the effects of seasons on the nocturnal rhythm of melatonin (Illnerová et al., 1985; Honma et al., 1992; Wehr et al., 1993; 1995; 2001; Vondrasová et al., 1997), on sleep (Wirz-Justice et al., 1991; Honma et al., 1992; Yoneyama et al., 1999; Lehnkering and Siegmund, 2007; Kantermann et al., 2007; Friborg et al., 2012), and on light exposure (Hébert et al., 1998; Thorne et al., 2009) have been investigated. Despite the attention given to this subject, an integrative inspection of melatonin, sleep, and light exposure responses to changes in seasons is lacking.

The biological clock uses the light/dark cycle as the environmental cue to entrain our body's physiology and behavior to the external 24-h day. Modern human subjects can self-select their exposure to light. Usually, in daytime they spend most of their time indoors with light levels of only a fraction of those found outdoors, while in the evening they switch on artificial light until they decide that it is time to sleep. This results in irregular light pattern exposures that also differ largely between subjects (Okudaira et al., 1983; Cole et al., 1995; Guillemette et al., 1998; Beersma et al., 1999; Dumont and Beaulieu, 2007). To understand entrainment, we need to explore the day-to-day variation in our exposure to light and the responses of the biological clock to these variations. Seasonal studies represent a valuable opportunity to assess these responses under at least two different light scenarios (e.g., long summer days- vs. short winter days).

Here we present an analysis of human melatonin rhythms in summer and winter at moderate latitude both under real life light conditions as well as under laboratory dim light conditions. We investigated changes in sleep-wake rhythms and subjective sleepiness throughout the day and we collected information on ambulatory white light exposure. In the Netherlands, daylight saving time (DST) is introduced the last weekend of March and ends the last weekend of October. DST imposes a shift in behavior by which we delay our social clocks by 1 hour from March to October. Assessing the effects of seasons on human behavior and physiology is therefore confounded by the in-

troduction of DST. The reports on seasonal changes in human behavior and physiology mentioned above have shown no consistency in reporting whether data was collected/analyzed under DST or wintertime. Given the impact that such decision has on the interpretation of the results we have opted to express and analyze our data as in Central European Time (CET) (i.e., also in summer, time is expressed as CET, not as Central European Summer Time). We will discuss our results with regard to previous reports keeping in mind our choice for analyzing behavioral and physiological timing according to CET.

Methods

Subjects

The study was carried out in Groningen, The Netherlands (53°12'N, 6°58'E). Healthy young subjects (n = 15, 8 females/7 males) aged between 25-34 years (average ± SD: 28 y ± 3 y), and with an intermediate chronotype (average midsleep on free days ± SD: 4: 47 h ±. 46 min) participated in this study. All subjects were non-smokers, free from prescribed medication, and reported neither medical disorders nor drug abuse as assessed by questionnaires. They all showed no sleep disorders (Pittsburgh Seep Quality Index ≤ 5), and did not suffer from (winter) depression (Beck Depression Inventory –II, Dutch version, BDI_II_NL ≤ 8, Beck et al., 1996, 2002). Subjects were also not color blind (Ishihara test) nor did they have any other visual impairment (assessed by the general health questionnaire) except for, in some cases, prescribed glasses or contact lenses. Subjects had not travelled across more than two time zones and/or worked night shifts during 2 weeks prior to taking part in the study.

All subjects gave their written informed consent and were paid for their participation. The Medical Ethics Committee of the University Medical Center of Groningen, The Netherlands, approved the experimental protocol.

Experimental Design

The study took place between June and August 2008 (further called "summer", sunrise range: 4:06 h – 5:20 h, sunset range: 21:03 h – 19:54) and between December 2008 and February 2009 (further called "winter", sunrise range: 8:29 h – 8:21 h, sunset range: 16:19 h – 17:14h). Measurements were, in winter, at least 28 days after the change to wintertime, and in summer measurements started at least 49 days after the change to summertime.

In a within subject design, subjects participated in summer and winter during two consecutive weeks. During those 2 weeks the following measurements were conducted:

Actigraphy: By means of actiwatches-L (Actiwatch®, Cambridge Neurotechnologies, UK) actigraphy data (1-min epoch) was recorded and sleep logs were completed on a daily basis.

Light profiles: Light exposure (lux) was collected by means of Actiwatches-L on a 1-min epoch basis. Careful instructions were given to the subjects to make sure their

clothes did not cover the light sensor.

Melatonin profiles: In randomized order subjects ended each week either with a laboratory condition (i.e., dim light) or a real-life condition (i.e., at home with no light restrictions). The laboratory condition was meant to assess melatonin as a marker of the clock with a method that is commonly used and reported in the literature. The real life condition was meant to assess melatonin as it naturally occurs. Nocturnal melatonin profiles were obtained on two consecutive nights (day 6 and 7) of each week. For the present report melatonin patterns of the two consecutive nights in the same condition were averaged per participant.

For the laboratory condition, subjects arrived at the lab at 18:00 h and left on the next day after 9:00 h (clock time). Laboratory light was below 5 lux at the level of the eye in the direction of gaze in all situations. Lights were off during sleep. From 19:00 h until 1:00 h (clock time) saliva samples were collected hourly. From 1:00 h until 9:00 h (clock time) samples were collected every 2 hours. Samples were centrifuged immediately after collection and stored at -20ºC until analysis. Subjects were allowed to watch videos, listen to music, read or work on their computers. Light intensities of all devices were adjusted to keep light exposure below 5 lux.

For the real life condition, saliva samples were collected at the same time points as in the laboratory condition, but at home, under natural conditions. Samples were kept in the fridge and on the next day they were brought to the laboratory, where they were immediately centrifuged and stored at -20ºC until analysis.

In both conditions, subjects were carefully instructed about the collection of saliva samples. Eating was restricted to the first 15 minutes after each sample. Chocolate, bananas, coffee and/or tea were not allowed during the whole sampling period. Ten minutes prior to each sample, subjects were asked to sit quietly to avoid any influence of posture (Deacon and Arendt, 1994).

Data Analysis

Note, as mentioned in the introduction all data on timing are expressed in Central European Time (CET). Also in summer, time is expressed as CET, not as Central European Summer Time.

The sleep analysis 5 software (Cambridge Neurotech Ltd, Cambridge, UK) set at medium sensitivity was used, together with sleep logs, to determine the timing of the sleep–wake rhythm. Sleep during the two nights in the lab was not included in the analysis. The Actiwatch algorithm scores wake or sleep per minute as follows. If a_i is the activity score in minute i, then $A_i = a_i+(a_i-1+a_i+1)/5$ is compared with a threshold value of T = 40. When Ai>T the bin is scored as wake and if Ai <T the bin is scored as sleep. Sleep onset is operationally defined as the first episode after bedtime of 10 consecutive sleep minutes with no more than 1 bin of wake within that time. The time from bedtime until sleep onset is recorded as sleep latency. Sleep end is the last sleep minute before a 10-minute consecutive period of wake

recorded from the get up time. Sleep duration was calculated as the difference between sleep offset and sleep onset. We also calculated the average activity of the 10 most active hours (M10) and of the 5 least active hours (L5) (Witting et al., 1990; Van Someren et al., 1999), and sleep efficiency (percentage of time spent asleep while in bed). M10 and L5 are expressed as counts/hour.

Salivary melatonin concentration was assessed by radio-immunoassay (RK-DSM, Bühlmann laboratories AG, Alere Health B.V. Tilburg, The Netherlands). All samples from one individual were analyzed within the same series. The limit of detection for the RIA was 0.3 pg/ml with an intra-assay coefficient of variability of 6.7% at a low melatonin concentration (mean 1.5 pg/ml, n = 30) and 6.5% at a high melatonin concentration (mean = 15 pg/ml, n = 30). Inter-assay coefficients of variability were 12.2% at low melatonin concentration (mean = 2.1 pg/ml, n =15) and 19.7% at high melatonin concentration (mean = 17.5 pg/ml, n = 16). The full melatonin profiles were fitted to a bimodal skewed baseline cosine function (i.e., a cosine function that allows for bimodality and differences in the steepness of the rising and declining parts of the melatonin rhythm) (Van Someren and Nagtegaal, 2007). As relevant parameters we estimated the onset and offset of the melatonin rhythm, which was defined as the time when the threshold at which 25% of the maximum value of the fitted curves was crossed for the increasing and decreasing part of the curve respectively, the amplitude of the rhythm calculated as the difference between the minimum and the maximum value of the fitted curve, the duration of the rhythm calculated as the difference between onset and offset, and the skewness of the fit. Melatonin concentration values are expressed as a fraction of the fitted maximal melatonin value during winter condition for every individual subject. Phase angle differences were calculated between midsleep on work days (MSW, virtually all melatonin assessment occurred on working days) and the onset and the offset of the nocturnal melatonin rhythm.

Light analysis 5 software (Cambridge Neurotech Ltd, Cambridge, UK) was used to calculate the average light intensity (lux), the maximum intensity and the time spent above 1000 lux, for all days together as well as for workdays and days off separately. For further analysis, data on light intensity per subject was log transformed and then averaged over 0.5-hour periods. Average log light intensities over four time sections of the day: (i.e., 8:00-12:00 h, 12:00-18:00, 18:00-00:00 h, and 00:00-08:00 h on the day after) were calculated in order to explore the relationship between average light exposure at different times of the day and the various sleep and melatonin parameters investigated in this study (i.e., sleep onset, sleep offset, sleep efficiency, sleep latency, DLMO, DLMOff, and the amplitude of the average melatonin rhythm). The intervals over which average light levels were calculated were chosen to roughly represent the phases of the day with advancing (morning), delaying (evening), suppressing (evening and night) or no (afternoon) expected effects of light on melatonin and sleep parameters.

Statistics

The effects of season on sleep and melatonin rhythms were tested via a repeated measures ANOVA. The factors: seasons, type of day (workdays vs. days off, for sleep rhythms), light conditions (dim vs. real-life, for melatonin rhythms) and the interaction effects between season and type of day/light condition were tested via a 2-way repeated measures ANOVA. The same approach was used to investigate the phase angle relationship between MSW and the onset and the offset of the melatonin rhythm. For the KSS scores a 3-way repeated measures ANOVA was used to test the effects of season, time of the day, day of the week, and the interaction effects between these three factors.

Differences in the average light exposure, maximum light exposure and time spent above 1000 lux was tested via a 2-way repeated measures ANOVA, for the factors: season, type of day and the interaction between season and type of day. Ambulant light exposure depends on both natural and behavioral factors. The role of these factors in the pattern of day-to-day difference in ambulant light exposure was assessed by means of a multilevel regression analysis using MLwiN software (MLwiN 2.23, Centre for Multilevel Modelling, Institute of Education, London, UK). We investigated how the predictors of season (summer = 1, winter = 0), type of day (workday = 1, day off = 0), time of day, and the interaction between these factors could explain the light profiles. The regression model was as follows:

$$\text{Log (light intensity)}_{ij} = \beta_{0ij} + \beta_1 \text{ x Season}_{ij} + \beta_2 \text{ x Type of day}_{ij} + \beta_3 \text{ x Time of the day}_{ij} + \beta_4 \text{ x (Season x Type of day)}_{ij} + \beta_5 \text{ x (Season x Time of the day)}_{ij} + \beta_6 \text{ x (Season x Type of day x Time of the day)}_{ij}$$

This analysis takes into account the hierarchical structure of the study: 0.5-h-light measurements (i) nested within subjects (j), accounting for the interdependency of the data points (Twisk, 2003).

A mixed effect regression analysis was also performed to explore the additional effect of changes in light exposure at different periods of the day (i.e., 8-12 h, 12-18 h, 18-0 h, 0-8 h) on the different sleep and melatonin parameters, on top of the effect of the factors season and type of day. The likelihood ratio test was used to compare nested models (i.e., the additional effect of changes in light exposure at different periods of the day), to distinguish the "best" model when light at more than one period of the day was shown to be a significant predictor. Only those parameters that contributed significantly to the model are described.

All p-values shown are two-tailed. Significance levels were set at 0.05.

Results

Sleep Timing and Sleep Characteristics

Table 1 describes the average (work and days off) sleep characteristics in summer and winter (nights at the lab are excluded). Overall we observed a significantly delayed pattern in winter as compared to the summer (Fig. 1A). On average the delay of the onset, mid-

sleep, and offset did not significantly differ from 1 h ($p = 0.33$, 0.51, and 0.9, $t(14) = -1.0$, -0.7, and -0.1 respectively). This difference could be explained by the 1 h change introduced due to DST. When we distinguish between work days and days off, a significant interaction effect between season and type of day is observed. The delays observed in winter are larger on days off as compared to workdays (table 2, figure B and C). These differences were shown to be significantly different from the 1 h expected due to DST. On workdays in winter, the delays observed for the onset and midsleep were significantly smaller than the expected 1 h ($p < 0.05$, $t(14) = -2.4$ and -2,7 respectively for the onset and midsleep), while on days off, the delays observed for the offset and midsleep in winter were significantly larger than 1 h ($p < 0.05$, $t(14) = 2.7$ and 2.1 respectively for offset and midsleep).

Sleep onset latency was about 5 minutes shorter in summer as compared to winter. This significant shortening of the sleep onset latency in summer was observed for both work days and days off. In general

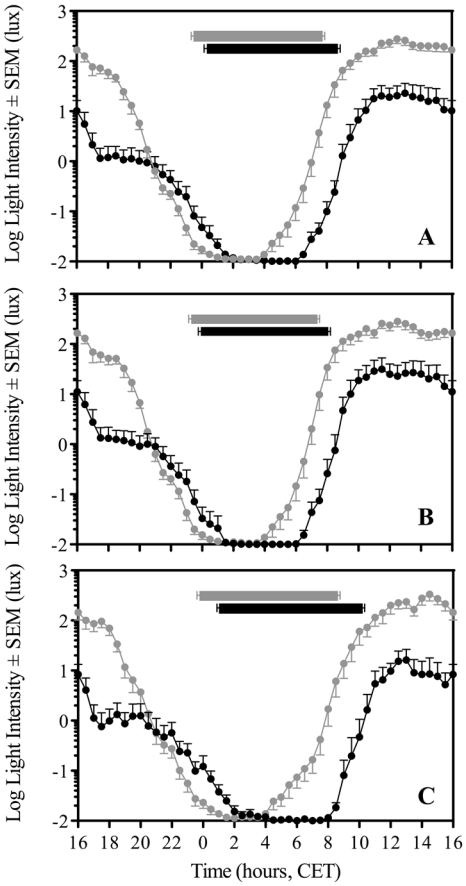


Figure 1. Overall light exposure and sleep profiles. The curves represent the average (±SEM) light exposure across the day in summer (grey) and winter (black). The bars represent the average (±SEM) sleep period in summer (grey) and winter (black). (A) Averaged over workdays and days off. (B) On workdays. (C) On days off.

Table 1. Overall Sleep Characteristics

	Summer	Winter	Δ Season
Bed Time	23:20 ± 31min	00:08 ± 48 min	*48 min**
Sleep Latency	8 min ± 0.5 min	14 ± 6 min	*6 min***
Sleep Onset	23:28 ± 32 min	00:21 ± 48 min	*53 min**
Sleep Offset	7:42 ± 39 min	8:43 ± 31 min	*1 h 1 min**
Get Up Time	7:48 ± 40 min	8:52 ± 33 min	*1 h 4 min**
Mid Sleep	3:18 ± 30 min	4:32 ± 32 min	*1 h14 min**
Sleep Duration	8:14 ± 40 min	8:22 ± 49 min	*8 min*
Sleep Efficiency	84.93 % ± 4.68 %	80.2 % ± 4.65 %	*-4.73 %**

All data shown as mean ± SD. Data is expressed as Central European Time. Δ Season refers to (winter – summer). *$p < 0.001$, **$p < 0.05$.

Table 2. Sleep characteristics on workdays and days off

	Summer Work Days	Summer Days Off	Δ Week Day Summer	Winter Work Days	Winter Days Off	Δ Week Day Winter	Δ Season Work Days	Δ Season Days Off
Bed Time	23:06 ± 25 min	23:44 min ± 58 min	*38 min**	23:41 ± 38 min	1:00 ± 1 h 20 min	*1 h 19 min**	*35 min**¶	*1 h 16 min**¶
Sleep Latency	9 min + 0.6 min	7 min ± 0.4 min	*-2 min*	14 min ± 9 min	11 min ± 8 min	*-3 min*	*5 min***	*4 min***
Sleep Onset	23:16 ± 29 min	23:51 ± 57 min	*35 min**	23:54 ± 40 min	1:12 ± 1 h 18 min	*1 h 18 min**	*38 min**¶	*1 h 21 min**¶
Sleep Offset	7:20 ± 38 min	8:32 ± 53 min	*1 h 12 min**	8:01 ± 43 min	10:08 ± 47 min	*2 h 7 min**	*41 min**¶	*1 h 36 min**¶
Get Up Time	7:26 ± 41 min	8:38 ± 54 min	*1 h 12 min**	8:08 ± 44 min	10:23 ± 51 min	*2 h 15 min**	*42 min**¶	*1 h 45 min**
Mid Sleep	3:18 ± 29 min	4:11 ± 43 min	*53 min**	3:58 ± 30 min	05:40 ± 56 min	*1 h 42 min**	*40 min**¶	*1 h 29 min**¶
Sleep Duration	8:05 ± 34 min	8:42 ± 1 h 8 min	*37 min**	8:07 ± 57 min	8:56 ± 1 h 5 min	*49 min**	*2 min*	*14 min*
Sleep Efficiency	84.62 % ± 5.3 %	85.15 % ± 4.3 %	*0.53 %*	80.96 % ± 4.76 %	78.92 % ± 4.87 %	*-2.04 %*	*-3.66 %**¶	*-6.23 %**

All data shown as mean ± SD. Data is expressed as Central European Time. Δ Week Day refers to (days off – work days), Δ Season refers to (winter-summer).
*$p < 0.005$. **$p < 0.05$, ¶significant interaction effect between season and day of the week all $p < 0.05$.

Table 3. Melatonin rhythms characteristics

	Dim Light			Real Life			Δ light conditions	Δ light conditions
	Summer	Winter	Δ Season	Summer	Winter	Δ Season	Summer	Winter
DLMO	21:36 ± 1 h 7 min	22:14 ± 1 h 9 min	*38 min**	21:55 ± 1 h10 min	22:33 ± 57 min	*38 min**	*19 min****	*19 min****
DLMOff	7:19 ± 1 h 59 min	8:37 ± 36 min	*1 h 18 min***	7:03 ± 1 h 8 min	8:29 ± 43 min	*1 h 26 min***	*- 16 min*	*- 8 min*
Duration	9 h 42 min ± 2 h 7 min	10 h 22 min ± 1 h 17 min	*40 min****	9 h 8 min ± 1 h 20 min	9 h 56 min ± 55 min	*48 min*	*- 34 min*	*- 26 min*
Amplitude (pg/ml)	22.82 ± 13.03	25.64 ± 15.17	*-2.82*	20.53 ± 18.71	20.51 ± 14.37	*-0.02*	*- 2.29**	*- 5.13**
Skewedness	0.035 ± 0.21	-0.17 ± 0.14	*	0.021 ± 0.18	-0.12 ± 0.17	*		

All data shown as mean ± SD. Data is expressed as Central European Time. Δ Season refers to (winter – summer).
Δ light conditions refers to (real life – dim light). *$p < 0.05$, **$p < 0.001$, ***$p = 0.07$.

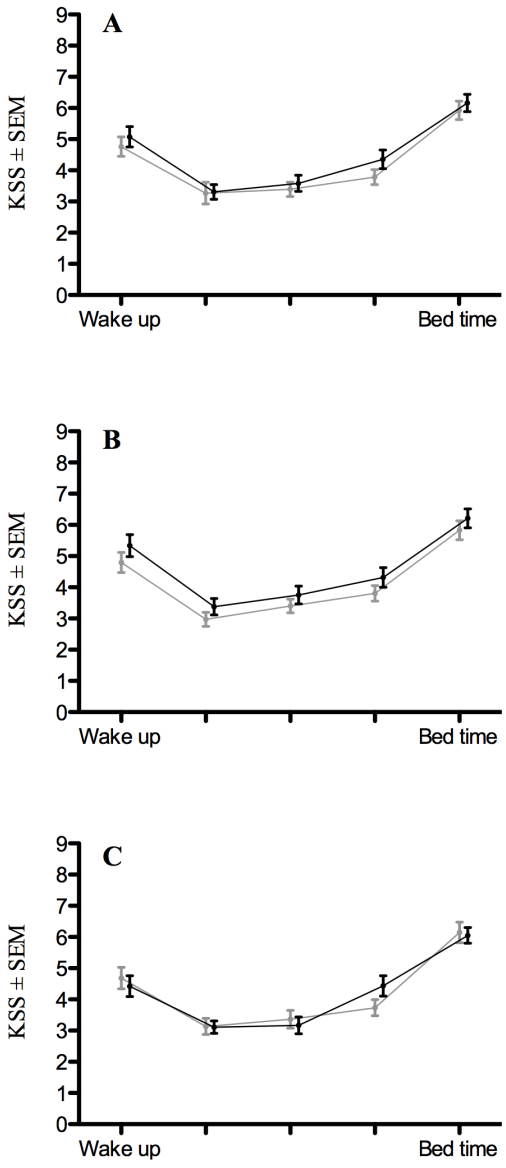


Figure 2. Subjective sleepiness (KSS). KSS values (±SEM) at wake up time, at three fixed time points separated by 4 hours across the day, and at bed time in summer (grey) and winter (black). (A) Averaged over workdays and days off and days off. (B) On workdays. (C) On days off. Average (±SEM) KSS scores measured at 16h, 20h, bed time, wake up time, and 12h (clock time) in summer (grey) and winter (black) on workdays (C) and days off (D).

sleep efficiency (i.e., the % of sleep while in bed), was reduced by about 5 % in winter. A significant interaction effect between type of day and season was also observed. Sleep efficiency was slightly increased (0.5 %) during days off as compared to workdays in summer, while in winter sleep efficiency was reduced by about 2 % during days off. Social jetlag (mid sleep on free days – mid sleep on workdays) was significantly larger in winter as compared to summer (average ± SD winter: 1:35 h ± 54 min; summer: 0:46 h ± 35 min, $F(1,14) = 18.82$, $p < 0.01$).

Average subjective sleepiness scores were slightly higher in winter than in summer, however, this differences were not significant (average ± SEM KSS rating, summer: 4.2 ± 0.2, winter: 4.6 ± 0.3; $F(1,14) = 3.51$, $p = 0.08$) (figure 2 A). When we distinguish between workdays and days off an interaction effect between seasons, time of the day of the score, and type of day is observed ($F(1,14) = 4.01$, $p < 0.05$; figure 2B and C). Specifically, it is during the morning hours on workdays that the differences in sleepiness between summer and winter are the largest with higher sleepiness ratings in winter.

Melatonin Rhythms

Table 3 summarizes the average characteristics of the melatonin rhythms in summer and winter under dim and real life light conditions. In winter we observed a delay in the onset of 38 min as compared to summer in both the dim and the real life light conditions. The offset of the melatonin rhythm was delayed in winter by 1 h and 18 min and by 1

h and 26 min in the dim and real life light conditions respectively. No significant effect was observed in the interaction between the factors season and light condition ($F(1,14) = 0.001$ and 0.089, $p = 0.97$ and 0.77 for the onset and offset of melatonin respectively).

The symmetry of the melatonin rhythm was significantly different. Whereas profile of the rhythm was almost symmetrical in summer, a negative skew was observed in winter.

The light conditions at which the melatonin was sampled had a significant effect on the amplitude of the melatonin rhythm: a smaller amplitude is observed in the real life light condition (average amp. = 20.5 pg/ml) as compared to the laboratory dim light condition (average amp. = 24.2 pg/ml). No effects of season or of light conditions were observed on the duration of the melatonin rhythm. The nocturnal melatonin profiles are shown in figure 3.

The phase angle difference between midsleep on workdays (MSW) and the onset of the melatonin rhythm was not significantly affected by the factor season (average ± SD: summer: -5:32 ± 1: 14h; winter: -5:34 h ± 58 min; $F(1,14) = 0.01$, $p = 0.9$). Season led, however, to a significantly earlier offset of the melatonin rhythm relatively to MSW (smaller phase angle difference), by an average of 42 minutes in summer ($F(1,14) = 5.92$, $p < 0.05$). Average dim and real life melatonin offset occurred 3:53 h after MSW in summer and 4:35 h after MSW in winter. The light condition factor (i.e., dim light, real life light) and the interaction between season and light showed no significant effect (figure 4).

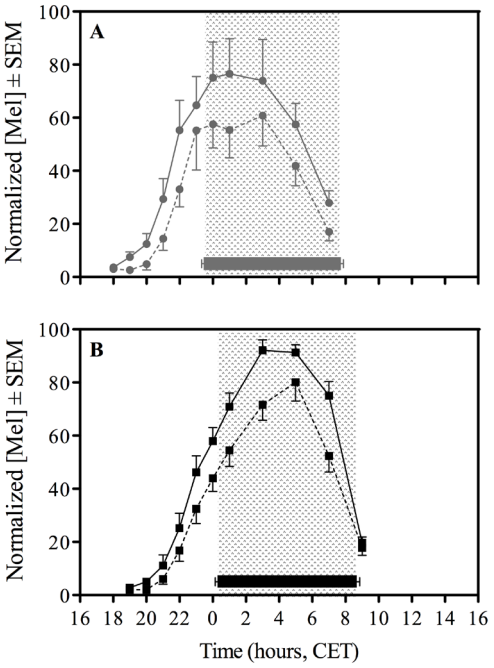


Figure 3. Melatonin profiles. Average (± SEM) melatonin profiles in summer (A) and winter (B) under dim (continuous line) and real life (dotted line) light conditions. Melatonin concentration values are expressed as a fraction of the fitted maximal melatonin value during winter condition for every individual subject.

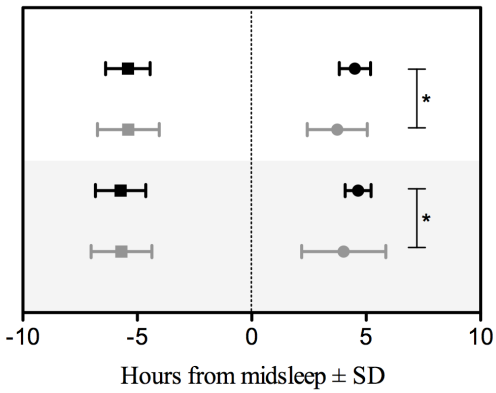


Figure 4. Phase angle between sleep and melatonin rhythms. Average (±SD) hours from midsleep on workdays and the onset (squares) and the offset (circles) of the nocturnal melatonin rhythms. Black and grey symbols refer to the summer and winter months respectively. The upper two traces refer to melatonin assessments under real life conditions, the lower traces (shaded box) to melatonin assessments under dim light condition in the laboratory.

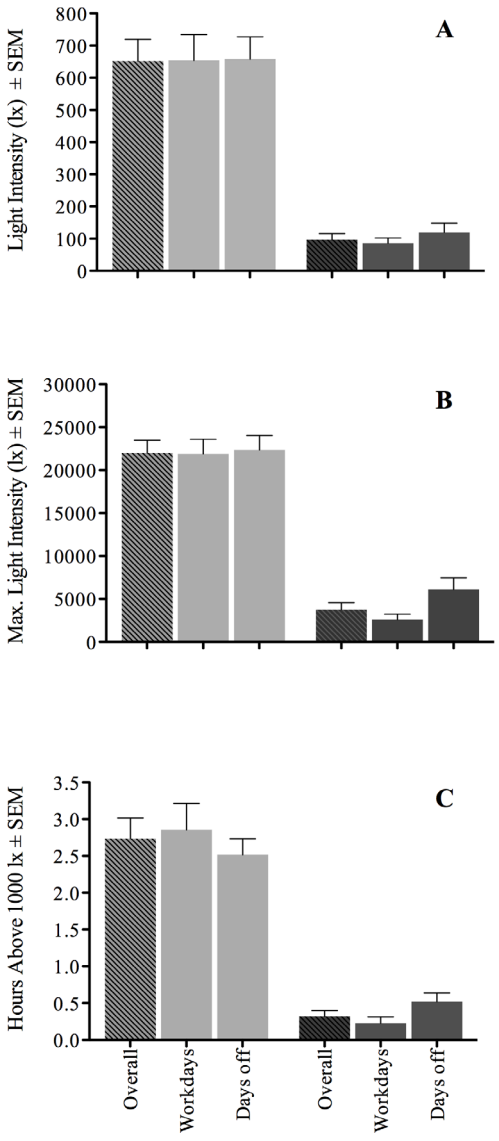


Figure 5. Light exposure general characteristics. (A) Average (± SEM), (B) Average maximum light exposure (± SEM), and (C) Average number of hours (± SEM) spent above 1000 lux light exposure, in summer (grey bars) and winter (black bars) across all days (dashed bars), and during work days and days off separately. Only the days outside the laboratory are included.

Ambulatory Light Exposure Levels

As expected, an effect of season was observed in the average ambulatory light exposure (average ± SD: summer: 652 lux ± 250 lux, winter: 99 lux ± 73 lux; $F = 97.7$, $p < 0.001$), the average maximum exposed light intensity (average ± SD: summer: 22013 lux ± 5560 lux, winter: 3882 lux ± 3185 lux; $F = 169.5$, $p < 0.001$), and the average time spent above 1000 lx across all days (average ± SD: summer: 2:44 h ± 1:02 h, winter: 19 min ± 17 min; $F = 92.1$, $p < 0.001$). The factors type of day (working day vs. day off) and the interaction between season and type of day had no effect on either variable (figure 5).

The multilevel regression analysis showed that the profiles in ambulatory light exposure levels correlate significantly with the factors season (β_1 estimate = 0.46 ± 0.14, $p < 0.001$), type of day (β_2 estimate = 0.29 ± 0.08, $p < 0.005$), time of the day (β_3 estimate = 0.02 ± 0.003 log (lux)-h, $p < 0.001$), and the interaction between season and time of day (β_5 estimate = 0.014 ± 0.005, $p = 0.005$). The interaction of season x type of day and the interaction of season x type of day x time were shown to be non significant ($p = 0.18$ and 0.4 respectively).

Table 4 summarizes the regression model estimates for the predictors of season, workdays/days off, and the average light exposure at different time-intervals, with regards to the observed daily changes in sleep. Only those predictors that were shown to be significant are presented in the table. On top of the negative relationship (advancing) between changes in the onset/offset of sleep and the summer season and working days, we observed that morning light also shows a negative correlation with the offset of sleep but does not significantly add to the effects of season and type of day for the onset of sleep. Furthermore, light during the rest of the day

Table 4. Model estimates for sleep rhythms

	Sleep Onset (β + SEM)	Sleep Offset (β + SEM)	Sleep Efficiency (%) (β + SEM)
Intercept	*00:59 ± 11 min*	*9:54 ± 9 min*	*80.15 ± 0.95*
Season	-55 min ± 10 min**	-51 min ± 8 min**	3.46 ± 0.81**
Day of the Week	-56 min ± 9 min**	-1 h 42 min ± 7 min**	-
Average Light Exposure 8-12h	-	-6 min ± 3 min/log(lx)[a]	0.59 ± 0.31 %/log(lx)[a]
Intercept	*00:48 ± 12 min*	*9:55 ± 10 min*	*80.23 ± 1.02*
Season	-1 h 09 min ± 11 min**	-57 ± 9 min**	4.19 ± 0.84**
Day of the Week	-57 min ± 9 min**	-1 h 44 min ± 7 min**	-
Average Light Exposure 12-18h	11 min ± 5 min/log(lx)*	-	-
Intercept	*1:04 ± 10 min*	*9:52 ± 9 min*	*80.52 ± 0.97*
Season	-1 h 6 min ± 8 min**	-59 ± 7 min**	4.11 ± 0.67**
Day of the Week	-54 min ± 8 min**	-1 h 44 min ± 7 min**	-
Average Light Exposure 18-0h	32 ± 5 min/log(lx)**¶	-	0.77 ± 0.45 %/log(lx)[b]
Intercept	*1:49 h ± 16 min*	*9:50 ± 14 min*	*89.1 ± 1.86*
Season	-1 h 3 min ± 8 min**	-59 ± 7 min**	2.35 ± 0.72*
Day of the Week	-58 min ± 8 min**	-1 h 44 min ± 7min**	
Average Light Exposure 0-8h	29 min ± 7 min/log(lx)**	-	4.76 ± 0.87 %/log(lx)**

*p < 0.05, **p < 0.01, ¶best model-lowest -2*log likelihood.
[a] p = 0.06, [b]p = 0.08.

Table 5. Model estimates for melatonin rhythms

	Onset (β + SEM)	Offset (β + SEM)	Amplitude (pg/ml) (β + SEM)
Intercept	*22:43 h ± 12 min*	*8:44 h ± 14 min*	*22.8 ± 2.61*
Season	-	-1 h 3 min ± 18 min**	-
Light Conditions	-	-	-
Average Light Exposure 8-12h	-15 min ± 6 min/log(lx)*[1]	-21 min ± 8 min/log(lx)**	-
Intercept	*22:54 h ± 15 min*	*8:58 ± 00:17*	*26.94 ± 3.17*
Season	-	-1 h 15 min ± 17 min**	-
Light Conditions	-	-	-
Average Light Exposure 12-18h	-17 min ± 8 min/log(lx)*[1]	-23 min ± 9 min/log(lx)*	-4.53 ± 1.66 (pg/ml)/log(lx)**
Intercept	*22:32 h ± 11 min*	*8:29 ± 00:13*	*21.08 ± 2.43*
Season	-34 min ± 13 min*	-1 h 33 min ± 16 min**	-
Light Conditions	-	-	-
Average Light Exposure 18-0h	-	-	-
Intercept	*22:22 h ± 25 min*	*7:47 ± 00:29*	*23.6 ± 5.38*
Season	-33 min ± 14 min*	-1 h 27 min ± 16 min**	-
Light Conditions	-	-	-
Average Light Exposure 0-8h	-	-	-

*p < 0.05, **p < 0.01, [1]no differences in -2*log likelihood.

has a positive (delaying) relationship with the observed changes in sleep onset on the subsequent evening. Sleep onset was positively correlated with the average intensity of prior evening light (18-0 h). Morning, evening, and night light exposure correlated positively with subsequent sleep efficiency. Morning light showed a non-significant negative correlation (shortening) with sleep latency during the subsequent night ($p = 0.07$).

Table 5 summarizes the regression model estimates for the predictors of season, dim/real life condition, and the average light exposure at different time-intervals in relation to the observed melatonin rhythm characteristics. The onset and offset of the melatonin signal on the subsequent night correlated negatively (advance) with light exposure in the morning and afternoon. A lower amplitude of the nocturnal melatonin rhythms correlated with higher average exposure to light in the prior afternoon between 12-18 h. Average light exposure between18-00 h did not correlate with the variation observed in the tested parameters of the melatonin rhythms.

Discussion

Our results show a delay in the winter months of both the sleep-wake as well as of the melatonin rhythms compared to summer. The Zeitgeber strength (day-night difference in light perceived) in the winter months is expected to result in a delay (Roenneberg et al., 2003). In interpreting the phase shifting effects, the consideration of DST in the analysis of the results is critical. We have presented our data in CET throughout. If season had no effect, and if subjects would adjust fully to DST, a 1-hour delay of behavior in winter should be observed. The average (work and days off) 50 minutes delay in sleep that we observe in winter is close to the expected time difference introduced by DST. Though, when we distinguish between work and days off we observed that, on workdays, the delay of the onset of sleep and midsleep are significantly smaller than 1h, suggesting that adjustment to DST is not achieved on work days. This could be due to difficulties in further delaying sleep in winter because of obligations on the next day, and/or to difficulties in advancing further during the long summer days, as a consequence of the presence of light until late in the evening. During days off, the delay in winter of the offset of sleep and midsleep are significantly larger than the 1h difference expected due to DST only. The additional, on average, 36 minutes that we observed could be attributed to seasonal effects, probably due to the lack of morning light in winter. The above findings are important, as the literature on sleep timing and seasons does not always distinguish between workdays and days off. Also, virtually nothing has been reported on researchers' approach to DST, and different parameters (i.e., onset, midsleep, offset, and duration of sleep) are often described across different studies. All of this makes comparing studies and gaining a complete picture of the effects of season difficult. Our results are in accordance with the delay observed on midsleep on free days (MSF) during winter compared to summer, reported by Kantermann et al. (2007). These authors also report their findings in Central European Time, allowing for straightforward comparisons between our

study and theirs. If we average Kantermann's data from December until March and from June until August (in the same way as we did in the present study), we find that MSF in winter is on average 1:15 h later than in summer. The small difference in the observed delay between both studies (1:29 h vs. 1:15 h) could be due to the later behavior that is observed in more western longitude locations within the same time zone (Netherlands in the present study vs Germany in Kantermann's study) (Roenneberg et al., 2007). Friborg and colleagues (2012) as well as Wirz-Justice et al. (1991), distinguish between week and weekend days. Although week and weekend days do not necessary mean with or without social obligations respectively, they are a good attempt to detach the influence of social Zeitgebers. While no differences in the onset or offset of sleep were observed on weekend days by Friborg and colleagues (2012), a small (~10 minutes), but significant, earlier onset and later offset of sleep was observed in the winter months by Wirz-Justice et al. (1991). In the latter study, sleep duration was increased by about 24 minutes, which implies that no actual shift occurred. An increase in sleep duration of 18 minutes, across all days (i.e., work and days off) was observed by Lehnkering and Siegmund (2007) in autumn as compared to spring but this was not long enough to result in significant differences in the onset or offset of sleep. No differences in the timing of sleep (onset, midsleep, and offset) were observed in Antarctica either (Yoneyama et al., 1999). The observed delay in our study therefore contradicts the absence of change observed by Friborg and collaegues (2012), Lehnkering and Siegmund (2007), Wirz-Justice et al., (1991), and Yoneyama et al., (1999). Assuming that the authors chose to present their data as a function of local clock time, and in view of DST being introduced at those locations, the lack of differences under clock time in the timing of sleep represents a 1-hour relative advance of sleep in summer. In this sense, overall a shift to a relatively late timing of midsleep is observed during the dark months across all studies. The size of this shift ranges from about 1 hour (Wirz-Justice et al., 1991; Yoneyama et al., 1999; Lehnkering and Siegmund, 2007; Friborg et al., 2012) to greater than an hour, as observed in the present study and by Kantermann and colleagues (2007). The introduction of DST has a confounding effect. We have assumed that adjustment to DST occurs, which should account for 1 hour difference between seasons, however, this is only an assumption and therefore the actual effect size of DST and of seasonal effects may deviate from the present figures with adjustment to DST to vary from full adjustment to no adjustment. In Japan where DST has been discontinued since 1951, Honma and colleagues observe about an 1-hour delay in the onset and offset of sleep in winter as compared to summer in humans (Honma et al., 1992). Sunrise in Sapporo ranges from about 4:00 to 4:30 in the months of June and July, while sunsets ranges from about 19:00 to 19:20. This earlier sunrise and sunset as compared to central European countries could facilitate a larger advance in the summer months.

We observed that the discrepancy between midsleep on work days (MSW) and midsleep on free days (MSF), known as social jetlag (Wittmann et al., 2006), was larg-

er in winter than in summer. This suggests a larger misalignment between internal and external timing in winter. In summer, as compared to winter, we observed a small increase in the efficiency of sleep as measured by actigraphy. This is in accordance with the suggestion that light intensity during the day could have a positive influence on sleep (Dumont and Beaulieu, 2007; Hubalek et al., 2010). The quality of sleep could also be related to the pattern of the nocturnal melatonin profile. The steeper increase of the nocturnal melatonin in summer could represent a more precise onset of the dark period. In winter the shape and onset of the melatonin rhythm is more skewed, probably as a consequence of a less strong light-dark signal. Whether this is the case and/or whether this is due to artificial lighting leading to a less clear difference between the day and night needs to be studied in more detail.

Lastly, we also observed differences in the subjective assessment of sleepiness. During workdays, subjective scores of sleepiness were higher during winter, especially immediately after awakening (within 15 minutes). Immediately after waking up sleep inertia (i.e., a transitory process of grogginess) may take place (Tassi and Muzet, 2000). In winter, probably because of the lack of light in the morning and/or a non-optimal phase to wake up on workdays, an increase in early morning sleepiness is feasible. It has been shown that sleep inertia could be improved with an increasing light signal before waking up (Giménez et al., 2010; Van De Werken et al., 2010). Sleepiness scores on days off during summer and winter do not differ. This could be the result of being exposed upon awakening on days off to higher light intensity levels (later in the morning hours) than during workdays and/or by waking up at a more optimal time.

Animal studies have revealed that information on changes in day length is (partially) encoded in the daily duration of melatonin secretion (i.e., longer duration in the winter months) (Menaker, 1997; Goldman, 2001; Lincoln et al., 2006; Hut and Beersma, 2011). Yet, only one study in humans has reported an increase in the duration of melatonin secretion at night after subjects were exposed to artificial long and short days (Wehr et al., 1993). In the present study no increase in the duration of the melatonin profile in winter was observed. As in the reported literature (Bojkowski and Arendt, 1988; Honma et al., 1992; Vondrasová et al., 1997; Yoneyama et al., 1999; Wehr et al., 2001), we also observe a delay of the melatonin rhythm in winter. The shift of the onset of the melatonin rhythm was similar to the onset of sleep observed on working days, whereas the shift of the offset was larger. From our model on melatonin timing we found that the offset of the melatonin, and not the onset, correlated significantly with the factor season and factor average light exposure. When assessing the different correlational models, the factor season and the factor average light exposure in a certain interval are certainly not independent and the addition of one affects the size, and therefore the significance of the other. Given the small number of subjects, this observation should be taken with caution. Yet, it is in accordance with the idea of humans being better followers of the dawn signal (Roenneberg et al.,

2007). Non-parallel responses to light of the onset and offset of melatonin rhythms, with larger differences for the offset, have been reported previously in rats (Illnerová and Vanecek, 1982) and humans (Buresová et al., 1991; Warman et al., 2003). The differential behavior of the onset and offset of the melatonin rhythm was interpreted as being due to an evening and morning oscillator, respectively (Illnerová and Vanecek, 1982). In the present study we showed that non-parallel responses to light of the onset and offset of melatonin rhythms are also observed under natural conditions.

Recent studies have reported that home-like-light intensities, between 150-200 lux, under laboratory controlled conditions can have a suppressing/delaying effect on melatonin during the same night (Gooley et al., 2011; Santhi et al., 2012). In our study, even during summer, subjects were exposed to intensities below 100 lux already at 18:00 h and up till 9:00 h (figure 1). Given these intensities, it is reasonable to conclude that the timing of the rhythm of melatonin is not different between the home and the lab situation. A circadian system that would be too sensitive to changes in light conditions would not result in a reliable clock. We only observed changes in amplitude of the melatonin rhythms between the dim and the real life light conditions. This was unexpected since at that time of the night, while sleeping, also in the natural situation it was dark. We do not think that the overall reduction in amplitude in the natural light condition is due to a difference in saliva collection methodology, although in this situation saliva was collected at home by the subjects themselves and samples were centrifuged only the next day. It has been shown that it is possible to keep samples at room temperature for at least 1 week without affecting the concentration of melatonin (Weber et al., 1997). Our light exposure model also indicates that midday light relates to the change in melatonin amplitude and not nighttime light. Hashimoto and colleagues observed no significant changes in the amplitude of the melatonin rhythm measured on night 4 after exposure to 3 consecutive days of 5000 lux between 11 h and 17 h as compared to exposure to 200 lux (Hashimoto et al., 1997). More systematic studies are needed in order to understand the relationship between daytime light exposure and melatonin amplitude.

The time spent at light intensities above 1000 lux has been used as an estimation of the time spent outdoors in previous studies (Cole et al., 1995; Guillemette et al., 1998; Goulet et al., 2007; Staples et al., 2009). We observed that on average 2 h 44 min/day were spent above 1000 lux in summer, while in winter, this value was only of about 19 min/day. These results fall within a range similar to previous studies on light exposure in summer and winter (Cole et al., 1995; Guillemette et al., 1998). Correlational models including light at different times of the day, throughout the whole day, showed that light has an effect on top of the effect of season, type of day, and light condition. For instance, afternoon-evening light contributes positively (delay) to the onset of sleep, which is opposite to the overall advancing relationship between summer and sleep onset. In a recent publication similar effects have been reported. It was observed that exposure to

bright light enriched with short wavelength of sufficient intensity during daytime could counteract the advancing effects of moving towards the long days season (Vetter et al., 2011). While increased light exposure during daytime is related to a delay in the onset of sleep, we observed a negative (advance) relationship with DLMO. This could mean that depending on the daytime light intensity, the day-to-day phase angle between sleep and melatonin rhythms may change. The present correlations are based on a small number of subjects. Increasing this number in further studies with an experimental design especially suitable to test the effects of daytime light exposure on sleep- and melatonin rhythms is desirable. If a causal relationship exists between phase of entrainment and day-to-day light exposure, this could have clear implications for light strategies to improve entrainment.

Aknowledgments

The authors thank Prof. Dr. Serge Daan for his insightful comments on the manuscript and Bühlmann laboratories AG for the direct saliva melatonin radioimmunoassay tests provided for this study. Our work is supported by the 6th Framework Project euclock (No. 0187410). §

Chapter 3

Effects of Artificial Dawn on Subjective Ratings of Sleep Inertia and Dim Light Melatonin Onset

Marina C. Gimenéz[1], Martijn Hessels[2], Maan van de Werken[1], Bonnie de Vries[1], Domien G. M. Beersma[1], Marijke C. M. Gordijn[1]

[1]*Department of Chronobiology, Center for Life Sciences, University of Groningen, The Netherlands and*
[2]*Medical Design Engineering, Amsterdam, The Netherlands*

Chronobiology International (2010), 27(6), 1219–1241.

Abstract

The timing of work and social requirements has a negative impact on performance and well-being of a significant proportion of the population in our modern society due to a phenomenon known as social jetlag. During workdays, in the early morning, late chronotypes, in particular, suffer from a combination of a nonoptimal circadian phase and sleep deprivation. Sleep inertia, a transient period of lowered arousal after awakening, therefore, becomes more severe. In the present home study, the authors tested whether the use of an alarm clock with artificial dawn could reduce complaints of sleep inertia in people having difficulties in waking up early. The authors also examined whether these improvements were accompanied by a shift in the melatonin rhythm. Two studies were performed: Study 1: three conditions (0, 50, and 250 lux), and Study 2: two conditions (0 lux and self-selected dawn-light intensity). Each condition lasted 2 weeks. In both studies, the use of the artificial dawn resulted in a significant reduction of sleep inertia complaints. However, no significant shift in the onset of melatonin was observed after 2 weeks of using the artificial dawn of 250 lux or 50 lux compared to the control condition. A multilevel analysis revealed that only the presence of the artificial dawn, rather than shift in the dim light melatonin onset or timing of sleep offset, is related to the observed reduction of sleep inertia complaints. Mechanisms other than shift of circadian rhythms are needed to explain the positive results on sleep inertia of waking up with a dawn signal.

Introduction

The 24-h sleep-wake cycle is controlled by circadian and homeostatic processes (Borbély, 1982; Daan et al., 1984). However, large individual differences are found between human subjects in their preferred (Tonetti et al., 2008) or actual sleep timing (Horne and Ostberg, 1976; Roenneberg et al., 2003). These differences are referred to as chronotypes. Chronotypes can be classified as early, intermediate, and late. Extreme early types are characterized by going to bed and waking up early, especially during free days (22:00 - 06:00 h), whereas late types do the opposite (04:00 - 12:00 h; based on Dutch general population data Zavada et al., 2005). Nowadays, work and social requirements impose difficulties, especially for subjects in both extreme ends of the distribution. This misalignment between internal and external timing is known as social jetlag. The amount of social jetlag correlates significantly with mental distress, and unhealthy behaviors, such as the tendency to smoke and to consume alcohol (Wittmann et al., 2006).

Early and late types not only sleep earlier and later, respectively, than intermediate types, but they also exhibit shifted rhythms in physiological and psychological parameters (Baehr et al., 2000; Duffy et al., 1999; Kerkhof and Van Dongen, 1996) as well as in alertness and mood (Kerkhof, 1998; Kerkhof and Van Dongen, 1996). Subjective alertness and calculation performance have been shown to cycle in a circadian manner (Johnson et al., 1992; Monk et al., 1985). As a possible consequence of the differences in phase, performance in the early hours is especially impaired in late chronotypes, whereas the opposite occurs in early types. Impaired performance, confusion, and sleepiness in the early morning after waking up are states that are experienced by most people to some extent. This transient period after sleep is known as "sleep inertia" (Akerstedt and Folkard, 1997; Dinges, 1990; Tassi and Muzet, 2000). Depending on sleep timing and sleep phase upon awakening, sleep inertia may differ (see review of Tassi and Muzet, 2000). However, it seems that the main factor influencing sleep inertia is the preceding amount of sleep (Achermann et al., 1995; Jewett et al., 1999). Sleep-inertia severity is increased under sleep-deprivation conditions (Balkin and Badia, 1988; Dinges et al., 1985). Subjects getting up early on work days at their non-optimal circadian phase and after being sleep deprived during previous working days may suffer from a combination of detrimental factors causing severe sleep inertia.

Lack of morning light during winter days may also worsen sleep-inertia complaints due to the absence of the phase-advancing stimulus of morning light (Beersma

and Daan, 1993; Gordijn et al., 1999; Honma, 1988; Khalsa et al., 2003; Minors et al., 1991) and the lack of its acute alerting effect (Cajochen et al., 2000; Campbell et al., 1995; Rüger et al., 2006; 2003). Because of the impacts that sleep inertia may have in our society when high performance and alertness are required in the early morning, diminishing complaints of sleep inertia is of great interest.

The two studies described here investigate whether it is possible, in a natural home set up, to reduce sleep-inertia complaints in persons who have difficulties waking up early by means of an artificial dawn during the dark winter months. Based on the hypothesis that a late circadian phase is one of the factors causing these difficulties in waking up in the morning by later types, it is also tested whether improvements are accompanied by a shift in the melatonin and/or the sleep-wake rhythm.

Methods

Subjects

Subjects, who were recruited by advertisement at the University of Groningen and public places, had to have a regular life style consisting of at least four days/wk when they had to rise earlier than on free days. They completed the Munich Chronotype Questionnaire (MCTQ, Roenneberg et al., 2003) as part of the selection process. From this questionnaire, data were obtained on sleep habits on work and free days. Other main selection criteria, also obtained from this questionnaire, were self-reported need for ≥60 min after awakening to feel fully alert in the morning and not to nap. After a general health screening by means of questionnaires, 92 subjects (51 and 41 subjects for Study 1 and 2, respectively) who did not suffer from winter depression (BDI-II-NL ≤8; Beck et al., 1996) enrolled in this home study. Subjects suffering from somatic and/or sleep disorders, or who used sleep medication or other drugs, were excluded

The study protocol was approved by the Medical Ethics Committee of the University Medical Center of Groningen, The Netherlands and conformed to international ethical standards (Portaluppi et al., 2008). All subjects signed a written informed consent form prior to their participation.

Study 1

Thirty-six subjects participated in the months of November-December 2006 (sunrise range: 07:32-08:49 h., sunset range: 16:25 - 17:02 h). The remaining 15 subjects participated in the months of January-February 2007 (sunrise range: 07:25-08:48 h, sunset range: 16:26-18:09 h). Although the number of subjects who dropped out was low (n = 3), several subjects failed to follow the protocol, for example; not all the questionnaires were completed for all conditions. Thus, complete data sets for all measured variables were obtained only from 23 subjects.

Study 2

Forty-one subjects participated in the months of January-February 2007 (sunrise

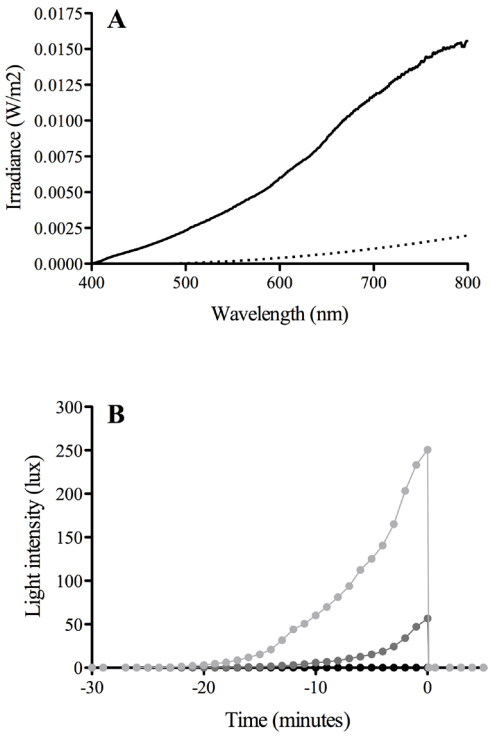


Figure 1 Wake-up light characteristics. (A) Irradiance (W/m2) as a function of wavelength (nm) of the wake-up light set at 250 lux and recorded at 40 cm distance (straight line) and after the filtering effects of the eyelid (dashed line) derived from Moseley et al. (1988). (B) Light intensity as a function of time during the 30 min before the alarm (alarm time = 0), for the modified wake-up light where the light turns off at the time of the alarm. Light grey circles: 250 lux; dark grey triangles: 50 lux; black circles: 0 lux.

range: 07:25-08:48 h, sunset range: 16:26-18:09 h). Because of violation of the protocol complete data sets were obtained only from 23 subjects.

Experimental Design

Instructions to subjects were given personally during a session when the experimental set up and methods of data collection were explained. After giving consent, subjects received an artificial dawn Wake-up Light with an incandescent 100W E27 Philips SOFTTONE softwhite 230 V T55 lamp (see Figure 1A for spectral composition) (Philips Wake-up Light by Philips DAP B.V., CoC Vitality Care, Drachten, The Netherlands) to be used at home. The increase in light intensity follows an exponential function where the ratio of the step to the absolute intensity is constant. The very beginning of the curve follows a linear function starting with 0 lux. Light intensity increases every 10 ms. The smallest step size is in the order of 0.01 lux, whereas the largest is in the order of 2.5 lux (Figure 1B). The experiment was conducted during the wintertime to avoid large differences in exposure to external natural light after waking up between conditions. Two different studies served to test the effects of this home-light system in subjects with difficulties in waking in the morning.

STUDY 1

The aim of Study 1 was to assess whether changes in sleep-inertia complaints depend on light intensity. Secondly, it was tested if those changes were accompanied by a phase advance of the biological clock estimated by the dim light melatonin onset (DLMO).

In a home study, a modified Wake-up Light (modified Philips Wake-up Light by Philips DAP B.V., CoC Vitality Care, Drachten, The Netherlands) was used by the subjects for 42 days (6 wks). In a within subject design, the maximum intensity of light reached during the 30 min dawn signal was varied every 2 wks between 0 lux (control,

no dawn signal), 50 lux (medium), or 250 lux (high). The order of the conditions was randomized between subjects. At the subject's defined time, this maximum intensity was reache and an audible alarm sounded. To avoid differences in light exposure after waking up between conditions within subjects, the Wake-up Light was modified so that light was turned off automatically when the audible alarm sounded. The snooze function was disabled.

Each condition lasted exactly 14 days. Within this period, subjects were free to either use or not use the artificial dawn, depending on whether they did or did not need an alarm clock. However, subjects were instructed to start and end this 14-day period during a working week, so assessment of the effects of the use of the artificial dawn took place during a span of structured social routine. On average (±SD) the artificial dawn was used 11.8 ± 1.7 days.

Study 2

Study 2 was conducted to investigate the range of light intensities preferred by people and whether those intensities, in comparison to an alarm -wake-up only (no dawn signal), led to a decrease of sleep-inertia complaints. A non-modified artificial dawn Wake-up Light with the snooze function available (Philips Wake-up Light by Philips DAP B.V., CoC Vitality Care, Drachten, The Netherlands) was used for 28 days (4 wks). Subjects were asked to select the intensity with which they felt most comfortable, ranging from 20 up to 400 lux. They were asked to determine their preferred intensity within the first three days and to retain it for the rest of the experimental condition. In a randomized order, subjects used for 2 wks either the self-selected intensity (dawn condition) or the 0 lux intensity (control condition, no dawn). As in Study 1, subjects were free to use or not use the artificial dawn, depending on their own needs, but were instructed to start and end the 14-day period during a span of structured social routine. On average (±SD) the artificial dawn was used 11.5 ± 2 days.

Measurements

Sleep-inertia duration and severity, well-being, and sleep parameters

Sleep inertia was characterized by means of subjective ratings, both prospectively and retrospectively. Sleep-inertia duration was defined as the amount of time required per subject to feel fully awake. An evaluation form was developed for the purpose of assessing general well-being. Several parameters, that is, wake-up quality, easy rising, energetic feeling, mood after waking up, social interactions, concentration, and productivity, relevant to describe general well-being and often used in chronobiological studies, were assessed (Norden & Avery, 1993). For each parameter a 1 to 10 rating was obtained, 1 being very bad and 10 being excellent. Sleep-inertia duration and general well-being were assessed retrospectively at the end of every 2-wk period.

The Karolinska Sleepiness Scale (KSS), an often-used questionnaire that has been validated against EEG parameters (Åkerstedt and Gillberg, 1990; Kaida et al.,

2006), was completed daily 5 and 30 min after rising. The KSS ranges from 1 to 9; the higher the reported value the greater the sleepiness. These values were used as a prospective measurement of sleep-inertia severity, that is, how sleepy the person felt within the first 30 min when the feelings of sleep inertia are common (Tassi and Muzet, 2000). The Groningen Sleep Quality Scale (GSQS; (Leppämäki et al., 2003) was also completed daily 30 min after awakening. The GSQS ranges from 0 to 14; the higher the value, the poorer the sleep quality.

Sleep timing, namely, bedtime, sleep onset, alarm time, sleep offset, and get-up time, was recorded daily. This allowed us to check for a regular sleep-wake schedule and to record the timing of the artificial dawn. For the present study, sleep offset is of particular interest due to its sensitivity to the phase-advancing effects of morning light (Gordijn et al., 1999).

The sleep diaries, including both prospective and retrospective measurements, were returned to the investigators at the end of every 2-wk condition. In this way, subjects did not have access to the estimations made in previous conditions.

Melatonin

In Study 1, subjects collected saliva samples at home at the end of every 2-wk condition to assess whether changes in sleep-inertia complaints were accompanied by a shift of the dim-light melatonin onset (DLMO). Saliva samples were collected hourly starting 5 h before subject's habitual bedtime and continued for 1 h after it. In total, saliva samples were collected at seven timepoints per experimental condition. Subjects were carefully instructed about the requirements of collecting saliva. No chocolate, bananas, artificially colored sweets, coffee, or tea were allowed during measurements. Eating and drinking were restricted to 15 min after the collection of saliva, and 45 min before each sample subjects were instructed to rinse their mouths with water. Brushing teeth with toothpaste was not allowed. Subjects were also asked to expose themselves to as little light as possible by keeping the curtains closed, using only small light bulbs, and wearing sun-glasses inside, commencing 1 h before the first sample was taken. Watching TV was allowed at a distance of ≥2 meters. Postural changes were not allowed during the 10 min period before and during saliva collection.

Saliva was collected using Sarstedt Salivettes with a cotton swab (Sarstedt B.V. Etten-Leur, The Netherlands). Samples were stored at 4°C until sent to our lab (the period between collection and arrival in the lab was ≤1 wk). Once the samples arrived, they were stored at -20°C. Melatonin concentration was assessed by radio-immunoassay (RK-DSM, Bühlmann laboratories AG, Siemens Medical Solutions Diagnostics, Breda, The Netherlands). All samples from one individual were analyzed within the same series.

Data Analysis

Sleep-inertia duration and severity, well-being, and sleep parameters

Depending on their obligations, subjects were free to choose when to use the artificial

dawn alarm clock. The analysis of the subjective ratings was conducted only for those days when subjects used the alarm clock. To avoid possible bias, data analysis was conducted on those subjects who completed all the three conditions for all the measured variables (n = 23). To check for robustness of the data, it was assessed whether the general pattern for each particular parameter remained if data of all available subjects were included.

Melatonin

The limit of detection for the RIA was 0.3 pg/ml with an intra-assay variation of 6.7% at a low melatonin concentration (mean = 1.5 pg/ml, n = 30) and 6.5% at a high melatonin concentration (mean = 15 pg/ml, n = 30). Inter-assay variation was 12.2% at low melatonin concentration (mean = 2.1 pg/ml, n = 15) and 19.7% at high melatonin concentration (mean = 17.5 pg/ml, n = 16).

The DLMO was used as a phase marker. To avoid differences in the DLMO between subjects due to variation in total amount of melatonin production, melatonin levels were normalized within subjects to the maximum value attained during any condition. DLMO was defined as the clock time when the melatonin values crossed a threshold of 15% of the maximum concentration. This value was chosen on the basis of a frequency analysis of percentage melatonin. It was the timepoint when changes in melatonin concentration became apparent (after 15% melatonin concentrations only increased up to their maximum and then started to decrease). DLMO was determined by linear interpolation between the last sample with a lower concentration and the first sample with a higher concentration than the threshold value.

Statistics

The use of the artificial dawn was expected to reduce sleep-inertia duration and severity in comparison with the non-dawn simulation condition (0 lux), as well as to improve well-being and sleep quality. Non-parametric tests were conducted due to the non-normal distribution of the sleep-inertia variables. In Study 1, one-tailed Friedman-tests were conducted to assess the main effects of condition, the main effects of time, and the interaction between both. In order to have an approach conceptually similar to a repeated-measures ANOVA (a test generally used to deal with this type of design), data were treated as follows. Data were averaged over conditions to investigate the main effect of time, and data were averaged over time to investigate the main effect of condition. After finding a significant main effect, comparisons between conditions were performed. In Study 2, a one-tailed Wilcoxon test was conducted. All tests were performed with $\alpha = 0.05$

The use of the artificial dawn was expected to advance the DLMO and/or sleep offset. A repeated-measures ANOVA was used to test for significant differences in DLMO and sleep timing between conditions in Study 1. The effects of the use of the artificial dawn in Study 2 as well as sleep timing were tested by means of a paired-t test.

To determine the role of DLMO and sleep offset as an alternative or additionally to the main effects of light on sleep-inertia duration, a multilevel analysis was conducted by means of MLwiN software (Centre of

Multilevel Modeling, Institute of Education, London, UK). The following model equation was used:

$$\text{Sleep-inertia duration} = \beta 0_{ij} + \beta 1_{ij} * \text{Condition} + \beta 2_{ij} * \text{DLMO} + \beta 3_{ij} * \text{SleepOffset}$$

where β0 represents the model intercept, β1 the main effect of condition, β2 the effect of DLMO (only tested in Study 1), and β3 the effect of the timing of sleep offset. ßs correspond to the slope of the correlations between the Y and X variables. The model takes into account the hierarchy of the protocol consisting of i = conditions nested in j = subjects. The regression coefficients were tested with a z-test.

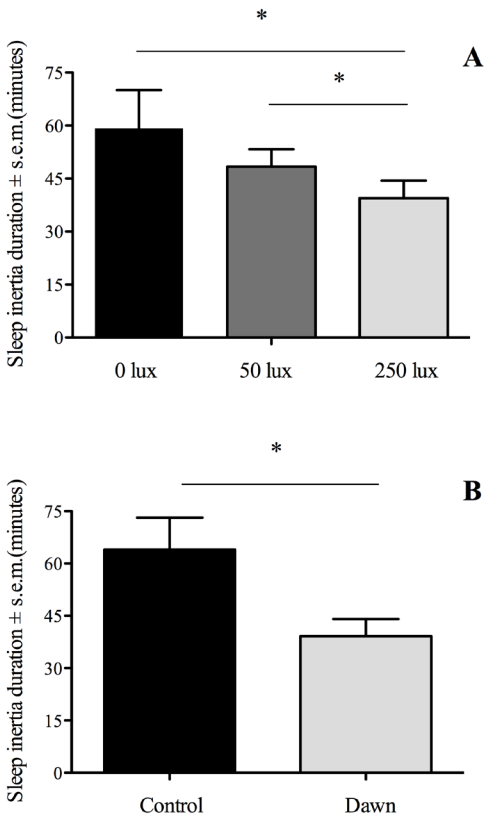


Figure 2 Sleep-inertia duration. Mean ± SEM values of subjective sleep-inertia duration measured as the amount of minutes needed to feel fully awake after waking up. (A) Study 1: significant 19.7-min and 8.9-min decreases were found between the 250 lux and the 0 lux (control) conditions, and between the 250 lux and 50 lux conditions, respectively. (B) Study 2: a significant 24.8-min decrease was found between the artificial dawn (self-selected intensities) and 0 lux (control) conditions.

Results

Subjects

The average mid-sleep time on free days (MSF) has been used to define chronotype (Roenneberg et al., 2003). The MSF observed among the 46 subjects (21 males/25 females, average age ± SD: 30 ± 11 yrs) who completed all conditions was on average (± SD) 04:56 h ± 49 min and 05:04 h ± 1:06 h for Study 1 and Study 2, respectively. Mid-sleep on work days (MSW) was on average (± SD) 03:15 h ± 41 min and 03:38 h ± 48 min for Study 1 and Study 2, respectively. Social jetlag, defined as the difference between mid-sleep on free and work days, was on average (± SD) 1:40 h ± 56 min (Study 1) and 1:26 h ± 39 min (Study 2), being relatively long. On average (± SD), subjects awoke 2:09 h ± 50 min later on free days.

Sleep-Inertia Duration and Severity, Well-Being, and Sleep Parameters

Study 1

Sleep inertia duration was estimated retrospectively as the amount of time needed to feel fully awake. Overall, a significant effect of condition was found ($n = 23$, $X^2 = 6.844$, $p < 0.05$). Further analysis revealed a significant reduction of sleep-inertia duration

Table 1A. Summary data on sleep inertia severity

	0 lux	50 lux	250 lux	*Average per time point*
5 min after rising	7. 07 ± 0.26	6.83 ± 0.23	6.92 ± 0.23	6.94 ± 0.21[a]
30 min after rising	5.36 ± 0.28	4.97 ± 0.21	5.19 ± 0.22	5.17 ± 0.22[b]
Average per condition	6.22 ± 0.26[*]	5.89 ± 0.12[§]	6.06 ± 0.21[*]	

Data shown as average ± SEM. Sleepiness ratings as obtained by KSS.

[a,b]Significantly different from each other ($p < 0.001$). [*,§]Significantly different from each other ($p < 0.001$). No significant interaction was found between condition and time ($p = 0.32$).

Table 1B. Summary data on sleep inertia severity

	0 lux	Artificial Dawn	*Average per time point*
5 min after rising	6.85 ± 0.18	6.43 ± 0.25	6.64 ± 0.12[a]
30 min after rising	5.26 ± 0.23	4.69 ± 0.23	4.98 ± 0.21[b]
Average per condition	6.05 ± 0.18[*]	5.56 ± 0.22[§]	

Data shown as average ± SEM. Sleepiness ratings as obtained by KSS.

[a,b]Significantly different from each other ($p < 0.001$). [*,§]Significantly different from each other ($p < 0.001$). No significant interaction was found between condition and time ($p = 0.1$).

of 19.7 min between the control and 250 lux condition ($n = 23$, $z = -2.311$, $p < 0.01$), and a smaller, but significant, reduction of 8.9 min between the 250 lux and 50 lux condition ($n = 23$, $z = -2.030$, $p < 0.05$). A 10.8 min difference, although not statistically significant, was found between the 50 lux and control condition ($n = 23$, $z = -0.769$, $p = 0.22$) (Figure 2A). When considering the maximum number of subjects, the significant effect of condition remained ($n = 33$, $X^2 = 7.600$, $p < 0.05$). Although differences in sleep-inertia duration became a bit smaller (15.8 min, 8.8 min, and 7.1 min between 250-0 lux, 250-50 lux, and 50-0 lux, respectively), the statistical significances between conditions remained the same ($z = -2.612$, -2.228, and -0.628, $p < 0.005$, $p < 0.05$, and $p = 0.26$ for the differences between 250-0 lux, 250-50 lux, and 50-0 lux, respectively).

Sleepiness scores obtained 5 and 30 min after waking-up by means of the KSS were used as an estimation of sleep-inertia severity (Table 1A). A significant main effect

of time was found. Sleepiness was reduced 30 min compared to 5 min after waking-up for all conditions considered together ($n = 23$, $z = -4.197$, $p < 0.001$). This change over time represents the expected reduction of sleep inertia in the period shortly after waking up. A significant effect of condition was also found ($n = 23$, $X^2 = 10.783$, $p < 0.001$). Further comparisons between conditions showed a significant reduction in sleep-inertia severity by the 50 lux compared to the control condition ($n = 23$, $z = -2.220$, $p < 0.05$) and compared to the 250 lux condition ($n = 23$, $z = -1.780$, $p < 0.05$). No differences were found between the 250 lux and the control condition. There was no significant interaction between condition and time ($n = 23$, $X^2 = 2.264$, $p = 0.32$). The significant main effects of time and condition remained for the maximum number of subjects that completed the questionnaires ($n = 42$, $z = -5.646$, $X^2 = 8.491$, $p < 0.001$, $p < 0.005$ for time and condition, respectively). Again, only the 50 lux condition was significantly different from the control condition ($n = 42$, $z = -1.888$, $p < 0.05$).

From the variables selected to assess general subjective well-being, waking-up quality, easy rising, energetic feelings, mood, social interactions, and productivity were significantly improved in the artificial dawn condition compared to the control condition ($n = 23$, X^2 for all variables between 6.677 and 12.030; both 50 and 250 lux, $p < 0.05$). No significant differences were observed between the two light intensities (Figure 3A). When considering the maximum number of subjects, the parameters social interactions, concentration, and productivity were no longer significantly different when the artificial dawn condition was compared to the control ($n = 33$, X^2 between 3.022 and 3.639; $p = 0.16$, $p = 0.10$, and $p = 0.11$, respectively). The pattern remained the same for the other variables.

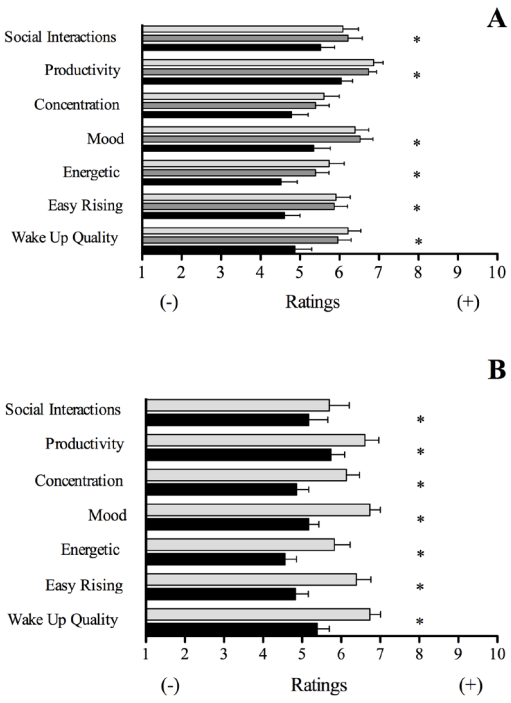


Figure 3. General well being. Mean ± SEM values for subjective ratings on different parameters to assess general well-being after waking up. (A) Study 1: a significant improvement in waking up quality, easy rising, energy, and mood was found in by the use of the artificial dawn at any light condition. Black, dark grey, and light grey columns represent the control, 50 lux, and 250 lux conditions, respectively. (B) Study 2: a further improvement in social interactions, concentration, and productivity was observed in the artificial dawn (self-selected intensities, light grey columns) condition as compared to the 0 lux (control, black columns) condition.

Sleep quality (GSQS) was relatively good in all conditions ($n = 23$, average ± SEM: 3.4 ± 0.2, 3.6 ± 0.3, 3.2 ± 0.3 for the 0, 50, and 250 lux condition respectively). No significant effects resulted from the use of

the artificial dawn alarm clock at any of the intensities compared to the control condition ($n = 23$, $X2 = 1.826$, $p = 0.2$). No significant differences were observed when considering the maximum number of subjects ($n = 42$, average ± SEM: 3.4 ± 0.3, 3.5 ± 0.2, 3.4 ± 0.2 for the 0, 50, and 250 lux condition, respectively).

Bedtime and sleep offset did not differ significantly between conditions (average ± SD: bedtime: 0 lux = 22:44 h ± 46 min, 50 lux = 23:11 h ± 56 min, 250 lux = 23:13 h ± 52 min; sleep offset: 0 lux = 07:09 h ± 42 min, 50 lux = 07:22 h ± 58 min, 250 lux = 07:04 h ± 43 min; $F = 2.512$, $p = 0.105$). Exposure to the artificial dawn always occurred during the dark span before sunrise, and its timing was not significantly different between conditions. The artificial dawn signal started on average (± SD) at 06:41 h ± 44 min for 0 lux , 06:30 h ± 49 min for 50 lux , and 06:41 h ± 45 min for 250 lux ($F = 0.502$, $p = 0.613$).

The multilevel analysis confirmed the significant main effect of condition on the reduction of sleep-inertia duration (ß1 estimate: -0.08 ± 0.04 min/lux, $p<0.05$). In addition, sleep offset was negatively and significantly related to sleep-inertia duration (ß3 estimate: -10.57 ± 5.33 min/h $p < 0.05$), indicating that the later people awoke the less they suffered from sleep inertia. The multilevel analysis revealed no effect of DLMO on the observed sleep-inertia duration (ß2 estimate: 6.03 ± 4.68 min/h, $p = 0.2$).

STUDY 2

In this study subjects chose their own individually preferred light intensity. The chosen light intensities ranged between 120 and 400 lux. On average, when considering the advised 40 cm distance to the artificial dawn alarm clock, the chosen light intensity was 264.7 ± 85.8 lux. The median light intensity was 280 lux.

Sleep-inertia duration, measured as the time needed to feel fully awake, was significantly decreased by 24.8 min in the dawn compared to the control condition (Figure 2B; $n = 23$, $z = -2.827$, $p < 0.001$). The effects on sleep-inertia duration remained when considering the maximum number of subjects available for this analysis ($n = 25$, $z = -3.138$, $p < 0.001$) and amounted to a difference of 25.8 min.

Sleep-inertia severity was estimated by means of the KSS at 5 and 30 min after waking-up (Table 1B). A significant main effect of time was found. Sleepiness was lower after 30 min compared to 5 min after waking-up ($n = 23$, $z = -4.199$, $p < 0.001$). The use of the artificial dawn at a self-selected intensity significantly reduced the complaints of sleep-inertia severity ($n = 23$, $z = -2.566$, $p < 0.005$). There was no significant interaction between condition and time ($n = 23$, $z = -1.620$, $p = 0.1$). The overall effects of time and condition were still present when considering the maximum number of subjects ($n = 26$, $z = -4.459$, 3.055, $p < 0.001$ and $p < 0.001$ for time and condition, respectively). Figure 3B shows the subjective measurements of well-being. Use of the artificial dawn lead to an improvement of all variables ($n = 23$,

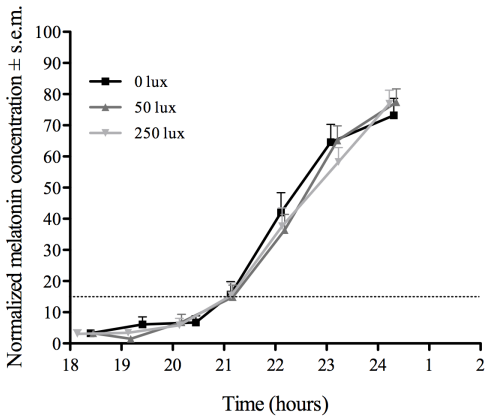


Figure 4. Melatonin profiles. Average melatonin profiles ± SEM for the three conditions. DLMO ± SD measured at 15% level (dotted line) showed no significant differences between conditions. Average DLMO (± SD) was 21:23 h ± 58 min for the control condition, 21:25 h ± 52 min for the 50 lux condition, and 21:23 h ± 57 min for the 250 lux condition.

z values between -2.194 and -3.265, all $p < 0.05$) except "social interactions" ($n = 25$, $z = -1.414$, $p = 0.08$). The results remained the same when calculated over the maximum number of subjects.

In Study 2, sleep quality was also relatively good in both conditions (mean ± SEM: 3.6 ± 0.3 and 3.1± 0.3 for the control and artificial dawn condition, respectively). Sleep quality did not significantly improve with the use of the artificial dawn ($z = -1.543$, $p = 0.06$). When considering the maximum number of subjects, the observed tendency becomes significant. The use of the artificial dawn improved, although to a small extent, the quality of sleep ($n = 26$, average ± SEM: 3.7 ± 0.3 and 3.1 ± 0.3 in the experimental and control condition, respectively, $z = -1.844$, $p < 0.05$).

Bedtime did not differ significantly between conditions (average ± SD: 0 lux = 23:13 ± 1:19 h versus artificial dawn = 23:37 h ± 57 min). Sleep offset was earlier in the artificial dawn condition (average ± SD: artificial dawn = 07:22 h ± 55 min versus control = 07:32 h ± 1:07 h, $p < 0.05$). Exposure to the artificial dawn occurred always during the dark before sunrise, and the timing did not differ significantly between conditions. The artificial dawn signal started on average (± SD) at: 06:59 h ± 51 min (0 lux) and 06:47 h ± 41 min (artificial dawn).

Multilevel analysis confirmed the significant main effect of condition on sleep-inertia duration. Use of the artificial dawn reduced sleep-inertia duration (ß1 estimate: -24.74 ± 10.25 min/lux, $p < 0.05$). Although sleep offset was earlier in the artificial dawn condition, when added to the model the timing of sleep offset did not contribute significantly to the observed sleep-inertia duration (ß2 estimate: 0.5 ± 5.38 min/h, $p = 0.93$).

Melatonin

Saliva samples were received from 41 subjects. However, nine subjects either did not produce complete data sets ($n = 5$) or the expected pattern in melatonin levels was not found ($n = 4$), suggesting these subjects did not follow the protocol. The curves were either completely flat with mostly zeros, started at high levels and became lower with time (opposite direction), or fluctuated rather randomly crossing the DLMO criterion value repeatedly. Only those 23 subjects that completed the subjective ratings on sleep inertia and well-being were included in the analysis. Figure 4 shows the average normalized melatonin curves for the three conditions.

The average DLMO did not differ significantly between the three conditions (average DLMO ± SD: 0 lux = 21:23 h ± 58 min; 50 lux= 21:25 h ± 52 min; 250 lux = 21:23 h ± 57 min; F = 0.039, p = 0.49). Time-of-year (first period in November/December, second period in January/February) when the samples were collected had no significant effect on the DLMO (between subjects comparison: F = 0.083, p = 0.77) or on the effect of the artificial dawn on the DLMO (F = 0.387, p = 0.68).

Discussion

By definition, sleep inertia is a severe subjective feeling of sleepiness and grogginess upon awakening (Tassi and Muzet, 2000); therefore, human subjects are the best models to investigate how these symptoms can be affected. In the present home study, we assessed the effects of the artificial dawn on sleep inertia in healthy subjects. Home studies may have disadvantages because of the absence of direct control over conditions and measurements. However, it constitutes a natural set up to assess the potential effects of this artificial dawn system in the way it will ultimately be used. Being a light device available on the market, studying its effects is of great relevance. In addition, the results of the present two studies provide insight on the awakening process.

For this purpose, subjects who suffered from sleep inertia were selected. The average MSF of the participating subjects was ~29 min later than the average found for a matched-aged-group of the Dutch population (Zavada et al., 2005). This can easily be understood by the fact that the later the chronotype, the more difficult it becomes to wake up early during workdays (Roenneberg et al., 2003). By waking up after being sleep deprived due to previous working days and after a relatively short sleep, late chronotypes are particularly prone to severe symptoms of sleep inertia during the early hours of the day. Therefore, although we did not choose for late chronotypes, due to our selection criteria, of ≥60 min needed to feel fully awake, later chronotypes are over-represented in our sample.

In the present study, as expected, sleep inertia was shown to decrease with time (Achermann et al., 1995; Jewett et al., 1999; Wertz et al., 2006). Moreover, also in accordance with previous studies in people suffering from winter depression (Avery et al., 2002) and subsyndromal winter depression (Norden and Avery, 1993), exposure to a 30 min artificial dawn signal before the alarm sounded led to lower subjective ratings of sleep inertia and to improvement of general well-being. Interestingly, self-selected intensities in combination with lights-on at the time the alarm went off (Study 2) led to an even larger decrease of sleep-inertia complaints. Light intensity, however, cannot explain the strengthening of the results. In Study 2, the chosen intensity (264.7 lux on average) was rather similar to the high light intensity used in Study 1 (250 lux). Persistence of light after the alarm went off, on the other hand, could explain this finding. Light is known to have direct activating and alerting effects both at night and during the daytime (Cajochen, 2007; Cajochen et al., 2000; Phipps-Nelson et al., 2003; Rüger et

al., 2006). Waking up in a dark in contrast to an illuminated room might increase subjective ratings of sleep inertia.

All retrospective and longitudinal measurements of sleep inertia showed an improvement in the artificial dawn condition. The congruency between these analyses is generally interpreted as supporting evidence of the findings. A dose-response relationship for the different light intensities, however, was only visible for the sleep-inertia duration measurements. Although measured on a daily basis, this suggests that the KSS might have not been sensitive enough to detect dose-dependent changes in sleep-inertia severity. The KSS is limited to a restricted range of values (Åkerstedt and Gillberg, 1990). In measuring sleep-inertia duration, on the other hand, there is more freedom to select for the number of minutes needed to feel fully awake, which could allow for more sensitive measurements. Similarly, no significant differences were detected between the 50 and 250 lux condition in the assessment of well-being. These results, together with the larger effect found in the second study in which subjects were asked to find their preferred intensities, seem to indicate that the mere presence of light does lead to beneficial results. Preferred intensities were ~250 lux, and although this intensity might not be necessarily needed to reduce the symptoms, the duration of sleep inertia after waking up was shortened with higher intensities.

Most studies conducted with dawn simulators were developed as an alternative to bright-light treatment for seasonal affective disorders (SAD). Interestingly, it has been shown that a simulated dawn was more effective than a square-wave-bright-light stimulus (light-on/lights-off) in treating SAD patients (Avery et al., 2001; Terman and Terman, 2006). This suggests that light exposure before consciously waking up exerts some effect that cannot be achieved even with exposure to bright light after awakening. The mechanism by which artificial dawn signals might work could be related to the gradual increase of light intensity, allowing for a gradual wake up in contrast to lights-on/lights-off. It has been shown that an abrupt wake up can negatively influence sleep inertia (Dinges, 1990; Dinges et al., 1985). Although we did not test the effects of lights–on/lights-off, most participating subjects experienced the artificial dawn during the winter mornings in a positive way. When subjects were asked for an internal evaluation to compare the use of the artificial dawn alarm clock with their normal alarm clock, by means of a 1 to 5 scale (1: worse and 5: better), 27% chose 5, 45% chose 4, 19% chose 3 (no difference), 6% chose 2, and 3% chose 1. Because the major part of the exposure to the natural dawn signal occurs while sleeping, with eyelids closed (Beersma et al., 1999) an alternative (or complementary) mechanism by which dawn signals could exert an effect is due to the transmittance characteristics of the eyelids; only light of longer wavelengths is transmitted (Ando and Kripke, 1996; Moseley et al., 1988).

During the past decade a new photoreceptor key in non-image-forming responses called the intrinsically photosensitive retinal ganglion cells (ipRGCs) was discovered (Berson et al., 2002; Hattar et al., 2002). Interestingly melanopsin, the photopigment

found in the ipRGCs, shows two states: the 11-cis-retinal state (rhodopsin, R state) and the all-trans-retinal state (metarhodopsin, M state). Under broadband natural or artificial light exposure, these two states exist in equilibrium. However, under monochromatic light exposure, it was shown that while especially short wavelengths initiated the phototransduction cascade (from R to M), long wavelengths could restore responsiveness by regeneration of the M to the R states (Melyan et al., 2005; Mure et al., 2009). The light intensities used in Mure's study to drive the M-state back to the R-state were quite high. Exposure to long wavelengths during dawn after a full night of darkness, however, could shift the equilibrium of the M and R states to a higher sensitivity for short wavelengths after waking up or even before, during the regular arousals that occur during sleep (Gordijn et al., 1999). More studies on how different mono- and polychromatic light sources can modulate melanopsin-dependent non-image-forming responses are needed.

A limitation of our study is the lack of performance measurements during the sleep-inertia period. In a laboratory study by our group using the same device during one day, similar improvements of sleepiness, but no clear effects on a simple reaction-time or addition task, were found (Van De Werken et al., 2010). In future field studies, it would be interesting to test whether other measures of sleep inertia, for instance more complex reaction-time performance, grip strength, or cognitive functioning, are improved with the long-term use of the artificial dawn. We cannot exclude a placebo effect of the use of the artificial dawn to explain the improvements in subjective ratings (Eastman, 1990b). In studies using light, subjects are always aware of the treatment. However, neither the existence of dose-response effects nor extra measurements can ever rule out the possibility of a placebo effect underlying the observed differences.

We hypothesized that the possible improvements in subjective ratings of sleep inertia could have been due to a shift towards a more optimal phase of the circadian system. Although exposure to the artificial dawn occurred during the advance portion of the PRC, ~9:22 h after the DLMO (Khalsa et al., 2003), no significant differences were found in the DLMO between conditions. One could argue that this lack of detection is due to the resolution of our saliva sampling frequency (one sample/h). However, because we were not able to detect even a trend, this is unlikely to be the case. An alternative possibility is that exposure to evening light prohibited a phase advance. Although season was not a factor, there was no difference in effects on the DLMO between the darker 1st period and the maybe somewhat more evening light-containing 2nd period, exposure to artificial light in the evening could, indeed, have prevented a phase advance to occur. Nevertheless, the main question whether a phase shift accompanies an improvement of sleep inertia can still be answered with "no", irrespective of the reason that no phase advance was observed. Earlier studies found a shift in the DLMO after exposure to an artificial dawn signal (Danilenko et al., 2000; Terman et al., 1989). The discrepancy with these studies can be easily explained by the differences in the experimental set up, in

the light intensities used, and/or in the duration of the artificial dawn signal. In our study, light was not only in the low range of intensities (Zeitzer et al., 2005), but it was also shifted to the long wavelength range of the visible spectrum. It has been extensively shown that the circadian system is more sensitive to short wavelengths (Brainard et al., 2001; Cajochen et al., 2005; Lockley et al., 2003; Revell et al., 2005a; 2006; Thapan et al., 2001). Higher intensities of long wavelengths light could have shifted the DLMO (Hanifin et al., 2006; Zeitzer et al., 1997), but they may also lead to undesired earlier wakefulness. The present study shows that an improvement in sleep inertia is possible without a shift of the onset of the melatonin rhythm. This leads to the conclusion that shifts in the underlying rhythms are not a prerequisite to obtain an improvement in waking up by an artificial dawn signal. A possible shift in the offset of the melatonin rhythm or suppression in the early morning was not measured. It could be hypothesized that morning light induces a larger phase advance in the offset than in the onset of the melatonin rhythm (Illnerová and Sumová, 1997; Warman et al., 2003; Wehr et al., 2001a). This effect, however, is thought to be transient and only present during the first days after morning light. In the present study, the artificial dawn effects on DLMO were assessed after 2 wks. A shift in morning decline is not expected in the absence of a shift in the onset. Furthermore, a possible suppression of melatonin by the artificial dawn signal was not expected. The dawn signal occurred during the last 30 min of sleep before waking up, more than 9 h after the DLMO, when the synthesis of melatonin is most likely already turned off (Lewy et al., 1999). The possible effects of the unmodified Wake-up Light (Study 2) on melatonin profile and suppression were not tested. Interestingly, it was found that the timing of sleep offset was earlier. The earlier sleep offset occurred after the alarm went off. Exposure to light prior to the alarm, therefore, was not longer in Study 2 compared to Study 1. By means of a multilevel analysis, the effects of DLMO and sleep offset on sleep-inertia duration were tested as an alternative or additional effect to the effects of the artificial dawn treatment. While the DLMO asserts no significant effect on the reduction of sleep inertia, sleep offset related negatively. This indicates that the later the sleep offset, the lower the suffering from sleep inertia. This is understandable in view of our relatively late chronotypes, who will suffer less from sleep inertia the later they wake up. The use of the artificial dawn, however, did not affect the timing of sleep offset. In Study 2, although sleep offset was earlier, the multilevel analysis revealed no effect of it on the duration of sleep inertia.

Taken all together, only the artificial dawn treatment is responsible for the reduction of sleep-inertia complaints. The activating effects of light have been shown to be present both during the night and daytime, indicating that melatonin suppression may not necessarily be a prerequisit to assert the effect (Cajochen et al., 2000; Campbell et al., 1995; Phipps-Nelson et al., 2003; Rüger et al., 2003; 2006). We hypothesize that this is the most likely mechanism by which complaints are reduced. In a recent review, Vandewalle and co-authors (2009) concluded that several brain structures, especially the

thalamus, might play a key role in the light-induced changes in alertness and cognitive functioning (Vandewalle et al., 2009). It would be interesting to measure the effects of an artificial dawn on brain activity shortly after waking up. A wide range of physiological changes accompanies the waking-up process in the morning, such as heat dissipation (Kräuchi et al., 2004), awakening cortisol response (Edwards et al., 2001), and changes in EEG spectrum (Tassi et al., 2006). The immediate effects of artificial dawn on these aspects were tested in the study of Van de Werken and co-authors under laboratory-controlled conditions. A faster decline in distal skin temperature after waking up and an increase in the number of arousals during the last 30 min of sleep have been shown to be related to a reduction in sleep inertia. These mechanisms, rather than a shift of circadian rhythms, may explain the positive effects of a dawn signal on sleep inertia.

Conclusion

Both studies clearly show that artificial dawn during the last 30 min of sleep exerts beneficial effects on subjective ratings of sleep inertia. However, as tested in the present study, no significant shifts in DLMO were observed. The artificial dawn signal, although not capable of having circadian effects, is hypothesized to assert an effect on physiological processes at waking up by activating/alerting the system.

Acknowledgements

Our work was supported by Philips DAP B.V., CoC Vitality Care, Drachten, The Netherlands and by the 6th European Framework EUCLOCK (018741). §

CHAPTER 4

IN VIVO QUANTIFICATION OF THE RETINAL REFLECTANCE SPECTRAL COMPOSITION IN ELDERLY SUBJECTS BEFORE AND AFTER CATARACT SURGERY: IMPLICATIONS FOR THE NON-IMAGE-FORMING EFFECTS OF LIGHT

MARINA C. GIMÉNEZ[1], MARTIJN J. KANIS[2], DOMIEN G. M. BEERSMA[1], BERT A. E. VAN DER POL[3], DIRK VAN NORREN[2], MARIJKE C. M. GORDIJN[1]

[1] Research Unit of Chronobiology, Centre for Life Sciences, University of Groningen, The Netherlands; [2] Department of Ophthalmology, University Medical Centre Utrecht, Utrecht, The Netherlands; [3] Department of Ophthalmology, University Medical Centre Groningen, Groningen, The Netherlands.

Journal of Biological Rhythms (2010), 25(2), 123–131

Abstract

Light is the signal that entrains the biological clock in humans to the 24 hours external time. Recently, it has been shown that short wavelengths play a key role in this process. In the present study we describe a procedure to measure, objectively and in a quick way, the spectral composition of the light reaching the retina in vivo. The instruments involved are the foveal reflection analyzer (FRA) and the macular pigment reflectometer (MPR). By making use of these reflectometers we show quantitatively that in subjects with cataract the light input is especially reduced in the short wavelength range. After cataract surgery during which the crystalline lens is replaced by a transparent artificial lens the transmittance of the short wavelengths (in the range between 420 and 500 nm) improved on average by a factor of 4. We conclude that this technique holds great promises for the chronobiological field because it allows for quantification of the spectral composition and light levels reaching the retina in vivo.

Introduction

The main role of the circadian pacemaker is to provide an internal representation of time, allowing organisms to anticipate the different demands of the day and night. In order to do this, the circadian system needs to be entrained to the 24 h light/dark cycle. Entrainment of the biological clock is important for human well-being; an out of phase internal clock leads to general discomfort (Rajaratnam and Arendt, 2001). Light is the main entraining signal in humans and photoreception is therefore an essential process between the external environment and the endogenous pacemaker. In mammals, photoreceptors are only found within the eye, in the retina.

With aging the circadian system, as well as the eye show changes. It has been reported that during aging several aspects of the circadian system show alterations e.g., less stable activity-rest cycles and reduction in the nocturnal secretion of melatonin (Dijk et al., 2000; 1999; Mishima et al., 2001; Nair et al., 1986; Sharma et al., 1989; Skene and Swaab, 2003; Touitou et al., 1981; Touitou, 2001; Van Someren, 2000). However, the cause and origin of the disturbances of circadian rhythms in aging are still unknown (Skene and Swaab, 2003). On the other hand, it is well known that one of the most conspicuous changes occurring in the eye is the development of a yellow pigmentation in the central part of the crystalline lens (nuclear sclerosis, nuclear cataract) that reduces light transmission in particular of the short wavelengths (Boettner and Wolter 1962; van Norren and Vos 1974). Light of short wavelengths has been shown to play a major role in both circadian (Brainard et al., 2001; Cajochen et al., 2005; Lockley et al., 2003; Thapan et al., 2001) and alerting responses (Cajochen et al., 2005; Lockley et al., 2006; Revell et al., 2006). A disrupted circadian system in the elderly may be, at least partially, the consequence of a weaker entraining signal due to the aging of the lens (Charman, 2003; Turner and Mainster, 2008).

The replacement of the yellowish cataractous lens by an artificial transparent one is one of the most common surgical procedures in the developed countries to improve vision in the elderly. If the increase in light input after cataract surgery is effective in stimulating the circadian system and improving rhythmicity this is of utmost importance both for understanding the disturbances in circadian rhythms in the elderly, and for the practical use of light in the aged population (Turner and Mainster, 2008).

The present report describes the use of an *in vivo* measurement of lens transmittance that is independent of conscious light perception. This method uses retinal spot reflectometers: the foveal reflection analyzer

(FRA) and the macular pigment reflectometer (MPR) (van de Kraats et al., 2006; van de Kraats and van Norren, 2008; Zagers et al., 2002). Data on lens transmittance before and after cataract surgery will be presented to illustrate the use of this methodology. This is of great relevance in chronobiological studies since it allows for quantification of the spectral composition and light levels of the light reaching the retina.

The Eye and Its Optical Characteristics

Light enters the eye through the transparent cornea, goes through the pupil surrounded by the coloured iris, and passes the lens. In an emmetropic eye, the cornea and crystalline lens focus the incident light on the central retina, the fovea centralis. In front of both the pupil and the iris is the cornea. The cornea is continued by the sclera, and these two together form the external layer of the eye. Under this external layer, the intermediate layer is found. This layer can be divided in two components: the anterior part formed by the iridal stroma and the ciliary body, and the posterior part formed by the vascular plexus of the choroid consisting of blood vessels. The retina is the most inner layer of the eye, containing the photoreceptors: rods, cones, and the intrinsically photosensitive retinal ganglion cells (ipRGCs). In between the lens and the retina lies the vitreous body that consists of a transparent gel-like substance. Light passes through the ganglion cell layer and other retinal layers with amacrine, bipolar, and horizontal cells. Eventually, light reaches the last retinal layer where the outer segments of the rods and cones are found. Behind the retina, light is strongly absorbed by the melanin-rich retinal pigment epithelium and choroid.

All structures in the eye can absorb, reflect, and/or scatter light depending on their optical characteristics (Van De Kraats et al., 1996). A very small portion of the incident light is reflected at the level of the retina and beyond. The reflected light escapes the eye via the pupil, and thus passes the lens twice. The difference between the spectral composition of light entering the eye and leaving it can be used to asses the transmittance properties of the lens and other structures of the eye. The vitreous body as well as the cornea are largely transparent in the visual spectrum and they both play a very minor role in changing the spectral composition of light reaching the retina.

The Effects of Aging on the Eye

An important aspect of the changes with aging of the eye is observed in the lens (Boettner and Wolter 1962; van Norren and Vos 1974). Recently, van de Kraats and van Norren (2007a) provided an extensive literature analysis on the optical density of the aging eye media. They provide a list of factors that explain the gradual increase with age in the absorption of the media between the cornea and the retina. While in a newborn the total density of the eye media at 400 nm is about 1.5, at the age of 80 the density of the media rises to 3.3. At 450 nm these numbers are even more striking; 0.3 for a newborn and 0.9 for an 80 year old man.

In individual cases the lens might

age more rapidly to such an extent that it is diagnosed as cataractous. Patients with cataract may have complaints of reduced visual acuity, photophobia caused by light scattering, and sometimes monocular diplopia altered by cortical lens densities. The physiological development of cataract can be accelerated by long-term exposure to UV light, by diabetes mellitus, and by smoking (Johnson, 2004; Robman and Taylor, 2005). Cataract is one of the most common causes of visual impairment. In developed countries however, the consequences of cataract are dramatically reduced due to surgery. Cataract surgery is conducted in large numbers every year. In 2008, more than 100,000 cataract operations were conducted in the Netherlands (for statistical information see: Prismant Institute, http://www.prismant.nl/Informatie-expertise/Thema's/Ziekenhuisstatistieken). Intraocular implant lenses often have a UV filter to protect the retina against harmful UV radiation (< 400 nm). Nevertheless, these implant lenses will increase the total amount of light reaching the retina relative to the cataractous lens, in particular in the short wavelength region. This increase in light input could hypothetically have effects on the health of the elderly population. The stronger the light input, the better the synchronization between the internal and external timing is. It has already been shown in elderly demented people that higher environmental light exposure can induce a more stable rest-activity rhythm and an increase in the nocturnal production of melatonin (Mishima et al., 2001; Van Someren et al., 2002). This suggests that the circadian system in the elderly retains its plasticity and has the capacity to function properly, as long as the input signal to entrain the clock, e.g., short wavelength light, is of sufficient intensity.

In collaboration with the University Medical Center in Utrecht (UMC Utrecht) and the University Medical Center in Groningen (UMCG), both in the Netherlands, the retinal reflectance of 14 elderly subjects before and after cataract surgery has been measured in the context of a larger study looking for a potential causal relationship between cataract and disturbed sleep-wake cycles and melatonin profiles. Data on the *in vivo* spectral composition and light levels of light reaching the retina before and after cataract surgery are presented in this paper together with a detailed description of the methods used for it and its relevance for further understanding of the non-image-forming effects of light.

Methods

Foveal Reflection Analyzer and the Macular Pigment Reflectometer

For the *in vivo* measurements of retinal reflectance before and after a cataract surgery two different spot reflectometers were used; the foveal reflection analyzer (FRA) (van de Kraats and van Norren, 2008; Zagers et al., 2002) and the macular pigment reflectometer (MPR) (van de Kraats et al., 2006). The instruments were developed for measuring directional sensitivity of the foveal cone-photoreceptors and absorption of the macular pigment, respectively. Both devices are capable of measuring the spectral composition of the reflected light in the living human

eye, and can thus be used in this study. The differences in the spectral composition of the reflected light, measured with this technique due to lens absorption, have already been validated in different age groups (Zagers et al., 2002).

Both instruments consist basically of a head-chin-holder and a direct vision prism for spectral decomposition of the light in a range from 400 to 950 nm (in practise, the wavelength range covered by the spectrograph is from 420 to 790 nm). Reflected light is observed with a charged-couple device (CCD) camera and analyzed with an imaging spectrograph. A Maxwellian view system is used for a controlled spot illumination on the retina. The incident light beam has an intensity level of more than 6 log Troland, enough to bleach approximately 97% of the visual pigments. The light source is a halogen lamp that emits a continuous spectrum in the visible range. Incident light is filtered by a GG395 UV cutoff filter (Schott, Mainz, Germany). Some fluorescence of the tissues in the eye cannot be excluded. However, due to the separate pathways of incoming and measured light in the frontal parts of the eye, combined with the nature 360 deg distribution of fluorescent light, any substantial influence can be neglected. Light entering the eye will be absorbed and reflected by the various components found in the eye. The spectral characteristics of these components will determine the spectral composition of the light that escapes the eye. Typically, above 600 nm retinal melanin is the only absorber while the choroid acts as the main reflector. Between 500 and 600 nm, melanin and hemoglobin from the choroidal circulation together with the photopigments are the main absorbers, while the cone outer segments, the retinal pigment epithelium and the superficial choroidal layers are the main reflectors. Going from wavelength values above 600 nm to values below 600 nm the reflected light in a healthy young subject is reduced by more than half. For values below 500 nm the reflected light is even further reduced due to the absorbance of the macular pigment. Below 430 nm absorption of the lens sets in, reducing the reflected light to very low values below 0.2 % (Zagers 2004). In the present study it is assumed that within each subject and in the short time interval of maximally 6 months between the two measurements, all absorbers and reflectors remain unchanged, independent of cataract surgery. Because the light beam is directed to the fovea it is only the number of cones reached by it that could influence our measurements. However, due to the light intensity cones are bleached and can no longer respond to light. Thus, all changes observed in the spectral composition of the light reaching the retina before, compared to after surgery, are due to the changes in the lens absorbance characteristics.

Measurements

Measurements were conducted before and after cataract surgery on both eyes of every subject. The fellow-eye was operated upon within maximally two months. The measurements after cataract surgery were performed at least one month after the second eye was operated.

Before each measurement a calibration was carried out obtaining white and

dark reference frames. The white reference frames allowed calibrating the output of the light source, the transmission of the optics, and the sensitivity of the CCD cameras. The dark frames served to account for stray light in the set-up and dark current in the CCD camera. The pupils of all subjects were dilated with tropicamide 5 mg/ml and phenylephrinehydrochloride 25 mg/ml drops approximately 10 minutes before the measurement took place. Once the pupils were dilated, subjects positioned their heads in the headrest and were asked to fixate at the centre of the light spot, allowing the light to be projected at the fovea. Focal adjustments were conducted in order to maintain a sharp image at the retina. The entrance beam was positioned in the pupil plane such that at 550 nm a maximum in the, continuously displayed, reflectance was found, and noise was minimal at around 400-430 nm. While only a vertical scan of the pupil is needed in order to find this peak, the extra horizontal scan achievable with the FRA makes it easier. When comparing measurements before and after surgery the FRA is expected to perform better (when compared to the MPR) because the position of the Stiles-Crawford peak (near the center of the pupil) in both measurements (before and after surgery) can be expected to be identical. The measurements were repeated five times in order to have more accurate estimations taking approximately 3-5 minutes. The maximal reflectance found was displayed and served as background for the new measurement in order to try to achieve such a maximum again or even higher reflectance whenever possible. Subjects' fixation was continuously monitored and measurements were taken at those moments when fixation was correct (*e.g.*, minimum standard deviation between the maximum found and the new measurements). Between measurements, subjects were allowed to blink. The whole procedure including alignment and calibration took about 10-15 minutes. All measurements were conducted in the morning.

Furthermore, considering that the main changes before and after surgery in the amount of light reaching the retina is due to the changes in the crystalline lens, it is possible to estimate the improvement factor at a certain wavelength (λ) of the transmittance of the lens after cataract surgery as follows:

$$\text{lens transmittance improvement factor}_{\lambda} = 10^{((\log(AS\lambda) - \log(BS\lambda))/2)}$$

where AS is the retinal reflectance after cataract surgery and BS is the retinal reflectance before cataract surgery. The division by 2 is due to the fact that in the retinal reflectance measurements the light is attenuated by the lens twice. Since we are interested in the amount of light reaching the retina, we only consider the improvement on the amount of incident light.

Subjects

Fourteen subjects between 66 and 87 year old (5 males and 9 females, average age ± sd: 77.9 ± 5.2 years) listed at the UMCG for a cataract surgery in both eyes participated in this within subject design study (two subjects however, ended up by having only one eye operated). For logistic reasons, seven subjects (2 males and 5 females, average age ± sd: 77.1 ± 6.5 years) were measured with

the FRA, while the other seven (3 males and 4 females, average age ± sd: 78.1 ± 3.9 years) were measured with the MPR. During cataract surgery 13 out of the 14 subjects received AMO AR40e implant lenses (UV absorbing hydrophobic acrylic) and one received an AMO Z9002 implant lens (UV blocking SLM-2 Silicone).

The study protocol was approved by the Medical Ethical Committee of the University Medical Center of Groningen, The Netherlands. All subjects signed a written informed consent form prior to their participation.

Results

In figure 1A the average percentage (± sem) of the retinal reflectance before and after cataract surgery against wavelengths of the fourteen subjects is shown (26 eyes, the non-operated eyes are not included). There is virtually no short wavelength-light transmitted to the retina before cataract surgery. After replacement of the lens an overall increment is visible across all wavelengths. In the range between 420 and 500 nm lens transmittance is improved on average (± s.e.m.) by a factor of 4.0 ± 0.3 after cataract surgery, while in the range between 505 and 750nm the average improvement is only 1.3 ± 0.1 (figure 1B). The use of the two devices did not assert a significant effect on the calculated improvement factors ($p = 0.25$). Individual changes in lens transmittance across the 420-500 nm range before and after cataract surgery are plotted in figure 2. Individual improvement factors range from 2.3 to 8.3.

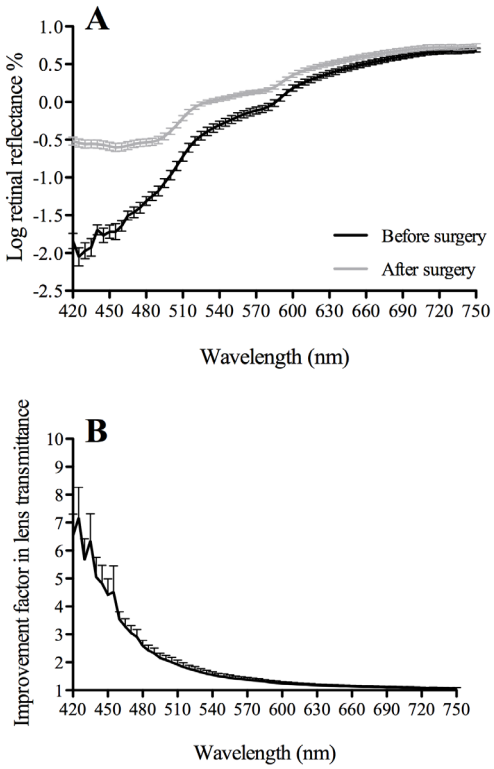


Figure 1. (A) Mean retinal reflectance percentage in log units (±SEM) per 5-nm bin wavelength before (black line) and after (gray line) cataract surgery (n = 14). (B) Mean improvement factor (±SEM) in light transmittance after cataract surgery (n = 14). The improvement factor at every wavelength was calculated as the following: $10^{((\log(AS\lambda) - \log(BS\lambda))/2)}$

The results indicate that light input in subjects with cataract is reduced especially in the short wavelengths and that replacing the crystalline lens by a transparent artificial implant can restore it.

Discussion

By using the spot reflectometer technique described in this article, we were able to quantify *in vivo* that there is a clear change in the intensity and spectral composition of the light reaching the retina after cataract

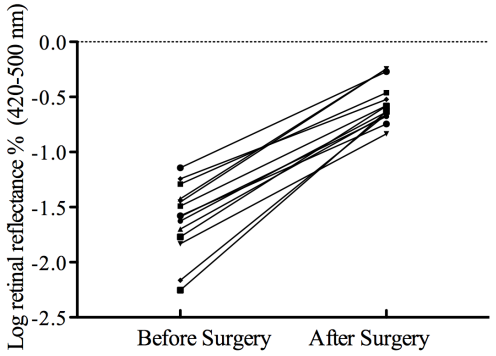


Figure 2. Individual responses to cataract surgery. Mean log retinal reflectance percentage between 420 and 500 nm before and after cataract surgery for all individual subjects.

surgery. The average improvement factor of 4 in the range of short wavelengths between a naturally aged lens and an artificial transparent lens may become an important value in future chronobiological studies.

Due to the effects of aging of the human lens and its impact on visual sensitivity (Alió et al., 2005; Pokorny and Smith, 1986) measurements of light being absorbed by the lens are widely conducted in the ophthalmologic field. However, it was not until the discovery of the photopigment melanopsin in the human retina (Provencio et al., 2000) and the existence of intrinsically photosensitive retinal ganglion cells (ipRGCs) (Berson et al., 2002; Hattar et al., 2002) with a peak sensitivity around 480 nm (Berson et al., 2002; Panda et al., 2005) that the chronobiological community became highly interested in the spectral composition of light. Short wavelengths have been shown to play a major role in the entrainment of the circadian system (Brainard et al., 2001; Cajochen et al., 2005; Lockley et al., 2003; Thapan et al., 2001) and in activating/alerting subjects (Cajochen et al., 2005; Lockley et al., 2006; Revell et al., 2006). The effects of the aged yellow lens on the circadian system and of cataract surgery after which the old yellowish lens is replaced by a lens that is transparent in the visible range has therefore intrigued the chronobiological field (Charman, 2003; Skene and Swaab, 2003). Some studies have tried to elucidate the effects of aging on non-image-forming responses. Herljevic et al. (2005) assessed the effects of short wavelengths in suppressing melatonin in young and elderly women. The authors found that the elderly group suppressed less melatonin, an indication that their system was less sensitive to blue light. Indirectly, this was associated with the aging effects of the human lens. It was also found that blue light had less alerting effects in elderly subjects (Sletten et al., 2009). No differences were found in the phase shifting effects of monochromatic blue light between the young and the elderly group (Sletten et al., 2009) however Duffy et al. (2007) found an age related reduction in sensitivity for phase shifting to polychromatic light. In order to assess what the effects are of the aging lens on non-image-forming responses, quantifying the amount of light reaching the retina along the visual spectrum is important. Furthermore, comparisons between age groups so far have been conducted with monochromatic light sources. Because of the additional involvement of rods and cones in entrainment of the circadian system (Aggelopoulos and Meissl, 2000; Drouyer et al., 2007), monochromatic light comparisons could lead to an overestimation of the role of the ipRGCs. In an extensive review conducted by Turner and Mainster (2008) it was shown that the most prominent differences

in circadian photoreception due to age are in the UV and short wavelength range. Retinal illumination was estimated by considering the effects of lens aging and pupil size. By means of these estimations and data available on the spectral sensitivity of melatonin suppression the effects of age on reduced circadian photoreception were established (Turner and Mainster, 2008). These estimations illustrate the relevance of the *in vivo* measurements of the actual amount of light reaching the retina that the present methodology allows for.

The effects of replacement of a yellowish lens for a transparent artificial one during cataract surgery are presently being studied in our lab. The results will increase our insight in the direct relationships between the aging process of the eye and the entrainment of the circadian system. There are some indications that sleep disturbances, assessed by means of questionnaires, are diminished after cataract surgery with UV-blockers artificial intraocular lenses (IOL) (Asplund and Lindblad, 2002). This is in accordance with the observed plasticity of the circadian system shown in elderly subjects after increasing their environmental light (Van Someren et al., 2002).

Currently there is debate on which spectral characteristics are optimal for the implanted artificial intraocular lenses after cataract surgery. In response to an excellent overview of the effects of blue-blocking IOLs on vision (Mainster and Sparrow, 2003), Van Gelder (2004) started to question the use of blue-blocking IOLs in view of circadian photoentrainment. The discussion is intensified but up till now inconclusive (Augustin, 2008; Cuthbertson et al., 2009; Henderson and Grimes, 2010; Mainster, 2006; Mainster and Turner, 2010; Patel and Dacey, 2009; Turner and Mainster, 2008; van de Kraats and van Norren, 2007b; Van Norren and Van De Kraats, 2007). Blue-blocking IOLs were developed due to indications that exposure to blue light could increase the risk of macular degeneration in the aging retina (age related macular degeneration: AMD). The benefits of the blue-blocking lenses on the macula remain unclear (Henderson and Grimes, 2010; Mainster and Turner, 2010). On the other hand, based on the knowledge of the short-wavelength sensitivity of the circadian system, blocking violet and blue light by 43-57% could have detrimental consequences for the biological clock (Cuthbertson et al., 2009; Mainster and Turner, 2010; Patel and Dacey, 2009; Turner and Mainster, 2008). The first attempt to quantify the effects of blue-blocking IOLs in comparison with UV-blocking IOLs on non-image-forming responses in a field study was conducted by Landers et al (2009). By means of the Pittsburgh Sleep Quality Index (PSQI) subjects who underwent cataract surgery were asked to rate the quality of their sleep. The measurements took place 12 months after surgery had been conducted on both eyes. The authors concluded that subjective ratings of sleep quality were not impaired by the use of blue-blocking light lenses. The study is however conducted on a small number of subjects, lacks basic information such as pre-surgery sleep quality ratings, and does not control for factors such as season, gender and light exposure. Commercially available blue-blocking light lenses reduce short wavelength light intensities mainly below 470 nm

(Mainster, 2006; Patel and Dacey, 2009; Augustin, 2008). Moreover, they have been shown to be comparable to the photoreception abilities of a 35-year-old man while having the photoprotective power of a 42-year-old man (Van Norren and Van De Kraats, 2007). The photoentrainment effectiveness of blue blocking-IOLs, as compared to exclusively UV filtering-IOLs was assessed by Patel and Dacey (2009). The authors show that the photoentrainment effectiveness of the two IOLs are similar. They conclude that effectiveness is considerably high, especially when in their calculation the action spectra for the non-image forming system obtained by Berson et al (2002) or Dkhissi-Benyahya et al (2007) are used. Effectiveness decreases if instead, the action spectra obtained by Brainard et al (2001) and Thapan et al (2001) are used. Also Turner and Mainster (2008) came to the conclusion that blue-blocking IOLs in elderly people will reduce circadian photoreception based on the two latter melatonin action spectra curves. In a later paper the same authors argue that blue-blocking IOLs not only reduce the amount of specifically blue light, but also overall reduce dim light visual abilities. Since with aging also pupil area diminishes they conclude that the unproven benefits of blue-blocking IOLs for eye diseases do not outweigh the possible disadvantages for dim light vision and circadian photoreception (Mainster and Turner, 2010). On the other hand, Augustin (2008) concludes that at 480 nm blue-light filtering lenses show transmission comparable to a young person. They expect no clinically significant effects of blue-blocking IOLs on circadian rhythms. The main discrepancies between the mentioned studies are because of the used action spectrum for non-image-forming photoreception with peak sensitivities either around 460 nm or 480 nm, and taking into account the additional effects of reduced pupil size. In view of the discrepancies between studies and the available literature on the non-image-forming effects of short wavelength (Brainard et al., 2001; Cajochen et al., 2005; Herljevic et al., 2005; Lockley et al., 2003; 2006; Sletten et al., 2009; Thapan et al., 2001) it is too early to conclude that blue-blocking IOLs will have no effect on photoentrainment. However, it is necessary to acknowledge that most studies on the effects of short wavelengths on non-image-forming responses are mainly based on monochromatic light comparisons. This is not only a non-natural scenario but can also, as stated before, overestimate the role of the ipRGCs (Aggelopoulos and Meissl, 2000; Drouyer et al., 2007). Further experiments are needed to investigate whether the transmission of short wavelengths through the blue blocking-IOLs is sufficient for the non-image-forming system. For that purpose knowledge of the actual amount of the different light spectra reaching the retina *in vivo* is crucial in order to have a better understanding of the light levels and spectral composition at which the circadian system is still functioning properly.

Different approaches can be taken in order to measure the absorbing characteristics of the lens that primarily includes physical and psychophysical methods. The reflectometry assessment is a physical method and it is an objective one. Psychophysical tests usually involve the assessment of scotopic or photopic thresholds, which imply vision,

properly functioning visual photoreceptors, and a reliable response of the subject. In certain populations, especially in some elderly or other populations like demented people and in children this may be difficult. Another disadvantage of the psychophysical tests is that they require dark adaptation, making this procedure a much longer one than the one presented here. Diurnal changes in psychophysical luminance sensitivity have also been shown (O'Keefe and Baker, 1987) and may be dependent on diurnal changes in the classical photoreceptor sensitivity (Roenneberg et al. 1992). Although the changes are rather small against the differences between subjects, it could be of importance to fix the psychophysical measurements to a certain time of the day for comparisons.

The method presented here easily allows the quantification of the spectral composition of light reaching the retina *in vivo*. It is an objective, quick and easy method. It might effectively be used to increase chronobiological knowledge regarding light intensity levels and its spectral composition at which a response can be triggered. Such knowledge is critical for instance for developing lighting conditions and light therapy treatments.

Acknowledgements

The authors thank the participants for their efforts to take part in the study. We acknowledge the help of R. Wieringa, E. van der Valk, M. Boon and G. Hooisma in recruiting subjects. We also like to thank Dr. J. van Kraats for his thoughtful contribution regarding the methodological aspects and Dr. R. A. Hut for his input. Our work is supported by the 6th Framework Project EUCLOCK (No. 018741). §

Chapter 5

Melatonin and Sleep-Wake Rhythms Before and After Ocular Lens Replacement in Elderly Humans

Marina C. Giménez[1], Domien G. M. Beersma[1], Serge Daan[1], Bert A. E. van der Pol[3], Martijn J. Kanis[2], Dirk van Norren[2], Marijke C. M. Gordijn[1]

[1] Research Unit of Chronobiology, Centre for Life Sciences, University of Groningen, The Netherlands; [2] Department of Ophthalmology, University Medical Centre Utrecht, Utrecht, The Netherlands; [3] Department of Ophthalmology, University Medical Centre Groningen, Groningen, The Netherlands.

Abstract

Light of short wavelengths has been shown to play a key role in non-image forming responses. Due to aging, the ocular lens becomes more yellow reducing the transmission of short wavelengths in the elderly. In the present study we make use of cataract surgery to investigate the effects of a relative increase of short wavelength transmission (n = 14). We observed, on average, a delay of both the sleep-wake and the nocturnal melatonin rhythms after cataract surgery. This delay is tentatively attributed to an effect of light transmittance in the evening hours that is increased compared to the effect under the higher light intensities in daytime. However, not all patients responded in the same way. A positive correlation was found between chronotype and the size of the phase delay. The later phase that we observed after cataract surgery (clear lens) as compared to the earlier phase observed before cataract (yellowish lens), is in agreement with the earlier phase reported in the elderly population.

Introduction

Humans display a multitude of circadian rhythms in physiology and behavior, each with their own specific timing with respect to day and night. Many of these rhythms are under (partial) control of a circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus. The SCN in turn is entrained to the systematic 24-h variations of the environment. In humans this is almost exclusively achieved by daily adjustments through exposure to the light-dark cycle. The spectral composition of light has been shown to be critical for these so-called non-image forming responses to light. Short wavelengths (circa 460-480 nm) in particular are of importance for inducing these responses (Brainard et al., 2001; Cajochen et al., 2005; Lockley et al., 2003, 2006; Revell et al., 2005; Warman et al., 2003). This is related to the role of intrinsically photoreceptive retinal ganglion cells (ipRGCs) containing the blue-sensitive pigment melanopsin. ipRGCs connect directly with SCN neurons (Berson et al., 2002; Hattar et al., 2002; Provencio et al., 2000, 2002) and other regions such as the ventrolateral preoptic area (VLPO), a key region for sleep regulation (Hattar et al., 2006).

There are some indications that in the elderly population the amplitudes of overt rhythms generated by the circadian system are reduced. This is shown, although not consistently across all studies, for instance in fragmented sleep-wake rhythms, lower amplitude of melatonin rhythms as well as reduced amplitude in rhythms already present at the level of the SCN such as vasopressin (for review see Van Someren, 2000). It is conceivable that this reduction in the strength of circadian expression is partly related to a reduced input of light. In particular, short wavelengths are filtered out as a consequence of a progressive yellowing of the ocular lens with age (Van Norren and Vos, 1974). Although it is a tempting hypothesis, there is no evidence for the idea that reduced circadian rhythmicity is indeed the result of such a reduction in light input. It is also undetermined whether replacement of the natural lenses by artificial ones after cataract surgery would partly reverse the effects. In The Netherlands, cataract surgery is conducted in large numbers (in the order of 100,000 lens replacements per year). In this study we exploit cataract surgery to investigate the effects of removing the age-related reduction of short wavelength transmission through the ocular lens on some overt circadian rhythms. If the observed changes in robustness of the circadian system are mainly due to the age-related changes of the ocular lens, the implantation of a new transparent lens is expected to increase the Zeitgeber strength and thereby affect the entrained

phase of sleep-wake and melatonin rhythms as well as restore their robustness. These hypotheses are tested in the present study.

Methods

Subjects

Subjects were recruited from the waiting list for cataract surgery on both eyes at the University Medical Center in Groningen (UMCG), The Netherlands. Subjects had to be older than 65 years and free of medical conditions. Subjects completed a general health questionnaire. Sleep characteristics were not used as a criterion for inclusion or exclusion. Those who had been traveling on transmeridian flights within a month before the initiation of the study protocol were excluded. All subjects gave written informed consent and were paid for participation. The experimental protocol was approved by the Medical Ethics Committee of the UMCG. The study took place between April 2007 and February 2009.

Experimental Design

Measurements were conducted before and after surgery. The first assessment started between one and two months before the replacement of the first eye lens. The operation on the second eye occurred maximally 2 months after the operation of the first eye. The second assessment was conducted at least 4 weeks after the operation on the second eye to allow for recovery.

The following measurements were made before and after bilateral lens replacement:

Actigraphy: During three consecutive weeks actigraphy data were collected (Actiwatch®, Cambridge Neurotechnologies, UK). Movements of the non-dominant arm were recorded per 1-minute bin. During those three weeks sleep-logs (going to bed and waking up times) were completed on a daily basis.

Melatonin profile: During the same three weeks, subjects came to the lab for one night to assess their salivary melatonin profile. In three cases subjects collected the saliva samples at home, either with strict instructions (n = 2) or under supervision (n = 1). In all cases, subjects stayed under dim light conditions (<5 lux) from 17:00 h local time at the date of collection, i.e., Central European Time (CET) in winter, and Daylight Saving Time (DST) = CET + 1 in summer. Sleep started at their usual bedtime. During sleep, all lights were off. Saliva samples were collected once per hour from 18:00 h till 1:00 h and once per two hours from 1:00 h till 9:00 h. Food intake was restricted to the first 15 minutes after each sample. Chocolate, bananas, coffee and tea were not allowed during the whole sampling period. Ten minutes prior to each sample, subjects were asked to sit quietly to avoid influence of posture (Deacon and Arendt, 1994). Saliva samples were centrifuged immediately after collection and stored at -20°C until analysis when samples were collected in the lab, or on the next day when samples were collected at subjects' home. Melatonin concentration was assessed by radio-immunoassay (RK-DSM, Bühlmann laboratories AG, Alere Health B.V., Tilburg, The Netherlands). All samples from the same

individual were analyzed within the same series. The limit of detection for the RIA was 0.3 pg/ml with an intra-assay variation of 6.7 % at a low melatonin concentration (mean = 1.5 pg/ml, n = 30) and 6.5 % at a high melatonin concentration (mean = 15 pg/ml, n = 30). Inter-assay variation was 12.2 % at low melatonin concentration (mean = 2.1 pg/ml, n = 15) and 19.7 % at high melatonin concentration (mean = 17.5 pg/ml, n = 16).

Lens transmittance: *In vivo* measurements of lens transmittance were obtained before and after surgery by means of spot reflectometry. In short, a beam of white light entered the eyes of the subjects and the spectral composition of the reflected light was measured. The technique has been described in detail in previous studies (Giménez et al., 2010; van de Kraats and van Norren, 2008;).

Data Analysis

For analysis all timing data were set to Central European Time (CET). Also in summer, time is expressed as CET, not as DST.

The sleep-wake rhythm was characterized from actigraphy data by means of sleep analysis 5 software (Cambridge Neurotech Ltd, Cambridge, UK) together with sleep logs. The Actiwatch algorithm scores wake or sleep per minute as follows. If a_i is the activity score in minute i, then $A_i = a_i+(a_{i-1}+a_{i+1})/5$ is compared with a threshold value T = 40. When A_i>T the bin is scored as wake; if A_i <T the bin is scored as sleep. Sleep onset is operationally defined as the first episode after bedtime of 10 consecutive sleep minutes with no more than 1 bin of wake within that time. The time from bedtime till sleep onset is sleep latency. Sleep end is the last sleep minute before a 10-minute consecutive period of wake from get up time. Sleep duration was calculated as the difference between sleep offset and sleep onset. Midsleep was used as phase marker (Roenneberg et al., 2003). We also calculated the average activity of the 10 most active hours (M10) and of the 5 least active hours (L5), which have been shown to be sensitive parameters to describe the rest-activity rhythm in the elderly (Van Someren et al., 1999; Witting et al., 1990), and sleep efficiency (percentage of time spent asleep while in bed). M10 and L5 are expressed as counts/hour.

A bimodal skewed baseline cosine function was fitted to the melatonin profiles before cataract surgery. This function allows for bimodality and skewness (Van Someren and Nagtegaal, 2007). The maximum of an individual's fitted curve before surgery was used to normalize the corresponding melatonin profile as well as the one obtained after cataract surgery. Dim light melatonin onset (Lewy et al., 1999) and dim light melatonin offset were defined as the times at which 25% of the maximum value was crossed in the increasing and decreasing part of the fitted curve respectively.

The phase angle differences (in hours) were computed between the average midsleep time over the three weeks and both DLMO and DLMOff.

Results

Subjects

Fourteen participants (5 males and 9 females) between 66 and 87 years old (average age ± SD: 77.9 ± 5.2 years) participated in the study. All participants were retired. The average chronotype (MSF, based on MCTQ data) was: 3:25 h ± 58 min (SD). Two subjects ended the study with only one eye operated. In one case the subject was satisfied with the result, in the other there was a medical reason. After cataract surgery, 13 subjects had received Sensar implant lenses (UV absorbing hydrophobic acrylic) and one received a Tecnis CL implant lens (UV blocking SLM-2 Silicone) (Abbott Medical Optics inc., Santa Ana, Ca, USA). Both lens types allow for short wavelength transmittance (as opposed to blue-blocking lenses). The single-eye operation subjects showed no deviating behaviours (i.e., values within 2 SD from the average behaviour) in the parameters assessed in this study and were therefore included in the analysis. In comparison to pre-surgery transmittance, on average the transmittance of short wavelengths (between 420–500 nm) for all subjects was found to increase by a factor of 4.0 after cataract surgery (Giménez et al., 2010).

The time of the year at which the measurements before and after cataract surgery occurred was not distributed evenly; by coincidence 10 subjects started during the summer months, in the presence of daylight saving time (DST) (before surgery measurements) and finished during the winter time (CET) months (after surgery measurements), while the other 4 subjects started in winter and finished during summer. It is conceivable that in addition to a possible effect of lens replacement, changes both due to DST and to other aspects of season influenced the results (Kantermann et al., 2007).

Sleep-Wake Rhythms

Data on average sleep onset, midsleep, and sleep offset before and after surgery of each individual are shown in Figure 1. We observe a slight tendency for the sleep-wake rhythm to lock onto dawn rather than dusk, i.e., both onset and end of sleep tended to be later in

Table 1. Sleep timing obtained by means of a combination of sleep diaries and actiwatch data

	Before Surgery	After Surgery	F	P	Difference
Sleep Onset	23:12 ± 1 h 6 min	23:28 ± 1 h13 min	5.8	< 0.05	16 min ± 25 min
Midsleep	03:10 ± 51 min	3:28 ± 59 min	10.3	< 0.01	18 min ± 19 min
Sleep Offset	7:08 ± 49 min	7:25 ± 50 min	10.7	< 0.01	17 min ± 10 min
Sleep Duration	7:56 ± 38 min	7:56 ± 40 min	0.006	0.94	0.5 min ± 24 min
Sleep Onset Latency	10.5 min ± 6 min	13 min ± 9.8 min	2.26	0.16	2.5 min ± 6 min

Data after subtracting seasonal/DST confounding effects (see text for description).

Data are shown as average ± SD. N = 14.

winter than in summer. Because conditions were not randomized across seasons, the comparison between before and after surgery is confounded with the seasonal change of phase, which itself is likely to be affected by the summer introduction of DST as shown by Kantermann and colleagues (2007) and in our own recent data (CHAPTER 2) . We used the data on changes in mid sleep on free days (MSF) across the year from Kantermann et al. 2007 to correct our sleep timing data for the confounding effect of season/DST. We first fitted a sine curve (circwave analysis tool, developed by Dr. R.A. Hut; available from http://www.hutlab.nl) through the midsleep data of Kantermann et al. and estimated for each day in the year a correction factor by calculating the deviation from the overall annual mean midsleep. Secondly, we corrected each participants' midsleep data by subtracting the appropriate correction factor for that date. The same procedure was followed for other parameters of sleep timing (sleep onset, offset).

Sleep timing after correction for seasonal/DST confounding effects is summarized in table 1. A significant delay of 18 minutes was observed for midsleep after surgery compared to before surgery. Not all subjects responded in the same way. To explore these individual differences we assessed how the observed differences correlated with, on the one hand chronotype (as assessed before starting the study) and on the other with the improved blue lens transmittance factor (average factor: 4.0 ± 1.5 SD) as a result of cataract surgery (Giménez et al. 2010). A significant correlation was found between the observed shifts in midsleep and chronotype; the later the chronotype the more midsleep delayed after surgery (Pearson's r = 0.55, $p < 0.05$) (figure 2B). Similar results were observed for the onset of sleep but not for the offset (figure 2A and 2C). No correlation was observed between the changes in

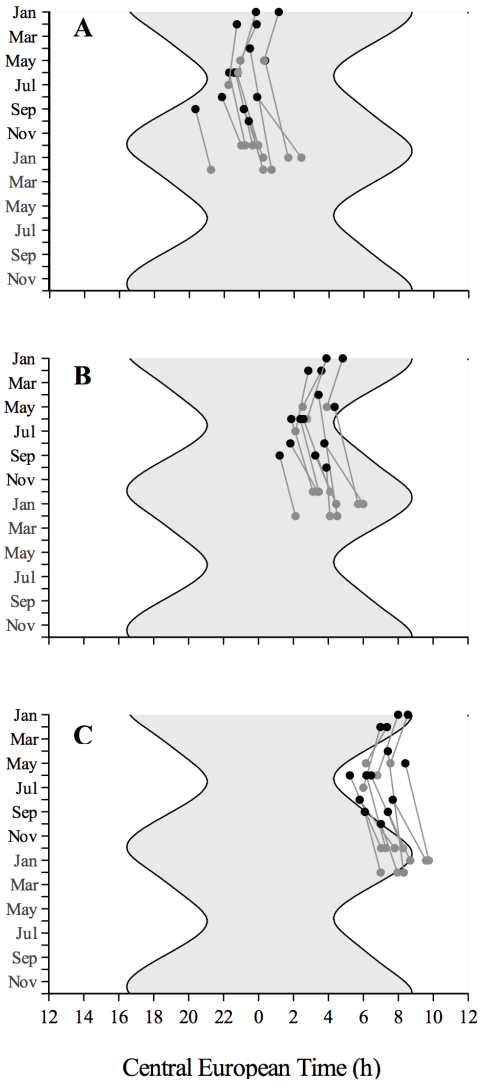


Figure 1. Sleep timing. Individual raw measurements of (A) sleep onset, (B) midsleep, and (C) sleep offset before (black circles) and after (grey circles) cataract surgery across the year as a function of Central European Time (CET). The grey area represents the nights.

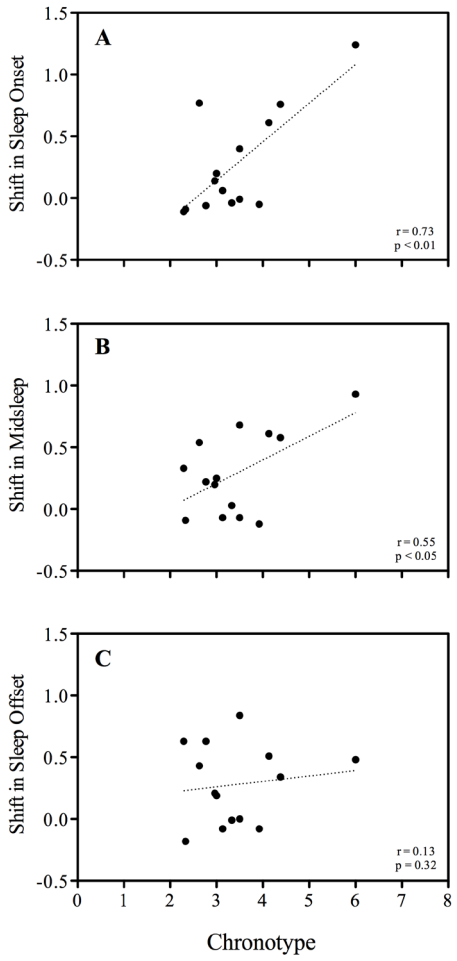


Figure 2. Correlation (Pearson) among chronotype (MSF) and the difference between the before and after cataract surgery measurements of (A) sleep onset, (B) midsleep, and (C) sleep offset.

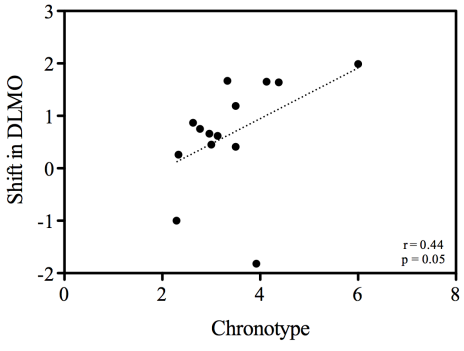


Figure 4. Correlation (Pearson) among chronotype (MSF) and the difference between the before and after cataract surgery measurements of dim light melatonin onset (DLMO).

the onset, midsleep, and offset of sleep, and the improvement factor in lens transmittance due to cataract surgery (Pearson's r = 0.093, 0.185, and 0.28; p = 0.75, 0.53, and 0.34 for the onset, midpoint, and offset of sleep respectively). Sleep duration was virtually the same before and after surgery and both the onset and offset of sleep were significantly delayed on average by about 17 minutes in response to cataract surgery. No significant differences were observed in the time it took the patients to fall asleep (sleep onset latency).

There is no data available in the literature of seasonal/DST effects on aspects of the sleep-wake cycle other than timing. To estimate the effects of surgery on sleep efficiency, M10, and L5 the following approach was taken. We hypothesized that the effect of season contributed positively to the total effect of season + surgery in those subjects who started during the summer months while season was hypothesized to contribute negatively in those subjects starting in the winter. We assumed that the effect of cataract surgery was not dependent on season. The hypothesized relationship is as follows:

Group 1 (n = 4, summer-winter):
average shift = effect of surgery
+ effect of season/DST

Group 2 (n = 10, winter-summer):
average shift = effect of surgery –
effect of season/DST

The effects of season and DST are hypothesized to go together. With two equations and two unknown variables, by entering the average shifts of group 1 and group 2 in the formulas we were able to estimate the av-

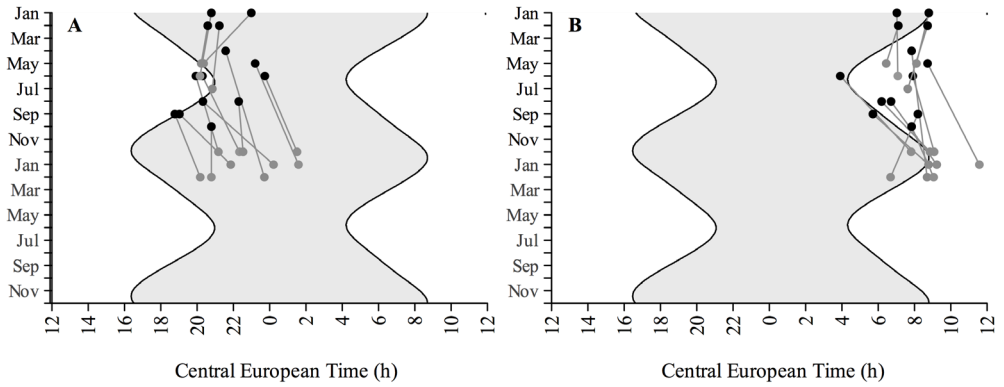


Figure 3. Timing of the melatonin rhythms. Individual raw measurements of (A) dim light melatonin onset, and (B) dim light melatonin offset before (black circles) and after (grey circles) cataract surgery across the year as a function of Central European Time (CET). The grey area represents the nights.

erage effect of surgery and of season/DST separately. The average season/DST effect is then used to correct individual data. The correction factors for season/DST we obtained were: -0.49, 0.14, and -0.68 for sleep efficiency, M10, and L5 respectively. The size of the factor indicates a very small effect of season/DST in these parameters. After correction of the individual data for this "season/DST" factor, effects of cataract surgery were observed neither in sleep efficiency (average ± SD: before surgery: 81.3 ± 8.1 % after surgery: 82.8 ± 8.2%, F = 1.27, n.s.), nor in M10 (average ± SD: before surgery: 53.4 ± 3.3 counts, after surgery: 53.6 ± 4.0 counts, F = 0.08, n.s.), nor in L5 (average ± SD: before surgery: 9.4 ± 4.9 counts, after surgery: 9.8 ± 5.6 counts, F = 0.91, n.s.).

Melatonin Rhythms

DLMO and DLMOff before and after surgery with respect to CET are shown in figure 3. In accordance with the sleep behaviour, we observe a tendency to lock onto dawn. DLMO and DLMOff tend to be later in winter than in summer. Considering MSF being a good phase marker for human subjects, we used the same seasonal information on the effects of midsleep and applied the same method of correction as we did for the sleep timing data to reveal the effects of surgery on DLMO and DLMOff (table 2). DLMO was significantly delayed by about 40 minutes after surgery ($F = 5.69$, $p < 0.05$). Not all subjects showed a phase delay in DLMO. A positive correlation between the shift in DLMO after surgery and chronotype was on the verge of significance (Pearson's $r = 0.52$, $p = 0.05$) (figure 4). The delay observed in DLMOff after surgery compared to before surgery (+ 47 minutes) was almost significant ($F = 4.57$, $p = 0.05$) and did not correlate with chronotype (Pearson $r = 0.23$, $p = 0.22$). Neither DLMO nor DLMOff correlated with the improvement factor in lens transmittance as a result of the cataract surgery (Pearson r = 0.33; 0.19; p = 0.25; 0.51 for DLMO and DLMOff respectively).

No significant differences were ob-

Table 2. Timing of dim light melatonin onset (DLMO) and offset (DLMOff)

	Before Surgery	After Surgery	F	P	Difference
DLMO (h:min)	21:14 ± 1 h 30 min	21:54 ± 1 h 41 min	5.7	< 0.05	40 min ± 1 h 30 min
DLMOff (h:min)	07:20 ± 1 h 12 min	08:07 ± 1 h 8 min	4.6	0.05	47 min ± 1 h 19 min

Data after subtracting seasonal/DST confounding effects (see text for description).

Data are shown as average ± SD. N = 14.

served between the mean phase angle differences between DLMO and midsleep before (-5:56 h; SD 1 h 32min) and after (-5:33 h; SD 1h 26 min) lens replacement (F = 2.55, p = 0.13). Phase angle between DLMOff and midsleep was also not significantly different between before (4:09 h; SD 1:24) and after replacement (4:41 h; SD 46 min) (F = 2.27, p = 0.16).

Estimations of the effect of season/DST on the amplitude of the nocturnal melatonin rhythm were calculated in the same way we calculated the effects for sleep efficiency, L5, and M10. After subtracting the effect of season/DST we observed no significant changes in the amplitude of the melatonin rhythm after cataract surgery (10.3 pg/ml; s.d. 7.4) as compared to before surgery (13.1 pg/ml; s.d. 10.6. F = 1.52, p = 0.24).

Discussion

In recent years several researchers have raised the question of possible effects of the aged lens and its replacement on the circadian system (Charman, 2003; Giménez et al., 2010; Turner and Mainster, 2008; Turner et al., 2010; Schmoll et al., 2011) and sleep in particular (Asplund and Lindblad, 2004; Kessel et al., 2011; Tanaka et al., 2010). We set out to answer this issue by assessing objective measurements of output parameters of the circadian system, such as the timing of the sleep-wake rhythm and the nocturnal melatonin rhythm as well as sleep quality before and after cataract surgery within the same individuals.

Unfortunately, due to our uneven distribution across seasons of the before and after surgery measurements, corrections had to be applied to our data. Corrections were based on the seasonal/DST effects on human sleep observed by Kantermann et al., (2007). Their data is mainly based on the German population, which is slightly earlier than the Dutch one (Roenneberg et al., 2007b). In view of the sample size and the time resolution of their study, and the fact that we use differences and not actual values for our corrections, our approach remains appropriate. After removing the confounding effects of seasons including the annual shift to DST, our study suggests that the implantation of new transparent lenses after cataract surgery leads, on average, to a delay of the sleep-wakefulness and nocturnal melatonin

rhythms. This was unexpected in view of the relative increase in Zeitgeber strength after cataract surgery that is supposed to lead to a phase advance (Roenneberg et al., 2003). Nonetheless, it is consistent with the later phase observed in the young (clear lens) population as compared to the elderly (yellowish lens) (Duffy et al., 1998; Miles and Dement, 1980; Myers and Badia, 1995; Roenneberg et al., 2007a). The mechanism behind the advanced sleep-wake cycle that is observed in the elderly is not known (Cajochen et al., 2006; Czeisler et al., 1999; Duffy et al., 1998; Duffy and Czeisler, 2002). It may be hypothesized that the aging of the lens contributes to it. Exposure to relatively larger amounts of especially blue light in the evening in comparison with the situation before surgery could cause the observed phase delay. During the evening hours, when light intensities are low compared to daytime, an increase in photons by a factor 4 (~ 0.5 log unit) could be sufficient to raise light levels just above a critical value to induce a shift in phase (Boivin et al., 1996; Zeitzer et al., 2005) and counteract the advancing effects of morning light. Alternatively, the shift to a later phase that we observed after the replacement of the cataractous lens for a transparent one may be explained by a reduction in sleep pressure. If subjects go to bed later due to the activating effects of light (Cajochen, 2007), this would lead to a later waking up time and consequently to a shift in the exposure to the light-dark cycle. The positive correlation between chronotype and phase shifting effects that we observed in our data suggests that evening light exposure may be responsible for the observed changes. Later chronotypes are exposed to more light in the evening allowing, possibly, for larger delays as compared to earlier chronotypes. Previous studies have shown that both in young subjects (Goulet et al., 2007) and in the elderly (Staples et al., 2009), early chronotypes are exposed to relatively more light earlier in the day than later chronotypes who are more exposed to light later in the day. Correlations between chronotype and phase shifting effects have previously been shown in the elderly. Benloucif et al (2006) reported that the phase delay in the melatonin rhythms of elderly subjects correlated with the offset time of sleep (Benloucif et al., 2006). Although sleep occurs at a later clock time after lens replacement, the lack of a change in phase angle difference between midsleep and DLMO before and after surgery shows that sleep occurs at a similar circadian time as before surgery. This could partially explain why we do not observe changes in the efficiency of the sleep (see below). The lack of correlation with the improvement factor in lens transmittance after surgery suggests that the observed changes are due to the relative increase in light input. Tanaka and colleagues recently reported no differences in the timing of sleep nor of the melatonin rhythms between before and after cataract surgery (Tanaka et al., 2010). Given the interindividual variation in timing, it would be good to take chronotype into account in the analysis.

We did not observe systematic differences in the parameters related to the amplitude of the circadian system (i.e., L5, M10, sleep efficiency, and melatonin amplitude). Unfortunately, there is no literature-based information to correct our data for the uneven distribution across seasons of the mea-

surements before and after lens replacement. We used our own records to estimate this effect, and after correction we observed no differences due to lens replacement. Asplund and Lindblad studied subjective sleep quality (i.e., poor sleep, frequent awakenings, and difficulty in falling asleep after nocturnal awakening) 1 and 9 months after lens replacement in Norway (Asplund and Lindblad, 2002; 2004). The authors did not quantify sleep quality but instead gave the proportion of subjects who reported an increase in sleep quality. From a database of 407 patients (35 % male), they report a decrease in the proportions for poor sleep from 1-month to 9-months after cataract surgery going from ~28 % to ~16 % in males and from ~37 % to ~31 % in females. Time of year was not taken into account in the analysis of Asplund and Lindblad and it is conceivable that, especially at high latitudes, changes in environment at different times of the year may have had an impact on sleep quality (Friborg et al., 2012; Pallesen et al., 2001).

The present study may serve as a first attempt to objectively assess the consequences of bilateral cataractous lens replacement for circadian sleep-wake and melatonin rhythms. It should promote awareness in considering the seasonal/DST influences when conducting a study in which the randomization of conditions is not feasible, as well as taking chronotype into account. Both are important factors that may impact entrainment in human subjects.

Acknowledgements

We acknowledge the help of R. Wieringa, E. van der Valk, M. Boon, and G. Hooisma in recruiting subjects, and the participants for their efforts in taking of this study. We thank Prof. Till Roenneberg for sharing the sleep timing data used in Kantermann et al., 2007. We thank Bühlmann laboratories AG (Basel) for the saliva melatonin radioimmunoassay tests used in this study. Our work is supported by the 6th Framework Project EUCLOCK (No. 0187410). §

Appendix

Table 1. Individual average sleep onset data before and after surgery with and without correction

Subject ID	Before Surgery	After Surgery	Before Surgery Corrected	After Surgery Corrected
01	24:20	25:40	24:25	25:01
02	22:17	23:13	22:49	22:46
03	22:43	23:58	23:06	23:31
04	22:39	23:38	23:02	23:14
05	23:49	22:47	23:22	23:22
06	25:07	24:17	24:28	24:25
07	23:52	22:56	23:14	23.29
08	22:44	22:15	22:08	22:17
09	23:28	24:43	23:20	24:06
10	21:53	22:59	22:31	22:24
11	23:53	26:25	24:31	25:46
12	23:07	24:14	23:38	23:36
13	20:21	21:15	20:41	20:36
14	23:25	24:14	23:29	23:38
	23:07	**23:45**	**23:12**	**23:28**

Table 2. Individual average midsleep data before and after surgery with and without correction

Subject ID	Before Surgery	After Surgery	Before Surgery Corrected	After Surgery Corrected
01	4:22	5:43	4:27	5:04
02	1:53	3:07	2:26	2:40
03	2:35	4:07	2:59	3:40
04	2:25	3:28	2:49	3:04
05	3:54	2:48	3:27	3:23
06	4:50	3:55	4:11	4:04
07	3:36	2:32	2:59	2:55
08	2:51	2:08	2:15	2:47
09	3:26	4:31	3:19	3:53
10	1:50	3:23	2:29	2:49
11	3:47	6:06	4:26	5:21
12	3:16	4:28	3:47	3:49
13	1:13	2:07	1:33	1:28
14	3:13	4:06	3:17	3:29
	3:05	**3:44**	**3:10**	**3:28**

Table 3. Individual average sleep offset data before and after surgery with and without correction

Subject ID	Before Surgery	After Surgery	Before Surgery Corrected	After Surgery Corrected
01	8:24	9:46	8:31	9:01
02	5:14	7:01	5:52	6:29
03	6:27	8:16	6:55	7:45
04	6:10	7:17	6:38	6:49
05	7:59	6:49	7:28	7:28
06	8:33	7:34	7:49	7:44
07	7:21	6:09	6:40	6:35
08	6:58	6:01	6:19	6:44
09	7:25	8:19	7:17	7:38
10	5:47	7:48	6:30	7:08
11	7:41	9:36	8:23	8:52
12	7:25	8:41	7:58	7:58
13	6:05	6:59	6:26	6:16
14	7:00	7:58	7:04	7:17
	7:02	**7:44**	**7:08**	**7:25**

Table 4. Individual average DLMO data before and after surgery with and without correction

Subject ID	Before Surgery	After Surgery	Before Surgery Corrected	After Surgery Corrected
01	23:12	1:35	23:17	00:56
02	23:44	1:31	00:17	1:03
03	20:18	22:20	20:42	21:53
04	19:56	21:11	20:20	20:47
05	20:48	20:10	20:20	20:45
06	22:59	20:22	22:20	20:31
07	20:36	20:14	19:50	20:36
08	21:14	20:50	20:38	21:30
09	21:35	23:43	21:28	23:05
10	22:19	22:32	22:57	21:57
11	20:56	00:14	21:35	23:34
12	19:02	21:52	19:33	21:13
13	18:47	20:02	19:06	19:22
14	20:47	22:09	20:52	21:32
	21:09	**22:12**	**21:14**	**21:54**

Table 5. Individual average DLMOff data before and after surgery with and without correction

Subject ID	Before Surgery	After Surgery	Before Surgery Corrected	After Surgery Corrected
01	8:44	11:35	8:49	10:56
02	3:55	7:49	4:28	7:22
03	-	-	-	-
04	7:55	9:05	8:18	8:41
05	7:59	7:05	6:34	7:40
06	7:02	8:07	8:08	8:15
07	8:47	6:27	6:29	6:49
08	7:07	7:38	8:08	8:18
09	8:44	8:42	7:43	8:05
10	6:11	8:52	6:50	8:16
11	6:43	9:14	7:21	8:34
12	5:43	8:47	6:14	8:08
13	8:11	6:41	8:32	6:01
14	7:50	9:04	7:55	8:26
	7:25	**7:56**	**7:20**	**8:07**

The grey are represents participants who started in winter (before surgery) and finished in summer (after surgery), while the white area represents the participants who started in winter and finished in summer. The bold figures represent the average value

Chapter 6

Effects of Reducing Short Wavelengths Input on Melatonin and Sleep Patterns in Humans: Evidence for Adaptation

Marina C. Giménez[1], Domien G. M. Beersma[1], Pauline Bollen[1], Matthijs L. van der Linden[2], Marijke C. M. Gordijn[1]

[1]Research Unit of Chronobiology, Center for Life Sciences, University of Groningen, The Netherlands,
[2]Oculenti, University Medical Center Groningen, The Netherlands

Submitted

Abstract

Light is an important stimulus for the entrainment of the circadian clock, and for increasing alertness. The intrinsically photosensitive ganglion cells in the retina play an important role in transferring light information for these non-image-forming responses to the brain. They are elicited in particular by short wavelength light. Exposure to short wavelengths is reduced, for instance, in elderly due to the yellowing of the ocular lenses. This reduction may be involved in the disrupted circadian rhythms observed in aged subjects. Here we tested the effects of reduced blue light exposure in young healthy subjects (n = 14) by using soft orange contact lenses (SOCL). We show (as expected) that a reduction in the melatonin suppressing effect of light is observed when subjects wear the SOCL. After continuous exposure to reduced (blue) light for 2 consecutive weeks we observed an increase in sensitivity. The response normalized as if it was a full spectrum light pulse. No differences were found in the dim light melatonin onset or in the amplitude of the melatonin rhythms after reduced blue light exposure during 2 weeks. The effects on sleep parameters were limited. Our results demonstrate that the non-image-forming light system of healthy young subjects is capable of adapting to changes in environmental light exposure, even in its spectral composition. The results emphasize the importance of considering long-term adaptation in addition to short-term effects of changes in environmental light characteristics.

Introduction

Light has a large impact on our everyday life. It does not only allow for vision but also for non-image-forming responses. Light is the environmental cue primarily responsible for the entrainment of the biological clock, *i.e.*, the synchronization of our physiological and psychological rhythms to the 24-h rhythm of the environment. Impaired entrainment can lead to discomfort and higher risks for diseases (Rajaratnam and Arendt, 2001; Rüger and Scheer, 2009; Pritchett et al., 2012). Light also has activating effects (Cajochen, 2007; Pritchett et al., 2012; Rajaratnam and Arendt, 2001; Rüger and Scheer, 2009; Rüger et al., 2006) and can acutely suppress the production of melatonin (Lewy et al., 1980).

In the late 90s a new photoreceptor with a key role in transferring light information for non-image-forming responses was discovered (Freedman et al., 1999; Lucas et al., 1999). This is the intrinsically photosensitive retinal ganglion cell (ipRGC) containing the photopigment melanopsin with a sensitivity peak at around 480 nm (Berson et al., 2002; Hattar et al., 2002; Provencio et al., 2000; 1998;). A similar sensitivity peak for non-image-forming responses was also observed in many human studies (Brainard et al., 2001; Cajochen et al., 2005; Lockley et al., 2003; 2006; Revell et al., 2005a; Thapan et al., 2001; Warman et al., 2003).

Aging is a natural process by which input of especially short wavelengths is reduced as a consequence of a denser ocular lens (Giménez et al., 2010a; Van Norren and Vos, 1974). Whether the weak and/or disturbed circadian rhythms observed in the elderly (i.e., fragmented sleep, early awakening, lower melatonin levels) (Van Someren, 2000a and 2000b) can be explained by this reduction in (short wavelength) light input needs further research.

The present study investigates the effects of a reduction in short wavelengths light input at the level of the lens on melatonin- and sleep rhythms, and on suppression of nocturnal melatonin in humans. By means of orange soft contact lenses (SOCL), we mimicked, to a certain extent, the aging effects of the lens in healthy young subjects. This allowed us to assess the effects of exposure to short wavelengths in a realistic natural scenario, as well as to separate the effects of altered lens transmittance from other aging effects. We hypothesized that reduced (blue) light input would result in a less stable activity-rest cycle (see review Dijk et al., 2000), a reduction in the nocturnal melatonin secretion (see review Skene and Swaab, 2003) as well as a reduction in the suppression of nocturnal melatonin by light (Brainard et al., 1997; Duffy et al., 2007; Herljevic et al., 2005). Knowledge of the response to changes in environmental light exposure will be relevant for understanding rhythm disturbances

in the elderly. It will also increase our understanding of the importance of changing the light environment in everyday life, a topic that is of interest to an interdisciplinary audience of health specialists, light industries and architects (Fournier and Wirz-Justice, 2010).

Materials and Methods

Subjects

50 subjects started the selection procedure for the study. Only those subjects between 18 and 30 years who were healthy, non-smoker, non-color blind (Ishihara test), and of an intermediate chronotype (midsleep on free days between 3.7 and 6.3 a.m. according to the Munich Chronotype Questionnaire (MCTQ; Roenneberg et al., 2003) were selected to participate. Subjects who had worked on night shifts or travelled across more than 2 time zones during the 2 weeks prior to the study were excluded.

The study required participants to wear SOCL during 2 consecutive weeks, 24 h per day in the experimental condition. To assess participants' eyes health condition a check-up by a contact lens specialist (coauthor ML vd L) was conducted at the University Medical Center of Groningen (UMCG), The Netherlands. After screening, 22 subjects were selected of whom 15 completed the study (7m: 8f, mean age ± sd: 24.5 ± 4.6 years). Most dropouts were due to irritations in one or both eyes and some due to discomfort. Special care was taken in order to exclude subjects who did not feel comfortable after wearing the orange lenses for 24 to 48 hours.

The Medical Ethics Committee of the University Medical Center of Groningen (UMCG) approved the study protocol. All subjects signed a written informed consent form prior to their participation. All subjects were financially compensated for their participation.

Soft Orange Contact Lenses

The SOCL (CE: 0120, with UV protection) were supplied by Ultravision International Ltd., UK. These lenses are normally used for medical purposes and are designed so that they can be continuously worn (24 h/day) for up to 3 consecutive months. The lenses reduce the overall light intensity, in particular of short wavelengths. Light transmittance in the visible range of the spectrum (from 420-700 nm) was reduced by 37%. In the short wavelengths range (420-500 nm) the reduction in transmittance was 53%. Figure 1 compares the transmission per wavelength (400-700 nm) of an average 25-year healthy subject without (Van de Kraats and Van Norren, 2007a) and with the SOCL, with the average transmission of cataractous eyes of 14 elderly subjects, where retinal light reflectance is severely reduced (data from Giménez et al., 2010a).

Experimental Design

In randomized sequence a control condition (13 subjects wore their own contact lenses and 2 subjects no lenses) and an experimental condition (all subjects wore the SOCL) were assigned to each of the 15 subjects (8 subjects started with the control and 7 sub-

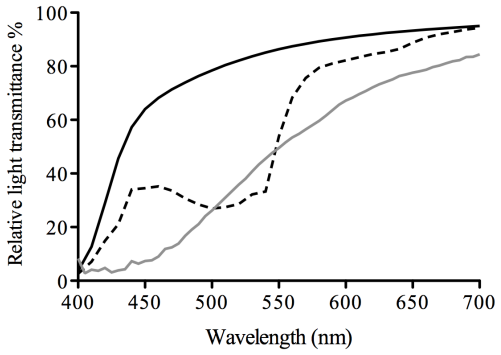


Figure 1. Relative light transmittance. Spectral composition of relative light transmittance through the ocular lens of an average 25-year-old subject with (dashed black line) and without the SOCL (continuous black line) in comparison with a cataractous eye (grey line).

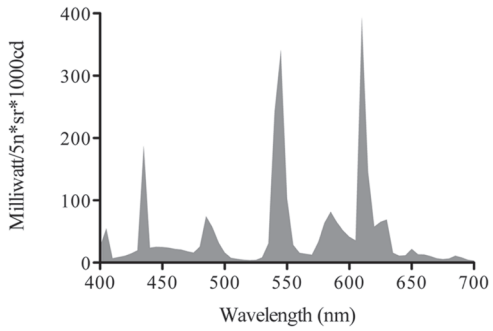


Figure 2. Spectral composition. Spectral composition of the Osram Dulux L 36W/835 tubes used in the Pharos Max light boxes.

jects started with the SOCL condition). The SOCL were adjusted according to the subjects' needs for visual corrections. Each condition lasted 16 days. They were timed at least 2 weeks apart to avoid potential carry-over effects, and for each subject they started on the same day of the week (this differed between subjects) in order to control for the possible pattern of behavior throughout the week within each subject.

Melatonin profiles were assessed on night 15. Subjects arrived at the lab at 18:00 h. Light levels were dimmed (<5 lux). Saliva samples were taken using cotton swabs (Sarstedt BV, Etten-Leur, The Netherlands) every hour from 19:00 to 00:00 h, then every half hour until 2:00 h, and every 2 hours from 3:00 until 9:00 h. On night 16 the suppression of nocturnal melatonin by light was assessed. For this purpose after the 00:00 h sample and until 2:00 AM subjects were asked to sit in front of two full spectrum white light boxes (600 lux, 190.5 μW/cm2, Pharos Max, Osram Dulux-L tubes, ©Lumie, see figure 2 for spectral composition). During these two hours, subjects watched a movie on a TV monitor situated in between both light boxes in order to keep the direction of gaze constant. Light intensity at eye level was regularly checked during the 2 hours and adjusted if necessary. The acute effect of the SOCL was assessed on a separate night not within 7 days after the 16 days of the experimental or control condition. For this purpose, the SOCL were worn only 30 minutes before the light pulse and during the 2-hour light pulse (in contrast with 16 days of continuously wearing the SOCL). Subjects were free to read or watch videos during the nights in the lab. Subjects were carefully instructed about the collection of saliva samples for melatonin assessment. Eating was restricted to the first 15 minutes after each sample. Chocolate, bananas, coffee and tea were not allowed during the whole sampling period. Ten minutes prior to each sample subjects were asked to sit quietly to avoid influence of posture (Deacon and Arendt, 1994). Samples were centrifuged immediately after collection and stored at -20°C until analysis.

Actigraphy data (1-min epochs) were collected continuously during the 16 days (Actiwatch®, Cambridge Neurotechnologies, UK) together with sleep logs. Subjective ratings of sleep quality were collected after waking up (1 to 10 scale, 1 = very bad, 10 = excellent). During the last five days, sleepiness ratings were assessed by means of the KSS (Åkerstedt and Gillberg, 1990) at five different time points: at waking up, at 12:00 h, at 16:00 h, at 20:00 h and at bedtime.

Light exposure (lux) was collected by means of Actiwatches-L on 1-min epoch basis. Careful instructions were given to the subjects not to cover the light sensor by sleeves.

Data Analysis

Salivary melatonin concentration was assessed by radio-immunoassay (RK-DSM, Bühlmann laboratories AG, Siemens Medical Solutions Diagnostics, Breda, The Netherlands). All samples from an individual were analyzed within the same series. The limit of detection for the RIA was 0.3 pg/ml with an intra-assay variation of 6.7% at a low melatonin concentration (mean 1.5 pg/ml, n=30) and 6.5% at a high melatonin concentration (mean=15 pg/ml, n=30). Inter-assay variation was 12.2% at low melatonin concentration (mean=2.1 pg/ml, n=15) and 19.7% at high melatonin concentration (mean=17.5 pg/ml, n=16).

The full melatonin profiles of the control condition were fitted to a bimodal skewed baseline cosine function (Van Someren and Nagtegaal, 2007). All melatonin values were expressed as a fraction of the maximal fitted value on the control night for the individual subject. DLMO was defined as the time when the threshold at 25% of the maximum value of the fitted curves was crossed. The suppressing effect of light on melatonin concentration during the 2 hours of light exposure was estimated for each subject as the difference between the area under the control curve and the curve during light exposure (AUC, pg.h/ml). The AUC was calculated from time point 00:30 until time point 2:00. The results are discussed as percentage of suppression relative to the control curve.

Sleep analysis 5 software (Cambridge Neurotech Ltd, Cambridge, UK) set at medium was used together with sleep logs. The Actiwatch algorithm looks at each data point from each epoch and those surrounding it and makes a total score based on these activity counts. The adjacent activity scores influence the total score in the following way: within 1 minute of the scored epoch activity levels are reduced by a factor of 5 in comparison to the epoch being scored and this value is added to the scored value of the epoch under consideration. When the total score is above the sensitivity threshold the epoch is designated as wake otherwise as sleep. For automatic determination of Sleep Start the algorithm looks for a period of at least 10 minutes of consecutively recorded immobile data, with no more than 1 epoch of movement within that time, following the bed time (sleep logs). The start of this defined period is classified as sleep start and the difference in this and bedtime is used to determine sleep latency. For sleep end the algorithm

looks for a 10-minute consecutive period of activity around the get up time (sleep logs) and then works back to find the last epoch of immobility before the start of such a sequence and classifies that as sleep end. Sleep onset, midsleep, and sleep offset were used to describe sleep timing. All timing variables are shown for work and days off as well as for the overall duration of the study. We further investigated sleep efficiency (percentage of time spent asleep while in bed), the average activity of the least active 5 hours, and the average activity of the 10 most active hours (Van Someren et al., 1999; Witting et al., 1990). The analysis of the sleep parameters is based on the first 14 days of each condition. The last two nights spent in the lab were excluded since sleep disturbances were introduced during the sampling of melatonin.

Light analysis 5 software (Cambridge Neurotech Ltd, Cambridge, UK) was used to calculate the average light intensity (lux), the maximum intensity and the time spent above 1000 lux

The effects on melatonin suppression, on DLMO and on the amplitude of melatonin were tested by means of a paired t-test. A 2-way ANOVA was used to test the effects on sleep for the factors: condition, work/day off, and the interaction effect. For the analysis of the KSS a repeated measurement ANOVA was conducted for the factors of condition: control and SOCL, for the factors of time: waking up, 12:00h, 16:00h, 20:00h and bedtime, and for the interaction. A paired-t test was used to evaluate the differences in light exposure between the control and the 16-days- SOCL condition.

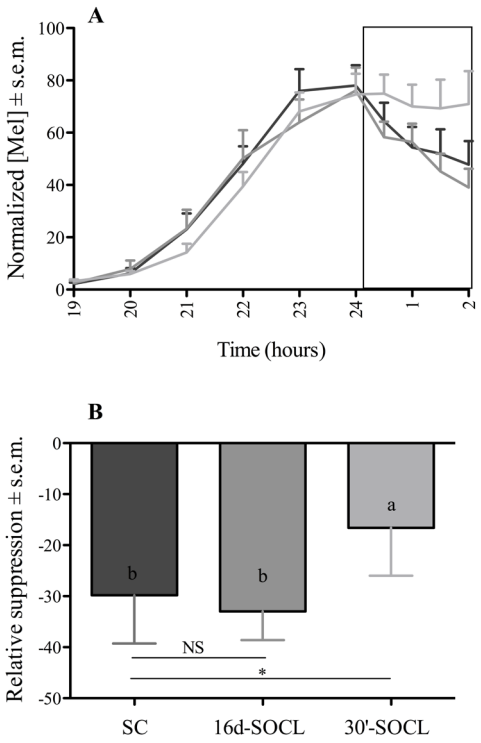


Figure 3. Melatonin Suppression. (A) Melatonin suppression curves. Dark, medium and light grey represent the suppression in the control condition (SC), the suppression after 16 days of wearing the SOCL (16d-SOCL), and the suppression after 30 min of wearing the SOCL (30'-SOCL) respectively. The block represents the time at which the 600 lux white light pulse is given. (B) Melatonin suppression (%) relative to the control melatonin profile values during the 2 hours of light exposure. Asterisks denote significant differences between suppressing effects of light in the 30'-SOCL and the suppressing effects of light in the 16d-SOCL as well as with the suppressing effects of light in the SC without SOCL.

Results

Melatonin Suppression by Light

The course of melatonin in the evening and the percentage of melatonin suppression during the light pulse relative to the control can be seen in figures 3A and 3B The melatonin suppression 30 minutes after plac-

ing the SOCL was significantly less (average ± SEM: 17% ± 9%) than in the control condition (average ± SEM: 30% ± 9%) ($t = -2.65$, $p < 0.05$). This result shows that the SOCL indeed filtered the light reaching the retina enough to reduce melatonin suppression. After wearing the SOCL for 16 days no differences were found in the suppression of melatonin (average ± SEM: 33 % ± 6, $t = 0.15$, $p = 0.88$) when compared to the suppression in the control condition. The suppression of melatonin in the 30-min SOCL condition was significantly less than in the 16-days SOCL condition ($t = -2.37$, $p < 0.05$). Two out of the 14 subjects showed no suppression of the nocturnal melatonin level to light at all in the control condition.

Melatonin Profile

No significant differences were found in the timing of the dim light melatonin onset (DLMO) between the control and the OL condition after 16 days (average ± SD: control: 21:50 ± 1 h 03 min; SOCL: 21:37 ± 1 h 35 min, $t = 0.831$, $p = 0.42$). No significant differences were observed in the amplitude of the melatonin rhythm (average ± SD: control: 95.4 % ± 3.7; SOCL: 100.7% ± 23, $t = 0.852$, $p = 0.41$).

Sleep Characteristics

Sleep timing for work and days off is summarized in table 1. Wearing the SOCL for 14 days had no significant effect neither in the timing of sleep nor on its efficiency or subjective quality. A main effect of day of the week was observed for all timing variables (all $p < 0.005$), except for sleep onset latency and for subjective sleep quality. No interaction effect between condition and day of the week was observed. Sleep onset latency was slightly shortened in the SOCL as compared to the control condition. While no differences were observed in the average activity during the least 5 active hours (L5) (average ± SD: control: 11.1 ± 3.2, SOCL: 11.8 ± 3.9, $t = -0.84$, $p = 0.41$), a small but significant ($t = 2.3$, $p < 0.05$) reduction in the average activity of the 10 most active hours (M10) was shown in subjects wearing the SOCL (average ± SD: 54.9 ± 3.2) in comparison to the control condition (average ± SD: 55.9 ± 2).

Karolinska Sleepiness Scale ratings (KSS) were analyzed to test whether condition had an effect in addition to the well-known effect of the time of day (a U-shaped curve with higher, more sleepy, values at waking up and before bedtime). No effect of the SOCL was found on the KSS ratings (average ± SD: control: 4.7 ± 1.2; SOCL: 4.8 ± 1.1, $F(1, 14) = 0.01$, $p = 0.94$). Only time-of-day contributed significantly to the explained variance (pattern over time: $F = 21.138$ (4, 11), $p < 0.001$, data not shown).

Light Exposure

No differences in the average light exposure, neither in the maximum, nor on the average light exposure duration above 1000 lux were observed between conditions (table 2).

Discussion

Since the discovery of the ipRGCs several

Table 1. Sleep characteristics. Sleep timing, efficiency and subjective quality obtained by a combination of sleep diaries and actiwatch data

	Work Days		Days Off	
	Control	16-day OL	Control	16-day OL
Bed Time[a]	00:25 ± 40 min	00: 05 ± 1 h 1 min	1:24 ± 11 min	1: 46 ± 1 h 28 min
Sleep Onset[a]	00:39 ± 40 min	00:18 ± 47 min	1:43 ± 1 h 9 min	1:59 ± 1 h 30 min
Sleep Offset[a]	7:46 ± 54 min	7:39 ± 47 min	9:22 ± 55 min	9:25 ± 1 h 23 min
Sleep Latency (min)[b]	14.4 ± 9	12.1 ± 7.3	19.1 ± 18.4	12.6 ± 8.9
Midsleep[a]	4:13 ± 39 min	3: 58 ± 42 min	5: 32 ± 52 min	5:42 ± 1 h 19 min
Sleep Efficiency (%)[a]	81.1 ± 4.4	80.1 ± 5.5	80.1 ± 6.2	79.8 ± 4.5
Subjective Sleep Quality[a]	6.4 ± 0.8	6.3 ± 0.9	7.2 ± 1	6.9 ± 0.8

Data are shown as average ± SD. [a] Significant main effect of day of the week ($p < 0.005$). [b] Main effect of condition $p = 0.06$. No significant effect of interaction between condition and day of the week was observed ($0.7 < p > 0.1$).

Table 2. Light exposure characteristics. Average light intensity, average maximum intensity, and average time spent above 1000 lux as obtained from actiwatch data

	Control	16-day OL	t	p
Average light intensity (lux)	552 ± 335	515 ± 340	0.41	0.69
Average max. light intensity (lux)	16945 ± 7860	15697 ± 7848	0.82	0.42
Average time spent above 1000 lux	2 h19 min ± 1 h 19 min	2 h 1 min ± 1 h 17 min	1.04	0.31

Data are shown as average ± SD.

studies have shown that short wavelengths play a major role in non-image forming responses. The spectral composition of light became a quality feature that was increasingly incorporated in the circadian field. The aim of the present study was to investigate the effects of exposure to diminished short wavelength light throughout the day as it occurs, for instance, in the elderly population on the suppression of the nocturnal melatonin by light and on melatonin and sleep-wake rhythms. The study yields three primary conclusions. 1) We found that melatonin suppression by light is sharply reduced when subjects wear the SOCL during the test (+ 30 minutes before). 2) This reduction disappears when subjects have worn the SOCL for 16 days continuously. 3) The use of the SOCL during 16 days had virtually no effect on circadian rhythms of sleep and melatonin. These conclusions are further discussed below. In view of these conclusions the implications of reduced exposure to blue light in the elderly population and in society in general are discussed.

1. Melatonin Suppression by Light

Light of short wavelength has been shown to have a larger suppressing effect on mel-

atonin concentrations when compared to larger wavelengths (Brainard et al., 2001; Thapan et al., 2001; Cajochen et al., 2005). Based on these results, studies where short wavelengths were blocked by means of goggles during a simulated night shift in bright light conditions, found nocturnal melatonin levels similar to those observed under dim light conditions (i.e., no significant suppression of the nocturnal melatonin) (Kayumov, 2005; Sasseville et al., 2006), without impairing performance, alertness or sleepiness (Kayumov, 2005). Our results are consistent with these studies in showing that melatonin concentrations in subjects wearing the SOCL during the 600 lux light pulse from midnight until 2 a.m. are not significantly different from the dim light melatonin values. A complete blockage of short wavelengths is most likely not needed to achieve this result, since our lenses cut down the irradiance in the short wavelengths range by about 50%. The advantages of the SOCL are the full coverage of the eye and their inconspicuousness. Alternatively, the room lights can be changed for reduced short wavelength output ones. In a recent study in our lab Van de Werken and colleagues showed that in a simulated shift work setup blue-reduced light leads to small larger melatonin suppression than dim light (Van de Werken et al., in press). Blue-depleted light may sometimes be an alternative when the burden of wearing goggles or contact lenses is larger for workers than the potential consequences of a small suppression (~6%) of melatonin.

In order to understand the effect of short wavelength reduction in a situation such as in the elderly another approach is needed. Here, the reduction of short wavelengths due to yellowing of their lenses is continuously present, 24 hours a day, and the long-term effects of this reduction need to be assessed. The results of this manipulation are discussed in the following sections.

2. Adaptation to Reduced (Blue) Light Exposure

After wearing the SOCL 24 h a day for 2 consecutive weeks the suppression of the nocturnal melatonin by light was as large as the suppression observed in the control condition (without the SOCL). Previous studies have shown that light history has a large impact on non-image-forming responses. In time frames ranging from hours up to a week, exposures to dimmer light conditions have lead to an increase in sensitivity of the biological clock system measured by means of melatonin suppression (Hébert et al., 2002; Jasser et al., 2006; Smith et al., 2004). Our findings do not only imply an increase in sensitivity but rather a restoration/normalization of the response to the levels of the control condition. We further found that wearing the SOCL for 2 weeks significantly affected neither DLMO nor the amplitude of the melatonin rhythm. Together these findings suggest that during these two weeks adaptation to the changes in the spectral composition of light occurred. Adaptation is the process that potentially compensates for light intensity differences. One could argue that differential exposure to light could have lead to the observed results. Subjects would have had to naturally, but systematically, expose themselves to just enough more light in order to compensate

for the difference between the control and the SOCL condition to restore melatonin suppression values to those observed in the control condition. Our light data exposure reveals no differences between both conditions, allowing us to discard differential light exposure as a key factor for our findings.

Neither the previous studies nor the present study were designed to precisely assess the temporal characteristics of adaptation. It would be valuable to develop an adaptation curve to changes in the spectral composition during the day (i.e., after how many hours/days of selective exposure to certain wavelengths during daytime is the melatonin suppression response restored to dim light melatonin levels again). Restoration after exposure to darkness should be considered. In mice circadian phase responses to light are reduced rapidly by prior light exposure and fully restored by prolonged (18 h) dark exposure (Comas et al., 2007). If the lenses have caused "dark adaptation", this would enhance sensitivity to entraining light stimuli and thus compensate for the reduced penetration of blue light to the ipRGC's. Alternatively, redistribution of sensitivity across photoreceptors could explain our observations. It is reasonable to surmise the occurrence of compensatory processes under the constant presence of relatively small changes in light intensity and spectral composition. The technique described in the present study (extended to different selections of spectral composition) would be suitable to construct such adaptation curves and assess the changes in relative contribution of the different photoreceptors.

3. Effects of SOCL on Circadian Rhythms of Melatonin and Sleep

We observed no differences in the timing of sleep or melatonin rhythms after wearing the SOCL for 2 weeks. Only slight changes in sleep latency a slightly and the reduced activity during the 10 most active hours were observed. The shorter sleep latency and less activity shown in the SOCL condition might indicate increased tiredness and/or less alerting/activating effects of light as expected after exposure to less blue light (Cajochen, 2007; Rüger et al., 2006). Sleep efficiency and sleep quality, although in the direction of a more disturbed sleep pattern, were not significantly affected by the SOCL. Studies in humans have shown that complete absence of short wavelength light before bedtime improves sleep (Burkhart and Phelps, 2009; Santhi et al., 2012), while its presence leads to the opposite effect (Münch et al., 2006; Santhi et al., 2012) in a blue-amount dependent manner (Santhi et al., 2012). The use of orange goggles during the morning hours (from awakening until ~15:00 h) lead to a phase delay of the DLMO (Figueiro and Rea, 2010). These studies tested the effect of (lack of) blue light at specific times of the day. Our study shows that sleep and melatonin rhythms after 2 weeks of continuous partial absence of blue light are not different from sleep and melatonin rhythms after 2 weeks of unfiltered light exposure.

It could be argued that the reduction of light exposure due to the SOCL was not large enough to induce sleep disturbances, or a shift in phase (both of sleep and/or melatonin rhythms) in these young people. But the melatonin suppression data indicate that

there is indeed a reduction in lens transmittance that leads to a reduction in melatonin suppression after wearing the lenses for 30 minutes. If changes in melatonin suppression are achieved by means of the SOCL, it is expected that those changes in light input are also capable of induce a shift in phase (Zeitzer et al., 2000). In this sense, adaptation to the new light environmental conditions seems a plausible explanation to the lack of effects observed after wearing the SOCL for 2 consecutive weeks.

Thus, neither the melatonin profile, nor the sleep characteristics suggest that entrainment of the circadian system is compromised by the long-term application of SOCL. The reduction we achieved by means of the SOCL is relatively comparable to that of a cataractous eye at about 480 nm at which the sensitivity of the ipRGCs peaks (Berson et al., 2003; Panda et al., 2005). At shorter wavelengths the discrepancy becomes larger, however this is to some extent caused by the peak in short wavelengths present in the light source we used in the present study (figure 3). If, as in young subjects (Revell et al., 2006), very short wavelengths lead to increase alerting effects in the elderly, this could have, in the long term, implications for the timing of sleep in the elderly. Further research is needed on the sensitivity to light of the elderly. The present study does not support the idea that the general circadian characteristics of the elderly can be explained by the aging characteristics of the lens based on a reduction in (blue) light transmittance only. The effects observed in the present study were marginal and probably of a transitional nature, whereas in the elderly the impaired circadian output remains. Healthy young subjects might have a more plastic non-image-forming system than elderly people. With pathological aging this might become less flexible and may lose its capability to fully adapt to changing situations. Still, exposure to bright light, exercise, and melatonin can promote restoration of diminished-non-image-forming responses in elderly subjects (for review see Van Someren et al., 2002). These improvements are mainly based on studies conducted on institutionalized subjects where light conditions are far from being optimal. Whether healthy old subjects can also show an improvement after increasing environmental light availability is not clear.

The present study differs from previous studies by the fact that subjects' exposure to light was not modified experimentally in any other way than by using the contact lenses. There were no behavioral restrictions, nor was there any unnatural situation. The study approaches the effects of light history on sensitivity of the circadian system in a rather realistic manner: the altered lens transmittance is continuously present, as it is in elderly people. The fact that the system in healthy young people is able to adapt to the spectral composition of the light is remarkable. The circadian system continues to function as a time keeping mechanism and it regulates entrainment and alertness as if nothing had changed. Through such adaptations, healthy humans may adjust to different life styles, such as living indoors or outdoors, and to seasonal and/or latitudinal changes.

Aknowledgments

The authors thank Prof. Dr. Serge Daan for his insightful comments on the manuscript and Bühlmann laboratories AG for the direct saliva melatonin radioimmunoassay tests provided for this study. Our work is supported by the 6th Framework Project EUCLOCK (No. 0187410) and ©Lumie (Outside In (Cambridge) Limited). §

Chapter 7

Epilogue

Marina C. Giménez

Entrainment is a key aspect of circadian rhythms. Entrainment allows for anticipation of the predictable changes that occur in the environment on a daily basis. The light-dark cycle is the main environmental cue that entrains our biological clock to the external 24 h day.

In this thesis, my coauthors and I have contributed to the circadian field by assessing how changes in light exposure affect human entrainment. To do so, studies were carried with humans in their natural every day life. No restrictions in behavior were imposed and visits to the lab were limited only to assessments of nocturnal melatonin rhythms in some cases. We took this approach because human entrainment has rarely been explored under natural conditions as compared to the extensive data available from controlled laboratory studies.

The sleep-wake alternation and melatonin rhythms have been the most common circadian variables previously measured in human subjects and they are also the main subjects of this thesis. We assessed sleep patterns by means of actigraphy. While polysomnography is the standard tool for the objective assessment of sleep, actigraphy has been shown to give valuable insights on sleep-wake rhythms of human subjects, it has been extensively used in human research (see Van Someren, 2011), and it is an optimal tool for long-term assessment of sleep in the field. In addition we also investigated the subjective feelings of sleepiness and well-being of our participants.

Field Studies

Laboratory studies have the great advantage that relevant parameters can be varied while possible confounding variables are controlled and kept constant. Laboratory studies are therefore excellent at giving a clear view of those parameters that can modify circadian phase (for instance) and improve alertness or well-being. Stepping out of the laboratory means dealing with natural fluctuations in behavior and light exposure and in real life a wide variety of confounding variables can be present. In the field, the modifiers of circadian phase, that are so carefully selected in the lab, may be overridden by other influences. Therefore, for a fuller understanding of entrainment we also need to address the question of how circadian overt rhythms respond to these, natural daily variations in light exposure. For this purpose field studies are indispensable.

The first section of this thesis deals with the natural seasonal variations in light that we are exposed to in modern life at a temperate latitude (53°13'N). In chapter 2 we compared sleep-wake and melatonin rhythms during the summer and winter months in healthy young subjects. Our goal was to explore these rhythms in an integrative way along with light exposure. Data were collected in the field, in line with the focus of the thesis. This includes the assess-

ment of nocturnal melatonin at home without restrictions on light exposure. A second set of nocturnal melatonin measurements were collected under dim light conditions in the lab in order to estimate circadian phase as it is generally done in human studies. These two types of melatonin profiles have not been measured within one study and compared intra-individually before. We observed that both the timing of the sleep-wake cycle and of the nocturnal melatonin rhythms were delayed in the winter months in comparison to summer. This delay is possibly the result of a lack of phase advancing morning light in the winter months and an endogenous period of the rhythm that is in general a bit longer than 24 hours (Wever 1979). When assessing seasonal effects it is important to pay attention to the introduction of daylight saving time (DST). Our results show that changes in the timing of sleep and of melatonin rhythms during workdays did not reach the 1-hour difference predicted solely on the basis of DST, while on days off the difference was larger than 1 hour. This shows the difficulties in the interpreting the influence of DST. For instance, assuming that full compliance with DST occurs, the 41 minutes advance of mid sleep on workdays (MSW) that we observe in summer implies a 19-minute delay. This is most likely due to the presence of light late in the day during the summer months, *i.e.*, the light- dark cycle in opposition to social Zeitgebers. We observed no systematic differences between the timing of the melatonin rhythms assessed in the lab and at home. This suggests that light exposure of our subjects at home was not sufficiently different from the lab as to induce a shift in phase. Correlations between changes in day-to-day variations in light exposure and the timing of sleep and of melatonin showed that, depending on light intensity and the timing of light exposure, the phase angle between sleep and melatonin may change. This observation has direct implications in the development of light strategies to enhance performance and well-being.

The dark winter months can also have a negative impact on the process of waking up. In CHAPTER 3 we tested the potential benefits of using a wake up alarm that provides an artificial dawn signal during winter. The device was designed and brought to the market with the intention of helping people to wake up easier. This is not trivial. To a certain extent everybody, but especially late chronotypes, experience some sort of grogginess after waking up, a phenomenon known as sleep inertia (Åkerstedt and Folkard, 1997; Dinges, 1990; Tassi and Muzet, 2000). We investigated the device's potential in reducing sleep inertia complaints in view of the impact that this could have on, for instance, early morning performance. A device that is ready to be used by people at home asked for research approaches both in the laboratory (Van De Werken et al., 2010) and in the field (Giménez et al., 2010). We setup the tests of the device at peoples' home, in the same way as it would be used by the final user. We observed a positive impact on subjective measurements of sleep inertia and well-being. This was, however, not accompanied by a shift in the dim light melatonin onset, although previous research has established the phase advancing effects of morning light (Dijk et. al., 1989). Recent research has followed up on our observations.

In a laboratory setup it was shown that use of the device (similar to the methods used by our own laboratory) helps to attenuate decrements in cognitive performance (objective measurements) and improve subjective well-being. In accordance with our results, this study did not show a shift in the melatonin rhythm (Viola et al., 2012). Exposure to dawn light simulation could be an option when only acute effects of light, but no phase shifting effects are desired.

Shining a New Light

In 1999 Russell Foster's group revealed that rods and cones were not the only photoreceptors in the mammalian retina (Freedman et. al., 1999). Within the ganglion cells layer in the retina a small subset of these cells were shown to be photosensitive (Berson et. al., 2002). This subset of cells are the intrinsically photosensitive retinal ganglion cells (ipRGCs). The photopigment present in these cells is melanopsin (known as Opn4) which shows a sensitivity peak in the short wavelength range (Hattar et al., 2002; Lucas et al., 2001; Provencio et al., 2000). Studies in human subjects have also revealed that the sensitivity to light of melatonin suppression could not be explained by the spectral characteristics of the traditional rods and cones photoreceptors. The maximum responses were shown for wavelengths around 460-480 nm (Brainard et al., 2001; Hankins and Lucas, 2002; Thapan et al., 2001), which correspond to the sensitivity of the ipRGCs. In the subsequent years several studies have been conducted in human subjects as well as in other species to assess the impact of short as compared to long wavelengths on the non-image-forming responses to light (see Brainard and Hanifin, 2005).

We were also intrigued by this new photoreceptor. The second section of this thesis focuses on the effects of changes in the spectral composition of light, particularly in the blue range. We set out to investigate the relatively long-term impact of reduced/enriched short wavelengths exposure under natural conditions.

In CHAPTER 4 and CHAPTER 5, we took advantage of cataract surgery to investigate the potential effects on entrainment of the age-related reduction of short wavelength transmission through ocular lenses. We first describe in CHAPTER 4 an objective technique that allows for the measurement of the spectral composition of the light reaching the retina *in vivo*. With a non-invasive procedure that does not take longer than 15 minutes we investigated the improvement factor in lens transmittance after removal of the cataractous lens and replacement by a transparent lens. Our results show that in the short wavelength range, between 420 and 500 nm, lens transmittance is improved by an average factor of 4.0 while in the long wavelengths (505 - 750 nm) the improvement was only a factor of 1.3. The difference is related to the gradual yellowing of the ocular lenses commonly observed in the elderly (Van Norren and Vos, 1974). In CHAPTER 5 we assessed how these changes in lens transmittance affected sleep and the nocturnal melatonin rhythms of those elderly who undergo cataract surgery. In CHAPTER 6, we took a methodological step further and, by means of soft orange contact lenses SOCL, we inves-

tigated in young healthy subjects the impact of long-term reduced input to the retina of short wavelengths on sleep-wake and melatonin rhythms as well as on acute melatonin suppression. By assessing these effects in young subjects we aimed to remove the confounding effects of aging and focus only on the spectral composition of light.

An overview of the effects observed on the timing of the sleep-wake and melatonin rhythms is shown in table 1. The interventions lead to different results. I first discuss the lack of phase shifts in sleep and melatonin rhythms that we observed in the young subjects in CHAPTER 6. This finding is in contrast with the phase delay we observed in the elderly in CHAPTER 5. Data collected on melatonin suppression in the young subjects showed that the suppression of melatonin after wearing the SOCL (reduced short wavelength input) for two weeks was not different from the suppression in the control condition. However, wearing the SOCL for only 30 minutes lead to less suppression, as expected for an input signal that contained less blue light (Brainard et al., 2001; Cajochen et al., 2005; Kayumov et al., 2005; Sasseville et al., 2006; Thapan et al., 2001). An increase in sensitivity seems to have taken place after wearing the orange lenses for 2 weeks. This may be explained by adaptation. Such adaptation may also be reflected in the absence of changes in the timing of sleep and melatonin rhythms between conditions. Adaptation could be, at least partially, the result of photostasis. This process accounts for retinal plasticity of, at least, the classical photoreceptors leading to a similar number of photons caught per day irrespective of the long-term light conditions. Photostasis has been observed both in rats (Penn and Williams, 1986) and in humans (Rufiange et al., 2007). This is one possibility in the many components of the circadian system that may contribute to adaptation. An obvious difference between both studies is the age of the groups participating. The observed differences may thus be age-related. In the elderly probably not only the aging of the lens plays a role, but the whole system, including the circadian pacemaker, is aged. With increasing age the number of rods and retinal ganglion cells in the retina decline (see review, Bonnel et al., 2003). Also, at the level of the SCN both neuronal activity and expression of arginine vasopressin (AVP), a major neuropeptide, drops (see review, Van Someren et al., 2002). There is also evidence of neural desynchrony in the SCN of aged mice (Farajnia et al., 2012). Thus the aged circadian system seems to become less robust. Considering the circadian system as a clock machinery that depends on external input for entrainment, a less robust system may become more responsive to changes in these inputs, changes that a young system may be able to filter out. The delayed phase after lens replacement involving increased exposure to blue light is at a first glance surprising. Exposure to a stronger (blue enriched) Zeigeber is expected to result in a phase advance (Roenneberg et al., 2003). On the other hand, it is a characteristic of the elderly (with aged lenses) to be relatively earlier than the young population (Duffy et al., 1998; Miles and Dement, 1980; Myers and Badia, 1995; Roenneberg et al., 2003). During a large part of the year, sunrise occurs before waking up for most people. If at wake-up time light is strong enough to

Table 1. Overview of changes in timing of sleep and melatonin rhythms and of phase angle between DLMO[a] and MSW, and DLMOff[b] and MSW from CHAPTERS 5 & 6

	Timing Blue Reduced → Blue Enriched	Phase angle Blue Reduced → Blue Enriched
CHAPTER 5		
Elderly subjects	(advance) → delay	-5:55 h ± 1 h 32 min → -5:33 h ± 1 h 26 min[a]
(before – after cataract surgery)	MSF + 18 min	4:09 h ± 1 h 24 min → 4:41 h ± 46 min[b]
	DLMO: + 40 min	
CHAPTER 6	n.s.	-6:22 h ± 1:44 h → -6:27 h ± 1 h 11 min[a]
Young subjects		3:26 h ± 1 h 46 min → 3:48 h ± 53 min[b]
(orange – clear lens)		

Data are shown as mean ± SD. MSF: midsleep on free days, MSW: midsleep on work days.
DLMO: dim light melatonin onset, DLMOff: dim light melatonin offset.

reset the circadian clock, a factor of 4 more photons (~ 0.5 log unit) in the short wavelengths due to cataract surgery, may not have a large impact in the morning. However, in the evening, when light levels are low, the effect may be strong enough to induce a shift in phase (Boivin et al., 1996; Zeitzer et al., 2005). The observation that phase advances, and not delays, are attenuated in the elderly compared to young subjects (Klerman et al., 2001) could enhance this delaying effect. It would therefore be interesting to systematically assess how light history affects the circadian system in the elderly.

Although the reduction in short wavelength input in the present studies was modest, these studies are among the first to investigate real-life changes in blue light exposure. Within these natural changes the phase relationship between sleep and melatonin rhythms remained within the range described for healthy subjects (Sletten et al., 2010) (see table 1).

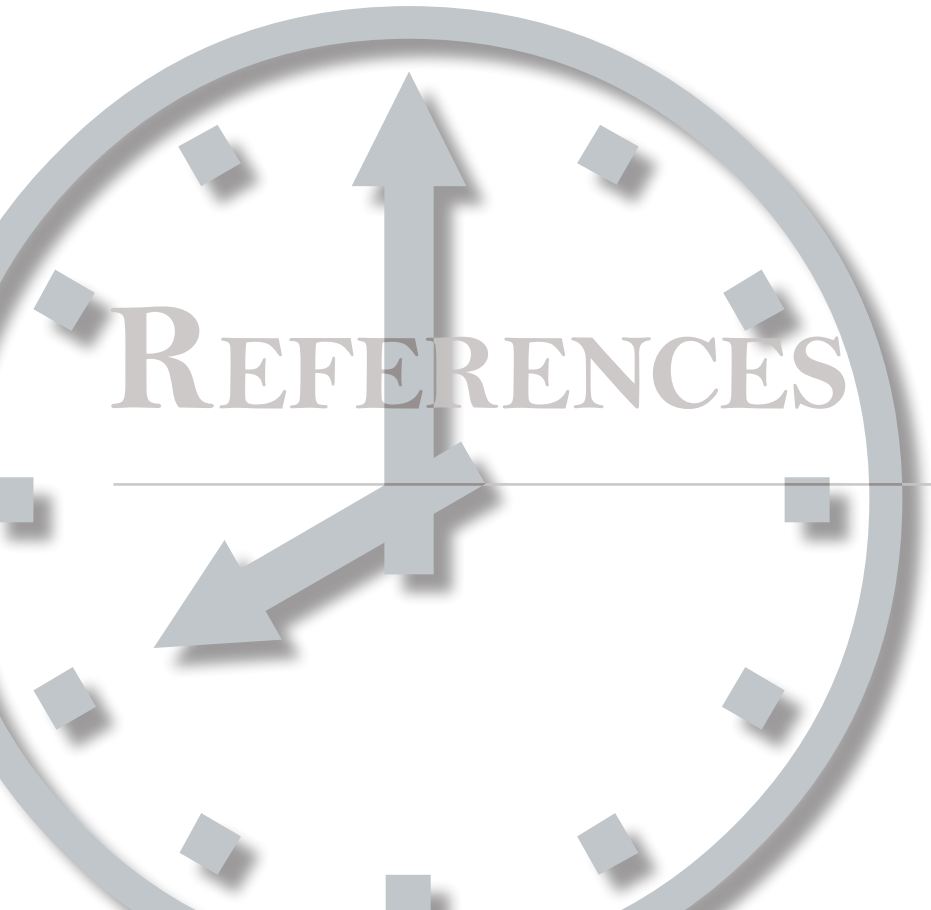
PERSPECTIVE

Understanding daytime light exposure is a crucial factor when moving from typical laboratory setups to the natural living situation and to long-term approaches. The suppression of nocturnal melatonin is the best described physiological non-image forming response to light in human subjects (Brainard et al., 2001; Thapan et al., 2001; West et al., 2011; Zeitzer et al., 2000). This is a nighttime response, usually measured after dim light adaptation. Previous studies (Phipps-Nelson et al., 2003; Rüger et al., 2006; Smolders and de Kort, 2012; Viola et al., 2008) have shown that increasing daytime light exposure can enhance performance and well-being, although disruption is also possible (Vetter et al., 2011). Our results also show that, depending on the population, light in the morning, daytime, and in the evening can have different impacts on sleep and melatonin rhythms. To optimize daytime light effects on people's performance and well-being, further research is needed to characterize the combina-

tions of color, intensity, timing and duration of light exposure needed for such responses. The methodology used in this thesis opens opportunities to follow up on this topic, and to explore through time – time in life, time of year, time of day - human responsiveness to changes in light and its spectral composition.

In this thesis we encountered many features inherent to real life studies in human subjects. It is good to emphasize several aspects of human research that are rarely taken into account, although they are known to play a role. Sleep timing on days off is less influenced by social Zeitgebers, leading to a better representation of the endogenous phase (corrected for sleep loss on work days) than during work days (Roenneberg et al., 2007). As shown by Roenneberg et al. (2007) and in chapters 2 and 6 of this thesis, human subjects tend to behave differently on days off as compared to work days. However, the distinction between work and days off is rarely made in human studies. We support Roenneberg et al (2007) in his encouragement that researchers do this distinction and take it into account in their studies, not only for phase assessment but also for describing physiological states associated to sleep-wake patterns.

Season is a modulator of circadian behavior. In CHAPTER 5 we had not explicitly taken season into account in the design of the study and we did not have an even distribution of subjects over the seasons. We therefore had to find a way to optimally cope with this seasonal effects. In assessing seasonal effects, the introduction of DST and how researchers deal with it should be explicitly reported. In studies where measurements of different subjects take place at different times of the year and randomization is not possible, or in one-directional studies where randomization of conditions is impossible (e.g., before and after cataract surgery) the impact of season/DST should be taken into account. To facilitate comparisons between studies and to characterize human behavior and/or physiology as accurately as possible, we hope to inspire other researchers to heed these recommendations. §



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SUMMARY

Virtually all places on Earth are exposed to a never-ending sequence of days and nights. This alternation between light and darkness occurs every 24 hours and is accompanied by predictable changes of the environment. The biological clock entrains to this light dark cycle synchronizing our internal biological rhythms with the external 24 h day. Physiological and behavioral functions associated with activity occur during daytime in diurnal organisms, whereas the physiology and behavior associated with rest are in phase with the nighttime. Entrainment largely depends on the quality of the light and darkness we are exposed to. This thesis focuses on investigating how changes in the quality of light (i.e., intensity, spectral composition, and timing) affect humans' rhythms of sleep and of melatonin in the natural environment. To achieve this, no restrictions were imposed upon the behavior of our participants. Visits to the laboratory were limited to the measurements of specific parameters such as the assessment of melatonin profiles. Most of the studied variables described in this thesis were measured in the field under natural conditions. Our observations were not restricted to acute effects of light, but rather focused on long-term effects

In chapter 2 we investigated day-to-day variations in light exposure as well as the patterns of sleep-wake and melatonin rhythms at two different light backgrounds, that is, during the long days of summer versus the short days of winter. At our latitude, both photoperiod and maximal natural light intensities differ throughout the seasons. Humans can however, self-select their light exposure, for instance by the use of curtains and artificial

light. Hence, we were curious to know how much of the natural variation in light is perceived by humans and to what extent the rhythms of sleep and melatonin respond to this variation. Our results show that the timing of sleep and of melatonin rhythms was delayed in winter compared to summer. The extent of this delay differed between workdays and days off and this was confounded by the introduction of daylight saving time (daylight saving time, DST). In the Netherlands, we delay our social clock 1 hour in March and we move it back in October imposing an advance in the timing of our activities relative to the day-night cycle. The delay of the rhythms of sleep and of melatonin secretion on days off in winter was larger than the 1-hour expected by DST, whereas the delay on workdays was smaller. This shows how DST impacts the adjustment of the circadian system. In this study melatonin was sampled not only under dim light conditions in the laboratory but also under real life conditions with no restrictions on light exposure. The timing of the melatonin rhythms did not differ between the laboratory and real life conditions, suggesting that light intensities in the laboratory and at home are not sufficiently different to induce an effect at the level of the melatonin signal. The amplitude of the rhythm i.e., the maximum level of melatonin at night, was however lower in the real life environment. We further used information on day-to-day variation in light exposure and tested by means of correlations how this variation, at specific times of the day, may affect (on top of the effects of season and whether it was a work day or a day off) the timing and quality of the sleep-wake and melatonin rhythms. The specific times of the day were chosen to roughly represent phases of the day with advancing (morning), delaying (evening), suppressing (evening and night) or no (afternoon) expected effects of light on sleep and melatonin parameters. Our results show that depending on the variable (i.e., timing of sleep, quality of sleep, timing of melatonin, amplitude of melatonin, etc.) and on the timing of light exposure, light can have different size and direction effects throughout the whole day. For instance, while increased light exposure during daytime is related to a delay in the onset of sleep, it was related to an advance in the onset of melatonin secretion. This could mean that depending on the daytime light intensity, the day-to-day phase angle between sleep and melatonin rhythms might change. If a causal relationship exists between day-to-day light exposure and phase of entrainment, this could have clear implications for light strategies to improve entrainment.

Waking up during the dark winter months can be challenging. In CHAPTER 3 we tested the potential benefits of reducing sleep inertia (i.e., impaired performance, confusion, and sleepiness after waking up) by an alarm that provides an artificial dawn-type light signal. The study was performed at the subjects' homes to achieve a realistic scenario of the final user. We observed that using the device for a 2-week period had a positive impact on subjective measurements of sleep inertia and well-being. In contrast with our hypothesis, this improvement was not accompanied by a shift in the dim light melatonin onset. Mechanisms other than an advance of circadian rhythms are needed to explain the positive results on sleep inertia of

waking up with a dawn signal.

In chapter 4 and 5, we took advantage of cataract surgery to investigate the potential effects on entrainment of a natural reduction of short wavelength transmission through the ocular lenses.

In chapter 4 we describe an objective technique that allows measuring the spectral composition of the light reaching the retina *in vivo*. With a non-invasive procedure that does not take longer than 15 minutes we investigated the improvement factor in lens transmittance after removal of the cataractous lens and replacement by a transparent lens. Our results show that in the short wavelength range, between 420 and 500 nm, lens transmittance is improved by an average factor of 4 whereas in the long wavelengths (505 - 750 nm) the improvement was only a factor of 1.3. It has been hypothesized that age related reduction in the transmission of short wavelength light may underlie sleep-wake rhythm disturbances in the elderly and that by restoring the light transmission of the lens, sleep-wake rhythms may recover.

In chapter 5 we assessed how the changes in lens transmittance affect sleep and nocturnal melatonin rhythms of those elderly people undergoing cataract surgery. We observed a delay of the sleep-wake and melatonin rhythms after surgery. The size of the delay correlated positively with chronotype. The later the chronotype, the larger the delay was after cataract surgery. At first sight, in view of the increase in Zeitgeber strength after cataract surgery, the delay was unexpected. Nonetheless, the recovering of a later phase that we observed after cataract surgery (clear lens) as compared to the earlier phase observed before cataract (yellowish lens), is in agreement with the advances of circadian phase reported with increased aging. We hypothesize that the delay can be attributed to an increase of the level of light transmittance in the evening hours; a time of the day where light exposure in people suffering from cataract might just be below the critical value to exert an effect. A factor 4 increase of short wavelength transmission during the day, when light intensities are already high, is thought to be insignificant for the circadian system.

In chapter 6, we studied the impact of long-term reduced input of short wavelengths to the retina on sleep-wake and melatonin rhythms in a different way. This time, the study was performed in young healthy subjects in order to assess whether the changes we observed in chapter 5 could be reproduced experimentally in young subjects. To achieve this, subjects wore soft orange contact lenses (SOCL) 24 h/day for two weeks. These lenses lead to a reduction in (blue) light exposure entering the eye. In this study we also assessed the effects of the SOCL on the suppression of melatonin. Melatonin suppression has been widely used as a parameter for describing the sensitivity of the circadian system. The most remarkable result was that suppression of melatonin in response to a light pulse after wearing the SOCL for two weeks was not different from suppression in the control condition, in the absence of lenses. The SOCL were effective in reducing short wavelength input, which was confirmed

by showing that wearing them for only 30 minutes leads to less suppression of melatonin in response to a light pulse. An increase in sensitivity seems to have taken place after wearing the SOCL for 2 weeks, which could reflect adaptation of the non-image forming system. Such adaptation may also explain the absence of changes in the timing of sleep and melatonin rhythms between conditions.

Ultimately, the knowledge gathered on non-image forming responses of light could serve to develop light solutions that positively affect human health and performance. The importance of aspects of light quality such as timing, intensity, and spectral composition is emphasized by our observations under natural conditions. Further, our results show that different outputs of the biological clock can be differentially affected by the same stimulus, and that, depending on characteristics of the target population, e.g., young versus old, these effects might, in the long-term, adapt to the new light environment. Developing light solutions is challenging but certainly a stimulating goal towards improving the quality of life of the population that encourages the active cooperation of scientists and companies. §

Sammenvating

Vrijwel alle plaatsen op aarde zijn blootgesteld aan een eeuwig durende afwisseling van dag en nacht. Deze afwisseling van licht en donker komt en gaat met een periode van 24 uur en gaat gepaard met voorspelbare veranderingen van de omgeving. De biologische klok loopt in de pas met de licht donker cyclus en synchroniseert op deze manier de interne biologische ritmes met de externe 24-uurs dag. Fysiologie en gedrag passend bij activiteit vindt in zogenaamde "dag-dieren" overdag plaats, terwijl de fysiologie en het gedrag dat hoort bij de rust in fase is met de nacht. "Entrainment", oftewel het in de pas lopen van de interne ritmes met de ritmiek in de buitenwereld, hangt grotendeels af van de kwaliteit van het licht en de duisternis waaraan we worden blootgesteld. In dit proefschrift ligt de focus op onderzoek naar hoe veranderingen in de kwaliteit van licht (dat wil zeggen, intensiteit, spectrale samenstelling en timing) ritmes van de slaap en van melatonine bij mensen beïnvloeden, in hun natuurlijke omgeving. Om dit te bereiken, werden geen beperkingen opgelegd aan het gedrag van onze deelnemers en bezoeken aan het lab werden zo veel mogelijk beperkt. Onze waarnemingen beperkten zich niet tot acute effecten van het licht, maar waren vooral gericht op lange-termijn effecten.

In hoofdstuk 2 onderzochten we variaties in blootstelling aan licht van dag tot dag en de patronen van slaap-waak en melatonine ritmes tegen de achtergrond van twee verschillende lichtomstandigheden; dat wil zeggen tijdens de lange dagen van de zomer ten opzichte van de korte dagen van de winter. Op onze breedtegraad verschillen zowel fotoperiode als de

maximale natuurlijke lichtintensiteit tussen de seizoenen. Mensen kunnen echter zelf hun lichtblootstelling kiezen door het gebruik van onder andere gordijnen en kunstlicht. Daarom waren we nieuwsgierig naar hoeveel van de natuurlijke variatie in licht wordt waargenomen door mensen en in hoeverre het ritme van melatonine en slaap reageert op deze variatie. Onze resultaten tonen aan dat de timing van de slaap en van de melatonine ritmes was vertraagd, met andere woorden was verschoven naar een later tijdstip, in de winter in vergelijking tot de zomer. De grootte van deze verschuiving varieerde tussen werkdagen en vrije dagen en dit proces werd verstoord door het ingaan van de zomertijd. In Nederland verzetten we onze sociale klok 1 uur in maart en zetten hem terug in oktober (zomertijd). Dit heeft tot gevolg dat onze activiteiten naar een vroegere fase worden verschoven ten opzichte van de dag-nacht cyclus. De fase verschuiving van het ritme van slapen en waken en van het melatonine ritme op vrije dagen in de winter naar een later tijdstip was groter dan de verwachtte één uur als gevolg van de zomertijd alleen, terwijl de fase verschuiving op werkdagen kleiner was dan één uur. Dit toont aan dat de zomertijd van invloed is op aanpassingen van het circadiane systeem. In deze studie werden melatonine waardes niet alleen onder de gebruikelijke lage lichtomstandigheden in het laboratorium verzameld, maar ook onder normale dagelijkse omstandigheden zonder beperkingen met betrekking tot blootstelling aan licht. De fase van de melatonine ritmes, verzameld in het laboratorium en onder normale omstandigheden, verschilde niet van elkaar. Dit suggereert dat lichtintensiteiten in het laboratorium en thuis niet voldoende verschilden om een effect te veroorzaken. De amplitude van het ritme, dat wil zeggen het maximale niveau van melatonine 's nachts, was echter kleiner in de thuissituatie dan in het laboratorium. Verder hebben we gebruik gemaakt van informatie van dag-tot-dag variatie in blootstelling aan licht. Met behulp van correlatie analyses werd gekeken hoe deze variatie, op specifieke tijdstippen van de dag, van invloed kan zijn op de timing en de kwaliteit van de slaap-waak en melatonine ritmes (bovenop het effect van seizoen en of het een werkdag of een vrije dag was). De specifieke momenten van de dag werden zodanig gekozen dat deze fasen van de dag vertegenwoordigden waarop licht de 24-uurs ritmes en andere parameters van slaap-waak en melatonine ritmes dan wel kan vervroegen (ochtend), vertragen ('s avonds), onderdrukken (late avond en nacht) of geen (' s middags) effect heeft. Onze resultaten tonen aan dat afhankelijk van de variabele (d.w.z. timing van slaap, slaapkwaliteit, timing van melatonine ritme, amplitude van melatonine ritme enz.) en afhankelijk van het tijdstip van belichting, het licht verschillende effecten kan hebben zowel voor wat betreft de grootte als ook de richting van het effect, gedurende de hele dag. Hoewel bijvoorbeeld verhoogde blootstelling aan licht overdag is gerelateerd aan een verschuiving van het in slaap vallen naar een later tijdstip, zagen we een verschuiving van de start van melatonine productie naar een vroeger tijdstip. Dit zou kunnen betekenen dat er afhankelijk van de lichtintensiteit overdag, variatie ontstaat in de fasehoek tussen slaap en melatonine ritmes van dag tot dag. Als er inderdaad een causaal verband bestaat tussen fase van "entrainment" en dag-tot-dag variatie in blootstelling aan licht,

zal dit duidelijke gevolgen hebben voor de keuzes van lichtaanpassingen voor het verbeteren van ritmes en slaap.

Wakker worden tijdens de donkere wintermaanden kan een uitdaging zijn. In HOOFDSTUK 3 testten we de potentiële voordelen van het verminderen van de slaap inertie (dat wil zeggen, verminderde prestaties, verwardheid, en slaperigheid na het ontwaken) door een wekker met licht, dat langzaam in intensiteit toeneemt zoals bij de ochtendschemering. De studie werd uitgevoerd bij de deelnemers thuis, zoals de wekker ook daadwerkelijk bedoeld is om te gebruiken. De resultaten lieten zien dat het gebruik van de wekkerlamp gedurende een periode van 2 weken een positief effect had op subjectieve scores van de slaap inertie en welzijn. In tegenstelling tot onze hypothese, werd deze verbetering niet verklaard door een verschuiving van het melatonine ritme. Er zullen dus andere mechanismen voor het verklaren van de positieve resultaten van de wekkerlamp op het wakker worden moeten worden gezocht dan een verklaring via de verschuiving van circadiane ritmes.

In HOOFDSTUK 4 en 5, hebben we ons voordeel gedaan met het klinisch uitvoeren van staaroperaties. We hebben deze ingreep gebruikt om de mogelijke effecten te onderzoeken van een natuurlijke vermindering van korte golflengte licht via de lens in ons oog.

In HOOFDSTUK 4 beschrijven we een objectieve techniek die het mogelijk maakt om de spectrale samenstelling van licht te meten dat het netvlies in werkelijkheid bereikt (*in vivo*). Met behulp van een niet-ingrijpende procedure die niet langer dan 5 minuten duurde, onderzochten we de verbeteringsfactor in doorlaatbaarheid van de lens na de operatieve ingreep waarbij de lens met staar werd verwijderd en vervangen door een transparante lens. Onze resultaten tonen aan dat in het gebied met korte golflengte licht (tussen 420 en 500 nm), de doorlaatbaarheid van de lens verbetert met een factor 4, terwijl in het gebied met lange golflengten (505-750 nm) de verbetering slechts een factor 1,3 bedraagt. Onze hypothese is dat de aan de leeftijd gerelateerde daling van de doorlaatbaarheid van de lens voor licht met een korte golflengte, een oorzaak zou kunnen zijn voor slaap-waak ritme stoornissen bij ouderen. Door het herstel van de doorlaatbaarheid van de lens voor licht na een staaroperatie zou het slaap-waak ritme kunnen verbeteren.

In HOOFDSTUK 5 onderzochten we hoe de veranderingen in doorlaatbaarheid van de lens na een staaroperatie de slaap en melatonine ritmes van de ouderen beïnvloedden. We zagen dat het slaap-waak ritme en melatonine ritme na de operatie naar een later tijdstip verschoven. De grootte van de vertraging was positief gecorreleerd met chronotype (zogenaamde avond- of ochtendtypes); hoe later het chronotype hoe groter de verschuiving naar een later tijdstip na een staar operatie. Aangezien de Zeitgeber "licht" na de operatie is toegenomen, lijkt deze fase verschuiving naar een later tijdstip op het eerste gezicht onverwachts. Echter het verschuiven naar een latere fase na een staaroperatie (transparante lens) vergeleken met voor de operatie (staar) kan gezien worden als herstel en komt overeen met het feit dat met het toene-

men van de leeftijd de fase over het algemeen vroeger wordt. Een mogelijke verklaring voor de faseverschuiving naar een later tijdstip met een meer transparante lens is dat dit vooral toe te schrijven is aan een verhoging van het niveau van licht dat het netvlies bereikt in de avonduren; een tijd van de dag waarbij de belichting bij mensen met staar misschien wel onder de kritische waarde voor een effect op het circadiane systeem uitkomt. Een factor 4 toename van de hoeveelheid korte golflengte licht die op het netvlies komt tijdens de dag, wanneer lichtintensiteiten al hoog zijn, wordt gedacht onbelangrijk te zijn voor het circadiane systeem.

In HOOFDSTUK 6 hebben we de gevolgen van een lange termijn reductie in korte golflengte licht op de slaap-waak en melatonine ritmes op een andere manier bestudeerd. Deze keer werd het onderzoek uitgevoerd bij jonge gezonde personen om te beoordelen of de veranderingen die we in hoofdstuk 5 zagen experimenteel konden worden gereproduceerd met jongere personen. Om dit te bereiken droegen de deelnemers zachte oranje contactlenzen (SOCh), 24 uur per dag gedurende twee aangesloten weken. Deze lenzen leidden tot een vermindering van (blauw) licht in het oog. Het meest opvallende resultaat was dat onderdrukking van melatonine in reactie op een lichtpuls na het twee weken dragen van de SOCh niet verschilde van onderdrukking in de controleconditie zonder de lenzen. De SOCh zelf waren wel effectief in het verminderen van korte golflengte input; dit werd bevestigd door aan te tonen dat melatonine suppressie tijdens een lichtpuls waarbij de SOCh 30 minuten werden gedragen leidde tot minder onderdrukking van melatonine dan in de situatie waarbij de SOCh niet werden gedragen. De conclusie is dat er een toename in gevoeligheid voor (blauw) licht lijkt te hebben plaatsgevonden tijdens het 2 weken dragen van de SOCh. Dit betekent een aanpassing van het systeem van niet-beeldvormende effecten van licht. Een dergelijke aanpassing kan ook verklaren waarom er geen veranderingen werden gevonden in de timing van slaap en melatonine ritmes bij deze jonge deelnemers.

Uiteindelijk dient de kennis die wordt verkregen over de niet-beeldvormende reacties op licht voor het ontwikkelen van lichtoplossingen die een positieve invloed kunnen hebben op de menselijke gezondheid en prestaties. Het belang van aspecten van lichtkwaliteit - zoals timing, intensiteit en spectrale samenstelling - wordt benadrukt door onze waarnemingen onder natuurlijke omstandigheden. Bovendien tonen onze resultaten aan dat verschillende ritmen die onder invloed staan van de biologische klok verschillend kunnen worden beïnvloed door dezelfde stimulus. Tevens is gebleken dat deze ritmes - afhankelijk van de kenmerken van de doelpopulatie, bijvoorbeeld jong versus oud - zich op lange termijn kunnen aanpassen aan een verandering in omgevingslicht. Het ontwikkelen van nieuwe lichtoplossingen voor het verbeteren van de kwaliteit van leven is een uitdaging, maar het is zeker een stimulerend doel dat de actieve deelname en samenwerking vraagt van wetenschappers en bedrijven. §

Resumen

Prácticamente todos los lugares en la Tierra están expuestos a una secuencia interminable de días y noches. Esta alternancia entre luz y oscuridad se produce cada 24 horas y está acompañada de cambios previsibles en el medio ambiente. El reloj biológico se ajusta al ciclo de luz-oscuridad sincronizando nuestros ritmos biológicos internos al día externo de 24 horas. De esta forma, en organismos diurnos, las funciones fisiológicas y de comportamiento asociados con la actividad ocurren durante el día, mientras que aquellas asociadas al descanso están en fase con la noche. Las propiedades de la luz y oscuridad a las que estamos expuestos afectan la calidad de sincronización del reloj biológico. El objetivo central de esta tesis es estudiar de qué manera las variaciones en las distintas propiedades que definen la calidad de la luz – intensidad, composición espectral, tiempo de exposición, etc. –afectan los ritmos de sueño y de melatonina en humanos en condiciones naturales. En el desarrollo de nuestros estudios no se impusieron restricciones en el comportamiento de los participantes. Las visitas al laboratorio fueron limitadas para la realización de registros específicos como la medición de melatonina. La mayoría de las mediciones que se describen esta tesis se hicieron en campo, representando la vida cotidiana de los sujetos. Nuestras observaciones no se limitaron a los efectos inmediatos de la luz, sino que se centraron en los efectos a largo plazo.

En el capítulo 2 se exploraron las variaciones diarias en la exposición a la luz (y oscuridad), así como también en los ritmos de sueño y de melatonina. Estas variaciones fueron estudiadas en dos entornos de luz diferentes; durante los días largos de verano y durante los días más cortos de invierno. En nuestra latitud, tanto el fotoperiodo como las intensidades

naturales máximas de luz difieren a lo largo de las estaciones. Los seres humanos pueden, sin embargo, modificar su exposición a la luz mediante – por ejemplo – el uso de cortinas o de luz artificial. En relación a dicha cuestión nos preguntamos hasta qué punto perciben los seres humanos esta variación natural de luz y oscuridad y en qué medida los ritmos de sueño y de melatonina responden a esta variación. Nuestros resultados muestran que la fase de los ritmos de sueño (comienzo del sueño, fase media del sueño y fin del sueño) y de melatonina (aumento y caída del nivel de melatonina en condiciones de iluminación tenue) ocurre más tarde en el invierno en comparación con el verano. La magnitud de este retraso es diferente entre los días laborables y los días no laborales y también se ve influenciada por la introducción del horario de verano. En los Países Bajos atrasamos nuestro reloj social 1 hora en marzo y lo volvemos adelantar en octubre, imponiendo de esta forma un avance en el horario de nuestras actividades en relación con el ciclo luz-oscuridad (día-noche). El retraso que observamos durante los días no laborales en invierno fue más grande que el retraso de 1 hora que hubiéramos esperado por el cambio del horario de verano, mientras que el retraso en los días laborables fue menor. Esto permitiría suponer que el ajuste al horario de verano puede ser un desafío para el sistema circadiano humano. En este estudio se tomaron muestras de la melatonina no sólo bajo condiciones de luz tenue en el laboratorio, sino también bajo condiciones naturales sin ninguna restricción en cuanto a la exposición a la luz. No se observaron diferencias entre los ritmos de melatonina medidos en el laboratorio y los medidos en el hogar de los participantes bajo condiciones naturales. Esto sugiere que la intensidad luminosa en el laboratorio y en el hogar no son lo suficientemente diferentes como para registrar diferencias en este marcador hormonal. La amplitud del ritmo, es decir, la diferencia entre el nivel máximo y mínimo de melatonina fue, sin embargo, más baja en condiciones naturales. Mediante estudios de correlación se analizó cómo la variación diaria en la exposición a la luz (y oscuridad), a determinadas horas del día, puede afectar (por encima del efecto de las estaciones y de si se trataba o no de un día laboral) la sincronización y la calidad de los ritmos de sueño y de melatonina. Los momentos específicos de la exposición a la luz fueron elegidos para representar aproximadamente las fases del día que llevan a un avance (por la mañana), a un retraso (por la noche), a la supresión (tarde y noche) o a ningún efecto (por la tarde) de los ritmos de sueño y de melatonina. Nuestros resultados muestran que, dependiendo de la variable (es decir, la sincronización y la calidad de los ritmos de sueño y de melatonina, la amplitud del ritmo de melatonina, etc.) y el momento del día al que nos exponemos a la luz, un mismo estímulo luminoso puede producir distintos efectos, tanto de tamaño como de dirección, a lo largo de todo el día. Por ejemplo, mientras una mayor exposición a la luz durante el día está relacionada con un retraso en el inicio del ritmo de sueño, observamos una correlación negativa (un avance) con el inicio del ritmo de melatonina. Esto podría significar que, dependiendo de la intensidad de la luz durante el día, el ángulo de fase entre los ritmos de sueño y melatonina podría cambiar de un día al otro. Si existiese una relación causal entre la fase de sincronización y la variación diaria en la exposición a la luz, esto podría tener claras implicaciones en el desarrollo de estrategias de luz para optimizar el ajuste del reloj biológico con el ciclo externo de luz y oscuridad.

Despertarse durante los oscuros meses de invierno puede ser un desafío difícil de cumplir. En el capítulo 3 se estimaron los potenciales beneficios de un reloj despertador que proporciona una señal luminosa que "simula" el amanecer, para reducir el fenómeno conocido como "inercia del sueño" (problemas en el funcionamiento, sentimiento de confusión y somnolencia inmediatamente después de despertarse). El estudio se llevó a cabo en los hogares de los participantes con el objetivo de testear el dispositivo en un escenario realista. El uso del dispositivo durante un período de 2 semanas tuvo un impacto positivo en distintas calificaciones subjetivas de la inercia del sueño y de bienestar de los participantes. En contraste con nuestra hipótesis, esta mejora no fue acompañada por un cambio en la fase de inicio del ritmo de melatonina. De esta forma, mecanismos distintos a un cambio a una fase más temprana de los ritmos de melatonina son necesarios para explicar el impacto positivo del dispositivo en la inercia del sueño.

En los capítulo 4 y 5 se hizo uso de la cirugía de catarata para investigar los potenciales efectos de una reducción natural en la transmisión de longitud de onda corta a través de las lentes oculares en la sincronización del reloj biológico.

En el capítulo 4 se describe una técnica objetiva que permite medir *in vivo* la composición espectral de la luz que llega a la retina. Con un procedimiento no invasivo que no toma más de 5 minutos se determinó el factor de mejora en la transmitancia de la lente después de la eliminación de la catarata y la sustitución de la vieja lente por una transparente. Nuestros resultados muestran que en el intervalo de longitud de onda corta, entre 420 y 500 nm, la transmitancia de la lente mejoró, en promedio, en un factor de 4, mientras que en las longitudes de onda largas (505 - 750 nm), la mejora fue sólo en un factor de 1,3. Una de las hipótesis que se ha planteado en la bibliografía es que esta reducción – relacionada con la edad – en la transmisión de luz de onda corta puede ser la causa de las alteraciones que se observan, en general, en los ritmos de sueño y de melatonina en los ancianos y que, por ende, la recuperación de transmitancia de luz de onda corta después de la cirugía de catarata debería restaurar los ritmos de sueño y de melatonina.

En el capítulo 5 se puso a prueba esta hipótesis. Se evaluó cómo los cambios en la transmisión de la lente de los ancianos sometidos a cirugía de cataratas afectan los ritmos de sueño y de melatonina. Nuestros resultados muestran un retraso de los ritmos de sueño y de melatonina después de la cirugía. El tamaño de este retraso muestra una correlación positiva con el cronotipo de los participantes. Cuanto más tardío el cronotipo, mayor fue el retraso después de la cirugía de cataratas. A primera vista, teniendo en cuenta el incremento en la fuerza del Zeitgeber después de la cirugía de cataratas, el retraso que observamos fue inesperado. No obstante, la recuperación de una fase más tardía después de la cirugía de catarata (lente transparente) en comparación con la fase más temprana que observamos antes de la cirugía (lente amarillenta), concuerda con los avances de fase de ritmos circadianos reportados con el incremento de edad. Probablemente, el retraso se pueda atribuir a un aumento del nivel de transmitancia de la luz en las horas de la tarde. En este horario, la exposición a la luz en aquel-

las personas que sufren de catarata podría ser inferior al valor crítico para ejercer un efecto. Un factor 4 de aumento de la transmisión de longitud de onda corta durante el día, cuando las intensidades de luz ya son altas, probablemente sea insignificante para el sistema circadiano.

En el CAPÍTULO 6 se continuó con la evaluación del impacto a largo plazo de una exposición reducida en longitudes de onda corta en los ritmos de sueño y de melatonina. También se evaluó el efecto en la supresión de melatonina. La supresión de melatonina ha sido extensamente utilizada como parámetro para describir la sensibilidad del sistema circadiano en humanos. Esta vez, el estudio fue realizado en participantes jóvenes con el fin de evaluar si los cambios que se observaron en el capítulo 5 son reproducibles de forma experimental en participantes jóvenes. Para lograr esto, los participantes usaron lentes de contacto blandas de color naranja 24 horas al día durante dos semanas consecutivas. El uso de las lentes permite reducir la exposición a la luz (de onda corta) que llega a la retina. El resultado más notable fue que la supresión de melatonina en respuesta a un pulso de luz después de usar las lentes por dos semanas consecutivas, no fue diferente de la supresión que observamos en el control. Las lentes fueron eficaces en reducir la entrada de longitud de onda corta, lo cual se confirmó a través de la reducción en supresión de melatonina que observamos cuando las lentes fueron usadas sólo 30 minutos antes de que comience el pulso de luz. Un aumento de la sensibilidad parece haber tenido lugar después de usar las lentes durante 2 semanas, lo que podría reflejar la capacidad de adaptación del sistema circadiano a un nuevo ambiente. Esta adaptación también podría explicar la ausencia de cambios de fase de los ritmos de sueño y de melatonina entre ambas condiciones experimentales.

En última instancia, el conocimiento adquirido en el campo de ritmos circadianos y de los efectos no visuales de la luz debería servir para desarrollar soluciones de iluminación que afecten positivamente a la salud y al rendimiento. La importancia de las distintas características relacionadas con la calidad fótica, tales como momento al cual uno se expone a la luz, la intensidad y composición espectral de la misma fue enfatizada por nuestras observaciones en condiciones naturales. Asimismo, nuestros resultados muestran que diferentes salidas (outputs) del reloj biológico puede ser afectadas en forma distinta por el mismo estímulo y que, dependiendo de las características de la población (por ejemplo, jóvenes vs. ancianos), estos efectos podrían - a largo plazo - adaptarse a las nuevas características de luz-oscuridad del medio ambiente. El desarrollo de soluciones de luz es definitivamente un desafío, pero al mismo tiempo un objetivo estimulante que fomenta la participación activa de científicos y de empresas en pos de una mejora en la calidad de vida de la población. §

Acknowledgments

It is time to end this book, but not without thanking all of those who, in one way or another, contributed to it.

I would like to start with a big collective thanks to all of my promoters. *Domien*, *Marijke*, *& Serge*, my most sincere gratitude to all of you for all your support and for being always available when needed, whether it was related to my PhD or not. Having three different people commenting on the manuscripts was not always easy, but it was certainly an enriching and exciting experience.

Domien, your kindness always made it so easy to just knock at your door and ask for your advice, not to mention your "there are no stupid questions" point of view. Thank you so much for your insights, your patience, your humor, your kindness, for opening the doors of your house for the group BBQs, for sharing your holidays' memories and your biking-thinking-processes. It was always, and still is, very enjoyable just to sit and chat with you. And thanks a lot for the last push. To have to send you an SMS during your holidays to tell you that the book was finished on the date we agreed on felt like a big commitment I did not want to fail to. But mostly, I would like to thank you for showing me that good science can be done in a humble and quiet way.

Marijke, your friendly attitude and smile were always so refreshing, something very much needed during a PhD journey. Thanks a lot for our discussions, your tons of emails in response to every single question I had, for being available almost 24/7 (no matter how unhealthy we know that is), for your help in the lab during the nightshifts or in getting ready for them, for de samenvatting, for the good times during our trips, and for the dinners at your place with your family. Let me also thank you for showing me about strength and about not

giving up, even when things get tough in this scientific world.

Serge, although you became actively involved at a later stage, your care and thoughtfulness about my PhD journey was shown on every single causal encounter we had. Thank you for your sharpness, for pointing me out how many times I used the word "however" or "moreover" in my manuscripts, for sharing your stories (it is always a pleasure to listen to you talking), for our discussions, and for the Tuesdays' meetings. I am very grateful that I had the chance to work with you.

Maan, it has been very nice to share this PhD experience with you. Thanks for your support in and outside work, for the trips we shared, the discussions, and for opening me the doors of your house and your family. Certainly the trip to Berlin was one of the funniest things we did together, I will never forget our conversation in front of that "fake old church". I am sure we will stay in touch once this journey is finished.

Roelof, I find no better way to thank you than by quoting yourself. During your presentation at Serge's valedictory lecture you said: "science is a serious business, but it should it also be a lot of fun". And that is what you are, serious-business-science and a lot of fun. Thanks for both!

Menno, thanks for the friendly and rich interactions and discussions we had.

Martha thanks for your sharing that enthusiasm you have whenever discussing science.

Debra, *Eus*, *Cees & Yvonne*, I am very grateful that you took the time to go through my thesis.

During this (long) journey lots of people entered and left the lab, making it a tricky task to write this section. Therefore, for all of those who I might forget in the next list, big thanks to you too.

Marian, people should not underestimate the pleasure that brings hearing ones' own language in a foreign country, even though sometimes we did not mean the same things. Thanks for the Spanish, for the dinners you organized, and specially for being there when I just started, getting me to dive into the chrono-literature. ¡Gracias! To the boys *Kamiel*, *Daan*, *Andrej*, it was a lot of fun to share the big room in Haren with you. Although noisy (oops), I have great memories of it. *Bonnie*, thank you for making those melatonin series analysis more enjoyable. *Marlies*, thanks for all you help on administrative issues throughout this time. *Gerard*, thanks for your help in the TVR.

I would also like to thank former and current colleagues. *Corrie*, *Susanne*, *Tim*, *Valeria*, *Violetta*, *Yao Yao*, *Kim*, *Margrien*, *Moniek*, *Connie*, *Jasper*, *and David*, it has been fun. *Petteri*, a special thank to you, I am glad we are still catching up now and then. *Melanie*, although we interact much more now than by the time we were in Haren I would like to thank you for your effort in trying to make me feel comfortable back then.

To all the *EUclock people*, the trip to and back from the island was always and adventure, but

once at the island the time spent there and the interactions with all of you were very inspiring and a lot of fun. *Vikki*, special big thanks to you! I have enjoyed your company very much throughout these years.

Luc & Vanja, I would like to thank you both for the nice company, for all you have taught me throughout the last years, for your support and for encouraging me in finishing this thesis. You both have been great colleagues and I am glad we are still interacting.

Diego (Golombek), me pone muy contenta el haberte conocido. Gracias por haberte tomado un tiempito para visitarnos acá en Groningen. Gracias por juntarte con nosotros cada año en Buenos Aires (en Quilmes para ser mas precisos) y mil gracias por ayudarme con el resumen en español de esta tesis que no fue nada fácil escribir. Pero más que nada muchísimas gracias por tu laburo, por lo que haces por la ciencia y por el país. En un honor para mi poder seguir interactuando con vos y con tus actividades. Ojala en algún momento podamos hacer algo juntos, aunque por ahora siga de este lado del océano.

I have been fortunate to find great friends in this country. It is not easy to live abroad but you all have made it, and still do, much easier. *Joost & Marijke, Eric Wout & Agnes*, thank you so much guys for the dinners, the games, the conversations, the movies, and for being there, both in the good and not so good times. *Michelle*, being in the Netherlands would have been a complete different experience had I not met you. Thanks for the trips we shared, the times at the gym followed, of course, by a nice taco or cantina moment, our skype meetings, thanks for the passion that you put on everything you do and that you share with us. Thanks for being there back at the time when I came back form Haren with my chronobiology syllabus, and from being here today during the final moments of this journey. Thank you so much for your friendship, you are an amazing friend! *Rie & Martina*, what can I say to you guys? (dance) (heidy) (poolparty) (h) (hug)! You are both pure awesomeness. *Ana & Diego*, que linda sorpresa que fue hacerse de buenos amigos a esta altura de la vida y a estas alturas grotianas. Sin dudas, ustedes fueron uno de los regalos más lindos que me dio Groningen. A vos en particular *Anita*, gracias por estar siempre ahí del otro lado del chat. Siempre (muy a tu pesar), valoro mucho tus palabras, y ni que hablar de tu humor. Gracias también por la ayuda que me diste con el resumen. *Ben & Betka*, thanks for the nice moments we spent and we are still spending together. *Juan*, gracias por las pelis, las comidas, los recitales y todos los lindos momentos que pasamos juntos.

Marcelito, no me da el espacio para nombrar todas las cosas por las que quisiera agradecerte. Simplemente gracias por estar absolutamente siempre y para todo. Sos casi un hermano (¡y hasta hermana a veces!) ¡Te adoro!

Distinguir entre amigos y familia en Argentina no es fácil. Cada viaje a Argentina es una fiesta para nosotros. Juntarnos con todos ustedes hacen que el viaje sea completo. Gracias *Abu & Manolo* por dejarnos invadirles la quinta una vez al año para disfrutar de momentos

inolvidables de asados, tortas, mates, y pizzas junto con amigos y familia. *Marta*, *Jorge*, *Paula*, *Dani & Ale*, gracias por los lindos momentos que compartimos en cada una de nuestras visitas a Argentina. *Juan & Juanito*, gracias por pasarse siempre a compartir un rato, generalmente de muchas risas, con nosotros. *Pato*, *Javier & flia*, un gracias enorme para ustedes por estar siempre al pie del cañón. *Gladys*, *Daniel & flia*, cada reunión con ustedes es una fiesta. Gracias por hacerse siempre de una tardecita para cocinarnos esas gloriosas pizzas y pasar un rato hermoso en su compañía. *Paula & Marta*, gracias por los mates, por su amistad incondicional, por las charlas, los recitales, las tardes en los parques, y por los viejos tiempos, también junto a Jorge. Estudiar en la FCEyN no solo me dejo llegar a este lugar sino que también me dejo grandes amigos con los que pasamos muchas horas de estudio (en la facu y en lo de la abuela), pero sobre todo, con los que pase hermosos momentos. *Diego & Male*, gracias por su amistad, por todos los momentos que pasamos juntos. Especialmente a vos *Dieguito*, un gracias enorme por todo el tiempo y experiencias que compartimos juntos y por todo lo que vendrá, ¡ahora con uno/a extra! *Esteban*, amigo, siempre dispuesto a acompañarnos a donde sea. Gracias por tu compañía, disfruto muchísimo del tiempo que paso con vos. *Ceci*, tu energía es tan refrescante. Mil gracias por siempre, no importa cuanto malabar tengas que hacer, encontrar una forma para que nos encontremos, inclusive cuando estuviste por acá. Siempre lo tengo muy presente y te lo agradezco infinitamente. *Marianita*, amiga, siempre estas ahí para darme tu consejo y brindarme tu ayuda cada vez que la necesito. Es hermoso que podamos hablar casi a diario, y que podamos compartir el crecimiento de la sobri, no sabes cuanto ayuda. *Meli*, vos siempre con una palabra dulce a mano para brindar, es un placer tenerte como amiga y me hace muy feliz compartir momentos con vos y ahora también con Violetita. *Barbi*, amiga del alma, aunque no hablemos tan seguido siempre que lo hacemos es como si el tiempo no hubiera pasado. Me encanta tenerte en mi vida, y ni hablar de Vicente y Maite. *Rotundo*, que suerte la mía al haberte conocido. Pase uno de los mejores momentos de mi vida entre monos y mosquitos junto a vos. Me hace muy feliz seguir en contacto con vos y con Ceci, los chat truchos son siempre una risa.

Pablo, me hubiera gustado estar mucho mas cerca tuyo. Se que el camino que estas transitando no fue, ni es, siempre fácil, pero confía en ese corazón hermoso que tenes. Sos una personita muy especial y me da mucho orgullo tenerte como hermano.

Geraldine, ¡sos un manojo de alegría! Gracias por poner una sonrisa en cada uno de nosotros.

Mamá y Papá, gracias por todo el apoyo y el amor que me dieron a lo largo de todos estos años, sin ellos nada de todo esto hubiera sido posible. Se que la distancia es difícil, pero a pesar de ella siempre los llevo muy cerquita mío en cada paso que doy.

Corrie, *Witske*, *Gijs*, *Henk & Rita*, hartelijk bedankt voor jullie steun en liefde. Het maakt me helemaal blij om zo een familie hier in Nederland te kunnen hebben.

Casper, nada de esto hubiera sido posible de no habernos encontrado. ¡Gracias! Cuantas sorpresas me regalo la vida al lado tuyo. Y aunque el camino no siempre fue fácil, estoy agradecida por absolutamente todo lo que hemos vivido, porque no hubo un solo acontecimiento que no haya traído algo hermoso. En cuanto a esta tesis, gracias por ponerme un deadline, fue el ultimo empujoncito que necesitaba para finalmente darle un fin a este proceso. ¡Gracias por TODO! Siempre para siempre.

May 2013, Groningen.

Now it is finished!