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Crosstalk between Synchronizers and the Human Circadian System

Interrelación entre los Sincronizadores y el Sistema Circadiano Humano

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CROSSTALK BETWEEN SYNCHRONIZERS AND THE HUMAN CIRCADIAN SYSTEM

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INTERRELACIÓN ENTRE LOS SINCRONIZADORES Y EL SISTEMA CIRCADIANO HUMANO

Memoria de Tesis Doctoral presentada por Antonio Martínez Nicolás para optar al grado de Doctor en Biología por la Universidad de Murcia

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Y aquí me veo escribiendo unos agradecimientos para mi tesis doctoral, que es algo que no va demasiado conmigo cómo ya sabréis. Sin embargo, una cosa que sí va conmigo es recordar. Y son muchas las cosas que recuerdo en este momento.

Recuerdo largas charlas sobre el sentido de la vida y el sexo de los ángeles.

Recuerdo también jornadas enteras arreglando, y si no cambiando, el mundo.

Recuerdo incontables negativas por mi parte a socializar.

Recuerdo gritos por los pasillos llamando a alguien que está a menos de 10 metros.

Recuerdo carreras por los pasillos.

Recuerdo pasos decididos, pies siendo arrastrados, taconeos característicos.

Recuerdo risas inconfundibles y conversaciones inconfesables.

Recuerdo frustración, tristeza y agobio inherentes a la creación.

Recuerdo envidias, temores, riñas y enojos por estupideces, como en toda familia.

Recuerdo abrazos, besos, cariños, sonrisas y miradas.

Recuerdo tantas cosas que no voy a seguir escribiendo. Y aunque en mi opinión sería mejor no haber escrito nada, mi naturaleza es complaciente y sé que una tesis se escribe para que se lean los agradecimientos.

Así que espero que os haya gustado porque no pienso repetirlo.

Y sobre todo…

… me llevo un buen recuerdo.

Index

1. Introduction

1.1. HISTORY AND PRINCIPLES OF CHRONOBIOLOGY

From its origin, Earth has been rounding on its axis and around the sun, generating daily and annual cycles, respectively. In its continued deceleration, the Earth reached its actual rhythmic condition, in which living organisms have evolved. In these conditions of daily light-dark oscillations and annual temperature and photoperiod oscillations, survival rate of organisms can be improved by predicting those periodic changes. Thus, possible injuries such as DNA damage produced by ultraviolet and more energetic electromagnetic waves can be diminished by shifting DNA replication to the dark period, antagonist metabolic processes can occur at the different time decreasing energetic wastes and cyclic events like breeding or hibernation can be prepared in advance. All oscillatory processes are studied by Chronobiology, including rhythms in ecology, behaviour, development, physiology, genetics and a long etcetera (DeCoursey, 2003).

In spite of this fact, modern physiology has been marked by the concept of homeostasis, described by Claude Bernard in 1865 although the word was coined by Walter Cannon in 1926. A homeostatic state means that all biological variables are maintained constant and every physiological process works to maintain this stability of the internal environment. The importance and success of this paradigm, delayed the establishment of Chronobiology as a scientific discipline until the middle of XX century, by means of the experiments of Colin Pittendrigh, Jürgen Aschoff and Erwin Bünning, known as Chronobiology's parents, thanks to their research about entrainment, relative independence of environmental temperature and phase response curve (Daan & Pittendrigh, 1976; Pittendrigh, 1954; Pittendrigh, 1958), synchronizers´ entrainment (Aschoff, 1969; Aschoff et al., 1967; Aschoff et al., 1969; Aschoff et al., 1972) and the biological rhythm period inheritability (Bünning, 1935; Bünning & Stern, 1930).

1.1.1. BIOLOGICAL RHYTHMS PROPERTIES

The main characteristics of biological rhythms are: they are repeated periodically, persist without external cues, are adjusted to an external synchronizer, and

are able to maintain the periodicity in a broad range of environmental temperatures (Pittendrigh, 1954).

The first main characteristic of a biological rhythm is its endogeneity, that is, the rhythm remains in absence of environmental time cues, although with a period slightly different from that for the ***zeitgeber*** (German word for synchronizer that means "time giver") period (**T**), known as free-running period (tau, **τ**). Tau can differ among species but it is around 24 hours (Aschoff, 1979). The second main characteristic is that an endogenous rhythm can be entrained to a *zeitgeber*, and in these conditions the period of the endogenous rhythm converges to the *zeitgeber's* period with a certain phase angle known as phase relationship (psi, **ψ**), that remains stable (**Figure 1**) (Pittendrigh, 1981). The last of the main characteristics is the temperature compensation, showing its ability to maintain a stable period despite temperature changes (Pittendrigh, 1960).

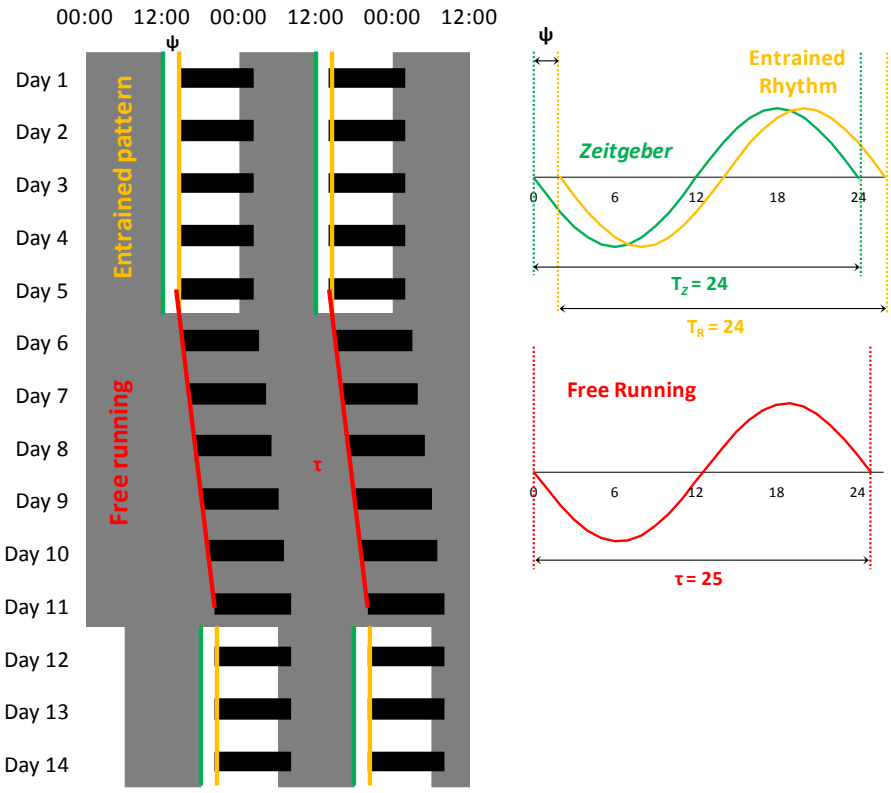

Figure 1. Entrainment. In a cyclic environment the rhythm exhibit a period of 24 h (T_R) because it is **entrained** by a ***zeitgeber*** with that period (T_Z) (in the example the light dark cycle) with a unique phase relationship (**ψ**) between the *zeitgeber* and the rhythm. However, in absence of external time cues, the rhythm change to his **free-running** period (**τ**) close to 24 h (τ=25 h in the example). *Modified from Pittendrigh, 1981.*

The biological rhythms are classified according to their period, or the duration of a complete oscillation, in three categories:

- **Infradian rhythms**: periodic endogenous oscillations with a frequency lower than one oscillation per day (period > 28 hours). This category includes:
    - Circalunar rhythms: those rhythms with a period of approximately 28 days, like the menstrual cycle.
    - Circannual rhythms: those rhythms occurring once in a year, for example, the breeding cycles in some species.
- **Circadian rhythms**: endogenous oscillation with a frequency of oscillation of around a cycle per 24 hours (from 20 to 28 hours). Most biological rhythms are included in this category, such as melatonin and cortisol secretion patterns, rest/activity and sleep/wake cycles, or mood and performance daily oscillations.
- **Ultradian rhythms**: endogenous cycles with a frequency higher than one complete oscillation per day (period < 20 hours). Numerous hormonal rhythms such as follicular stimulant and luteinizing hormones, physiological processes as heart and respiratory rates, or REM/NREM (Rapid Eye Movement/Non-Rapid Eye Movement) rhythms during the sleep are ultradian. One subgroup merits special attention: the tidal rhythms, with a period of circa 12.4 hours (dependent of the transition from low to high tide) usually present in maritime or coastal life.

Despite that classification, some variables display complex rhythms which are included in more than one category, for example, reproductive hormones present ultradian, circadian and infradian variations while melatonin secretion shows both circadian and circannual oscillations (Simmoneaux et al., 2006).

1.1.2. MASKING

Most of circadian variables (if not all) are influenced by exogenous factors, which exert a superimposed effect (masking) over the endogenous pattern driven by the central pacemaker (Minors & Waterhouse, 1989; Rietveld et al., 1993), generating a reactive response to concrete stimuli that the organism is not able to predict. However,

masking response can be easily differenced from the endogenous rhythm since it disappears when the exogenous factor does.

1.2. PROCEDURES & TOOLS FOR BIOLOGICAL RHYTHMS STUDIES

When studying a biological rhythm it is necessary to characterize a series of parameters which define the wave form. Firstly, periodogram analysis will provide the main periods of time series. When there is a period statistically significant, next step is to consider if the waveform fits or not to a cosine wave, thus a parametrical analysis or non-parametrical analysis, respectively must be performed.

1.2.1. PARAMETRICAL ANALYSIS

Adjusting to a cosine function allows defining waveform with a few parameters. These parameters are calculated by Cosinor's method and are well known and widely used in the field due to its simplicity. These parameters are **MESoR** (**M**idline **E**stimating **S**tatistic **o**f **R**hythm), **amplitude** (difference between maximum, or minimum, of the adjusted cosine wave and the mesor), **acrophase** (timing of maximum in the adjusted cosine wave) and the percentage of variance (**%V**) explained by the model. However, if there is more than one period in the rhythm, **Fourier's analysis** is needed, which uses shorter cosine waves dividing the 24h one (first harmonic) by two, three, four and so on to obtain the second, third and fourth harmonics which are added to the first harmonic, improving the adjustment of the model but sacrificing simplicity. At last, but not less important, Rayleigh's vector can be calculated for the daily acrophases to provide a measure of phase stability (**Figure 2**)(Refinetti et al., 2007).

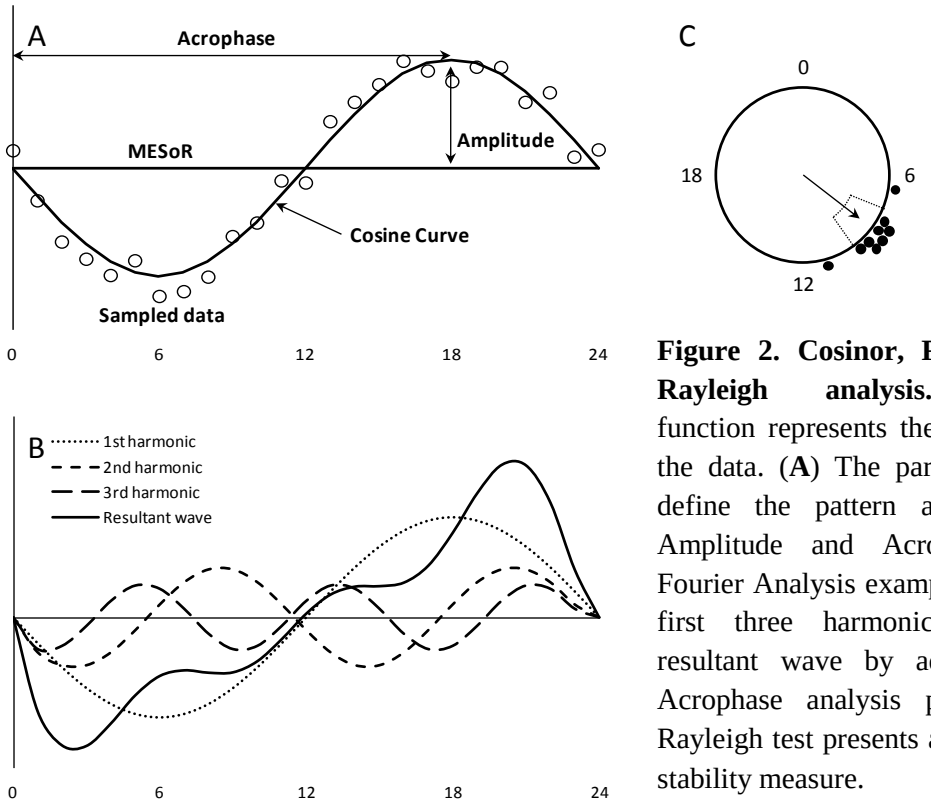

Figure 2. Cosinor, Fourier and Rayleigh analysis. Cosinor function represents the best fit for the data. (**A**) The parametres that define the pattern are MESoR, Amplitude and Acrophase. (**B**) Fourier Analysis example using the first three harmonics and the resultant wave by addition. (**C**) Acrophase analysis provided by Rayleigh test presents an acrophase stability measure.

1.2.2. NON-PARAMETRICAL ANALYSIS

Non parametrical analyses are convenient when the raw data does not fit a cosine wave. These analyses describe similar parameters avoiding adjustment to a sinusoid. It includes the calculation of the degree of pattern repeatability among different days (interdaily stability, IS); the fragmentation of rhythmic data (intradaily variability, IV), which measures if the pattern is a sinusoidal or square wave (very low fragmented) or gaussian noise (highly fragmented); the relative amplitude (RA), which is calculated as the difference between M10 (average for the 10 consecutive hours with the maximum value) and L5 (average for the 5 consecutive hours with the minimum value), divided by the sum of M10 and L5 (Van Someren et al., 1999). However, for those variables whose acrophase occurs during the rest period, these calculations were modified and the ten consecutive hours of minimum values (L10) and the five consecutive hours of maximum values (M5) were used. In addition, the timing when these maximum or minimum values occur avoiding sleep-wake transitions (TM or TL, respectively) can be used as phase measurements (Martinez-Nicolas et al., 2011).

1.2.3. DEMASKING

Masking has an important influence in biological rhythms. Because of that, two main procedures have been developed to reveal the endogenous pattern. The first one requires laboratory conditions and it is known as "constant routine", which consists in eliminating every time course signal of the environment, resulting on a constant ambient with darkness or dim light, constant environmental temperature, very frequent meals and water intake, no activity and lying position (Duffy & Dijk, 2002; Mills et al., 1978). However, this protocol has some limitations, since generates its own masking effect and requires active collaboration from the subject, who has to lye during days in bed (Minors & Waterhouse, 1989; Rietveld et al., 1993; Weinert & Waterhouse, 2007). Another method, known as demasking, consist in the mathematical subtraction of the masking effects from the pattern registered under free living conditions (**Figure 3**), thus collaboration from experimental subjects is not necessary (Minors & Waterhouse, 1989; Minors & Waterhouse, 1992; Waterhouse et al., 2000; Waterhouse et al., 2001; Waterhouse et al., 2005; Weinert et al., 2003; Weinert & Waterhouse, 1998). However, it needs long recordings and it becomes more complicated if more than one masking effect is present (Martinez-Nicolas et al. 2013).

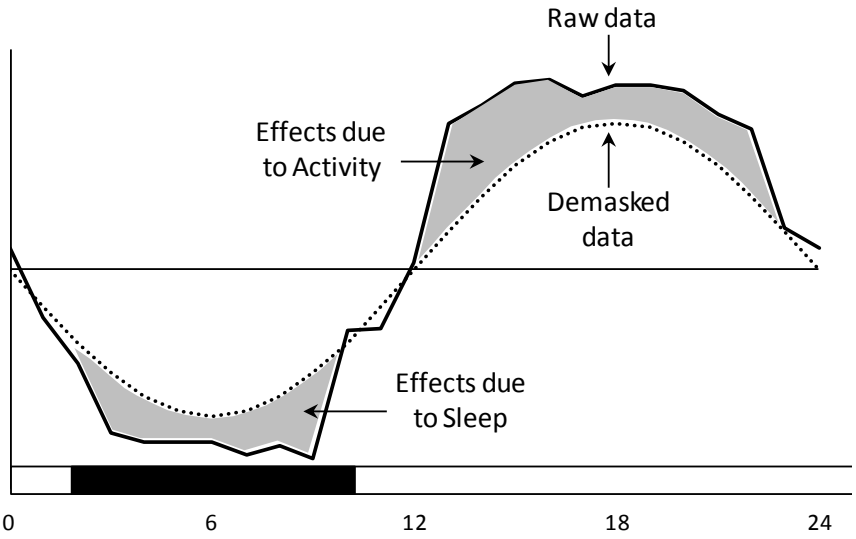

Figure 3. Demasking. Illustration of a demasking process from raw data to obtain the endogenous pattern (demasked data) subtracting the effects of rest-activity pattern. *Redrawn from Reilly & Waterhouse, 2009.*

1.3. HUMAN CIRCADIAN SYSTEM

The temporal organization and the environmental synchronization in the organism are driven by the circadian timekeeping system, which is constituted by a set of structures hierarchically organized. This system is structured like a clock, with a series of inputs to wind-up the clock, the main pacemaker as the central machinery, and the circadian rhythms as the clock hands. The central pacemaker is located in the hypothalamic suprachiasmatic nuclei of the hypothalamus (SCN). This is the structure where the information received from the environmental cues or *zeitgebers* is processed and a signal is sent to the organism for the coordination of physiology and behaviour. However, some overt rhythms are able to modify and synchronize the circadian clock, constituting a feedback signal, and some external variables can exert a direct influence on the overt rhythm in a process known as masking (see part 1.2.3).

1.3.1. INPUTS

Although light is the strongest *zeitgeber* for human circadian clock, non-photic synchronizers send also information to the central pacemaker in order to maintain the synchronization and phase relationship with the *zeitgebers* (Morin & Allen, 2006).

Circadian photoreception occurs mainly by a subgroup of ganglion cells in the retina which are intrinsically photosensitive (ipRGCs) due to the presence of melanopsin (Provencio et al., 2000; Ruby et al., 2002), an opsin linked to 11-*cis*-retinal (based on vitamin A) with a maximum sensitivity *in vivo* from 440 to 480 nanometres (for humans and mice, respectively) (Peirson & Foster, 2010). The melanopsin signalling pathway for light is initiated with the activation of the photoreceptor by light exposure, then it interacts with a G protein that activates a phospholipase C (PLC); PLC releases inositol trisphosphate and diacyl-glycerol, which ultimately modulate a transient receptor potential canonical channel, probably through the activation of a protein kinase C (Hankins et al., 2008; Peirson et al., 2007; Sekaran et al., 2007).

In addition, ipRGCs receives also information from cones and rods (Freedman et al., 1999; Hattar et al., 2003) completing light information, which is sent to the central clock (Berson et al., 2002; Güler et al., 2008; Hattar et al., 2002). Although ipRGCs are the main mediators, cones and rods are necessary for a complete circadian response in

all physiological and behavioural tests. It was demonstrated by experiments that ablated either cones and rods or ipRGCs and showed animals with merely attenuated circadian responses, and only when both, classical and novel photoreceptors were eliminated, the circadian response was completely abolished (Hattar et al., 2003).

Light information from ipRGCs is transmitted to the SCN thanks to the retinohypothalamic tract (Berson, 2003; Gooley et al., 2003; Hirota and Fukada, 2004). However, other targets of the ipRGCs have also a very important function such as the intergeniculated leaflet (Hattar et al., 2002; Morin et al., 2003), which also participates in the circadian photoentrainment (Harrington, 1997), the olivar pretectal nucleus involved in the pupillary reflex (Hattar et al., 2002; Morin et al., 2003; Trejo and Cicerone, 1984; Clarke and Ikeda, 1985a; Clarke and Ikeda, 1985b; Young and Lund, 1994), and others more recently discovered nuclei or areas as the ventral lateral geniculate nucleus, superior colliculus, hypothalamic subparaventricular zone and preoptic region (**Figure 4**) (Hattar et al., 2002; Gooley et al., 2003; Morin et al., 2003; Hannibal and Fahrenkrug, 2004) (for review see Hattar et al., 2006).

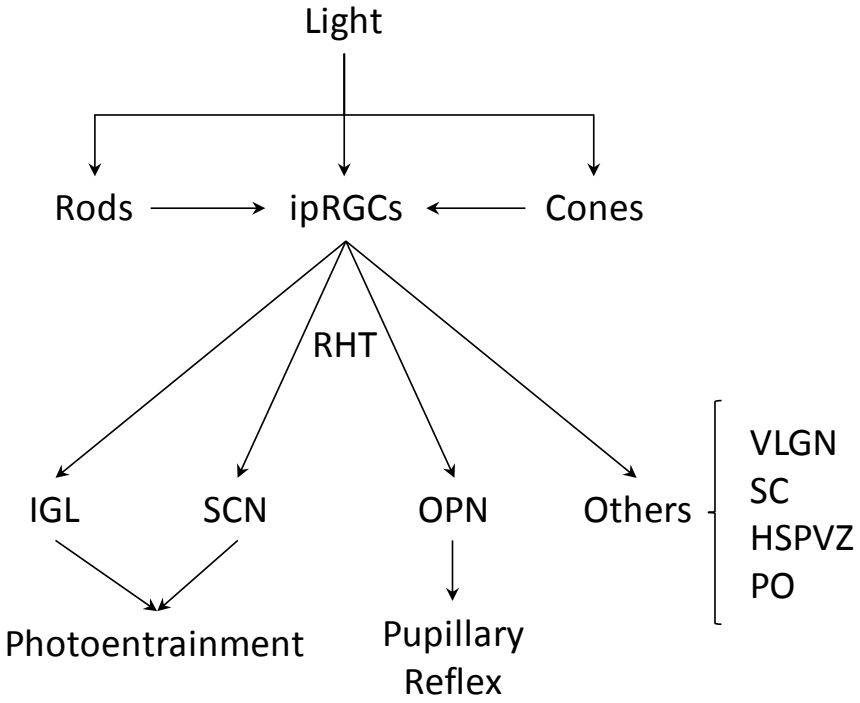

Figure 4. Schematic light input to Suprachiasmatic Nucleus (SCN) and other brain nuclei with functional correlations. ipRGCs: intrinsecally photosensitive Retinal Ganglion Cells, RHT: Retinohypothalamic Tract, IGL: Intergeniculated Leaflet, SCN: Suprachiasmatic Nucleus, OPN: Olivar Pretectal Nucleus, VLGN: Ventral Lateral Geniculated Nucleus, SC: Superior Colliculus, HSPVZ: Hypothalamic Subparaventricular Zone, PO: Preoptic Region.

Thermocycle is also an important *zeitgeber* to the circadian system, which is capable to entrain cellular cultures *in vitro* (Brown et al., 2002) and core body temperature of mice *in vivo* (Refinetti, 2010).

In addition, some overt rhythms present also a synchronizer effect such as scheduled sleep, activity and feeding (Atkinson et al., 2007; Mendoza, 2007; Mistlberger & Skene, 2004). These physiological and behavioural variables with both components (synchronizer and overt rhythm) are known as ***Zeitnehmer*** ("time taker" in German), which is defined as an input pathway that is itself rhythmically regulated by feedback from an oscillator. It will create a rhythmic input to the SCN even in constant conditions (Roenneberg & Merrow, 1998).

Finally, social contacts were considered long time as a variable with synchronizing effect due to classical experiments (Aschoff et al., 1971). However, it was not enough to entrain subjects in recent researches and it is now not considered as a synchronizer (Mistlberger & Skene, 2004; Mistlberger & Skene, 2005).

1.3.2. MACHINERY

The discovery of the central pacemaker in mammals was delayed due to the complexity of the brain. However, in the 1960s Ritcher provoked a series of brain lesions in rats and narrowed down the studied area to the hypothalamus (Richter, 1967). In 1972, two simultaneous publications pointed to the suprachiasmatic nuclei (Moore & Eichler, 1972; Stephan & Zucker, 1972). Afterwards, disappearance of rhythms by SCN ablation, rhythmic electrophysiological SCN recordings and rhythmic properties of isolated SCN in the brain gave more strength to this idea (Inouye & Kawamura, 1979). However, circadian mutant animals provided the final evidence, since the transplant of the SCN from a homozygous mutant animal to a wild type and *vice versa* demonstrated that circadian periodicity was determined by the donor (Ralph et al., 1990).

The central pacemaker resides in the suprachiasmatic nuclei of hypothalamus, located on top of optic chiasm bilaterally to the third ventricle. The human SCN consist of around 20000 heterogeneous and small neurons together with different populations of glial cells. Each single suprachiasmatic nucleus is formed by two regions in terms of

anatomical and physiological differences, the ventrolateral "*core*" and the dorsomedial "*shell*" (Antle et al., 2003; Moore et al., 2002; Morin, 2007). The dorsomedial area expresses as main neurotransmitter arginine-vasopressin (AVP) and has a low neuronal density compared to the ventrolateral area, which is highly packed and expresses as main neurotransmitter vasoactive intestinal polypeptide or VIP (Moore et al., 2002). In fact, the retinal inputs to SCN are mainly located in the ventrolateral region, where immediate genes are induced by light, while dorsomedial region is more involved in gene expression, therefore it is the place of reception of ventral projections and the origin of projections to other brain areas. Thus, ventrolateral area is considered as responsible for light synchronizing and dorsomedial one as more involved in the output modulation (Abrahamson & Moore, 2001; Hattar et al., 2002; Moore et al., 2002).

In the SCN, each neuron is an independent oscillator with its own period, although all together are coupled to show a unique periodicity (Honma et al., 1998; Silver et al., 1990; Webb et al., 2009; Welsh et al., 1995). Inside of each neuron there is a molecular clock, which consists in a series of genetic positive and negative autoregulated transcriptional and translational feedback loops (TTFL), showing recurrent 24 hour rhythms in mRNA and protein levels of key clock components. The transcription factors BMAL1 and CLOCK (alternatively NPAS2 in the SCN) constitute a heterodimer, which activates the expression of clock genes *Period*, *Cryptochromes* (*Per* and *Cry*, respectively) and *Rev-Erbα*, as well as other other clock controlled genes (represents approximately a 10% of the complete genome), by binding to E-box enhancement elements (Reppert & Weaver, 2002). PER and CRY dimerize and inhibit their own expression by translocation into the nucleus, interfering with the CLOCK:BMAL1 heterodimer with a delay of several hours (Vanselow & Kramer, 2010). REV-ERB proteins repress *Bmal1* transcription (Kornmann et al., 2007; Preitner et al., 2002), while ROR proteins activate it (Guillaumond et al., 2005; Sato et al., 2004) (**Figure 5**). However, oscillation in the transcription of the gene *Clock* is very weak if not arrhythmic in the mammalian circadian system (Rippeger & Brown, 2010). Several isoforms of Casein Kinase1 (CK1), which are involved in the regulation of many cellular processes, promotes the degradation of PER by the proteasome, regulating the negative TTFL (Eide et al., 2005).

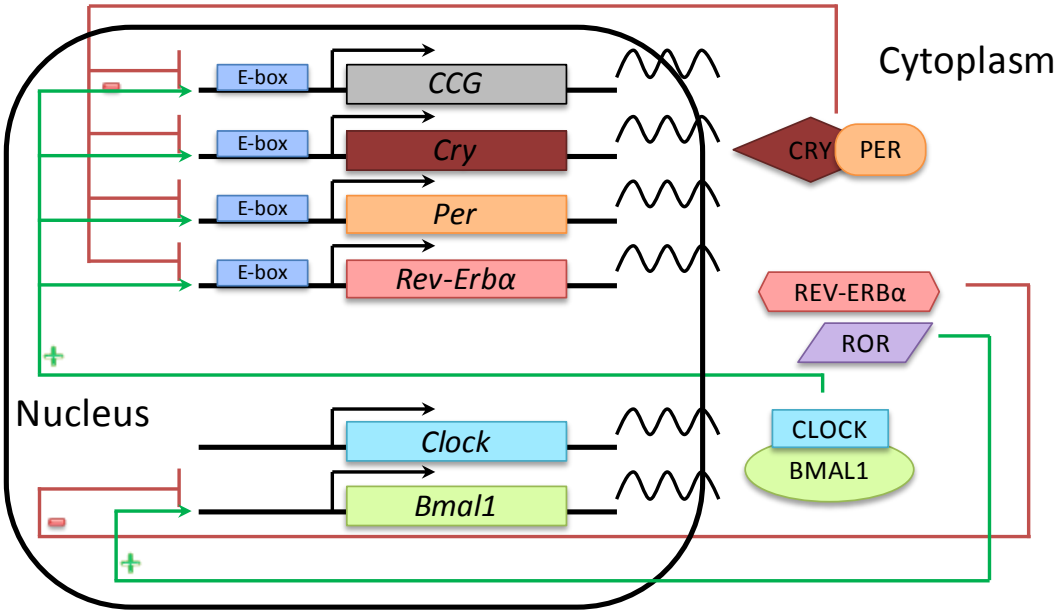

Figure 5. Simplified model of the mammalian molecular clock. The clock comprises of the interaction between two feedback loops: one positive (**green**) including CLOCK and BMAL1 proteins as main components promoting *Per*, *Cry*, *Rev-Erbα* and clock controlled genes (*CCG)* transcription and ROR as *Bmal1* transcription activator, and two negative (**red**) which involve PER-CRY heterodimer which inhibits *Per*, *Cry* and *Rev-Erbα* transcription while REV-ERBα proteins inhibit *Bmal1* transcription. See the text for more details.

1.3.3. Outputs

The SCN drives the complete organism as an orchestra conductor by neural or humoral eferences to other regions inside and outside the brain. Among the humoral mediators, some of them discovered by a technique where the SCN was encapsulated in a semi-permeable membrane avoiding neural connections (Silver et al., 1996), the most known are prokineticin-2 (Cheng et al., 2002), cardiotropin (Kraves & Weitz, 2006) and AVP. The SCN have dense neural connections to the dorsal and ventral subparaventricular zone (dSPZ and vSPZ, respectively), dorsomedial hypothalamus (DMH) and paraventricular nucleus (PVN) (Saper et al., 2005). The dSPZ regulates circadian body temperature by projections to the medial preoptic area (MPO) (Kalsbeek et al., 1993). vSPZ sends the connections mainly to DMH, which controls sleep-wake pattern through projections to the ventrolateral preoptic area (VLPA) (Moore, 2007), and also sleep, feeding and activity patterns by connecting with the lateral hypothalamus (LH) and the orexygenic system (Abrahamson et al., 2001; Deboer et al., 2004; Saper et al., 2001). In addition, SCN have preautonomic neurons, which innervate

LH and PVN regulating the sympathetic-parasympathetic balance (Buijs et al., 2003). At last, SCN drives the release of a variety of releasing hormones as corticoid, gonadotropin and thyrotropin releasing hormones (**Figure 6**) (Buijs and Kaalsbek, 2001).

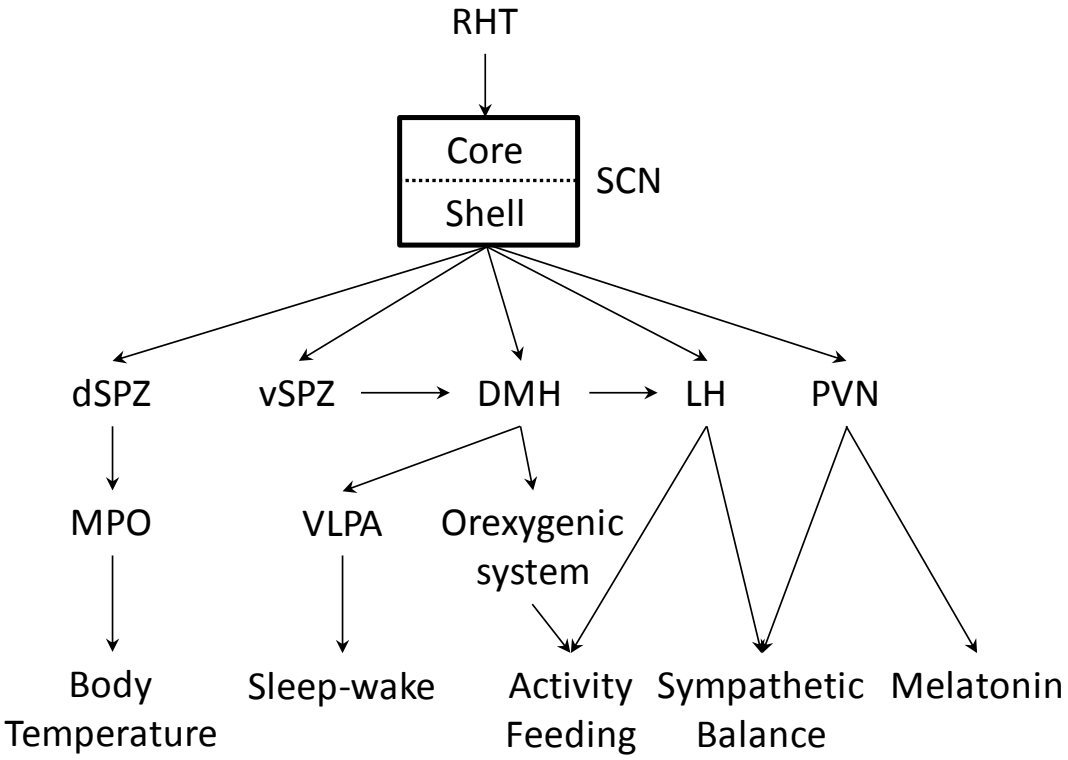

Figure 6. Schematic outputs from the Suprachiasmatic Nucleus (SCN) and projections to control different body functions. dSPZ: Dorsal Subparaventricular Zone, vSPZ: Ventral Subparaventricular Zone, DMH: Dorsomedial Hypothalamus, LH: Lateral Hypothalamus, PVN: Paraventricular Nucleus, MPO: Medial Preoptic Area, VLPA: Ventrolateral Preoptic Area.

For the study of the human circadian clock it is not possible a direct measure of SCN, thus, a reliable overt rhythm is necessary for assessing the SCN functionality (known as circadian marker rhythm). From the wide range of circadian rhythms only a few accomplish the required characteristics (high amplitude, reliable, easy-measure and specific phase relationship with the circadian clock). The most commonly used marker rhythms are core body temperature, motor activity, and cortisol and melatonin secretion patterns (Benloucif et al., 2005; Hofstra & de Werd, 2008; Mormont et al., 2002;

Touitou & Selmaoui, 2012; Van-Someren, 2000). From these, the melatonin pattern is considered the gold standard because its synthesis and release is directly dependent of the SCN (Van Someren & Nagtegaal, 2007) (detailed in part 1.3.3.1). Cortisol secretion shows also a circadian pattern with the peak in the morning related to the usual awakening time and has a stable phase relationship with melatonin. Core body temperature nadir coincides with the peak of melatonin (Hofstra & de Werd, 2008) whereas activity is more variable and subjected to voluntary activity of subjects' will (Acebo et al., 1999). Although distal skin temperature shows its evening increase coinciding with the onset of melatonin release (Bonmati-Carrion et al. 2014), it depends directly on circadian clock by the sympathetic balance (Buijs et al., 2003), favours the heat loss contributing to core body temperature rhythm and sleep onset (Kräuchi & Deboer, 2010; Kräuchi & Wirz-Justice, 2001; Van Someren, 2000) and presents a high amplitude rhythm, it is not considered as a marker rhythm yet.

1.3.3.1. MELATONIN

From the main hands of the human circadian clock, melatonin is the most important. Melatonin is a pineal hormone in charge of the timekeeping signal transmission (Pevet & Challet, 2011). The route for melatonin synthesis begins with L-Tryptophan, which in two steps becomes serotonin. Next step from serotonin to N-acetyl serotonin catalysed by arylalkilamine N-acetil transferase (AA-NAT) is the key regulated step. Finally, N-acetyl serotonin is methylated by Hydroxyindole-O-methyltransferase (HI-OMT) to melatonin. The key enzyme in the regulation (AA-NAT) is circadianly modulated by nervous input from SCN to the pineal gland through PVN, the intermediolateral column of the spinal cord and superior cervical ganglion (Benarroch, 2008; Pandi-Perumal et al., 2006). PVN neuron activity during night promotes melatonin secretion while light increases SCN electrical activity, which in turn, inhibits PVN neurons and, thus, melatonin secretion (Buijs & Kalsbeek, 2001; Teclemariam-Mesbah et al., 1999). Thus, melatonin is produced during the subjective night whenever light is absent. These characteristics allow organisms to use the melatonin as and endocrine clock and calendar for knowing when night-time occurs and which season is. The main property of melatonin is its chronobiotic effect producing a phase advance when it is administrated during late evening and a phase delay when it is

administrated during early morning (Lewy et al., 1998), but it possess other properties like its immunomodulatory (Haldar & Ahmad, 2010), antioxidative (Reiter, 1995), anti-inflammatory (Escames et al., 2006), neuroprotectinve (Reiter et al., 1998) and antitumoral effects (Erren et al., 2003) (for review see Hardeland et al., 2011). In addition, when light is present at night melatonin secretion is inhibited. However, this suppression depends of spectrum (Brainard et al., 2001; Thapan et al., 2001), intensity (McIntyre et al., 1989), duration (Czeisler, 1995), timing (Skene, 2003) and subject's previous light history (Smith et al., 2004) (for review see Duffy & Wright, 2005). Bluer lights, higher intensities, longer exposures, midnight exposures or dim light during previous days are related to higher reductions in the melatonin secretion pattern.

1.3.3.2. THERMOREGULATION

Almost all the systems in the body are implicated in the thermal regulation, from the skin to the cardiovascular and respiratory systems. The core temperature is the consequence of the heat produced, stored and lost by the body and reflects the heat gain/loss balance. Generally, heat is actively gained by catabolic processes, then, it is distributed by conduction and circulatory convection to the periphery and through the skin it is dissipated by conduction, convection and radiation whenever the environment is cooler than the skin. However, if the ambient is warmer than the skin, the process is inverted (body gains heat from the environment) and the only way to dissipate heat is by sweat evaporation. In the thermoneutral zone, core temperature regulation occurs by vasomotor changes in the periphery without affecting the metabolic rate. The thermic controller is located in the hypothalamus with a set point temperature (Hammel et al., 1963), which is an integration structure of signals from internal and cutaneous thermoreceptors. In case of a deviation from the set point a comparator sends an error signal proportional to the deviation measured. This set point is modulated by the SCN provoking higher core body temperatures during wake phase and lower core body temperatures during sleep phase (Aschoff, 1983; Gilbert et al., 2000). These changes are not produced by changes in heat gain (basal metabolic rate is relatively constant) but by changes in heat loss due to differences in peripheral blood flow (Bach et al., 2011, Blatteis, 2012). Thus, when core body temperature reaches its acrophase the distal skin temperature will be close to a nadir and the inverse is also true, but distal skin

temperature is phase advanced by around 100 minutes to core body temperature (**Figure 7**) (Kräuchi et al., 2006, Sarabia et al., 2008).

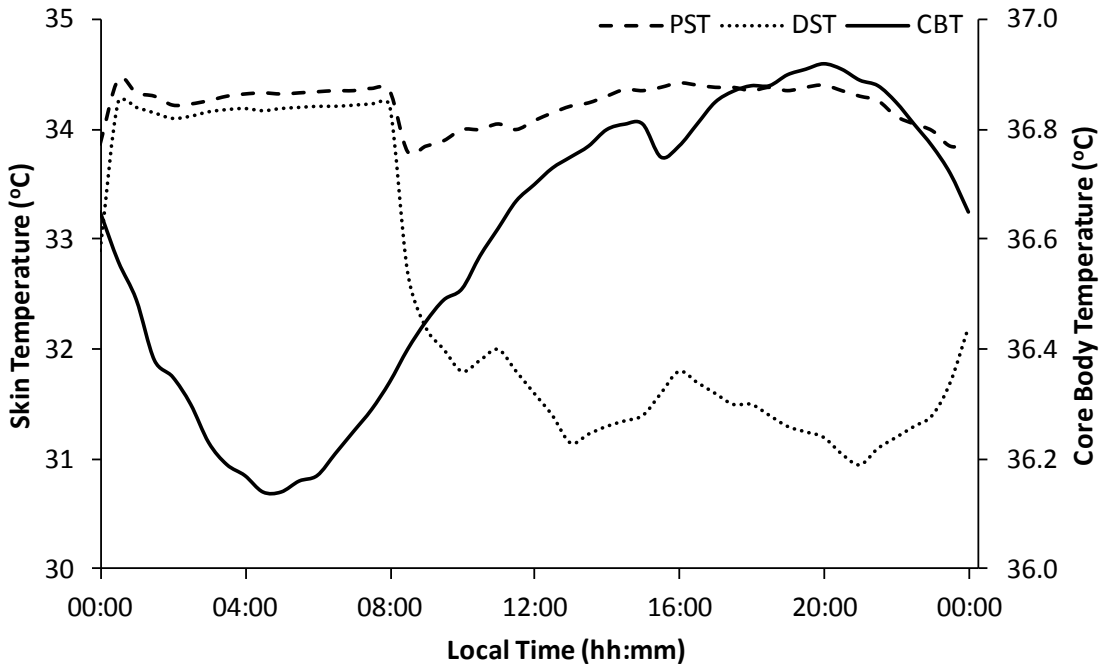

Figure 7. Circadian pattern of Core Body Temperature and Distal and Proximal Skin Temperature (DST and PST, respectively). Circadian pattern of CBT shows its nadir at night while the DST is in its acrophase. The inverse occurs during daytime. PST exhibits an intermediate behaviour between both, DST and CBT, with a pattern similar to DST during nighttime and more similar to CBT during the active phase.

1.3.3.3. CARDIOVASCULAR SYSTEM

The cardiovascular system is the major effector of thermal changes in thermoneutrality. In thermoregulatory terms, blood means heat and cutaneous circulation is the variable heat insulator underneath the skin, which determines, depending on the skin proximity, the heat transference velocity. The cutaneous circulation is regulated by vessels patency that is controlled by the autonomic nervous system. Ambient temperature changes are translated into blood redistribution, if weather becomes cool, blood will be stored in the "core" (trunk) to diminish heat loss by the "shell" (extremities), whereas in warmer conditions blood is redistributed toward the periphery to dissipate heat from the core (**Figure 8**), which is the same that occurs in wake and sleep conditions (Krauchi, 2007). This blood redistribution is controlled by sympathetic nervous system, which dilates and constricts peripheral vessels in general, and arteriovenous anastomoses more specifically (which are abundant in glabrous skin and are widely innervated by sympathetic nerves).

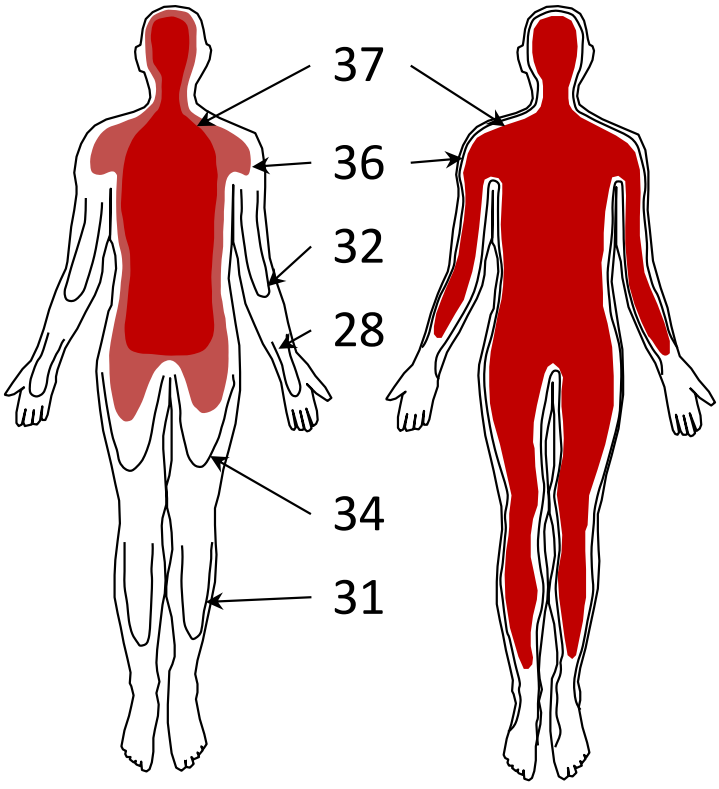

Figure 8. Heat distribution depending on room temperature. Schematic diagram showing temperature distribution from a human in cool (20°C) and warm (35°C) ambient (left and right, respectively). *Modified from Aschoff, 1971*.

In addition, cardiovascular system shows, as it has long been known, circadian modulation in blood pressure and heart rate (Blazquez et al., 2012; Kräuchi et al., 2012; Veerman et al., 1995). Recently, circadian rhythms have also been discovered in vascular tone and cardiac output (Veerman et al., 1995). All these rhythms have a similar pattern with high values during daytime and low values during nighttime. Nowadays, it is suggested that all these rhythms are a consequence of a circadian pattern in the sympathetic tone instead of the sleep-wake or rest-activity cycle dependence (Furlan et al., 1990; Yamasaki et al., 1996). The sympathetic activity pattern is reflected in the heart rate variability rhythm with an inverse pattern, that it is, variability is higher during rest phase (lower sympathetic activity) and lower during activity phase (higher sympathetic activity) (for review see Guo & Stein, 2003).

1.3.4. DEVELOPMENT AND AGEING

The human circadian system, like every cell, organ or system in the organism, presents a process of maturation and ageing. The maturation begins in the maternal uterus, where the synchronizers came from the mother in form of hormones, nutrients and uterine movements (Seron-Ferre et al., 1993). Suprachiasmatic nuclei can be detected in the middle of the pregnancy period (18^{th} week) and in the 32^{nd} week the retinohypothalamic tract is complete and functional (Seron-Ferre et al., 2001). However, it is not known if in this moment the circadian system is also able to respond to a light stimulus (Seron-Ferre et al., 2001). The foetus shows circadian rhythms from the 20^{th} week of pregnancy with circadian modulation of breathing and heart rate, while hormonal rhythms, like cortisol and melatonin patterns appear around 35^{th} week (Garcia et al., 2001; Seron-Ferre et al., 2001).

However, the birth is a hard process for the newborn because he/she needs to adapt him/herself to the new environment with different synchronizers. Because of that, the newborn lose almost all the circadian rhythms (Mirmiran & Kok, 1991; Seron-Ferre et al., 2001). In the first years of life the suprachiasmatic nuclei grow until almost the adult size (the number of neurons reaches around 80% of the adult neurons), and most circadian rhythms appear (or reappear) during the first year of life (Anders, 1982). For example, temperature, breathing and heart rate patterns appear around the 4^{th} month whereas hormones as melatonin, cortisol and growth hormone circadian rhythms are

manifested around the 8th month (Anders, 1982; Seron-Ferre et al., 2001; Zornoza-Moreno et al., 2011).

In the adolescence, the endogenous period is elongated and the adolescent shows a marked eveningness tendency appears (Garcia et al., 2001). Melatonin pattern is delayed and dampened in the adolescence, and it is not due to a behavioural change or because a pineal impairment but part of the normal growth process (Garcia et al., 2001). This stage finishes with adulthood, described in detail in other sections.

Ageing process in the circadian system alters all the structures, from the inputs to the outputs through the circadian clock, and affects all levels: morphological, physiological and biochemical (Myers & Badia, 2005; Turner & Mainster, 2008). The senescence impairs the inputs to the circadian system by age-related pupillary myosis and reduced crystalline lens transmission, particularly of blue light, weakening circadian system inputs, and there is also a reduced exposure to bright light (Turner & Mainster, 2008). In addition, alteration of the molecular clock, biochemistry and morphology of the suprachiasmatic nuclei occurs and the main output, melatonin, is dampened by pinealocyte receptors changes and pineal calcification and size reduction (Kunz et al., 1999; Myers & Badía, 1995; Schmid et al., 1994). In the overt rhythms, the main changes observed are a phase advance, rhythm fragmentation, amplitude dampening and period shortening (Myers & Badía, 1995). All these changes are provoked by normal ageing, thus aged people need longer and brighter light exposure to synchronize their rhythms to 24 hours. In fact, aged people who show this circadian impairment expose themselves to darker days and/or brighter nights, with lower melatonin levels (Touitou, 2001; Turner et al., 2012). However, regarding circadian period there is no total agreement in literature, so it has been reported that period length is similar to young people in forced desynchrony protocol for melatonin and core body temperature (Dijk et al., 1999).

1.4. CIRCADIAN HEALTH

In addition to the rhythmic patterns generation, the circadian system is in charge of maintaining the phase relationship between physiological and behavioural variables, which is considered necessary to maintain a good health status (Waterhouse &

DeCoursey, 2004). As every rhythm in the organism has to be in a certain phase with the others and with the environment, when impairment of this rhythmic harmony occurs, chronodisruption appears (Erren & Reiter, 2009b). However, this knowledge allows us to create strategies for circadian system enhancement by strengthening inputs for the circadian system (Martinez-Nicolas et al., 2014).

1.4.1. CHRONODISRUPTION

When a misalignment between the internal clock and *zeitgebers* occurs or the phase between biological rhythms is wrong and/or unstable, chronodisruption appears (a combination of them is also possible). This is a recently described syndrome, whose appearance has been favoured by artificial light (Rajaratnam & Arendt, 2001). This pathology is frequent in people exposed to bright light at night or darkness during daytime, shift-work and chronic and/or social jet-lag. However, other input anomalies as frequent snaking or meal shift may result in an internal misalignment (Erren & Reiter, 2009a). In addition, ageing or lesions of the suprachiasmatic nuclei can also produce chronodisruption, suppressing many overt rhythms, from activity and meal to hormones and body temperature (Moore & Eichler, 1972; Stephan & Zucker, 1972). Among people suffering from chronodisruption a predisposition to cognitive and affective impairments, metabolic syndrome, cardiovascular diseases, sleep disorders, premature aging, prostatic, mammary and colorectal cancer and, in general, higher mortality occurs (Davis & Mirick, 2006; Garaulet & Madrid, 2010; Gronfier et al., 2007; Karlsson et al., 2001; Middleton et al., 2002; Pauley, 2004; Rodrigues Menezes et al., 2004; Schernhammer et al., 2003).

1.4.2. CHRONOENHANCEMENT

The appearance of chronodisruption as a new health concern in the XXI century generates the necessity of preventive or therapeutical measures to counteract its impact on human health. The main strategy to prevent chronodisruption consists in empowering circadian inputs, increasing day-night contrast. Because of that, the number of possible strategies is as large as the number of circadian system inputs:

1. Light exposure: as main *zeitgeber*, light shows a strong effect on the circadian system and when it is applied at appropriate moments produces an increase in amplitude and stability (Martinez-Nicolas et al., 2011). However, sometimes solar light exposure is not feasible and it is necessary to increase the time of bright light exposure. In fact, bright light therapy has been demonstrated to reduce cognitive and affective disorders (Even et al., 2008; Dowling et al., 2008).
2. Darkness: in antagonism to light, darkness is also necessary for entraining. In fact, constant light at night disrupts the circadian system provoking arrhythmic patterns in short term (Grone et al., 2011; Leloup & Goldbeter, 2001; Ohta et al., 2005) and its chronic exposure is related to some types of cancer (Anisimov et al., 2012). In addition, melatonin (the "chemical darkness") has similar effects to darkness, in addition to afore mentioned properties, from antioxidant to neuroprotective (see section 3.3.1). However, its administration has to be during the subject's night to avoid providing contradictory information to the circadian system (Stevens, 2006).
3. Regular exercise: this *zeitnehmer* synchronizes the human circadian system and improves physical health and affective disorders (Atkinson et al., 2007).
4. Meal schedule: main *zeitnehmer* for most peripheral clocks, it is able to increase the synchronization between physiological and behavioural rhythms in animals (Mendoza, 2007). In addition, light dinners are recommendable due to glucose metabolism impairment during late evening or at nightin order to avoid high blood glucose levels (Saad et al., 2012; Van Cauter et al., 1989).
5. Environmental temperature: it has been described as a *zeitgeber* able to entrain living beings (Refinetti, 2010), but in humans its influence is restricted to enhancement of core body and skin temperature rhythms, urinary 6-hydroximelatonin sulphate excretion and rest sensation in a warm-cold day-night cycle (Kondo et al., 2007; Wakamura & Tokura, 2002).
6. Sleep habits: described as a *zeitnehmer* with a weak synchronizing power, sleep is able to determine light exposure and to drift core body temperature and melatonin secretion by a fixed schedule (Danilenko et al., 2003).
7. Social interaction: although with its influence is difficult to discern from other circadian inputs effect, as exercise or meal times, because they usually

occur at the same time (Mistlberger & Skene, 2004), maintain social contacts should also be desirable.

2. Objectives

The overall objective of this Doctoral Thesis was:

To establish the distal skin temperature pattern as marker rhythm to comfortably and reliably assess the human circadian system robustness. For this, thefollowing specific objectives were approached:

1. To obtain the endogenous circadian pattern of wrist temperature rhythm by mathematical removing light and temperature exposure, sleep, activity and body position effects and to determine the effect of these variables on wrist temperature rhythm.
2. To describe distal skin temperature pattern according to maturation and aging processes and to identify those rhythmic parameters that characterise different epochs of these processes.
3. To characterize light exposure naturalistic regimen and its influence on the human circadian system assessed by wrist temperature rhythm recordings.
4. To analyze the effect of environmental temperature seasonality on thermophysiological and cardiophysiological variables in young subjects.
5. To assess the lifestyle, synchronizers exposure and their effect on the circadian system in healthy elders comparing with young people and to evaluate possible chronodisruption in these age groups.
6. To study the influence of day/night contrast in synchronizers and lifestyle variables with synchronizer effect on human circadian system and propose a method for assessing circadian system aging without taking into account the biological age but the circadian pattern characteristics.
7. To create a healthy circadian lighting design to improve light exposure during daytime and to reduce harmful light effects during night-time.

3. Experimental Chapters

3.1. EXPERIMENTAL CHAPTER 1

UNCOVERING DIFFERENT MASKING FACTORS ON WRIST SKIN TEMPERATURE RHYTHM IN FREE-LIVING SUBJECTS

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3.1. UNCOVERING DIFFERENT MASKING FACTORS ON WRIST SKIN TEMPERATURE RHYTHM IN FREE-LIVING SUBJECTS

ABSTRACT

Most circadian rhythms are controlled by a major pacemaker located in the hypothalamic suprachiasmatic nucleus. Some of these rhythms, called marker rhythms, serve to characterize the timing of the internal temporal order. However, these variables are susceptible to masking effects as the result of activity, body position, light exposure, environmental temperature and sleep. Recently, wrist skin temperature has been proposed as a new index for evaluating circadian system status. In light of previous evidence suggesting the important relationship between wrist temperature and core body temperature regulation, the aim of this work was to purify the wrist temperature pattern in order to obtain its endogenous rhythm with the application of multiple demasking procedures. To this end, 103 subjects (18-24 years old) were recruited and their wrist temperature, activity, body position, light exposure, environmental temperature and sleep were recorded under free-living conditions for 1 week. wrist temperature demasking by categories or intercepts was applied to simulate a "constant routine" protocol (awakening, dim light, recumbent position, low activity and warm environmental temperature). Although the overall circadian pattern of wrist temperature was similar regardless of the masking effects, its amplitude was the rhythmic parameter most affected by environmental conditions. The acrophase and mesor were determined to be the most robust parameters for characterizing this rhythm. In addition, a circadian modulation of the masking effect was found for each masking variable. wrist temperature rhythm exhibits a strong endogenous component, despite the existence of multiple external influences. This was evidenced by simultaneously eliminating the influence of activity, body position, light exposure, environmental temperature and sleep. We therefore propose that it could be considered a valuable and minimally-invasive means of recording circadian physiology in ambulatory conditions.

Keywords: Masking, Purification, Wrist temperature, Human circadian system, Free-living conditions, Endogenous component, 24-h ambulatory monitoring, demasking.

INTRODUCTION

The circadian system is organized into a hierarchical network of structures that are responsible for the generation of circadian rhythms and their synchronization to environmental factors. This system includes a central pacemaker (the suprachiasmatic nucleus of the hypothalamus, SCN), several peripheral clocks, inputs and outputs, SCN pathways and the connections between them (Buijs & Kalsbeek, 2001; Strattmann & Schibler, 2006).

Certain circadian outputs, known as circadian marker rhythms, are being used to assess the overall status of the circadian system. These marker rhythms are variables that can be used to characterize the timing of the internal temporal order. To be considered a circadian marker rhythm, the variable must be easily measurable over long periods of time, preferably using non-invasive methods. To date, the most widely used marker rhythms are core body temperature (CBT), and plasma or salivary melatonin (Mormont et al., 2002; Van-Someren, 2000).

The circadian rhythm of CBT is determined by changes in heat production and heat loss (Kräuchi, 2007), which is delayed with respect to heat gain (Aschoff, 1983). Therefore, distal skin temperature (DST) is very important in the regulation of CBT as evidence has recently suggested. Changes in CBT are preceded by opposite changes in DST or wrist temperature (WT) (Gradisar & Lack, 2004; Sarabia et al., 2008). DST is becoming more widely used because it is less invasive, more comfortable, easy to use and stable under a constant routine as reflected by an increasing body of literature (Anders et al., 2010; Blazquez et al., 2012; Gomper et al., 2010; Kräuchi & Wirz-Justice, 2001; Martinez-Nicolas et al., 2011; Ortiz-Tudela et al., 2010; Raymann et al., 2008; Romejin & Van Someren, 2011; Sarabia et al., 2008; Zornoza-Moreno et al., 2011). However, like CBT, DST is subject to many environmental and physiological influences that mask its rhythms. These include physical activity, body position, light exposure, environmental temperature and sleep (Cajochen et al., 2000; Kräuchi, 2007; Kräuchi et al., 2005; Kräuchi & Wirz-Justice, 2001; Reilly & Waterhouse, 2009; Scheer et al., 1999; Wakamura and Tokura, 2002; Waterhouse et al., 1999).

Both variables (CBT and WT) are the result of two sets of influences: one endogenous, directly driven by the SCN (Kräuchi et al., 2005; Kräuchi & Deboer, 2010; Moore & Danchenko, 2002), and the other exogenous, exerting a masking effect and

superimposed onto the endogenous factors. Activity and sleep are the masking factors that are most frequently studied (Minors & Waterhouse, 1989; Waterhouse et al., 2000; Weinert & Waterhouse, 2007). However, other masking factors have been reported to include environmental temperature (Wakamura & Tokura, 2002), body position (Kräuchi et al., 2005), environmental light (Cajochen et al., 2000; Scheer et al., 1999) and even menstrual cycle, which affects mainly CBT, but not DST (Shechter et al., 2011).

Several methods have been described to suppress masking effects on the CBT rhythm. The most widely used method is a protocol that reduces the masking effect by submitting the subjects to a constant routine. Under this routine, sleep is forbidden, and the subject is kept in bed in a semirecumbent body position, with constant mental activity and dividing food and drink into frequent small portions of constant composition, evenly distributed throughout both day and night (Duffy & Dijk, 2002; Mills et al., 1978). However, this protocol introduces its own masking effects, as it is stressful, unpleasant and quite unsuitable for repeated assessments (Minors & Waterhouse, 1989; Rietveld et al., 1993; Weinert & Waterhouse, 2007). Moreover, these experimental conditions with constant light and temperature produce negative consequences for the subject's physiology and are very artificial, as people tend to live according to rhythmic conditions (Reiter et al., 2007). Another disadvantage of this protocol is that it does not provide information about the effects of the environment on circadian rhythms (Weinert & Waterhouse, 2007). A second method used to obtain the endogenous timing is forced desynchronization. In this case, subjects are exposed to 20-h or 28-h days, which are beyond entrainment limits. As a result, the circadian system reveals its endogenous timing, with similar results for both conditions (20-h and 28-h days) (Czeisler et al., 1999). However, this method fails to show the organism's internal timing under normal living conditions (24-h day).

To eliminate masking effects in free-living subjects, most authors use mathematical tools. The first method developed is referred to as purification by categories, and it has mainly been applied to activity and heart rate. According to this method, CBT values are classified by activity levels, which are divided into categories and then used to analyze CBT data. The advantage of this approach is that by selecting temperature values corresponding to the lowest categories, researchers can simulate a "constant routine" approach to compensate for these masking factors (Minors &

Waterhouse, 1989; Minors & Waterhouse, 1992; Waterhouse et al., 2000; Weinert & Waterhouse, 1998).

A second mathematical procedure to eliminate masking under free-living conditions is a method called purification by intercepts, or its more complicated version ANCOVA (Waterhouse et al., 2001). This method uses a regression analysis to calculate the core temperature that would correspond to zero activity or a low heart rate for each time bin, obtaining an approximate "constant routine" temperature curve (Waterhouse et al., 2001; Weinert et al., 2003).

Although some authors did not achieve good results when applying demasking techniques in forced desynchrony protocols (Klerman et al., 1999), it is a reliable option to obtain the endogenous pattern under normal free-living conditions (Waterhouse et al., 2001; Waterhouse et al., 2000). These methodologies have been tested to unmask the core body temperature rhythm, and the results obtained from each method are quite similar (Waterhouse et al., 2000). Moreover, the data purified by mathematical procedures and the "constant routine" protocol yield similar results (Waterhouse et al., 2005). However, to date, these procedures have not been applied to unmask variables other than CBT.

In light of the fact that the DST rhythm is subject to several environmental and behavioral masking effects, and since an increasing number of papers are focused on DST, the aim of this work was to obtain, for the first time, the endogenous circadian pattern of this rhythm by mathematical procedures for simultaneously removing the masking effects of light exposure, environmental temperature, sleep, activity and body position and to determine the influence of these masking variables on the WT rhythm.

MATERIAL AND METHODS

Subjects

For the present study, 103 undergraduate student volunteers (48 men and 55 women, 18-24 years old) residing in Murcia, Spain (latitude 38º 01' N) were recruited. All the recordings were made in November. The overall mean (±SEM) environmental temperature was 16.3 ± 0.5ºC and the natural photoperiod was between sunrise at 07:28-

07:49 and sunset at 17:51-18:09 (Data obtained from the University of Murcia weather station: https://estacion.um.es/). Participants were instructed to complete a sleep diary designed by the Chronobiology Lab at the University of Murcia and were encouraged to maintain their habitual life style. The diary compiled information regarding sleep periods, time the subject went to bed and the time he or she got up. The chronotype of all participants was assessed using the morningness-eveningness questionnaire (Horne & Östberg, 1976).

The study abides by the bioethical principles set out by the Declaration of Helsinki. Data from the volunteers were included in a database and were protected according to Spanish Law 15/1999 from 13 September. All participants received the appropriate information about the characteristics of the study and signed an informed consent form before their inclusion in the study (Portaluppi et al., 2010). The study was approved by the Ethical Review Committee from the University of Murcia. No research was conducted outside our country of residence.

Wrist temperature measurement

All subjects wore a Thermochron iButton DS1921H (Maxim Integrated Products, Sunnyvale, California, USA) that measured their wrist skin temperature with a precision of ±0.125ºC. This temperature sensor was placed on the wrist of the non-dominant hand over the radial artery and isolated from the environmental temperature by a double-sided cotton sport wrist band, as previously described (Martinez-Nicolas et al., 2011; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). Temperature sensors were programmed to sample every 10 minutes over the course of an entire week.

Body position and activity monitoring

Body position and activity rhythms were assessed every 30 seconds using a HOBO Pendant G Acceleration Data Logger UA-004-64 actimeter (Onset Computer, Bourne, Massachusetts, USA) positioned on the non-dominant arm by means of a sport band. These data were then averaged for 10-minute intervals, allowing for WT comparisons. The manufacturing specifications and the method used to obtain these variables have already been described in a previous work (Ortiz-Tudela et al., 2010). Activity was measured as the rate of change in degrees per minute, and body position

was calculated as the angle between the axis of the acelerometer parallel to the humerous bone and the horizontal plane.

Environmental temperature and light exposure recording

In addition, all subjects were required to wear a HOBO Pendant Temperature/Light Data Logger UA-002-64 (Onset Computer, Bourne, Massachusetts, USA) on a necklace close to eye level during waketime and to put it on the bedside table during the sleep time to record environmental temperature and light exposure. Manufacturing specifications, memory, spectrum and accuracy were as described in a previous work (Martinez-Nicolas et al., 2011). This device records light intensity at regular intervals that have been previously programmed (in this experiment, every 30 seconds). These data were also averaged over 10-minute intervals to obtain the same sampling frequency as for WT.

Data analysis

WT data were filtered in order to eliminate artifacts such as those produced by temporarily removing the temperature sensor. To that end, the interquartile distance (from Q1 to Q4) was calculated and each datum whose rate of change with respect to the previous value was higher than the interquartile distance was eliminated (Sarabia et al., 2008; Van Marken Lichtenbelt et al., 2006). Sleep-wake information was converted into binary values by assigning a value of 1 when the subjects declared they were asleep and 0 when awake, as has been previously described. Sleep probability indicates the percentage of individuals asleep at any given time, as already described (Martinez-Nicolas et al., 2011; Ortiz-Tudela et al., 2010; Sarabia et al., 2008).

For purposes of comparison, WT was purified for activity by means of two standard methods: categories and intercepts. The first procedure based on categories, as described by Waterhouse et al., 2000. To this end, individual activity was divided into terciles, and the values corresponding to the lower activity category (lower tercile of activity for each subject) were averaged for hourly intervals, and then the corresponding synchronous temperature values were used to reconstruct the WT mean rhythm.

The second procedure to demask WT was the purification by intercepts method (Weinert & Waterhouse, 1998). This procedure was performed using hourly intervals of activity and its corresponding temperature, which were linearly correlated. The

extrapolated temperature associated with zero activity was then assigned to the initial time-point of the hourly interval. The procedure was repeated to account for all 24 hours.

We also propose an extension to the purification by categories method originally reported by Waterhouse and col. (Waterhouse et al., 2000) to determine the masking effect produced on WT by each individual variable. The environmental temperature categories used were cool (12-19ºC), warm (19-26ºC) and hot (26-33ºC). In the case of activity, the categories were low (up to 33% activity for each individual subject, as previously described), medium (from 33% to 66%) and high (above 66%). To categorize body position, three intervals were considered: lying down (0-30º), leaning (30-60º) and standing (60-90º). Light exposure was divided in two categories: dim light (less than 10 lux) and non-dim light (more than 10 lux). Finally, the sleep variable was classified as sleep or wake state. Separate temperature curves for each category and variable (environmental temperature, light exposure, activity, body position and sleep) were obtained. Only those time points that included more than 15 subjects were considered.

To characterize the WT endogenous component under a protocol simulating a "constant routine", we performed a multiple demasking procedure by categories or intercepts, simultaneously considering the masking variables of light exposure, environmental temperature, sleep, activity and body position. For purification by categories, individual WT data were selected to calculate an hourly interval waveform only when the following conditions were met: wake period, dim light (less than 10 lux), warm environmental temperature (19-26ºC, a range matching that used in constant routine protocols, according to Graw et al., 1998 or Jasper et al., 2010), body position between 0 and 30º (according to Cajochen et al., 2001), and low activity (less than 33%). For the purification by intercepts, we performed a stepwise multiple regression for each subject, for one-hour periods to identify the intercepts for WT rhythm and thus to educe WT values under conditions of wakefulness, recumbent body position, absence of activity, dim light and three different environmental temperatures (15, 20 and 25ºC). The equation applied to each of the 24 hour periods for this demasking procedure is as follows:

$$WT = aET+bLE+cP+dA+eS+Constant$$

Where ET: Environmental Temperature; LE: Light Exposure; P: Body Position; A: Activity; S: Sleep. The polynomial coefficients of each variable are a, b, c, d and e. These coefficients were represented per time point in order to establish the time-dependent influence of each masking variable on WT. In addition, the constant is also represented, and corresponds to the extrapolation to an environmental temperature of 0ºC, 0 lux, 0 grades of position, 0 activity and no sleep.

To test whether the demasked WT rhythm allows for detecting differences in circadian phase for human chronotypes, two subgroups of 12 people each (belonging to higher and lower decile) were selected using the Horne-Östberg morningness-eveningness questionnaire (Horne & Östberg, 1976). A Student's t-test was performed to compare morning and evening types, before and after the demasking procedure.

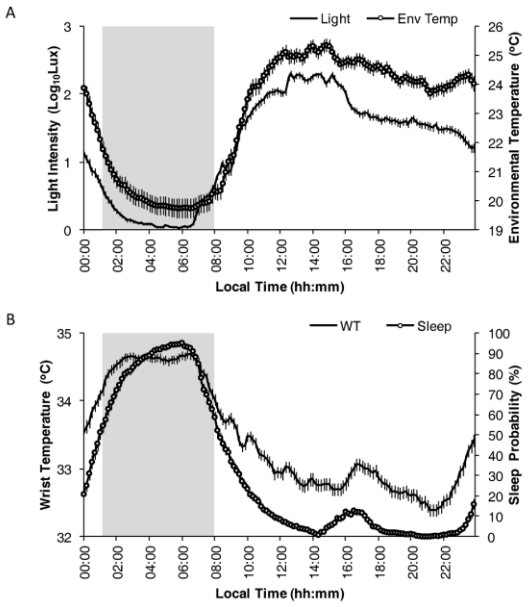
The WT, ET, LE, P, A and S rhythms were characterized using cosinor analysis. Rhythm parameters estimated from the cosinor procedure included its mesor (24 h rhythm-adjusted mean of the cosine curve fitted to the data), amplitude (difference between the maximum and the cosine calculated mesor) and acrophase (peak of the fitted cosine curve). All data are expressed as a mean with a 95% confidence interval. This inferential statistical method also provides the percent rhythm (%V; percentage of overall variance attributed to the best fitted cosine curve with reference to total variability of experimental data made equal to 100%) and a probability or p value that indicates the statistical significance of the fitness of the cosine curve to the data (rhythm detection level) and it was determined using the integrated package for temporal series analysis “El Temps” (A. Díez-Noguera, Universitat de Barcelona, 1999). The data were processed using Microsoft Office Excel 2007. The remaining data are expressed as mean ± SEM.

RESULTS

Mean patterns

The mean waveform of all recorded variables is shown in Figure 1. To facilitate the description of results, when more than 50% of volunteers were asleep, that period was considered the sleep period (01:10 h to 08:00 h), whereas when less than 50% were asleep, this was referred to as the wake period (08:10 h to 01:00 h). As can be observed

in Figure 1A, the light exposure rhythm exhibited minimum mean values (7.95 ± 1.59 lux) from 02:00 to 06:50 h, maximum mean values (162.19 ± 1.12 lux) from 12:00 to 15:50 h, and reached a plateau of 38.02 ± 1.12 lux from 17:00 to 22:50 h. The lowest mean values (20.16 ± 0.32ºC) of the environmental temperature rhythm coincided with the sleep period, while the highest mean values (24.08 ± 0.24ºC) occurred during the wake period. The minimum values for WT were observed from 20:10 h to 22:10 h (Figure 1B), coinciding with minimal sleep probability, a period known as the wake maintenance zone (32.48 ± 0.09ºC and 0.34 ± 0.19% for WT and sleep probability, respectively). Both variables showed the highest values (82.22 ± 1.90% for sleep probability and 34.54 ± 0.07ºC for WT) during the sleep period. In addition, a secondary peak in sleep probability (9.04 ± 1.45%) was obtained at the postprandial time (15:00 to 17:50 h) with a slightly delayed (16:00 h to 18:50 h) increase in WT (32.90 ± 0.09ºC). As expected, activity and body position showed low, stable values during the sleep period (22.85 ± 0.74º/min and 20.77 ± 0.93º, respectively) and higher, more variable values (66.62 ± 1.04º/minute and 46.56 ± 1.04º, respectively) during the wake period. Again, a small decrease in both variables was observed coinciding with the postprandial dip.



D

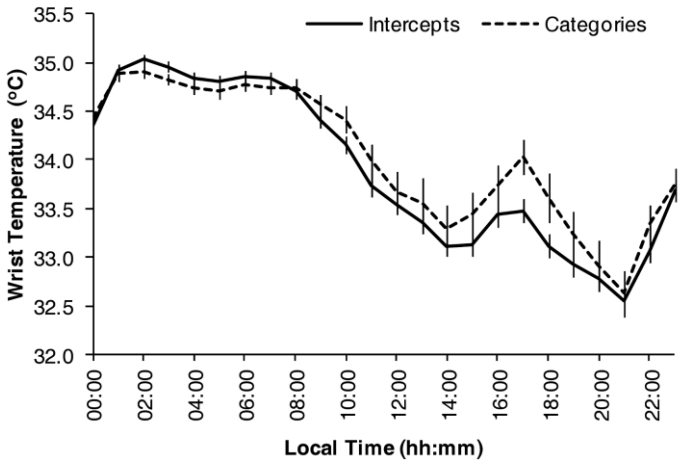
	Mesor	Amplitude	Acrophase (hh:mm)	%V
Env Temp (ºC)	22.94 ± 0.14	2.63 ± 0.25	16:47 ± 00:21	83.68*
Light (log_{10}lux)	1.24 ± 0.05	1.00 ± 0.09	15:47 ± 00:19	85.98*
Wrist Temp (ºC)	33.45 ± 0.06	1.02 ± 0.10	05:05 ± 00:22	82.74*
Sleep (%)	32.12 ± 2.61	43.79 ± 4.61	05:03 ± 00:24	79.86*
Activity (grades/min)	53.86 ± 1.37	29.05 ± 2.42	17:01 ± 00:19	86.21*
Position (grades)	39.04 ± 0.86	16.72 ± 1.53	16:39 ± 00:21	83.78*

Figure 1. Study population mean-waveforms. Mean-waveforms for light exposure (Light) and environmental temperature (Env Temp) (A), wrist temperature (Wrist Temp) and sleep (B), and activity and position (C). The shaded area shows the mean sleep period. All variables are expressed as mean ± SEM (n=103). The mean values (± 95% Confidence Interval) for Mesor, Amplitude and Acrophase, as well as the %V as calculated by the cosinor analysis for the above-mentioned variables, are shown in D. * indicates p<0.001 according to the cosinor analysis.

The cosinor analysis of all rhythmic variables is shown in Figure 1D. A coincidence was detected between the acrophases of WT and sleep probability and the bathyphases (minimum value of the cosinor curve) of activity (05:01 ± 00:19 h), body position (04:39 ± 00:21 h) and environmental temperature (04:47 ± 00:21 h). However, it should be noted that the light exposure bathyphase showed a slight phase advance (03:47 ± 00:19 h) with respect to the other variables.

Comparison between intercepts and categories method

The influence of activity on WT was determined by two demasking procedures: intercepts and categories (Figure 2). Both demasked curves present a roughly similar pattern, composed of three characteristic periods: high values during the sleep period (01:00 h to 08:00 h), a secondary postprandial peak (16:00 h to 18:00 h) and low values during the wake maintenance zone (20:00 h to 22:00 h). The two demasking procedures yielded significant rhythms ($p<0.001$) with no significant differences in mesor, amplitude or acrophase (Figure 2).



	Mesor (°C)	Amplitude (°C)	Acrophase (hh:mm)
Intercepts	33.91 ± 0.15	1.04 ± 0.26	05:13±00:57
Categories	34.04 ± 0.16	0.83 ± 0.29	05:31±01:21

Figure 2. WT pattern purified for activity. Demasked WT pattern, expressed as mean ± SEM after application of the purification by intercepts or categories method (correcting for the effect of activity). The shaded area shows the mean sleep period. The table below the graph shows the corresponding Mesor, Amplitude and Acrophase as well as the %V for WT, demasked by means of the purification by categories or intercepts method, (data are expressed as Mean ± 95% Confidence Interval). * indicates $p<0.001$ according to the cosinor analysis.

Effect of variables on WT

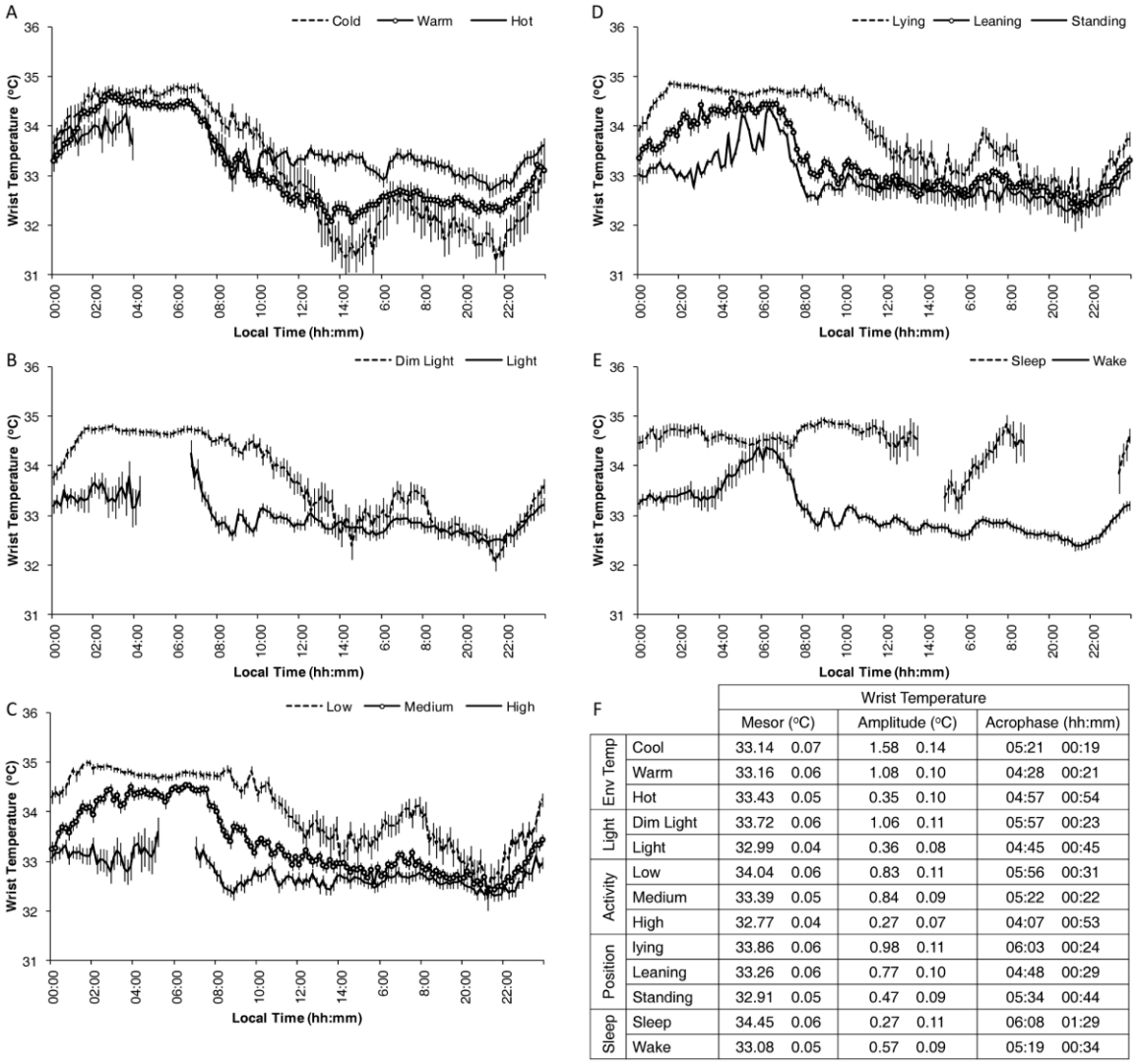
The category procedure was used to determine the contribution of each individual variable to WT (Figure 3).

The contribution of environmental temperature to WT is shown in Figure 3A. Although the mesor of the WT rhythm remained unaffected, its amplitude was significantly reduced as the environmental temperature increased (Figure 3F, cosinor analysis), with increased wake time values and slightly decreased sleep time values. Non-significant differences in demasked WT pattern acrophases were observed among environmental temperature categories. In addition, the wake maintenance zone was present in all curves, although high environmental temperatures increased WT even in this zone.

The demasking to remove the effects of light exposure is shown in Figure 3B. WT shows higher values with dim light than with non-dim light, except during two short periods at noon and again in the wake maintenance zone. Non-dim light reduces both nocturnal WT and its postprandial increase and, therefore, the mesor and amplitude of the WT pattern (Figure 3F, cosinor analysis).

Demasking, thereby eliminating the effects of activity and body position are shown in Figures 3C and 3D, respectively. WT presents similar patterns despite activity level or body position (higher nighttime and lower daytime values, and the acrophase from 04:00 to 06:00 h). However, higher activity levels or positions flatten the WT pattern and reduce WT values, reducing its mesor, except in the wake maintenance zone, in which all activity and body position categories displayed similar WT values (Figure 3F, cosinor analysis).

Sleep demasking is shown in Figure 3E. In spite of the large number of subjects recruited, the WT sleep-demasked curve presents two periods with an insufficient number of sleeping subjects (from 13:40 to 14:50 h and 18:50 to 23:10 h). Sleep increases WT values regardless of the time when sleep occurs (increasing mesor values, Figure 3F), with the exception of around 06:00 h when sleep and wake curves rendered similar values.



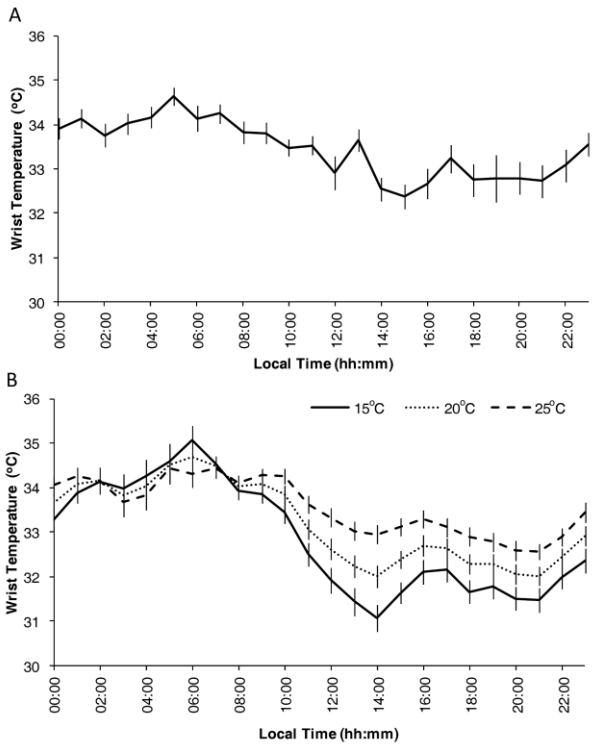
F

		Wrist Temperature		
		Mesor (°C)	Amplitude (°C)	Acrophase (hh:mm)
Env Temp	Cool	33.14 0.07	1.58 0.14	05:21 00:19
	Warm	33.16 0.06	1.08 0.10	04:28 00:21
	Hot	33.43 0.05	0.35 0.10	04:57 00:54
Light	Dim Light	33.72 0.06	1.06 0.11	05:57 00:23
	Light	32.99 0.04	0.36 0.08	04:45 00:45
Activity	Low	34.04 0.06	0.83 0.11	05:56 00:31
	Medium	33.39 0.05	0.84 0.09	05:22 00:22
	High	32.77 0.04	0.27 0.07	04:07 00:53
Position	lying	33.86 0.06	0.98 0.11	06:03 00:24
	Leaning	33.26 0.06	0.77 0.10	04:48 00:29
	Standing	32.91 0.05	0.47 0.09	05:34 00:44
Sleep	Sleep	34.45 0.06	0.27 0.11	06:08 01:29
	Wake	33.08 0.05	0.57 0.09	05:19 00:34

Figure 3. WT pattern purified for each studied masking variable. Demasked WT pattern, expressed as mean ± SEM, following the application of the purification by categories method according to environmental temperature level (A), light exposure (B), activity (C), position (D) and sleep status (E). The shaded area shows the mean sleep period. Note that in the case of the lowest level of activity, the same data set as for Figure 1 has been used, although in this case, with intervals of ten minutes instead of one hour. See the material and methods section for more details. The values for Mesor, Amplitude and Acrophase as well as the %V of each demasked wrist temperature pattern are expressed as Mean ± 95% Confidence Interval, and are included in F. * indicates $p<0.001$ according to the cosinor analysis.

Mathematical simulation of Constant routine

Figure 4 shows the WT patterns obtained after mathematical simulation of the "constant routine" protocol based on either categories (Figure 4A) or stepwise multiple regression intercepts (Figure 4B). The effect and the modulation by masking factors were quantified by the polynomial coefficient itself. If modulation did not occur, a constant coefficient would be obtained over the 24 h period. The constant routine by categories method yielded a WT pattern similar to that obtained by the intercepts method at 20ºC and 25ºC (see also the cosinor analysis at the bottom of the graph. Interestingly, environmental temperature seems to exert an effect during the activity phase, but not during the rest phase.



	Mesor (ºC)	Amplitude(ºC)	Acrophase (hh:mm)
Categories	33.44±0.13	0.80±0.22	04:51±01:02
Intercepts 15ºC	32.86±0.20	1.60±0.35	05:03±00:51
Intercepts 20ºC	33.21±0.15	1.18±0.27	05:20±00:53
Intercepts 25ºC	33.56±0.13	0.77±0.21	05:55±01:10

Figure 4. WT pattern after constant routine approach. Demasked WT waveforms obtained using the constant routine approach (see the material and methods section for details), employing either purification by categories (A) or by intercepts (B); in the latter case, three different environmental temperatures (15º, 20º and 25ºC) are considered. The shaded area shows the mean sleep period. Data are expressed as Mean ± SEM. The values for Mesor, Amplitude and Acrophase as well as the %V of wrist temperature demasked by the simulated constant routine are expressed as Mean ± 95% Confidence Interval and are included at the bottom of the graph. * indicates $p<0.001$ according to the cosinor analysis.

When this mathematical simulation was applied to morning and evening subgroups, acrophases were stable before and after demasking (03:49 ± 00:15 and 04:12 ± 00:37 for morning type, and 05:50 ± 00:35 and 06:23 ± 00:33 for evening type, respectively) and their corresponding phase difference was maintained between types ($p<0.05$ for both raw and demasked data).

Chronomodulation of masking

Circadian modulation of the masking effects induced by each variable is shown in Figure 5. The circadian pattern in light exposure masking indicates that exposure to high levels of light reduced WT from 00:00 to 12:00 h, but it had no effect from noon to midnight (Figure 5A). However, higher environmental temperature values failed to mask WT from 01:00 to 08:00 h, but they increased WT throughout the rest of the day (Figure 5A). The influence of body position was also modulated during the circadian cycle, with two main masking periods being evident around the usual times for going to bed (at midnight) and getting up in the morning (Figure 5B). When subjects lay down, their WT increased; on the other hand, when they got up, their WT decreased. In the case of activity, WT generally decreased, with the maximum influence exerted during the sleep period. It seemingly had no effect in the wake maintenance zone, however.

Sleep is related with higher WT values throughout the day, but this increase was lower at times when the volunteers were usually sleepy (in the postprandial zone and the maximum sleepiness zone around 06:00 h, as shown in Figure 5C).

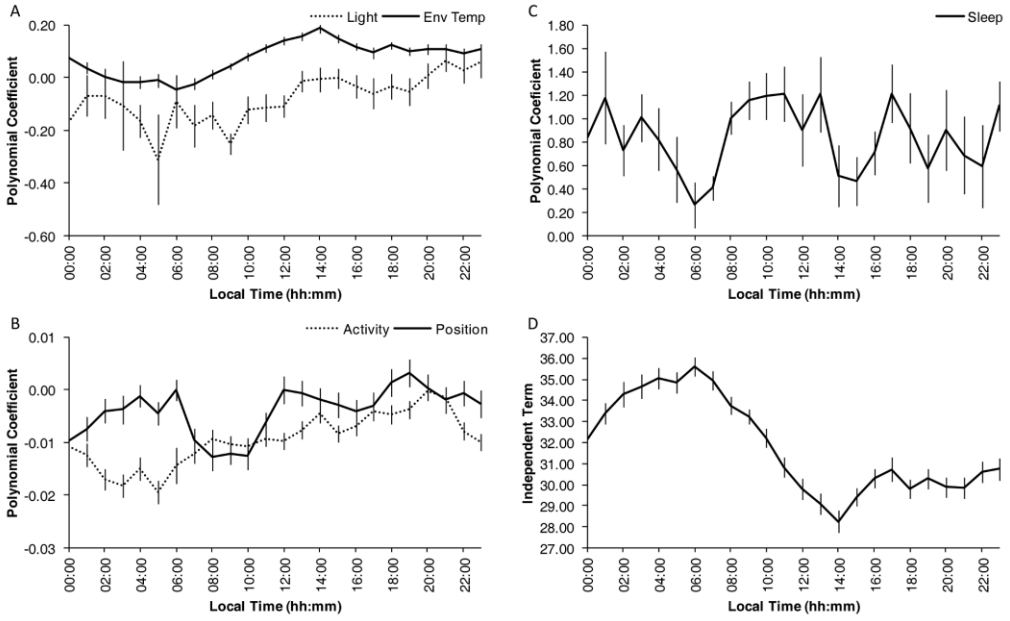


Figure 5. Circadian modulation of masking variables. Circadian modulation of the polynomial coefficients for the different masking variables: (A) light and environmental temperature (Env Temp), (B) activity and position and (C) sleep. The independent term for WT is represented in D. The shaded area shows the mean sleep period. Note that the independent term corresponds to an environmental temperature of 0ºC. All values are expressed as Mean ± SEM.

When all these masking influences were removed by representing the polynomial independent term (that is, the WT pattern under the specific condition where the rest of variables are zero), WT exhibited a roughly sinusoidal pattern (Figure 5D), with its acrophase at 04:40 ± 00:53, amplitude of 2.91 ± 0.66ºC and mesor of 31.82 ± 0.37ºC.

DISCUSSION

Our results show that despite the existence of multiple external influences on the wrist skin temperature rhythm, it exhibits a strong endogenous component which can be uncovered by using different demasking procedures to eliminate the influence of activity, body position, light exposure, environmental temperature and sleep. Although the overall circadian pattern is similar for both the masked and unmasked WT, there are changes in individual rhythmic parameters. Amplitude was most affected by environmental conditions, while the acrophase and mesor were the most stable and robust parameters for characterizing the circadian rhythm. These results suggest that the WT rhythm may prove to be valuable and minimally-invasive means of assessing circadian phase in ambulatory conditions, once further research determines appropriately consistent and accurate correlations with other well-established marker rhythms, for example dim light melatonin onset (DLMO).

To the best of our knowledge, this is the first time that demasking procedures have been applied to any variable other than CBT or that multiple masking factor influences (environmental temperature, light exposure, activity, sleep and body position) have been simultaneously removed by applying these mathematical techniques to a rhythmic variable.

Both demasking methods (intercepts and categories) produce similar WT mean waveforms and yield unmasked WT rhythms with characteristics similar to those of the raw WT circadian pattern reported in this paper and by others (Blazquez et al., 2012; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). Thus we can conclude that the endogenous (unmasked) WT rhythm has the same three characteristic stages of the raw WT rhythm in subjects under free-living conditions (Sarabia et al., 2008) and for DST in subjects under a constant routine (Kräuchi, 2007), which are: high nocturnal values, a

secondary peak in the postprandial region, and the lowest values before sleep onset (a period known as the "wake maintenance zone" (Lavie, 1985; Münch et al., 2005). As previously demonstrated for CBT, the WT rhythm seems to be the result of two sets of influences: endogenous, such as autonomic balance directly controlled by the SCN (Buijs et al., 2003), and exogenous, attributable to variables such as light exposure (Cajochen, 2007; Cajochen et al., 2000; Rüger et al., 2006), environmental temperature (Kondo et al., 2007; Wakamura & Tokura, 2002), activity (Reilly & Waterhouse, 2009) and sleep (Franken et al., 1992).

Despite the fact that modern humans live most of the time in artificial environments, all masking variables recorded here exhibit daily rhythms. Light exposure presented a maximum value at midday, coinciding with a break at work, as has already been observed by other authors (Goulet et al., 2007; Hebert et al., 1998; Heil & Mathis, 2002; Martinez-Nicolas et al., 2011; Okudaira et al., 1983; Savides et al., 1986). In our study, activity and body position exhibited similar patterns, with higher values during the period when the subjects were awake, a slightly postprandial decrease and lower values during the sleep period, coinciding with other reports (Huang et al., 2002; Ortiz-Tudela et al., 2010). With regards to environmental temperature, there are no previously published data on the environmental temperature rhythm to which subjects are exposed under free-living conditions, but our data reflects that this rhythm shows colder temperatures during the night and warmer temperatures during the day, with a slight delay with respect to light pattern.

The WT rhythm displays high values when environmental temperatures are low and low values when environmental temperatures are high consistent with other evidence that WT does not respond passively to environmental temperature but rather is regulated to preserve the CBT and brain temperature rhythms (Van Someren, 2000).

It has been published that exposure to hot environments during sleep periods (at or above 25ºC) reduces the amplitude of the CBT rhythm, as it increases nighttime CBT values (Kondo et al., 2007; Wakamura & Tokura, 2002). Exposure to colder environments during the night (below 25ºC), on the other hand, causes a more pronounced decreased in CBT during sleep (Wakamura & Tokura, 2002). As expected, we found that environmental temperature has a strong influence on WT, but only during daytime, when higher environmental temperatures increase WT values. In addition,

more robust WT rhythms are obtained under low environmental temperatures, as opposed to medium or high temperatures. Based on these considerations, exposure to moderately cold environmental temperatures may be advisable at night to allow for heat loss through peripheral skin and to contribute to CBT reduction at the beginning of the night, thus facilitating sleep onset (Kräuchi & Deboer, 2010; Kräuchi & Wirz-Justice, 2001; Van Someren, 2000).

Bright light during the night reduces the nocturnal increase in WT. This fact is consistent with previously published data (Kim & Tokura, 2007; Rüger et al., 2006) showing a lower decrease in CBT in response to bright light exposure at night. In addition, an acute increase in light intensity decreases distal skin temperature (Cajochen et al., 2005; Martinez-Nicolas et al., 2011). Unlike laboratory conditions, however, ambulatory conditions do not allow for the separate analysis of exposure to diurnal light, being awake and being in a vertical position. This therefore makes multiple demasking procedures desirable.

Moderate levels of physical activity are associated with reduced WT because such activity produces heat that increases CBT (Weinert & Waterhouse, 1998) and with it peripheral skin vasoconstriction that decreases WT.

The orthostatic reflex is presumably the mechanism responsible for the effect of body position on WT (Blazquez et al., 2012). The vasoconstrictor reflex reduces WT while subjects are standing, and increases it when they lie down as a secondary consequence of the vasodilator reflex gated to compensate for the blood pressure increase (Blazquez et al., 2012).

As some authors have demonstrated, sleep and distal skin temperature are closely related. So distal temperature, including WT, increases during sleep, only to decrease during waking hours (Kräuchi & Deboer, 2010; Kräuchi &Wirz-Justice, 2001; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). However, distal temperature can increase, although with lower values, during rest periods without sleep, probably due to relaxation or a recumbent position (Kräuchi & Deboer, 2010; Kräuchi &Wirz-Justice, 2001). However, around 06:00 h, the temperature values of subjects who are awake become similar to those observed in individuals who are asleep, which indicates that this period coincides with that described as the maximum sleepiness zone (Kräuchi et al., 2000).

Demasking by categories with the constant routine protocol yielded similar results to those considering multiple intercepts at 20 and 25ºC, probably due to the environmental temperature range selected (from 19 to 26ºC) for category approximation. All constant routine approximations show similar characteristics to those of the original WT pattern, highlighting the endogenous origin of the WT rhythm.

When the influence of each masking variable on WT is considered individually, there is the possibility that mixed confounding influences from other variables may be at work. Therefore, stepwise multiple regression methods may be of interest in order to unmask WT and other rhythms. The intercepts method has yet to be used with simultaneous multiple regression. However, this method allows us to simulate WT changes in response to environmental variables and circadian patterns of sleep-wake or rest-activity rhythms. This model has revealed the existence of a phase-dependent masking effect for each variable, specifically that: a) high environmental temperatures affect WT during the wake period, but not the sleep period, whereas high and low environmental temperatures respectively increase and decrease CBT during sleep (Kondo et al., 2007; Wakamura & Tokura, 2002); b) bright light reduces WT from the beginning of the sleep period until noon, as it is the case for distal temperature in accordance with the findings of Kräuchi (2007); c) activity decreases WT throughout the day, except during the wake maintenance zone; d) body position modifies WT, but its effect is restricted to the usual times of the main changes in body position, such as awakening and sleep onset; and e) sleep increases WT, as previously described [8], but its effect is the lowest around 06:00 h, the time of maximum sleepiness. Additionally, it is worth noting that variables other than those considered here could contribute to masking and thus affect the unpurified WT pattern.

Our results point to the potential value of WT rhythm in assessing differences in circadian phases in real life conditions. Despite the very homogeneous subject pool of our study, the demasking procedure revealed significant differences in phasing between two chronotype subgroups characterized by different morningness scores. Nonetheless, validation of WT ambulatory recordings to provide clinically useful circadian phase data must come from comparing results from WT to other phase marker rhythms (CBT, melatonin) while contrasting subjects suffering from a number of circadian abnormalities. For example, by studying patients with problems of depression or sleep quality and timing and by examining the responses of subjects to the disruption of the

physiological nexus between internal and external times, as occurs in social jet-lag or shift work.

In conclusion, the stepwise multiple regression method allowed us to reduce the masking influence on WT of all four recorded variables by using the independent term to unmask the endogenous circadian component of the WT circadian rhythm. This rhythm has a strong endogenous component, in spite of the influence of different masking variables, each of which affects WT in a phase-dependent manner. However, further experiments will be required to determine whether WT can be established as a marker rhythm for the circadian system under a normal range of environmental and behavioral situations. A further benefit of this achievement would be the suggestion that the multiple demasking procedure used here could become a useful tool for demasking other rhythmic variables, thus providing a new more readily employed standard for circadian system assessment under normal living conditions.

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REFERENCES

Anders D, Vollenweider S, Cann J, Hofstetter M, Flammer J, Orgül S, Kräuchi K. (2010). Heart-rate variability in women during 40-hour prolonged wakefulness. Chronobiol Int. 27:1609-28.

Aschoff J. (1983). Circadian control of body temperature. J Therm Biol. 8:143-7.

Blazquez A, Martinez-Nicolas A, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity and body position as determinants of the circadian pattern of blood pressure. Chronobiol Int. 29:747-56.

Buijs RM, la Fleur SE, Wortel J, Heyningen C, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol. 464:36-48.

Buijs RM, Kalsbeek A. (2001). Hypothalamic integration of central and peripheral clocks. Nature Rev Neurosci. 2:521-6.

Cajochen C. (2007). Alerting effects of light. Sleep Med Rev. 11: 453-64.

Cajochen C, Knoblauch V, Kräuchi K, Renz C, Wirz-Justice A. (2001). Dynamics of frontal EEG activity, sleepiness and body temperature under high and low sleep pressure. Neuroreport. 12:2277-81.

Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, Orgül S, Wirz-Justice A. (2005). High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 90: 1311-6.

Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. (2000). Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res. 115:75-83.

Czeisler CA, Duffy JF, Shanahan TL, Brown EN, Mitchell JF, Rimmer DW, Ronda JM, Silva EJ, Allan JS, Emens JS, Dijk DJ, Kronauer RE. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. Science. 284:2177-81.

Duffy JF, Dijk DJ. (2002). Getting through to circadian oscillators: why use constant routines? J Biol Rhythms. 17:4-13.

Franken P, Tobler I, Borbély A. (1992). Sleep and waking have a major effect on the 24-hr rhythm of cortical temperature in the rat. J Biol Rhythms. 7:341-52.

Gomper B, Bromundt V, Orgül S, Flammer J, Kräuchi K. (2010). Phase relationship between skin temperature and sleep-wake rhythms in women with vascular dysregulation and controls under real-life conditions. Chronobiol Int. 27:1778-96.

Goulet G, Mongrain V, Desrosiers C, Paquet J, Dumont M. (2007). Daily light exposure in morning-type and evening-type individuals. J Biol Rhythms. 22:151-8.

Gradisar M, Lack L. (2004). Relationships between the circadian rhythms of finger temperature, core temperature, sleep latency, and subjective sleepiness. J Biol Rhythms. 19:157-63.

Graw P, Haug HJ, Leonhardt G, Wirz-Justice A. (1998). Sleep deprivation response in seasonal affective disorder during 40-h constant routine. J Affect Disord. 48:69-74.

Hebert M, Dumont M, Paquet J. (1998). Seasonal and diurnal patterns of human illumination under natural conditions. Chronobiol Int. 15:59-70.

Heil DP, Mathis SR (2002). Characterizing free-living light exposure using a wrist-worn light monitor. Appl Ergon. 33:357-63.

Horne JA, Östberg O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. Int J Chronobiol. 4:97-110.

Huang YL, Liu RY, Wang QS, Van Someren EJW, Xu H, Zhou JN. (2002) Age-associated difference in circadian sleep-wake and rest-activity rhythms. Physiol Behav. 76:597-603.

Jasper I, Roenneberg T, Häubler A, Zierdt A, Marguardt C, Hermsdörfer J. (2010). Circadian rhythm in force tracking and in dual task costs. Chronobiol Int. 27:653-73.

Kim HE, Tokura H. (2007). Influence of two different light intensities from 16:00 to 20:30 hours on evening dressing behavior in the cold. Collegium Anthropologicum. 31: 145-51.

Klerman EB, Lee Y, Czeisler CA, Kronauer R. (1999). Linear demasking techniques are unreliable for estimating the circadian phase of ambulatory temperature data. J Biol Rhythms. 14:260-74.

Kondo M, Tokura H, Wakamura T, Hyun KJ, Tamotsu S, Morita T, Oishi T. (2007). Physiological significance of cyclic changes in room temperature around dusk and dawn for circadian rhythms of core and skin temperature, urinary 6-

hydroxymelatonin sulfate, and waking sensation just after rising. J Physiol Anthropol. 26:429-36.

Kräuchi K. (2007). The human sleep–wake cycle reconsidered from a thermoregulatory point of view. Physiol Behav. 90:236-45.

Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. (2000). Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regulatory Integrative Comp Physiol. 278:R741-8.

Kräuchi K, Cajochen C, Wirz-Justice A. (2005). Thermophysiologic aspects of the three process model of sleepiness regulation. Clin Sports Med. 24:287-300.

Kräuchi K, Deboer T. (2010). The interrelationship between sleep regulation and thermoregulation. Front Biosci. 15: 604-25.

Kräuchi K, Wirz-Justice A. (2001). Circadian clues to sleep onset mechanisms. Neuropsychopharmacology. 25:S92-6.

Lavie P. (1985). Ultradian rhythms: Gates of sleep and wakefulness. Exp Brain Res. 12:148-64.

Martinez-Nicolas A, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. Chronobiol Int. 28:617-29.

Mills JN, Minors D, Waterhouse J. (1978). Adaptation to abrupt time shifts of the oscillator[s] controlling human circadian rhythms. J Physiol. 285:455-70.

Minors D, Waterhouse J. (1989). Masking in humans: the problem and some attempts to solve it. Chronobiol Int. 6:29-53.

Minors D, Waterhouse J. (1992). Investigating the endogenous component of human circadian rhythms: a review of some simple alternatives to constant routines. Chronobiol Int. 9:55-78.

Moore RY, Danchenko RL. (2002). Paraventricular-subparaventricular hypothalamic lesions selectively affect circadian function. Chronobiol Int. 19:345-60.

Mormont MC, Langouët AM, Claustrat B, Bogdan A, Marion S, Waterhouse J, Touitou Y, Lévi F. (2002). Marker rhythms of circadian system function: A study of

patients with metastatic colorectal cancer and good performance status. Chronobiol Int. 19:141-55.

Münch M, Knoblauch V, Blatter K, Schröder C, Schnitzler C, Kräuchi K, Wirz-Justice A, Cajochen C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. Neurobiol Aging. 26:1307-19.

Okudaira N, Kripke DF, Webster JB. (1983). Naturalistic studies of human light exposure. Am J Physiol Regul Integr Comp Physiol. 245:R613-5.

Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. (2010). A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comp Biol. 6:e1000996. doi:10.1371/journal.pcbi.1000996.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 25:1911-29.

Raymann RJEM, Swaab DF, Van Someren EJW. (2008). Skin deep: enhanced sleep depth by cutaneous temperature manipulation. Brain. 131:500-13.

Reilly T, Waterhouse J. (2009). Circadian aspects of body temperature regulation in exercise. J Thermal Biol. 34:161-70.

Reiter RJ, Tan D, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. (2007). Light at Night, Chronodisruption, Melatonin Suppression, and Cancer Risk: A Review. Crit Rev Oncog. 13:303-28.

Rietveld W, Minors D, Waterhouse J. (1993). Circadian rhythms and masking: an overview. Chronobiol Int. 10:306-12.

Romejin N, Van Someren EJW. (2011). Correlated fluctuations of daytime skin temperature and vigilance. J Biol Rhythms. 26:68-77.

Rüger M, Gordijn MCM, Beersma DGM, de Vries B, Daan S. (2006). Time-of-day-dependent effects of bright light exposure on human psychophysiology: comparison of daytime and nighttime exposure. Am J Physiol Regul Integr Comp Physiol. 290:R1413-20.

Sarabia JA, Rol MA, Mendiola P, Madrid JA. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate of new index of the circadian system. Physiol Behav. 95:570-80.

Savides TJ, Messin S, Senger C, Kripke D (1986) Natural light exposure of young adults. Physiol Behav 38:571-4.

Scheer F, Doornen L, Buijs R. (1999). Light and diurnal cycle affect human heart rate: Possible role for the circadian pacemaker. J Biol Rhythms. 14:202-12.

Shechter A, Boudreau P, Varin F, Boivin DB. (2011). Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. J Biol Rhythms. 26:260-70.

Stratmann M, Schibler U. (2006). Properties, entrainment, and physiological functions of mammalian peripheral oscillators. J Biol Rhythms. 21:494-506.

Van Marken Lichtenbelt WD, Daanen HAM, Wouters L, Fronczek R, Raymann RJEM, Severens NMW, Van Someren EJW. (2006) Evaluation of wireless determination of skin temperature using iButtons. Physiol Behav. 88:489-97.

Van Someren EJW. (2000). More than a marker: interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. Chronobiol Int. 17:313-54.

Wakamura T, Tokura H. (2002). Circadian rhythm of rectal temperature in humans under different ambient temperature cycles. J Thermal Biol. 27:439-47.

Waterhouse J, Edwards B, Mugarza J, Flemming R, Minors D, Calbraith D, Williams G, Atkinson G, Reilly T. (1999). Purification of masked temperature data from humans: some preliminary observations on a comparison of the use of an activity diary, wrist actimetry, and heart rate monitoring. Chronobiol Int. 16:461-75.

Waterhouse J, Kao S, Weinert D, Edwards B, Atkinson G, Reilly T. (2005). Measuring phase shifts in humans following a simulated time-zone transition: agreement between constant routine and purification methods. Chronobiol Int. 22:829-58.

Waterhouse J, Nevill A, Weinert D, Folkard S, Minors DS, Atkinson G, Reilly T, Macdonald I, Owens D, Sytnik N, Tucker P. (2001). Modeling the effect of spontaneous activity on core temperature in healthy human subjects. Biol Rhythm Res. 32:511-28.

Waterhouse J, Weinert D, Minors DS, Folkard S, Owens D, Atkinson G, Macdonald I, Sytnik N, Tucker P, Reilly T. (2000). A comparison of some different methods for purifying core temperature data from humans. Chronobiol Int. 17:539-66.

Weather station of Murcia University's. https://estacion.um.es/.

Weinert D, Nevill A, Weinandy R, Waterhouse J. (2003). The development of new purification methods to assess the circadian rhythm of body temperature in Mongolian gerbils. Chronobiol Int. 20:249-70.

Weinert D, Waterhouse J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. Physiol Behav. 63:837-43.

Weinert D, Waterhouse J. (2007). The circadian rhythm of core temperature: Effects of physical activity and aging. Physiol Behav. 90:246-56.

Zornoza-Moreno M, Fuentes-Hernández S, Sánchez-Solis M, Rol MA, Larqué E, Madrid JA. (2011). Assessment of circadian rhythms of both skin temperature and motor activity in infants during the first 6 months of life. Chronobiol Int. 28:330-7.

3.2. EXPERIMENTAL CHAPTER 2

EL RITMO DE TEMPERATURA PERIFÉRICA DISTAL COMO UN ÍNDICE PARA EVALUAR LA ONTOGENIA Y EL ENVEJECIMIENTO DEL SISTEMA CIRCADIANO HUMANO

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RESUMEN

En términos circadianos, la ontogenia humana se caracteriza por la emergencia del patrón diario, a partir del patrón ultradiano previo, en los seis primeros meses de vida para la mayoría de las variables. El envejecimiento circadiano en humanos se acompaña de un avance de fase, y fragmentación y aplanamiento del ritmo. A pesar de la creciente bibliografía centrada en la temperatura periférica distal, existe muy poca información disponible acerca de su ontogenia y prácticamente nada sobre los cambios que experimenta esta variable con el avance de la edad. Por tanto, el propósito de este trabajo fue evaluar el grado de maduración y envejecimiento del sistema circadiano humano, utilizando el patrón de temperatura periférica distal para identificar aquellos parámetros que se modifican a lo largo de la vida, permitiendo diferenciar a los sujetos de acuerdo a su edad. Para ello se monitorizó el ritmo de temperatura periférica distal en 197 voluntarios (55% mujeres), incluyendo bebes de 15 días (30 sujetos), un mes (28 sujetos), 3 meses (31 sujetos) y 6 meses (10 sujetos) y adultos jóvenes de 19 años (37 sujetos), de mediana edad con 46 años (27 sujetos) y ancianos de 72 años (34 sujetos). La maduración del sistema circadiano se asoció con un incremento en la amplitud del ritmo circadiano y una reducción de la temperatura periférica distal durante el sueño. En la etapa adulta, las mujeres mostraron un patrón más robusto (menor fragmentación, mayor temperatura periférica distal nocturna, amplitud, índice de funcionamiento circadiano y potencia del primer armónico); sin embargo, estas diferencias se pierden con la edad, un periodo de la vida que está consecuentemente asociado consistentemente a un avance de fase del ritmo. En resumen, el patrón de temperatura periférica distal puede utilizarse como una variable robusta para evaluar el grado de maduración y envejecimiento del sistema circadiano humano.

Palabras clave: temperature de la piel distal, ritmo circadiano, ontogenia, humano, envejecimiento.

INTRODUCCIÓN

El sistema circadiano (SC) consiste en una red de estructuras que participan en la generación, mantenimiento y sincronización de los ritmos circadianos (Buijs & Kalsbeek, 2001; Duguay & Cermakian, 2009; Stratmann & Schibler, 2006). Como en otros sistemas, el SC madura durante la ontogenia para degenerar durante el envejecimiento (McGraw et al., 1999; Turek et al., 1995).

La mayor parte de los ritmos circadianos comienzan a aparecer antes de los 6 meses de edad (McGraw et al., 1999; Mirmiran et al., 2003; Serón-Ferré et al., 2001; de Weerth et al., 2003) con un período de alrededor de 2-4 horas, que es resultado de la expresión de un marcapasos circadiano desacoplado. La ritmicidad circadiana emerge cuando SC madura (Mirmiran et al., 2003; Moore, 1991). El envejecimiento se asocia con un avance de fase, fragmentación y aplanamiento de los ritmos (Hardeland et al., 2011; Hofman & Swaab, 2006; Van Someren et al., 1999; Weinert, 2010).

La importancia del sistema circadiano está siendo cada vez más reconocida en el ámbito de la medicina. En recién nacidos y lactantes, el retraso en la maduración circadiana puede ser debido a una deficiencia en la exposición a sincronizadores ambientales o por alteración del desarollo neural (Rivkees, 2003; Worobey et al., 2009).

En adultos y ancianos, la disrupción circadiana se asocia a un aumento de la morbilidad y la mortalidad debido al envejecimiento y a ciertas patologías, como síndrome metabólico, cancer, deterioro cognitivos, alteraciones del sueño y eventos cardiovasculares (Erren & Reiter, 2009; Garaulet et al., 2010; Reiter et al., 2007; Turner et al., 2010). Por tanto, el desarrollo de técnicas no invasivas para evaluar el sistema circadiano ambulatoriamente se ha convertido en un imperativo.

Para evaluar el sistema circadiano, se han propuesto ritmos marcadores controlados por el marcapasos central, que pueden ser medidos comodamente durante un período de tiempo prolongado. Entre los ritmos comúnmente utilizados destacan el de melatonina y cortisol, temperatura central (CBT), y actividad (Mormont et al., 2002; Van Someren, 2000). Por otra parte, la temperatura de la piel distal (DST) ha sido propuesta recientemente como una herramienta no invasiva, robusta, cómoda, y fácil de registrar (Bonmati-Carrion et al., 2014; Martinez-Nicolas et al., 2011; Martinez-Nicolas et al., 2013; Ortiz-Tudela et al., 2010; Sarabia et al., 2008; Zornoza-Moreno et al., 2011;

Zornoza-Moreno et al., 2013). Además, la DST regula la CBT, ya que la DST es el resultado de una vasodilatación-vasoconstricción periférica en respuesta al balance simpatico-parasimpático (Blazquez et al., 2012; Buijs et al., 2003; Kräuchi et al., 2000; Kräuchi & Deboer, 2010; Martinez-Nicolas et al., 2011).

Existe un aumento de la evidencia que indica que la DST es una herramienta no invasiva, cómoda, y sencilla de utilizar para evaluar el sistema circadiano bajo diversas condiciones, clínicas o no, y para recién nacidos, sujetos jóvenes sanos, y sujetos que sufren de obesidad, síndrome metabólico, y síndrome vasoespástico (en el caso de las mujeres) (Bandín et al., 2012; Blazquez et al., 2012; Gompper et al., 2010; Sarabia et al., 2008; Zornoza-Moreno et al., 2011). Sin embargo, existe poca información sobre su ontogenia y casi nada sobre los cambios en relación con la edad de la DST.

El objetivo de este studio fue determinar si el ritmo de temperatura distal podia ser utilizado como herramienta para evaluar el grado de maduración y el envejecimiento del sistema circadiano humano, y para identificar los parámetros rítmicos del ritmo de temperatura que permitan diferenciar los sujetos de acuerdo a su edad.

MATERIAL Y MÉTODOS

Participantes

Se reclutaron 197 voluntarios, desde recién nacidos (15 días de edad) hasta ancianos (85 años de edad) de ambos sexos (55% mujeres). La población fue dividida en 8 grupos de edad: 1) recién nacidos de 15 días de edad (15 varones y 15 mujeres); 2) bebés de un mes (13 varones y 15 mujeres); 3) niños de 3 meses (14 varones y 17 mujeres); 4) niños de 6 meses (4 varones y 6 mujeres); 5) adultos jóvenes (19 ± 1 año de edad), 18 varones y 19 mujeres; 6) adultos (46 ± 2 años), con 6 varones y 21 mujeres; 7) ancianos (72 ± 1 años), con 20 varones y 14 mujeres. Todos eran sanos, sin condiciones físicas que alterasen su sueño (ej. asma, síndrome de piernas inquietas, apnea del sueño, etc.). Durante los experimentos, que tuvieron lugar en las ciudades de Murcia y Toledo (España), los participantes mantuvieron su estilo de vida. El estudio cumplió los principios bioéticos de la Declaración de Helsinki. Los datos de los voluntarios se incluyeron en una base de datos y protegidos de acuerdo a la ley de protección de datos Española 15/1999 del 13 de Septiembre.

Medición de la temperatura

Todos los adultos llevaban un Thermochron iButton DS1921H (Dallas, Maxim) para la medición de la temperatura de la piel de la muñeca, con una precision de ±0.125ºC, que fue colocado en la muñeca de la mano no dominante sobre la arteria radial, y aislado del ambiente mediante una muñequera deportiva de algodón (Sarabia et al., 2008). Los bebés tenían el dispositivo dentro de un calcetín (Zornoza-Moreno et al., 2011). Todos los dispositivos fueron programados para medir una vez cada 10 minutos, durante un período de 3 días consecutivos (bebés), o 7 días consecutivos (resto).

Análisis de datos

Se utilizo Circadianware™ v7.1.1 (Campos et al., 2010) para analizar el ritmo de temperatura. Este software permite el cálculo de los principales parámetros de una serie temporal. Brevemente, se utilizó el cosinor para calcular mesor, amplitude, acrofase, y porcentaje de la varianza explicado (%V) junto al test de Rayleigh, periodograma de chi-cuadrado (Schwarzenberg-Czerny, 1998), análisis de Fourier con los primeros 12 armónicos y el Indice de Circadianidad (relación entre el primero y los 12 primeros armónicos). Como el ritmo de temperatura de la muñeca (WT) tiene una forma que dista de una curva sinusoidal en muchos sujetos se analizo el análisis no paramétrico (Van Someren et al., 1999), que permite cálcular la estabilidad interdiaria (IS; estabilidad); la variabilidad intradiaria (IV); fragmentación); la media horaria durante dos horas consecutivas, junto a su valor mínimo (L2) y el momento en el que ocurre (TL2); la media horaria durante 5 horas consecutivos, junto a su valor máximo (M5) y el momento en el que ocurre (TM5); la media horaria de 10 horas consecutivas, junto a su valor mínimo (L10) y el momento en el que ocurre (TL10), y la amplitud relativa (RA) determinada por la diferencia entre M5 y L10, dividida entre la suma de M5 y L10, y el índice de función circadiana o CFI, como descrito previamente (Martinez-Nicolas et al., 2011; Martinez Nicolas et al., 2013; Ortiz-Tudela et al., 2010).

Para distinguir entre grupos de edad, se calcularon dos matrices de correlación, y se seleccionaron las variables con una relación más fuerte con la edad cronológica. Además, para ser consideradas en los diagramas, estas variables debían medir diferentes aspectos del patron circadiano; por ejemplo, L10, M5 y el mesor son tres medidas del nivel de temperatura, por lo que solo una de ellas puede ser considerada. En este caso,

se utilizó la siguiente variable en términos de fuerza de correlación que no estuviese relacionada con el nivel de temperatura (por ejemplo, amplitud).

Finalmente, se utilizó el software estadístico R 3.0.0 para hacer una ANOVA de una vía para evaluar diferencias entre los grupos de edad, seguida de un análisis post-hoc de Bonferroni para comparaciones de pares, si necesario. Para analizar el efecto del sexo y la edad, y cualquier possible interacción entre ellos, se realizó una ANOVA de dos vías. Cuando solo se compararon dos grupos (por ejemplo, para evaluar las diferencias por género en cada grupo de edad), se realizó un test de T de Student. En todos los casos, una $p<0.05$ fue considerada estadísticamente significativa.

RESULTADOS

Comparación entre índices paramétricos y no paramétricos

Para analizar la correspondencia entre índices paramétricos y no paramétricos, se hicieron análisis de correlación, que no encontraron equivalencia en la regularidad del patron rítmico, ya que la Estabilidad Interdiaria (IS) se correlacionó solo ligeramente con el test de Rayleigh ($r=0.27$, $p<0.001$). La fragmentación rítmica, calculada como la Variabilidad Intradiaria (IV), se correlacionó significativamente con el Indice Circadiano ($r=0.40$, $p<0.001$). La amplitud relativa mostró una alta correlación con la amplitud del cosinor ($r=0.98$, $p<0.001$), mientras que el tiempo de M5 (TM5) se correlacionó estrechamente con la acrofase del cosinor ($r=0.65$, $p<0.001$). Por tanto, los pares IV-Indice Circadiano, RA-amplitud del cosinor, y TM5-acrofase del cosinor pueden ser considerados como índices similares para procedimientos no paramétricos y de ajuste sinusoidal, respectivamente. Sin embargo, hay que destacar que el test de Rayleigh y el IS miden diferentes aspectos de la regularidad.

Descripción de patrones

La temperatura periférica de la piel mostró un ritmo diario desde los primeros días de vida (Figura 1). A partir de los tres meses de vida, esta variable mantiene un patron diario, generalmente muy estable a lo largo de la vida. Este ritmo se caracteriza por cuatro fases principales: i) plateau nocturno (23:00-08:00 h) de TM elevada, y que se asocia con el período principal de sueño; ii) una disminución matutina (08:00-15:00 h) que comienza inmediatamente después del despertar; iii) un pico secundario en la tarde, que coincide con el período postprandial (15:00-18:00 h); y iv) una disminución en la tarde-noche (18:00-23:00 h), asociada con la "zona de mantenimiento de la vigilia". Durante la ontogenia, se produce un aumento progresivo en la amplitud del ritmo circadiano (Figura 1A-D), mientras que el envejecimiento se caracteriza por un avance gradual de fase y la pérdida de la caída de la tarde-noche asociada con la zona de mantenimiento de la vigilia (Figura 1E-G).

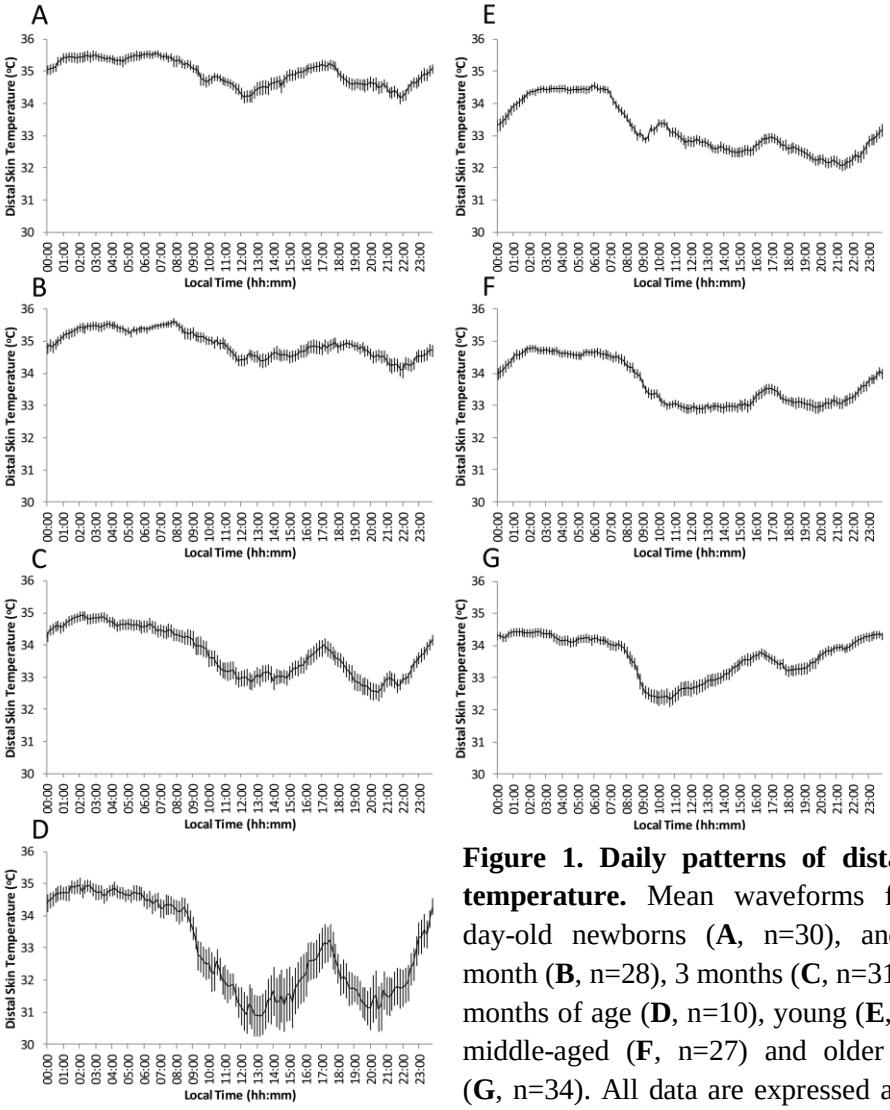

Figure 1. Daily patterns of distal skin temperature. Mean waveforms for 15-day-old newborns (**A**, n=30), and at 1 month (**B**, n=28), 3 months (**C**, n=31) and 6 months of age (**D**, n=10), young (**E**, n=37), middle-aged (**F**, n=27) and older people (**G**, n=34). All data are expressed as mean ± SEM.

La maduración circadiana entre los 15 días de vida y los 6 meses de edad se asoció con una disminución progresiva en el mesor de la WT, junto con una reducción nocturna significativa del M5, y más pronunciada del L10 diurno. La ontogenia de la WT se asoció también con un aumento significativo en la amplitud del componente circadiano y una reducción en la fuerza de los armónicos ultradianos (de 12 a 2 h). Se observó también un aumento significativo en el caso de la IS, junto con una disminución de la fragmentación rítmica (IV). Sin embargo, no se encontraron cambios significativos en ninguno de los marcadores de fase circadiana: TM5, acrofase del cosinor, o TL2 (Table 1, Figure 1A-D).

	0.5 months	1 month	3 months	6 months
Mesor	34.945 ± 0.089^{a}	34.950 ± 0.090^{a}	33.774 ± 0.142^{b}	33.030 ± 0.251^{c}
Amplitude	0.545 ± 0.083^{a}	0.558 ± 0.089^{a}	0.991 ± 0.108^{b}	1.682 ± 0.234^{c}
Acrophase	04:56 ± 00:40	05:16 ± 00:35	03:28 ± 00:39	03:03 ± 01:10
Rayleigh Vector	0.718 ± 0.040	0.763 ± 0.041	0.834 ± 0.037	0.743 ± 0.079
%V	12.704 ± 2.790^{a}	12.231 ± 2.307^{a}	23.070 ± 2.658^{b}	30.268 ± 4.772^{b}
IS	0.468 ± 0.027^{ab}	0.443 ± 0.021^{b}	0.562 ± 0.023^{c}	0.597 ± 0.048^{ac}
IV	0.218 ± 0.015^{ab}	0.290 ± 0.023^{a}	0.237 ± 0.020^{ab}	0.146 ± 0.016^{b}
RA	0.017 ± 0.002^{a}	0.017 ± 0.002^{a}	0.029 ± 0.003^{b}	0.049 ± 0.006^{c}
M5	35.664 ± 0.089^{a}	35.620 ± 0.074^{a}	34.942 ± 0.118^{b}	34.898 ± 0.163^{b}
L10	34.466 ± 0.127^{a}	34.468 ± 0.139^{a}	32.973 ± 0.188^{b}	31.685 ± 0.422^{c}
L2	33.587 ± 0.164^{a}	33.626 ± 0.160^{a}	32.035 ± 0.215^{b}	30.700 ± 0.502^{c}
TL10	17:12 ± 00:57	18:00 ± 00:53	16:49 ± 00:54	15:34 ± 01:31
TM5	05:13 ± 00:39	06:38 ± 00:50	05:03 ± 00:40	03:53 ± 00:38
TL2	17:31 ± 00:49	17:34 ± 00:40	15:51 ± 00:40	16:00 ± 00:25
CFI	0.511 ± 0.016^{a}	0.489 ± 0.015^{a}	0.581 ± 0.018^{b}	0.670 ± 0.036^{b}
Circadianity Index	24.903 ± 4.199^{a}	33.520 ± 4.789^{ab}	43.601 ± 3.689^{b}	52.664 ± 6.829^{b}
Qp	204.210 ± 12.685^{a}	222.630 ± 7.658^{ab}	248.903 ± 9.897^{b}	264.100 ± 26.617^{b}

Table 1. Caracterización de la ontogenia. Mesor, amplitude, acrofase, vector de Rayleigh, varianza explicada por onda sinusoidal (%V), estabilidad interdiaria (IS), variabilidad intradiaria (IV), amplitude relativa (RA), valores de mantenimiento de noche, día y despertar (M5, L10 y L2 respectivamente) y sus correspondientes tiempos (TM5, TL10 y TL2 respectivamente), indice de función circadiana (CFI), índice de circadianidad, y el poder del periodograma de la chi cuadrado para un período de 24 horas (Qp) para los 4 grupos de neonatos (15 días, 1 mes, 3 y 6 meses). Todos los índices fueron expresados como media ± SEM, y diferentes letras indicant diferencias significativas entre los grupos de edad (ANOVA una vía y comparación por parejas de Bonferroni post hoc, $p<.05$).

Opuestamente a lo observado en la ontogenia, el envejecimiento se caracteriza por un avance de fase, junto a un aumento en la estabilidad, sin que se afecten amplitud ni fragmentación (Tabla 2, Figura 1E-G). Se produjo un avance de fase significativo en ancianos en la acrofase del cosinor y la no paramétrica calculada como TM5. Otro cambio significativo asociado con la edad es la pérdida de la disminución vespertina en la WT, junto con una disminución más acentuada de temperatura de la muñeca (WT) durante la mañana. Estos cambios afectan a TL2, que se desplaza de las horas de la tarde en jóvenes y adultos a las horas de la mañana en ancianos. En consecuencia, el tiempo de L10 también muestra un avance de fase con la edad. Se observó también un aumento en IS, reflejando la regularidad de los estilos de vida de la gente anciana con respecto a jóvenes y adultos de edad media (Tabla 2).

	Young	Adults	Older
Mesor	33.220 ± 0.075^{a}	33.689 ± 0.086^{b}	33.643 ± 0.112^{b}
Amplitude	1.086 ± 0.097	0.943 ± 0.105	0.894 ± 0.078
Acrophase	04:51 ± 00:22^{a}	03:12 ± 00:18^{b}	00:37 ± 00:26^{c}
Rayleigh Vector	0.690 ± 0.032	0.768 ± 0.030	0.729 ± 0.039
%V	21.512 ± 2.310	18.260 ± 2.709	24.669 ± 2.270
IS	0.357 ± 0.024^{a}	0.297 ± 0.028^{a}	0.452 ± 0.029^{b}
IV	0.184 ± 0.013	0.226 ± 0.020	0.208 ± 0.014
RA	0.032 ± 0.003	0.027 ± 0.003	0.025 ± 0.002
M5	34.573 ± 0.088	34.793 ± 0.107	34.582 ± 0.094
L10	32.399 ± 0.129^{a}	33.065 ± 0.157^{b}	32.981 ± 0.166^{b}
L2	31.774 ± 0.147^{a}	32.449 ± 0.141^{b}	32.101 ± 0.203^{ab}
TL10	17:38 ± 00:41^{a}	14:44 ± 00:54^{b}	11:11 ± 00:29^{c}
TM5	04:11 ± 00:16^{a}	04:07 ± 00:19^{a}	01:23 ± 00:28^{b}
TL2	17:14 ± 00:24^{a}	15:11 ± 00:30^{b}	12:36 ± 00:30^{c}
CFI	0.433 ± 0.010^{ab}	0.404 ± 0.012^{a}	0.457 ± 0.011^{b}
Circadianity Index	56.260 ± 3.461	60.320 ± 4.143	54.415 ± 3.248
Qp	372.184 ± 21.196^{a}	548.333 ± 54.886^{b}	447.029 ± 27.462^{ab}

Table 2. Caracterizacion de la edad. Mesor, amplitude, acrofase, vector de Rayleigh, varianza explicada por onda sinusoidal (%V), estabilidad interdiaria (IS), variabilidad intradiaria (IV), amplitude relativa (RA), valores de mantenimiento de noche, día y despertar (M5, L10 y L2 respectivamente) y sus correspondientes tiempos (TM5, TL10 y TL2 respectivamente), indice de función circadiana (CFI), índice de circadianidad, y el poder del periodograma de la chi cuadrado para un período de 24 horas (Qp) para los 3 grupos de adultos (jóvenes, edad media, ancianos). Todos los índices fueron expresados como media ± SEM, y diferentes letras indicant diferencias significativas entre los grupos de edad (ANOVA una vía y comparación por parejas de Bonferroni post hoc, $p<.05$).

Ontogenia y caracterización de la edad

La matriz de correlación primero indicó una disminución en el nivel de temperatura durante la ontogenia (evaluado sea pour el mesor, L10, M5 o L2) con una relación negativa de moderada (M5, r=-0.501, p<0.001) a fuerte (mesor, r=-0.730, p<0.001; L10, r=-0.726, p<0.001; L2, r=-0.682, p<0.001). El paso siguiente encontró un aumento significativo en la medición de ambas amplitudes (amplitude y RA), con una relación moderadamente positiva (amplitud, r=0.557, p<0.001; RA, r=0.599, p<0.001) con el aumento de la edad. Usando un modelo bidimensional de diagrama para representar los dos mejores parámetros del nivel y la amplitud de la temperatura (mesor vs RA), fue posible discriminar los grupos de edad de acuerdo al patron general de maduración del ritmo de temperatura (Figure 2A). Este diagrama mostró que los niños de 15 y 30 días tienen patrones bastante similares, que los niños de 3 meses muestran un patron más maduro, y que los niños de 6 meses no muestran diferencias con respecto a las tres categorías de adultos (Figure 2A and 2B).

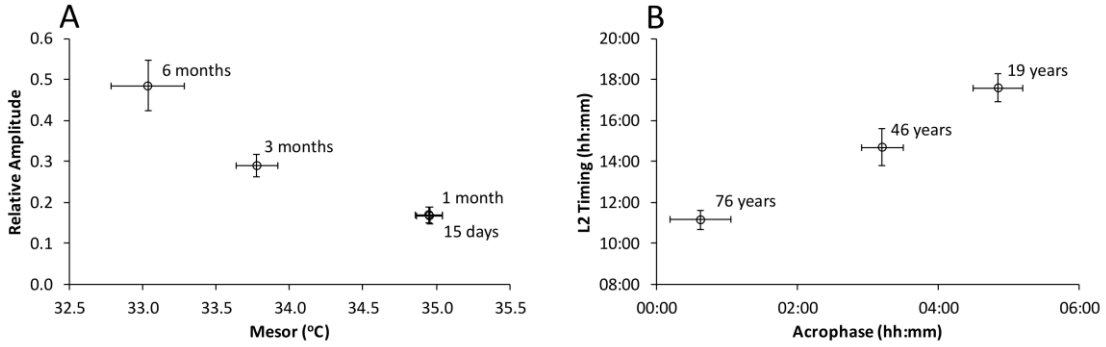

Figure 2. *Scatter plot* para ontogenia y envejecimiento del patron de temperature de la muñecapara. Las fases de ontogenia (**A)** pueden ser diferenciadas por el mesor y la amplitud relativa, mientras que en el envejecimiento (**B),** pueden ser diferenciadas pour la acrofase y el tiempo de L2. Los datos se expresan como media ± SEM.

Para distinguir entre los diferentes grupos de edad (jóvenes, edad media, adultos mayores), se realizó una matriz de correlación. Esta mostró que la acrofase, y los marcadores de fase TL2, TM5 y TL10 se correlacionaban mejor con la edad, evidenciando una correlación fuerte (acrofase, r=-0.641, p<0.001; TL10, r=-0.601, p<0.001) y moderadamente negativa (TL2, r=-0.576, p<0.001; TM5, r=-0.479, p<0.001). Por tanto, un diagrama bidimensional fue de nuevo realizado usando los dos mejores marcadores de fase, la acrofase y el tiempo L2, para diferenciar ancianos del resto de grupos de edad (Figure 2B).

Diferencias Edad-Género

En cuanto a las diferencias por género, la M5 fue la única variable que mostró un aumento significativo en mujeres, mientras que para el resto de variables las diferencias eran debidas a la edad (Table 3). Sin embargo, cuando al considerar edad y género, se observan diferencias significativas por género únicamente en adultos jóvenes (Table 3). En general, todos los índices asociados con la robustez del sistema circadiano (ej. amplitud de la función coseno, %V, IV, RA, M5 y CFI) mostraron valores más fuertes en mujeres jóvenes que en hombres jóvenes, y la mayor parte de los parámetros circadianos (mesor, amplitud, acrofase, IV, RA, L10, L2, TM5, TL10 y TL2) tenían diferencias significativas entre mujeres y hombres jóvenes. En hombres, todos estos marcadores de fase (TL2, TM5, TL10) mostraron un avance de fase con la edad, y el IS, CFI y Qp fueron estadísticamente mayores ($p<0.05$) en hombres ancianos.

	Young Men	Older Men	Young Women	Older Women	Global differences
Mesor	33.174 ± 0.030	33.511 ± 0.028	33.266 ± 0.029*	33.833 ± 0.040*	Age (F=11.71, p=0.001)
Amplitude	0.871 ± 0.028#	0.949 ± 0.026	1.300 ± 0.027*#	0.817 ± 0.037*	
Acrophase	04:50 ± 00:08*	24:06 ± 00:07*	04:51 ± 00:07*	01:20 ± 00:10*	Age (F=54.54, p<0.001)
Rayleigh Vector	0.728 ± 0.012	0.719 ± 0.011	0.652 ± 0.011	0.742 ± 0.015	
%V	17.008 ± 0.733#	24.173 ± 0.678	26.016 ± 0.714#	25.378 ± 0.969	
IS	0.312 ± 0.057*	0.438 ± 0.082*	0.403 ± 0.076	0.472 ± 0.104	Age (F=6.97, p=0.01)
IV	0.212 ± 0.004#	0.202 ± 0.004	0.157 ± 0.004*#	0.217 ± 0.006*	
RA	0.026 ± 0.007#	0.026 ± 0.007	0.038 ± 0.007*#	0.022 ± 0.011*	Age (F=6.11, p=0.02)
M5	34.242 ± 0.027#	34.505 ± 0.025	34.905 ± 0.026#	34.692 ± 0.035	Sex (F=13.22, p=0.01)
L10	32.471 ± 0.047	32.759 ± 0.044	32.328 ± 0.046*	33.298 ± 0.062*	Age (F=9.16, p=0.03)
L2	31.827 ± 0.056	31.840 ± 0.052	31.720 ± 0.055*	32.473 ± 0.074*	
TL10	17:52 ± 00:12*	10:42 ± 00:11*	17:24 ± 00:11*	11:50 ± 00:16*	Age (F=53.89, p<0.001)
TM5	04:25 ± 00:07*	01:02 ± 00:07*	03:56 ± 00:07*	01:54 ± 00:10*	Age (F=26.17, p<0.001)
TL2	17:07 ± 00:09*	11:59 ± 00:08*	17:22 ± 00:08*	13:27 ± 00:11*	Age (F=50.13, p<0.001)
CFI	0.411 ± 0.033*#	0.454 ± 0.031*	0.454 ± 0.032#	0.462 ± 0.045	
Circadianity Index	49.010 ± 1.066#	56.710 ± 0.986	63.510 ± 1.037#	51.138 ± 1.408	
Qp	333.895 ± 7.784*	430.650 ± 7.197*	410.474 ± 7.576	470.429 ± 1.282	Age (F=5.23, p=0.03)

Table 3. Diferencias Edad-Género. Mesor, amplitud, acrofase, vector de Rayleigh, varianza explicada por onda sinusoidal (%V), estabilidad interdiaria (IS), variabilidad intradiaria (IV), amplitude relativa (RA), valores de mantenimiento de noche, día y despertar (M5, L10 y L2 respectivamente) y sus correspondientes tiempos (TM5, TL10 y TL2 respectivamente), indice de función circadiana (CFI), índice de circadianidad, y el poder del periodograma de la chi cuadrado para un período de 24 horas (Qp) para jóvenes y ancianos, separados por género. Todos los índices fueron expresados como media ± SEM, * indica diferencias estadísticamente significativas de edad para cada sexo, mientras que # indica diferencias estadísticamente significativas de género para cada grupo de edad, verificado mediante una T de Student ($p<.05$). Los principales efectos por edad y género fueron estudiados por ANOVA de dos vías (última columna, "global differences").

DISCUSION

Hemos mostrado que la temperatura de la piel distal puede usarse como un procedimiento fiable y simple para determinar la maduración y el envejecimiento del sistema circadiano. La maduración del ritmo circadiano en bebés se caracteriza por una disminución progresiva de la DST durante el período de descanso, junto con un aumento en la amplitud del ritmo, pero sin cambios en los marcadores de fase. Sin embargo, avances fuertes de fase sin cambios significativos en la amplitud del ritmo son característicos del envejecimiento. Se encontraron diferencias de género en adultos, exhibiendo las mujeres una mayor robustez circadiana que los hombres; sin embargo, estas diferencias de géneros desaparecían en ancianos.

Nuestros resultados indican que la amplitud de la onda coseno y el RA pueden ser usados indistintamente, y producen un resultado equivalente. Igualmente, la acrofase del coseno y el TM5 aportan un buen nivel de concordancia cuando se usan como marcadores de fase, y el Indice de Circadianidad y el IV pueden ser considerados como índices adecuados para la fragmentación del ritmo. Además, la débil correlación observada entre el test de Rayleigh y el IS era esperada, ya que el test de Rayleigh es una medida de la estabilidad de la acrofase, mientras que el IS cuantifica la consistencia del patron entre los días.

La temperatura de la piel distal está sujeta a control endógeno por el marcapasos circadiano (Bonmati-Carrion et al., 2014; Kräuchi et al., 2000; Martinez-Nicolas et al., 2013), aunque enmascarada por influencias exógenas (Martinez-Nicolas et al., 2013). El DST refleja el ritmo endógeno de la activación simpática-parasimpática de la luz vascular, en relación al día-actividad y noche-reposo, respectivamente (Charkoudian et al., 2003; Gompper et al., 2010).

Aunque el ritmo de 24-h está ya presente en recién nacidos a los 15 días y al mes de edad, la predominancia de la ritmicidad ultradiana (medida mediante el Indice de Circadianidad) y la baja amplitud de este ritmo sugieren que los efectos enmascarantes podrían ser primariamente responsables del ritmo de DST. La luz y la temperatura ambientales, junto con las influencias comportamentales del cuidado parental, podrían provocar la debilidad del ritmo circadiano observada en estos recién nacidos. A los 6 meses de edad, la mayoría de los niños muestran un robusto patron diario, que se asemeja al de los adultos. Este se caracteriza por una amplitud e Indice de

Circadianidad mayores que el de los niños de 15 días y un mes de edad, alcanzando valores similares a los observados en adultos. A los tres meses de edad, los niños muestran algunas características de ritmos circadianos inmaduros, y otras características que los hacen más similares a los niños de 6 meses, sugiriendo que este período puede ser considerado como el punto de inflexion de la maduración del sistema circadiano. De hecho, el período de tres meses de edad se caracteriza por una elevada variabilidad en la tasa de maduración del sistema circadiano. Se ha sugerido que estas diferencias en la maduración del sistema circadiano dependen de los ritmos de cuidado parental y de condiciones ambientales (Rivkees, 2003; Worobey et al., 2009). Es decir, es probable que la exposición del niño a luz brillante durante el día, a la oscuridad durante la noche, y a la regularidad de las claves ambientales favorace una aparición más robusta y precoz de la ritmicidad circadiana (Rivkees, 2003).

Algunas de las características más evidentes del ritmo de DST en jóvenes son las diferencias significativas observadas en la mayoría de los parámetros rítmicos asociadas con el género, con las mujeres exhibiendo los mayores niveles de regularidad, amplitud, Indice de Circadianidad y de CFI, y una menor fragmentación que los hombres. Sin embargo, estas diferencias entre géneros desaparecen en ancianos. Se ha documentado también la existencia de diferencias en los hábitos de sueño y las escalas de cronotipo entre hombres y mujeres jóvenes, con patrones de sueño más sanos y puntuaciones de matutinidad más altos en mujeres que en hombres (Azevedo et al., 2008; Roenneber et al., 2007). Las variaciones en el estado hormonal podrían ser responsables de tales diferencias, aunque otros factores relacionados con el estilo de vida de los jóvenes podrían también ser responsables (Roenneber et al., 2007).

Aunque hay una tendencia a reducer la amplitud y a aumentar la fragmentación de los ritmos en ancianos en comparación con adultos jóvenes, las diferencias no fueron estadísticamente significativas; sin embargo, efectos significantes y consistentes fueron observados para todos los marcadores de fase circadianos, con un avance progresivo de fase con el envejecimiento de la persona. Una disminución en la regularidad de los estilos de vida, y por tanto en el IS, era también esperable; sin embargo, nuestros resultados y los de otros (Minors et al., 1998) fallan para corroborar esta expectativa. Los ancianos intentan adoptar estilos de vida regulares de forma creciente con el envejecimiento, lo que contrarresta el deterioro del sistema circadiano que ocurre con la edad.

Como se ha mencionado antes, el DST falló en mostrar la reducción de la amplitud esperable en ancianos. Se ha publicado que diversos ritmos circadianos muestran una disminución de su amplitud con la edad durante condiciones de rutina constante (Dijk et al., 2000; Harper et al., 2005). Esta disminución podría ser el resultado de un decremento en los componentes endógenos de este ritmo. El hecho de que nuestros resultados no muestren una reducción significativa de la amplitud podría ser explicado por el efecto compensatorio de un aumento de la regularidad de los estilos de vida que se asocia al envejecimiento.

Juzgando nuestros resultados, y los de otros, está claro que los individuos ancianos muestran una tendencia a ser más matutinos que los individuos jóvenes (Roenneberg et al., 2007). De nuevo, se puede argumentar que las contribuciones endógenas (reloj interno) o exógenas (estilo de vida), o una combinación de ambos, podría explicar este avance de fase. En términos del componente endógeno, existe evidencia de la existencia de un ritmo de curso libre de temperatura central más corto en ancianos durante protocolos de rutina constante (Dijk et al., 2000; Harper et al., 2005). Sin embargo, otros estudios han fallado en encontrar un avance significativo de fase en personas ancianas durante estas condiciones experimentales (Kendall et al., 2001). Aunque no podemos descartar la posibilidad de que su reloj interno simplemente avanza más rápido, explicaciones más creíbles tienen en cuenta la contribución exógena al sistema circadiano. Es probable que los individuos ancianos vayan antes a la cama por: a) su vision empobrecida, que limita lo que pueden hacer, b) quizás están expuestos a menores intensidades de luz durante la tarde y la noche; c) están aburridos más fácilmente durante la segunda mitad del día; d) el declinar de sus facultades mentales implica que se aburren más fácilmente (Waterhouse et al., 2012).

Sea una causa endógena o exógena la responsable de la alteración del sistema circadiano en ancianos, es claramente beneficioso incluir en su estilo de vida procedimientos que aumenten la dicotomía o el contraste entre las actividades diurnas durante la fase activa y promover el sueño durante la fase de reposo. Con este fin, actividades como la exposición a luz brillante, la actividad física en el exterior y las actividades mentales durante el día, y un aumento de los niveles de luz artificial (particularmente durante la tarde y el inicio de la noche) deberían ser programadas. Además, se debería educar a los ancianos para irse a dormir en completa oscuridad (la luz solo debería estar disponible si es necesaria por seguridad o para cuidados), y

permanecer en condiciones de oscuridad o luz tenue cuando deben ser despertados por la noche.

En resumen, el presente trabajo muestra que la maduración del sistema circadiano se asocia a un aumento en la amplitud del ritmo circadiano, y una disminución de la temperatura de la piel durante el sueño, mientras que el envejecimiento se asocia de forma consistente con un avance de fase. La temperatura de la piel distal puede ser utilizada como una variable robusta para evaluar el grado de maduración y envejecimiento del sistema circadiano humano.

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REFERENCES

Azevedo CVM, Sousa I, Paul K, MacLeish MY, Mondéjar MT, Sarabia JA, Rol MA, Madrid JA. (2008). Teaching Chronobiology and Sleep Habits in School and University. Mind Brain Educ. 2:34-47.

Bandín C, Martinez-Nicolas A, Ordovás JM, Ros Lucas JA, Castell P, Silvente T, Madrid JA, Garaulet M. (2012). Differences in circadian rhythmicity in CLOCK 3111T/C genetic variants in moderate obese women as assessed by thermometry, actimetry and body position. Int J Obesity. 37:1044-50.

Blazquez A, Martinez-Nicolas A, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity, and body position as determinants of the circadian pattern of blood pressure. Chronobiol Int. 29:747-56.

Buijs RM, la Fleur SE, Wortel J, Van Heyningen C, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol. 464:36-48.

Buijs RM, Kalsbeek A. (2001). Hypothalamic integration of central and peripheral clocks. Nature Rev Neurosci. 2:521-6.

Bonmati-Carrion MA, Middleton B, Revell V, Skene DJ, Rol MA, Madrid JA. (2014). Circadian phase assessment by ambulatory monitoring in humans: Correlation with dim light melatonin onset. Chronobiol Int. 31:37-51.

Sosa M, Mondéjar MT, Martinez-Nicolas A, Ortiz-Tudela E, Sarabia JA, Sosa J, Otalora B, Rol MA, Madrid JA, Campos M, Marín R. (2010). Circadianware. Spain Patent 08/2010/183.

Charkoudian N. (2003). Skin blood flow in adult human thermoregulation: How it works, when it does not, and why. Mayo Clin Proc. 78:603-12.

Dijk DJ, Duffy JF, Czeisler CA. (2000). Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep. Chronobiol Int. 17:285-311.

Duguay D, Cermakian N. (2009) The crosstalk between physiology and circadian clock proteins. Chronobiol Int. 26:1479-513.

Erren TC, Reiter RJ. (2009). Light Hygiene: Time to make preventive use of insights-old and new-into the nexus of the drug light, melatonin, clocks, chronodisruption and public health. Med Hypotheses. 73:537-41.

Garaulet M, Ordovás JM, Madrid JA. (2010). The chronobiology, etiology and pathophysiology of obesity. Int J Obesity. 34:1667-83.

Gompper B, Bromundt V, Orgül S, Flammer J, Kräuchi K. (2010). Phase relationship between skin temperature and sleep-wake rhythms in women with vascular

dysregulation and controls under real-life conditions. Chronobiol Int. 27:1778-96.

Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. (2011). Melatonin-A pleiotropic, orchestrating regulator molecule. Prog Neurobiol. 93:350-84.

Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A. (2005). Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. Am J Geriatr Psychiatry. 13:359-68.

Hofman MA, Swaab DF. (2006). Living by the clock: The circadian pacemaker in older people. Ageing Res Rev. 5:33-51.

Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. (2000). Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol. 278:R741-8.

Kräuchi K, Deboer T. (2010). The interrelationship between sleep regulation and thermoregulation. Front Biosci. 15:604-25.

Kendall AR, Lewy AJ, Sack RL. (2001). Effects of aging on the intrinsic circadian period of totally blind humans. J Biol Rhythms. 16:87-95.

Martinez-Nicolas A, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. Chronobiol Int. 28:617-29.

Martinez-Nicolas A, Ortiz-Tudela E, Rol MA, Madrid JA. (2013). Uncovering different masking factors on wrist skin temperature rhythm in free-living subjects. PLoS One. 8:e61142. doi:10.1371/journal.pone.0061142.

McGraw K, Hoffman R, Harker C, Herman JH. (1999). The development of circadian rhythms in a human infant. Sleep. 22:303-10.

Minors D, Atkinson G, Bent N, Rabbit P, Waterhouse J. (1998). The effects of age upon some aspects of lifestyle and implications for studies on circadian rhythmicity. Age ageing. 27:67-72.

Mirmiran M, Maas YGH Ariagno RL. (2003). Development of fetal and neonatal sleep and circadian rhythms. Sleep Med Rev. 7:321-34.

Moore RY. (1991). Development of the SCN. Introduction. In: Klein D, Moore R, Reppert S, editors. Suprachiasmatic nucleus. The mind's clock. New York: Oxford University Press. p. 375-90.

Mormont MC, Langouët AM, Claustrat B, Bogdan A, Marion S, Waterhouse J, Touitou Y, Lévi F. (2002). Marker rhythms of circadian system function: A study of patients with metastatic colorectal cancer and good performance status. Chronobiol Int. 19:141-55.

Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. (2010). A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comput Biol. 6:e1000996.doi:10.1371/journal.pcbi.1000996.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 27:1911-29.

Reiter RJ, Tan DX, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. (2007). Light at night, chronodisruption, melatonin suppression, and cancer risk: A review. Crit Rev Oncog. 13:303-28.

Rivkees SA. (2003). Developing circadian rhythmicity in infants. Pediatrics. 112:373-81.

Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, Merrow M. (2007). Epidemiology of the human circadian clock. Sleep Med Rev. 11:429-38.

Sarabia JA, Rol MA, Mendiola P, Madrid JA. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate of new index of the circadian system. Physiol Behav. 95:570-80.

Schwarzenberg-Czerny A. (1998). The distribution of empirical periodograms: Lomb-Scargle and PDM spectra. Mon Not R Astron Soc. 301:831-40.

Serón-Ferré M, Torres-Farfán C, Forcelledo ML, Valenzuela GJ. (2001). The development of circadian rhythms in the fetus and neonate. Semin Perinatol. 25:363-70.

Stratmann M, Schibler U. (2006). Properties, entrainment, and physiological functions of mammalian peripheral oscillators. J Biol Rhythms. 21:494-506.

Turek FW, Penev P, Zhang Y, van Reeth O, Zee P. (1995). Effects of age on the circadian system. Neurosci Biobehav Rev. 19:53-8.

Turner PL, Van Someren EJW, Mainster MA. (2011). The role of environmental light in sleep and health: effects of ocular aging and cataract surgery. Sleep Med Rev. 14:269-80.

Van Someren EJW. (2000). More than a marker: Interacion between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. Chronobiol Int. 17:313-54.

Van Someren EJW, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. (1999). Bright light therapy: improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int. 16:505-18.

Waterhouse J, Fukuda Y, Morita T. (2012). Daily rhythms of the sleep-wake cycle. J Physiol Anthropol. 31:5. doi: 10.1186/1880-6805-31-5.

de Weerth C, Zijl RH, Buitelaar JK. (2003). Development of cortisol circadian rhythm in infancy. Early Hum Dev. 73:39-52.

Weinert D. (2010). Circadian temperature variation and ageing. Ageing Res Rev. 9:51-60.

Worobey J, Vetrini NR, Rozo EM. (2009). Mechanical measurement of infant activity: A cautionary note. Infant Behav Dev. 32:167-72.

Zornoza-Moreno M, Fuentes-Hernández S, Prieto-Sánchez MT, Blanco JE, Pagán A, Rol MA, Parrilla JJ, Madrid JA, Sánchez-Solis M, Larqué E. (2013). Influence of gestational diabetes on circadian rhythms of children and their association with fetal adiposity. Diabetes Metab Res Rev. 29:483-91.

Zornoza-Moreno M, Fuentes-Hernández S, Sánchez-Solis M, Rol MA, Larqué E, Madrid JA. (2011). Assessment of circadian rhythms of both skin temperature and motor activity in infants during the first 6 months of life. Chronobiol Int. 28:330-7.

3.3. EXPERIMENTAL CHAPTER 3

CROSSTALK BETWEEN ENVIRONMENTAL LIGHT AND INTERNAL TIME IN HUMANS

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3.3. CROSSTALK BETWEEN ENVIRONMENTAL LIGHT AND INTERNAL TIME IN HUMANS

ABSTRACT

Daily exposure to environmental light is the most important *zeitgeber* in humans and all studied characteristics of light pattern (timing, intensity, rate of change, duration, and spectrum) influence the circadian system. However, and due to lack of current studies on environmental light exposure and its influence on the circadian system, the aim of this work is to determine the characteristics of a naturalistic regimen of light exposure and its relationship with the functioning of the human circadian system. 88 undergraduate students (18-23 years) were recruited in Murcia, Spain (latitude 38° 01' N) to record wrist temperature, light exposure and sleep for 1 week under free-living conditions. Light-exposure timing, rate of change, regularity, intensity, and contrast were calculated, and their effects on the sleep pattern and wrist temperature rhythm were then analyzed. In general, higher values for interdaily stability, relative amplitude, mean morning light, and Light Quality Index (LQI) correlated with higher interdaily stability and relative amplitude, and phase advance in sleep plus greater stability in wrist temperature and phase advance of the wrist temperature circadian rhythm. On the other hand, a higher fragmentation of the light-exposure rhythm was associated with more fragmented sleep. Naturalistic studies using 24-h ambulatory light monitoring provide essential information about the main circadian system input, necessary for maintaining healthy circadian tuning. Correcting light-exposure patterns accordingly may help prevent or even reverse health problems associated with circadian disruption.

Key words: Light exposure, Wrist temperature, Sleep-wake cycle, Free-living conditions, Human circadian system, Light quality index, 24-h ambulatory monitoring.

INTRODUCTION

The mammalian circadian system is composed of a hierarchically-organized network of structures responsible for generating circadian rhythms and synchronizing them to the environment. It includes a central pacemaker (suprachiasmatic nucleus of the hypothalamus, SCN), several peripheral clocks, and input and output pathways that are responsible for environmental entrainment and generating circadian rhythms in the organism, respectively (Buijs & Kalsbeek, 2001; Duguay & Cermakian, 2009; Strattmann & Schibler, 2006).

Under natural conditions, the SCN is reset every day by periodical light input from the retina through the retinohypothalamic tract (Güler et al., 2008; Moore et al., 2002), by means of cones, rods, and intrinsically photoreceptive melanopsin ganglion cells (Dijk & Archer, 2009; Güler et al., 2008). Although other periodic cues, such as scheduled exercise, social contacts, sleep habits, and feeding time can also entrain the circadian system (Atkinson et al., 2007; Mendoza, 2007; Mistlberger & Skene, 2004), daily exposure to environmental light is the most important *zeitgeber* in humans (Brainard et al., 1997; Skene et al., 1999). Based on this, it may be that inappropriate light exposure is involved in the pathophysiology of circadian disorders, and, therefore, contributes to a misalignment of the endogenous circadian clock and the voluntary rest-activity cycle.

Light-pattern timing, intensity, rate of change, duration, and spectrum are the major characteristics relevant to circadian entrainment by light (Pauley, 2004). Under laboratory conditions, it is known that light pulses during the first part of the night or at the end of the night, induce phase delay or phase advance of the circadian pacemaker, respectively (Khalsa et al., 2003). Moreover, insufficient light intensity during the day can cause phase instability or even free-running circadian rhythms (Gronfier et al., 2007; Middleton et al., 2002), a very important issue to consider in certain populations, such as elderly (Turner & Mainster, 2008). The threshold luminance required to prevent free-running is normally higher in the elderly, due to crystalline lens transmittance decline and pupil area reduction (Turner & Mainster, 2008). In addition, young people with normal vision free-run with weak non-photic *zeitgebers* and room light intensities <200 lux (Middleton et al., 2002). Similarly, astronauts show free-running rhythms with light exposures <80 lux (Gronfier et al., 2007). Regarding the duration of bright light

exposure, it is interesting to note that young adults from industrialized areas rarely receive more than 20-120 mins of daily light exposure >1000 lux (Espiritu et al., 1994, Hebert et al., 1998; Savides et al., 1986), whereas in the elderly exposure time is only ~30-60% of this value (Campbell et al., 1988; Mishima et al., 2001). Some studies show that progressive appearance of light (similar to natural dawn) may be more effective than "square-wave" light onset (Kavanau, 1962; Tang et al., 1999). It seems the biological clock is not only sensitive to level of light luminance, but also to its rate of change. Finally, the synchronizing power of light is dependent on its light spectrum, with light enriched in blue wavelengths (460-480 nm) being more effective than wavelengths outside this band (Rahman et al., 2008).

Several circadian rhythms under the control of the SCN are commonly used to evaluate the status of the circadian system. These rhythms, called circadian marker rhythms, serve to characterize the timing of the internal temporal order. A marker rhythm should be able to be easily measured over long periods using non-invasive methods. The most frequent human marker rhythms include salivary melatonin or cortisol, urinary 6-sulfatoxymelatonin, actimetry, and core body temperature (CBT) (Mormont et al., 2002; Van Someren, 2000). Recent evidence suggests sleepiness may be more closely linked to increased peripheral skin temperature than to a core temperature drop, and distal skin temperature seems to be correlated and phase-advanced with respect to CBT, suggesting heat loss from extremities may drive the circadian CBT rhythm (Krauchi et al., 2000; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). The wrist temperature (WT) rhythm, recorded on the radial artery on the non-dominant hand, exhibits an inverse phase relationship with CBT, and it has recently being proposed as a non-invasive, robust, and easy-to-register index of the circadian system (Ortiz-Tudela et al., 2010; Sarabia et al., 2008). WT rhythm integrates endogenous and exogenous influences, thus presenting considerable advantages for evaluating the effects of synchronizing agents, such as light exposure, on circadian function.

To date, most studies dealing with the effects of light exposure on the circadian system have been conducted under strictly laboratory-controlled conditions, using light stimuli that are too far from the natural light characteristics; thus, naturalistic studies, using 24-h ambulatory light monitoring, may provide very important information about the characteristics of the light-dark cycle that are essential to the maintenance of a

healthy circadian system. In fact, this is one of the first studies to simultaneously register the main input (light exposure) of the circadian system, along with two markers of circadian function, WT and sleep patterns, for a duration of 1 week. Considering the importance of light as a *zeitgeber* and the lack of current studies on light exposure in healthy young subjects and its influence on circadian rhythms, the aim of this work is to determine, for the very first time, the characteristics of a naturalistic light-exposure regimen and its relationship with the human circadian system, as assessed by WT and sleep diary recordings.

MATERIAL AND METHODS

Subjects

For the present study, 88 undergraduate volunteers (36 males and 52 females, 18-23 years of age) residing in Murcia, Spain (latitude 38^{o} 01' N) were recruited. All recordings were made in November. The mean environmental temperature was 12.3 ± 0.4°C (mean maximum temperature 19±0.6°C, mean minimum temperature 7.4±0.4°C), with sunrise occurring between 07:31 and 07:54 h, and sunset between 17:46 and 18:03 h (data obtained from the University of Murcia's weather station: https://estacion.um.es/).

The study abides by the bioethical principles set out by the Declaration of Helsinki. Data from the volunteers were included in a database and were protected according to Spanish Law 15/1999 of 13th December. All participants received adequate information about the study characteristics and signed an informed consent form before their inclusion in the study (Portaluppi et al., 2010).

Participants were instructed to complete a sleep diary designed by the Chronobiology Lab at the University of Murcia, and they were encouraged to maintain their normal lifestyle. The diary compiled information regarding sleep onset, sleep offset, and the time and duration of naps.

Environmental light exposure recording

All subjects were required to wear a HOBO® Pendant Temperature/Light Data Logger UA-002-64 (Onset Computer Corporation, Bourne, Massachusetts, USA) on a

lanyard close to their eyes to record light exposure. According to the manufacturer specifications, the data logger has a measurement range between 0 and 320,000 lux, memory capacity for up to 28,000 values taken at regular, previously programmed intervals (in our case, every 30 s), and light-spectrum wavelength recording capacity of 150-1200 nm, which is broader than the sensitivity of the human eye. In order to validate the light sensor, a LX 101 lux meter (3E NDT, Pasadena, Texas, USA) was used to make a set of simultaneous recordings in different environments (data not shown). Readings from both devices demonstrated a strong, significant positive correlation at different intensities ($r=0.997$, $p<0.01$). A high degree of repeatability of sensor measurements was observed when recordings were simultaneously performed with two different Hobo sensors ($r=0.998$, $p<0.01$). Participants were instructed to wear the lux meter over clothing and to leave it on the bedside table during sleep. To compare the light exposure of our subjects to the natural sunlight cycle, environmental light intensity was recorded for 1 wk in the same area and for the same experimental period. To do this, a Hobo sensor was placed outdoors, facing North in a shady location to avoid direct light irradiance.

Wrist skin temperature measurement

In addition to the Hobo sensor, all subjects wore a Thermochron iButton DS1921H (Maxim Integrated Products, Sunnyvale, California, USA) for WT measurement with a precision of ±0.125°C. This temperature sensor was placed on the wrist of the non-dominant hand over the radial artery and isolated from the environmental temperature by means of a double-sided cotton sport wrist band, as previously described (Sarabia et al., 2008). The sensor was programmed to sample every 10 min throughout the entire week. Both the light and temperature sensors were worn simultaneously, as shown in Figure 1.

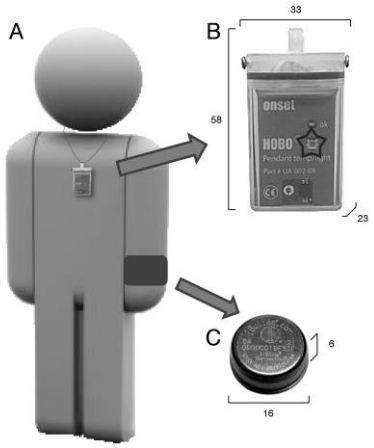

Figure 1. Body location of light and temperature sensors. The light data logger is hung around the neck on a lanyard, and the temperature data logger is placed on the wrist of the non-dominant hand (**A**). **B** shows the HOBO data logger, with a mark indicating the exact position of the light sensor. The iButton data logger is shown in **C**. Sensor measurements are expressed in millimeters.

Data analysis

To facilitate the determination of light exposure, the presence/absence of natural light (solar day and night, respectively), and the light intensity analysis, the following ranges were established: very dim light (<10 lux), indoor dim light (10-500 lux), indoor bright light (500-1000 lux), and outdoor bright light (>1000 lux), as described in a previously conducted study (Turner & Mainster, 2008). Environmental light intensities in lux were converted into logarithmic units and averaged every 10 mins to allow comparisons with temperature data.

WT data were filtered to eliminate artifacts, such as those produced by temporarily removing the temperature sensor. Sleep-wake information was converted into binary values by assigning a value of 1 when the subjects declared they were asleep and 0 when awake. Mean WT and sleep- and light-exposure patterns were calculated per individual and group. Sleep probability indicates the percentage of individuals asleep at any given time, as already described (Sarabia et al. 2008).

To characterize WT, light exposure, and declared sleep rhythms, a non-parametrical analysis was performed. This analysis determined the following parameters: Interdaily Stability (the constancy of the 24-h rhythmic pattern over days, IS); Intradaily Variability (the rhythm fragmentation, IV); average in 10-min intervals for the 5 h with the maximum temperature (M5) and its timing (TM5); average in 10-min intervals for the 10 h with the minimum temperature (L10) and its respective timing (TL10), and the relative amplitude (RA), which was determined by the difference between M5 and L10 divided by the sum of M5 and L10 as previously done by Van Someren et al. (1999). For sleep, in addition to the time of sleep onset and offset, the duration and the time of the midpoint were also calculated. With respect to light exposure, we calculated the mean intensity (MI) over the entire 24-h period, as well as the mean light intensity during the morning (from 08:00 to 15:50 h), evening (from 16:00 to 23:50 h), and night (from 00:00 to 07:50 h).

In order to classify individuals according to their circadian system functionality, we used a new scoring index, the circadian function index (CFI), which was previously proposed by our laboratory (Ortiz-Tudela et al., 2010). This index was calculated by averaging three non-parametric indices (IS, IV, and RA) for temperature and sleep data. Before averaging, all these indices were normalized between 0 and 1, (IV was inverted,

since its values are opposite to those for IS and RA). Accordingly, the CFI oscillates between 0 (gaussian noise) and 1 (a sinusoidal wave).

Similarly, we propose a new index for evaluating the quality of light exposure during the day, which we call the Light Quality Index (LQI). This index considers the time spent in >500 lux, minus the time spent in <10 lux, divided by the time spent in >500 lux plus the time spent at <10 lux, and oscillates between +1 (all daytime exposed to >500 lux) and -1 (all daytime exposed to <10 lux).

Changes in WT associated with acute changes in light exposure were examine by correlating positive and negative changes in light intensity (in logarithmic interval units: 0-1, 1-2, 2-3, and 3-4) with the corresponding changes in WT for a given time point in 12 subjects chosen randomly (6 men and 6 women). In order to eliminate the influence of sleep and body position, the sleep periods and 30 mins before and after sleep were excluded from the analysis. All these spontaneous changes in light exposure were balanced among subjects, and a correlation analysis was then performed between the rate of change for light intensity and that for WT. To determine the long-term effects of light exposure on circadian rhythmicity, Pearson correlations were calculated between each of the light-dark cycle features (IS, IV, RA, MI, morning, evening, and night light exposure, and LQI) and sleep probability (IS, IV, RA, CFI, and sleep midpoint), and WT (IS, IV, RA, CFI, and TM5) parameters. In addition, a classification of individuals according to the light-pattern features that most affected their sleep and WT rhythms was performed. For this purpose, we first classified subjects into quartiles based on their individual light-dark pattern stability, morning light exposure, day/night contrast (or RA for light), and LQI. The corresponding 24-h mean waveform (for temperature, sleep, and light) per quartile was then calculated. The first quartile (Q1) represents the lowest values, while the fourth quartile (Q4) represents the highest values. Similarly, we also classified the subjects according to their WT pattern quartiles and analyzed their light-pattern characteristics.

All data are expressed as mean ± SEM. The data were processed using Microsoft Office Excel 2007, and all statistical analyses (repeated-measures ANOVA followed by *post-hoc* pairwise comparisons using a Bonferroni test and Pearson correlations using Bonferroni correction) were performed with SPSS version 15.0 (SPSS, Inc. Chicago,

IL, USA). Values of $p<0.01$, or $p<0.001$ for Pearson correlations, were considered to be statistically significant.

RESULTS

Light-Exposure Pattern

A weekly record and its corresponding mean waveform from a representative subject is shown in Figure 2. WT showed a characteristic rhythm with stable values above 34.5°C during sleep time, and low and highly variable values during the active period. The light intensity and timing of exposure during the day also displayed a very high variability, with values of up to 70,000 lux, but the resting period was mostly spent in darkness. In addition, a high degree of irregularity for sleep onset, offset and duration can be observed.

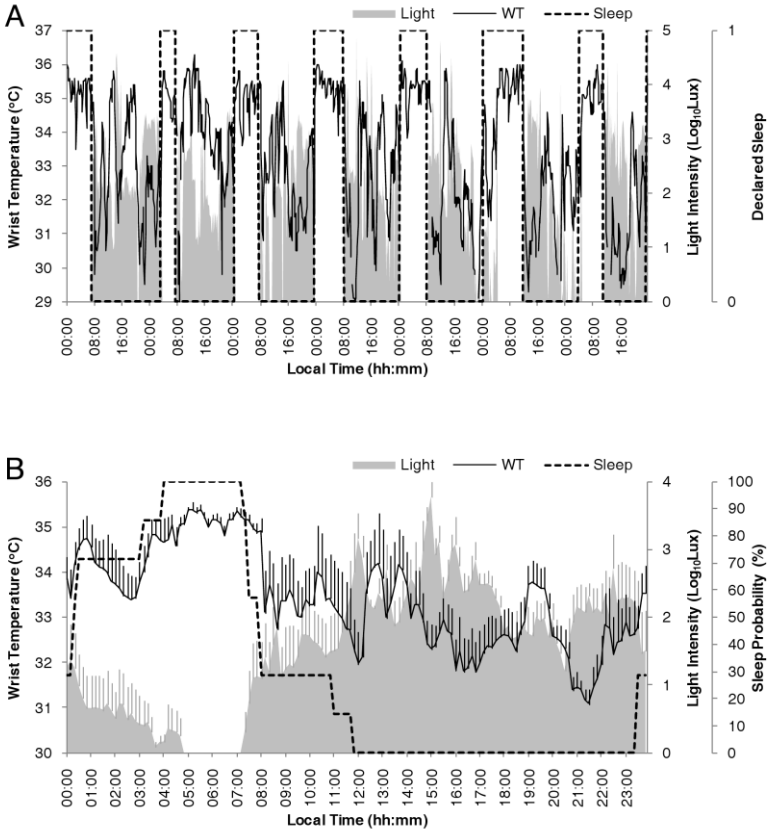

Figure 2. Individual wrist temperature, light intensity, and sleep pattern from a representative subject. One-week of continuous recordings (A) and daily mean waveforms (B) for wrist temperature (black line), light intensity (grey area), and sleep probability (dotted line) from a representative subject. In the latter case, the values are expressed as mean ± SEM.

3.3. Light exposure influences on the internal time

Figure 3 represents the average 24-h mean pattern for light exposure, environmental light cycle, WT, and sleep probability, averaged over 7 days. WT exhibited a daily rhythm that matched the sleep probability rhythm. Maximum mean light exposure during the natural day was ~200 lux, which is much lower than the maximum potential light exposure in the shade (10,000 lux). On the contrary, light intensities were stronger than natural during the evening and night. Light exposure seemed to show a roughly inverse relationship to WT. WT increased in anticipation of sleep onset, maintained a high level during the sleep period, and then dropped immediately after awakening. A secondary peak was observed in the afternoon, while the 24-h minimum occurred between 20:00 to 22.00 h, a period known as the "wake maintenance zone", due to the low probability of finding adults asleep at that time. The light-exposure pattern showed values <10 lux between 00:00 to 08:00 h, >100 lux at midday, and in the range of 10-100 lux during both the morning (08:10 to 12:30 h) and evening (16:00 to 00:00 h), all of which are consistent with artificial light and/or lighting inside buildings.

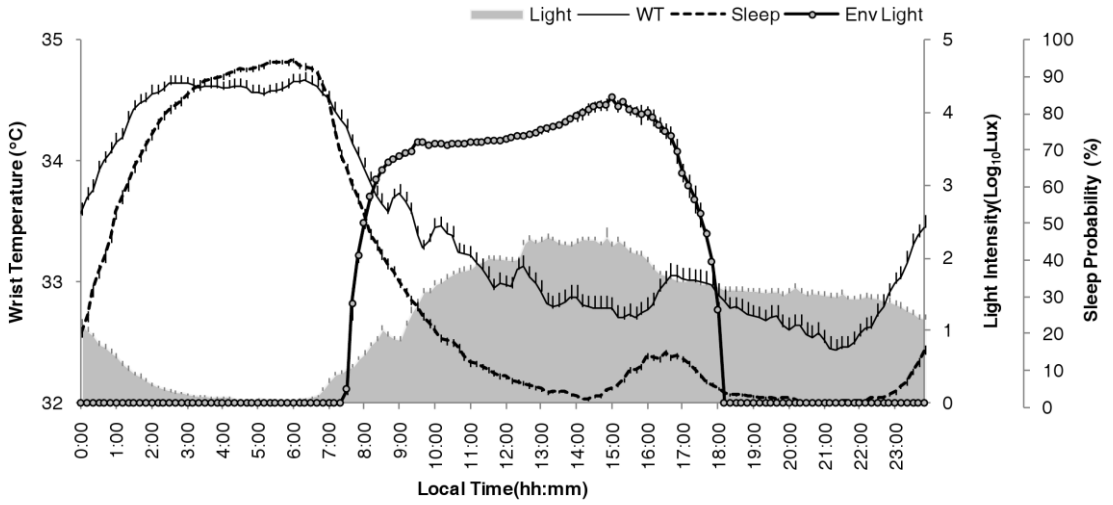

Figure 3. Average mean waveform for wrist temperature, **light intensity, and sleep pattern.** Average daily evolution of light exposure (grey area), wirst temperature (black line), sleep pattern (dotted line), and environmental light (grey circles) for all experimental subjects (n=88). The data shown have been obtained by averaging all individual mean waveforms. All variables are expressed as mean ± SEM.

Table 1 shows main characteristics of the circadian pattern in light exposure, WT, and sleep. Minimum light-exposure values were recorded at 04:35 ± 00:05, coinciding with TM5 and Midpoint of sleep.

	Light Exposure		Temperature		Sleep
IS	0.53 ± 0.10	IS	0.45 ± 0.02	IS	0.69 ± 0.01
IV	0.25 ± 0.07	IV	0.18 ± 0.01	IV	0.08 ± 0.00
RA	0.94 ± 0.07	RA	0.03 ± 0.00	RA	0.99 ± 0.00
MI	1.22 ± 0.22	CFI	0.58 ± 0.01	CFI	0.88 ± 0.00
Morning	1.84 ± 0.31	TM5	04:21 ± 00:09	Onset	01:16 ± 00:30
Evening	1.56 ± 0.40	TL10	17:21 ± 00:14	Offset	08:40 ± 00:37
Night	0.28 ± 0.16	M5	34.76 ± 0.06	Midpoint	04:58 ± 00:29
LQI	-0.18 ± 0.31	L10	32.61 ± 0.09	Duration	07:31 ± 00:37

Table 1. Mean values for light exposure, temperature, and sleep pattern characteristics. Main characteristics for light exposure, wrist temperature, and sleep 24-h patterns: Interdaily Stability (IS), Intradaily Variability (IV), Relative Amplitude (RA), and Circadian Function Index (CFI). In light exposure pattern, MI indicates mean intensity throughout the 24-h period; Morning, Evening, and Night indicate the mean light exposure between 08:00-15:50 h, 16:00-23:50 h, and 00:00-07:50 h, respectively; LQI is the value for the Light Quality Index. Wrist temperature rhythm uses the midpoint of the 5 h of maximum values (TM5) and the midpoint of the 10 h of minimum values (TL10) as phase markers, and its mean value (M5 and L10, respectively). Sleep-wake pattern Onset and Offset indicate night sleep start and end; duration and midpoint of the night sleep are indicated as Duration and Midpoint, respectively. All the values are expressed as mean ± SEM.

Short-term effects of light exposure

A common feature of natural light exposure is the very high variability in light intensity hitting the retina. Using the spontaneous changes in light exposure, it was possible to analyze the influence of acute light changes on WT. Figure 4 shows two examples that demonstrate this association. In the case depicted in panel A, when light intensity decreased, WT immediately increased. Similarly, panel B shows how a transient increase in light intensity was mirrored by a decrease in WT that was also transient. Using these spontaneous changes in light exposure, selected in a balanced manner among subjects and considering only the wakefulness period, a significant inverse relationship between rate of change in light exposure and rate of change in WT (Figure 4C), with a slope of -0.14°C per logarithmic unit, of light intensity was obtained.

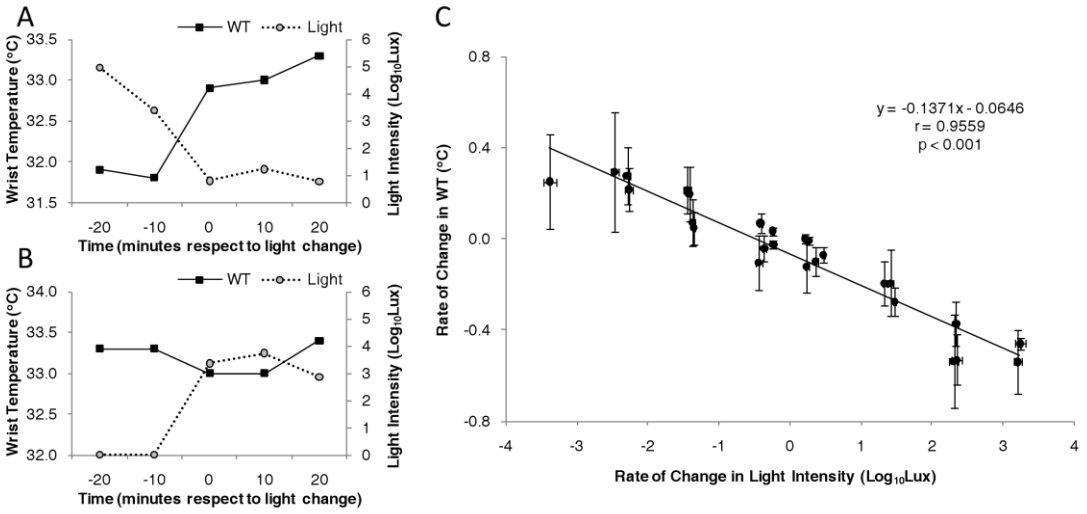

Figure 4. Acute effects of light on wrist temperature. Two examples of wrist temperature changes produced by negative (**A)** and positive (**B**) changes in light exposure. The rate of change for wrist temperature in response to changes in light exposure (in logarithmic units) is shown in **C**. This graph shows the average of all WT changes for any given light-intensity change and subject (in this case, only 6 men and 6 women were studied). The values are expressed as mean ± SEM.

Long-term effects of light exposure

Duration of light exposure

As shown in Table 2, individuals spent most of their time at low light intensities. In fact, on average over the 24-h period, they exposed themselves to intensities of <500 lux for 21 h: 27 min ± 23 min, and to intensities >1000 lux for only 1 h:43 min ± 16 min. But what is more important, during the photophase of the natural day, the subjects only received 1 h:18 min of bright light, while they were exposed to >1000 lux for 26 min during the scotophase of natural day. This short diurnal bright-light exposure period occurs in addition to relatively long exposures (2 h: 46 min) to intensities <10 lux during the natural day.

	Day	Night	P
<10 lux	2:46 ± 0:07[a]	8:26 ± 0:08[a]	0.01
10-500 lux	5:10 ± 0:07[b]	4:55 ± 0:08[b]	n.s.
500-1000 lux	0:35 ± 0:02[c]	0:24 ± 0:02[c]	0.01
>1000 lux	1:18 ± 0:04[d]	0:26 ± 0:04[d]	0.01

Table 2. Duration and intensity of light exposure. Duration and intensity of light exposure during the photophase and scotophase for all the subjects (n=88). Duration is expressed as mean ± SEM (h:min). Different letters in the same column indicate significant differences (p<0.05). The last column indicates the p value when comparing day and night.

Association between long-term light exposure, WT, and sleep

Significant correlations were found between light exposure and WT rhythmic parameters. The CFI for WT indicates that the light pattern feature that is associated with better WT rhythmicity is IS ($r=0.304$, $p<0.05$). Light exposure IS and RA were positively correlated with wrist temperature IS ($r=0.394$, $p<0.01$ and $r=0.326$, $p<0.05$, respectively). In addition, higher light intensities during the morning were correlated with phase advance (measured as TM5) in WT ($r=-0.311$, $p<0.05$)

Highly significant correlations were also observed between most of the light parameters and the sleep declared by subjects (Table 3). In contrast with the positive, significant correlation between light exposure parameters (IS, RA, MI, morning, evening, and light LQI) and the sleep IS and CFI, intradaily variability in the light pattern and light at night were associated with worse sleep characteristics.

		Sleep				
		IS	IV	RA	CFI	Midpoint
Light Exposure	IS	0.484**	-0.164	0.297*	0.502**	-0.495**
	IV	-0.313*	0.227	-0.113	-0.319*	0.151
	RA	0.559*	-0.286*	0.292*	0.577**	-0.341*
	MI	0.343*	-0.069	0.085	0.330*	-0.425**
	Morning	0.437**	-0.108	0.204	0.439**	-0.651**
	Evening	0.304*	-0.136	0.106	0.303*	-0.287*
	Night	-0.206	0.265	-0.310*	-0.262	0.241
	LQI	0.420**	-0.201	0.229	0.435**	-0.571**

Table 3. Relationship between light exposure and sleep. Correlation coefficients and level of significance (*p<0.01, **p<0.001) for the correlation analysis between light exposure and sleep variables: Interdaily Stability (IS), Intradaily Variability (IV), Relative Amplitude (RA), Circadian Function Index (CFI). Midpoint indicates middle of the night sleep. MI indicates mean intensity throughout the 24-h period; Morning, Evening, and Night indicate the mean light exposure between 08:00-15:50 h, 16:00-23:50 h, and 00:00-07:50 h, respectively. LQI expresses the values obtained for the Light Quality Index.

3.3. Light exposure influences on the internal time

It has been proposed that the WT rhythm shows a clear relationship with a well-established marker rhythm: the sleep-wake cycle. In agreement with this, a statistically significant correlation was observed between WT and sleep IS, CFI, and phase markers (Table 4).

		Temperature				
		IS	IV	RA	CFI	TM5
Sleep	IS	+0.394**	-0.057	+0.140	+0.243	-0.368**
	IV	-0.127	+0.301*	-0.189	-0.194	+0.048
	RA	+0.093	-0.141	+0.093	+0.107	-0.097
	CFI	+0.380**	-0.098	+0.157	+0.252	-0.351*
	Midpoint	-0.427**	+0.116	-0.144	-0.266	+0.421**

Table 4. Relationship between wrist temperature and sleep. Correlation coefficients and level of significance (*p<0.01, **p<0.001) between sleep and wrist temperature variables: Interdaily Stability (IS), Intradaily Variability (IV), Relative Amplitude (RA), Circadian Function Index (CFI), Midpoint of the 5 h of maximum values (TM5). Midpoint indicates middle of night sleep.

To further analyze the influence of light intensity on circadian system functionality, the four light-exposure parameters that showed the most significant association with sleep and WT were used as criteria for classifying individuals into quartiles, and then to calculate their corresponding WT and sleep average mean waveform. However, and to facilitate comparison, only the first and fourth quartile (Q1 and Q4) for light-exposure patterns are shown.

The first classification criterion was light interdaily stability (Figure 5A). The light pattern for the subjects with the highest and lowest light IS values are shown in Figure 5B. Sleep and wrist temperature rhythms for those same subjects are shown in Figure 5C and 5D, respectively. The highest degree of light regularity (Q4) was associated with higher values for both IS (0.75 ± 0.02 *vs.* 0.60 ± 0.03, p<0.001) and RA (1.00 ± 0.00 *vs.* 0.99 ± 0.01, p<0.05) in the sleep pattern, along with a phase advance in sleep midpoint (04:35 ± 00:10 h *vs.* 05:41 ± 00:12 h, p<0.001). In addition, the Q4 group presented higher values for IS (0.56 ± 0.03 *vs.* 0.41 ± 0.03, p<0.001), RA (0.04 ± 0.00 *vs.* 0.03 ± 0.00, p<0.05), CFI (0.67 ± 0.02 *vs.* 0.56 ± 0.02 p<0.01), and reduction in IV (0.12 ± 0.01 *vs.* 0.18 ± 0.02, p<0.01) for WT, as compared to Q1 subjects.

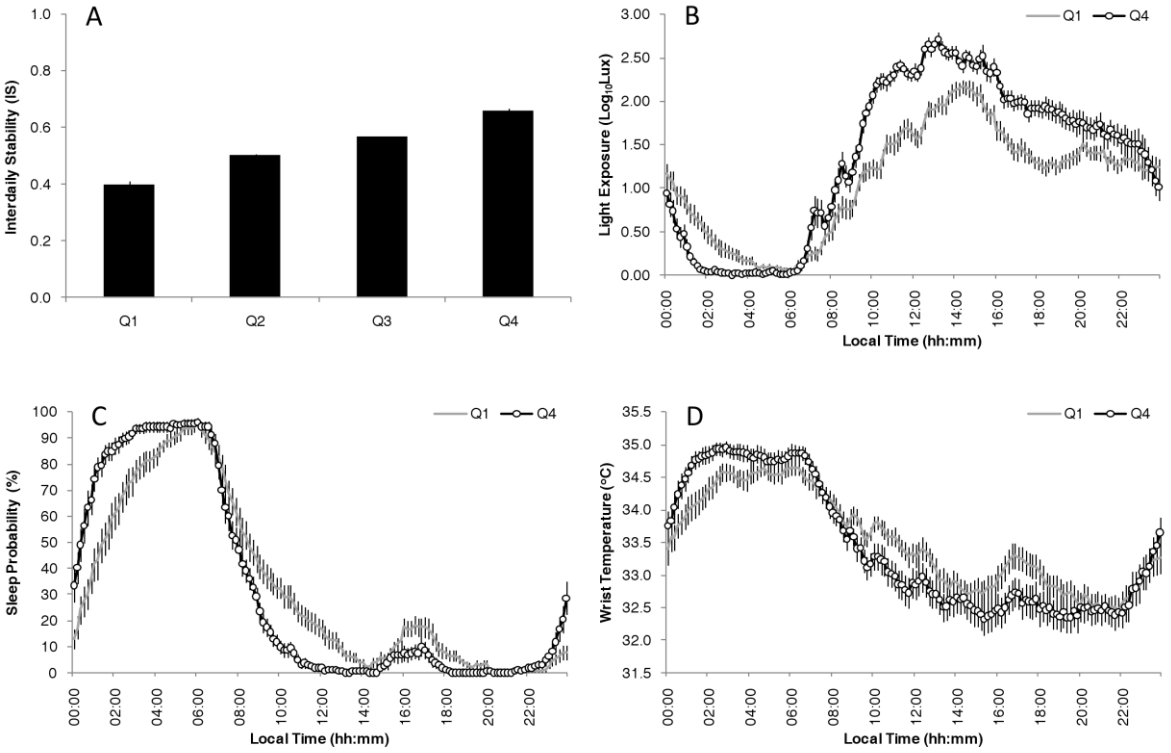

Figure 5. Quartile distribution according to the interdaily stability of light exposure. (**A**) Subjects distribution into quartiles using the interdaily stability of light exposure as the main classifying criterion (n=88). Values are expressed as mean ± SEM. (**B**) Mean light-exposure pattern for quartile 1 (Q1, grey line) and 4 (Q4, black line with white circles) subjects. Their corresponding sleep and wrist temperature rhythms are shown in **C** and **D,** respectively.

Studies involving both laboratory and free-living conditions have shown the timing of bright-light exposure to be a key factor for circadian synchronization. Figure 6 shows light exposure, sleep, and wrist temperature daily patterns for individuals classified according to the morning-light intensity they received between 08:00 and

16:00 h (Figure 6A). Light-exposure waveforms for both quartiles (Q1 and Q4) are shown in Figure 6B. Stronger morning-light exposure was associated with higher IS (0.76 ± 0.02 *vs.* 0.61 ± 0.03, p<0.001), RA (1.00 ± 0.00 *vs.* 0.99 ± 0.00, p<0.01), and CFI (0.91 ± 0.01 *vs.* 0.85 ± 0.01, p<0.001) values of sleep patterns (Figure 6C), as well as phase advance measured in terms of sleep midpoint (04:21 ± 00:09 h *vs.* 05:53 ± 00:11 h, p<0.001). Similarly, stronger morning light was associated with a wrist temperature phase advance measured at the midpoint of M5 (03:47 ± 00:12 h *vs.* 05:19 ± 00:22 h, p<0.001) (Figure 6D).

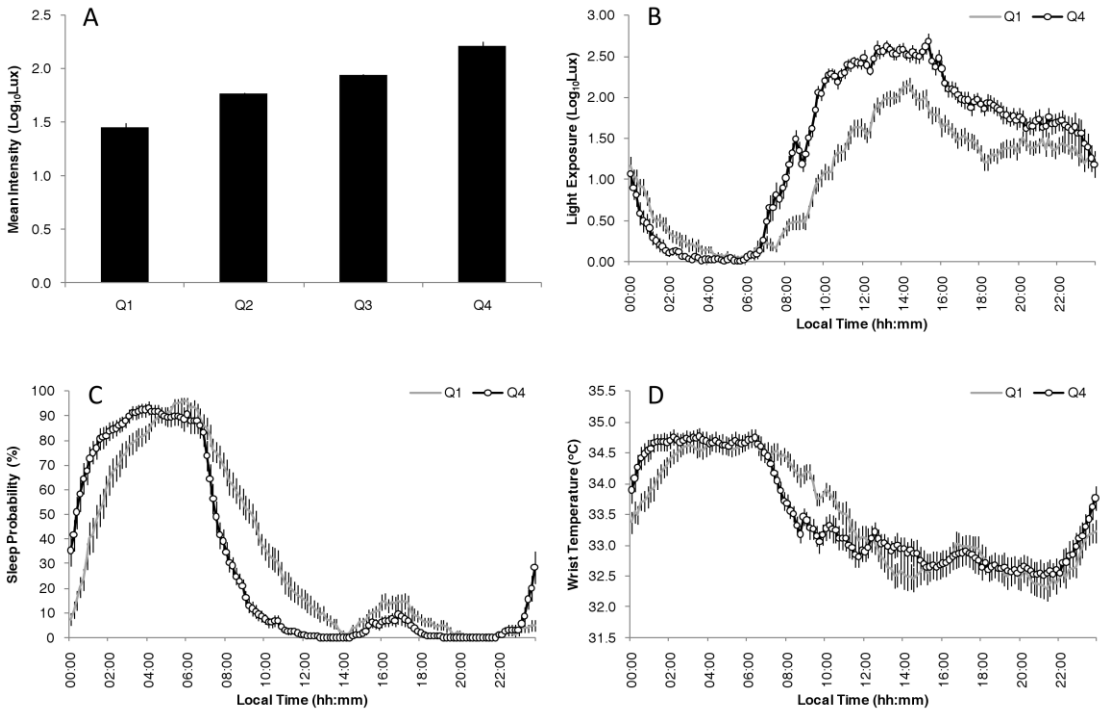

Figure 6. Quartile distribution according to the mean intensity of light exposure during the morning. (**A**) Subjects distribution into quartiles using the mean intensity of light exposure during the morning (08:00-16:00 h) as the main classifying criterion (n=88). Values are expressed as mean ± SEM. (**B**) Mean light exposure pattern for quartile 1 (Q1, grey line) and 4 (Q4, black line with white circles) subjects. Their corresponding sleep and wrist temperature rhythms are shown in **C** and **D**.

Another important characteristic of the light-dark exposure that affects WT and sleep rhythms is RA, or the contrast between daytime and nighttime light. In Figure 7, subjects are classified into quartiles according to their RA light exposure value (Figure 7A). The corresponding Q1 and Q4 light-exposure rhythms are shown in Figure 7B. The high day-night contrast of Q4 was associated with higher IS (0.78 ± 0.02 *vs.* 0.60 ± 0.03, p<0.001), RA (1.00 ± 0.00 *vs.* 0.99 ± 0.01, p<0.05), and CFI (0.91 ± 0.01 *vs.* 0.85

± 0.01, p<0.001), plus lower sleep IV (0.09 ± 0.00 *vs.* 0.08 ± 0.00, p<0.05), and phase advance in sleep midpoint (04:37 ± 00:10 h *vs.* 05:33 ± 00:11 h, p<0.001) (Figure 7C). In the case of WT (Figure 7D), greater day-night contrast was associated with higher values for IS (0.52 ± 0.02 *vs.* 0.35 ± 0.03, p<0.001), RA (0.03 ± 0.00 *vs.* 0.02 ± 0.00, p<0.05), and CFI (0.62 ± 0.03 *vs.* 0.52 ± 0.02, p<0.01).

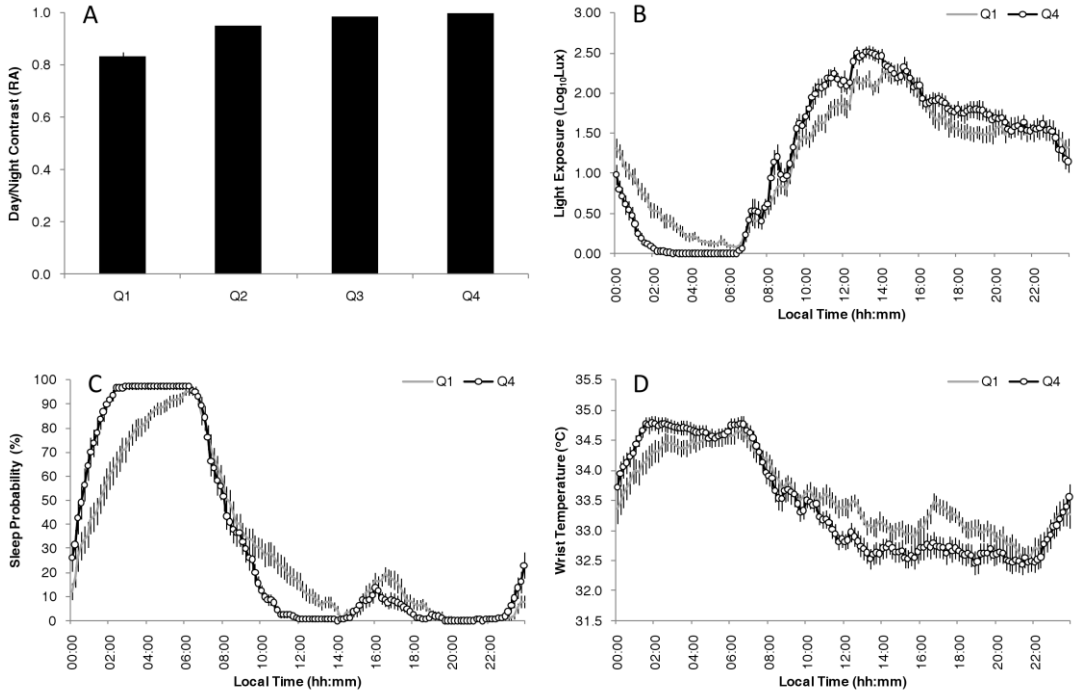

Figure 7. Quartile distribution according to the relative amplitude of light exposure. (**A**) Subjects distribution into quartiles using the relative amplitude of light exposure as the main classifying criterion (n=88). Values are expressed as mean ± SEM. (**B**) Mean light-exposure pattern for quartile 1 (Q1, grey line) and 4 (Q4, black line with white circles) subjects. Their corresponding sleep and wrist temperature rhythms are shown in **C** and **D**.

The final characteristic of light exposure that may be important for WT and sleep is the Light Quality Index (LQI). High LQI represents daylight exposure during the environmental daytime, while low LQI is a sign of nighttime light exposure during the environmental daytime. LQI scores for the different quartiles are shown in Figure 8A, with the corresponding light exposure for quartiles 1 and 4 in Figure 8B. High LQI values were associated with higher values for sleep IS (0.75 ± 0.02 *vs.* 0.64 ± 0.03, p<0.01), RA (1.00 ± 0.00 *vs.* 0.98 ± 0.01, p<0.01), and CFI (0.90 ± 0.01 *vs.* 0.86 ± 0.01, p<0.01) (Figure 8C), as well as phase advance measured in terms of sleep midpoint (04:23 ± 00:10 h *vs.* 05:40 ± 00:13 h, p<0.001). Similarly, a high LQI is associated with

a higher IS (0.49 ± 0.03 *vs.* 0.40 ± 0.03, p<0.05) and phase advance, measured as M5 time (03:53 ± 00:12 h *vs.* 04:57 ± 00:22 h, p<0.05), in the WT rhythm (Figure 8D).

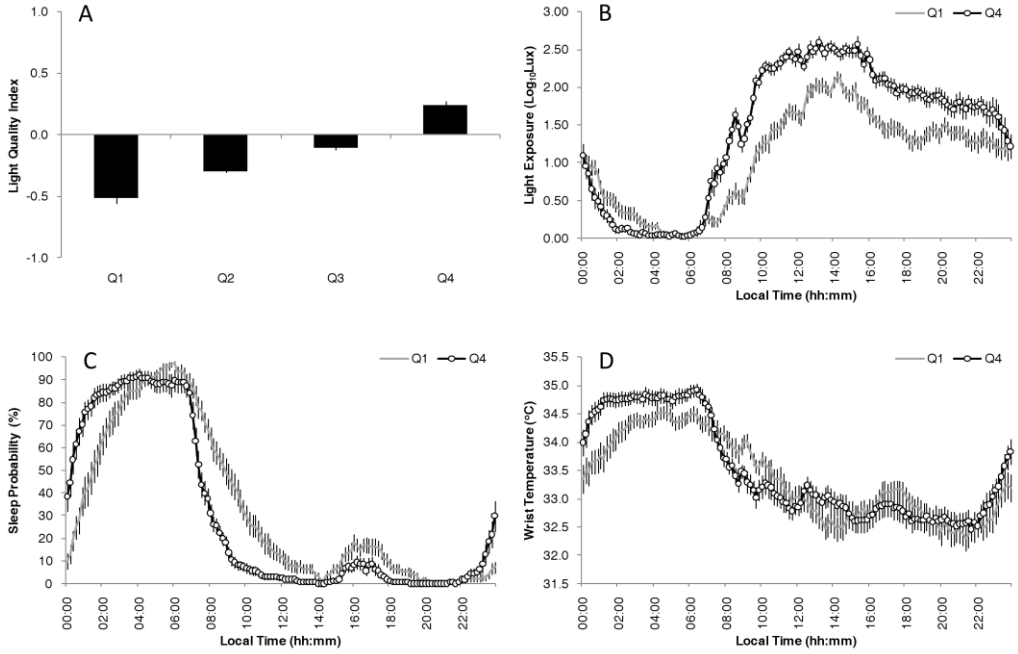

Figure 8. Quartile distribution according to the Light Quality Index. (**A**) Subjects distribution into quartiles using the Light Quality Index as the main classifying criterion (n=88). Values are expressed as mean ± SEM. (**B**) Mean light-exposure pattern for quartile 1 (Q1, grey line) and 4 (Q4, black line with white circles) subjects. Their corresponding sleep and wrist temperature rhythms are shown in **C** and **D**.

When only considering the Q1 and Q4 quartiles of WT characteristics (IS, IV, RA, and CFI) no statistical differences were observed between them in their light-exposure pattern. However, when subjects were sorted by phase marker (TM5), higher mean light intensity during the morning (1.95 ± 0.07 *vs.* 1.72 ± 0.06, p<0.05) was observed in those subjects presenting more phase advance.

DISCUSSION

To our knowledge, the present study is the first attempt to simultaneously measure during an extended span of time (1 week) an input (light exposure) and two circadian outputs (WT and sleep) in young, healthy subjects under normal-living conditions. We, therefore, propose the use of a Temperature/Light Data Logger to measure light exposure by means of a non-invasive, inexpensive and non-disruptive method. Acute changes in light intensity exhibit an inverse relationship with WT, while

regularity, light exposure timing, day-night light-exposure contrast, and the newly proposed LQI are the main parameters associated with significant changes in WT and sleep rhythms.

The classical conception of light as a mere input to the circadian system should be questioned in humans, considering the fact that contemporary humans spend most of their time indoors, with very low levels of natural light and with artificial light sources providing illumination both day and night. As a result, light exposure is voluntarily and also unconsciously manipulated to match rest-activity rhythms, as well as working and leisure activities. The rhythm of light exposure should, therefore, be considered simultaneously as an input, and, in some aspects, a result of the circadian system function, which in turn provides feedback to the suprachiasmatic nuclei (SCN). Unlike our ancestors, who lived in natural environments, the last five generations of people residing in developed countries have been able to self-select their light-dark cycle. The main differences between these two lifestyles with regard to light exposure are an overall decrease in light intensity and regularity; a modification in light timing, with delayed and reduced exposure during the day, and increased light at night; alterations in the rate of change over time of light exposure, and shift in the light spectrum towards artificial light sources. These changes in light input are, in part, the reason why it is hypothesized a large proportion of people suffer from some degree of circadian rhythm disruption (or chronodisruption) in modern society (Erren et al., 2009; Francis et al., 2008; Mottram et al., 2010; Reiter et al., 2007). There is currently a growing body of scientific evidence that links chronodisruption to increased risk of developing certain diseases, and to worsening of pre-existing medical conditions, such as cancer, metabolic syndrome, insomnia, affective disorders, cognitive impairment, and cardiovascular diseases, as well as premature aging (Erren et al., 2009; Garaulet & Madrid, 2010; Reiter et al., 2007; Turner et al., 2010).

Wrist skin temperature, recorded under ambulatory conditions with minimal discomfort by means of an iButton sensor, has previously been proposed by our group as an index of circadian system function (Ortiz-Tudela et al., 2010; Sarabia et al., 2008). To complement this information with the most important environmental *zeitgeber*, the light-dark cycle, we propose to use a small lux meter for the ambulatory monitoring of light exposure. The use of a lanyard to place the light sensor close to the eyes, as previously described Smith and Eastman in 2009, provides more representative data for

light irradiance received by the eyes than a wristband, as has been proposed previously (Cole et al., 1995; Emens et al., 2009; Francis et al., 2008; Goulet et al., 2007; Mottram et al., 2011; Okudaira et al., 1983).

When subjects are indoors, most light intensities are <500 lux, as occurs in those subjects working in a Antarctic station during winter (Francis et al., 2008; Mottram et al., 2010), and values between 50 and 200 lux were quite frequent in our recordings. These intensities are close to the circadian threshold (Duffy & Wright, 2005). In contrast, sunlight, even on very overcast days, is >2000 lux. Thus, we can consider 1000 lux as a reliable level to differentiate between artificial and natural light. It was surprising that our young subjects were only exposed to light brighter than 1000 lux for 1 h:18 min during the day, and 20 mins during the natural night. These values are in the range of light exposure values for young adults in industrialized countries, most of whom typically receive only 20-120 mins of daily light exposure >1000 lux (Espiritu et al., 1994; Hebert et al., 1998; Mishima et al., 2001; Savides et al., 1986).

The 24-h pattern of light exposure was similar to that previously described (Emens et al., 2009; Goulet et al., 2007; Hebert et al., 1998; Savides et al., 1986), with maximum light exposure occurring at midday, which coincides with a break at work, as has previously observed by other authors (Heil & Mathis, 2002; Okudaira et al., 1983). WT shows a circadian rhythm with higher values during sleep and lower values during waking hours, as has also already been described (Ortiz-Tudela et al., 2010; Sarabia et al., 2008). In fact, WT is known to oscillate in parallel to sleep probability (Sarabia et al., 2008). However, simultaneous recording of all three variables showed light exposure displays a daily pattern with an approximately inverse relationship to that observed for WT and sleep probability.

A common characteristic of natural light exposure is the high variability of its intensity, which contrasts with the more constant values observed under laboratory conditions or artificial lighting. Acute changes in light exposure are associated with transient changes in WT in the opposite direction. Thus, positive changes in light intensity diminish WT, and consequently sleepiness, and *vice versa.* This inverse relationship could be explained by the alerting properties of light through sympathetic activation, inducing blood-vessel constriction and, in turn, reduction in skin temperature (Buijs et al., 2003). Other authors have demonstrated the short-term effect of bright-

light exposure on reducing melatonin and sleepiness and on increasing alertness, heart rate, and core temperature (Cajochen et al., 2005; Ishibashi et al., 2010; Phipps-Nelson et al., 2009). Laboratory studies have suggested that short, intermittent periods of exposure to bright light, such as those characteristic of our modern life style, may have a much greater impact on circadian entrainment than has previously been recognized (Hebert et al., 1998; Okudaira et al., 1983; Savides et al., 1986).

What can be considered as a healthy circadian pattern is very difficult to define, and is still a matter of discussion, although most authors agree with regard to which parameters can characterize a healthy circadian rhythm. They include: circadian pattern regularity across different days (high interdaily stability), reduced fragmentation (or low intradaily variability), high amplitude, a period close to 24 h, and a circadian phase correctly aligned with environmental cues (Myers & Badia, 1995; Van Someren et al., 1999). Recently, in order to classify individuals according to their circadian system functionality, we proposed a scoring index (Ortiz-Tudela et al., 2010) we call the circadian function index (CFI), which combines three non-parametric indices, stability, fragmentation, and amplitude, as previously proposed by Van Someren and coworkers (1999).

In the present work, we have found the best circadian rhythms for WT and sleep are associated with high regularity in the light-dark cycle, high RA, and high LQI, whereas high fragmentation and nocturnal light exposure are associated with the worst circadian sleep patterns. From these results, it seems that it may be possible to modify specific characteristics of the light-dark exposure in order to improve the organization of the circadian system. For example, improvement in the CFI for WT can be achieved by an increase in light regularity. But if the objective is to induce a phase change, morning light is associated with a phase advance.

It has been reported that environmental illumination is inversely correlated with insomnia (Hood et al., 2004; Mishima et al., 2001). According with our results, some cyclic parameters of light exposure are associated with the sleep-wake 24-h rhythm. Thus, the light-dark pattern stability, day-night contrast, morning light, evening light, and LQI are associated with better sleep-wake characteristics, whereas nocturnal light exposure and fragmentation is related to phase delay and worse sleep-wake pattern characteristics. These results confirm previous observations indicating that natural

bright-light exposure improves sleep quality and mood (Dumont & Beaulieu, 2007; Wirz-Justice et al., 1996), as previously demonstrated in light-controlled studies with blue-enriched white light (Glickman et al., 2006; Viola et al., 2008) and bright-light therapy (Even et al., 2008; Kirisoglu & Guilleminault, 2004). However, our results show the circadian clock is influenced by the overall pattern of light-dark exposure, and not merely by isolated instances of bright light, as it was thought from laboratory studies. Our results also confirm the importance of prior light history when analyzing subsequent light synchronizing effects, as already suggested (Smith & Eastman, 2009).

Because modern humans spend most of their time indoors with only short, intermittent periods of exposure to natural sunlight, and since artificial light sources have a spectral distribution unlike that of natural light, it would be important to record the spectral composition of light to which the subjects are exposed using ambulatory devices, in order to know how it affects the human circadian system, as seems to be the case (Duffy & Wright, 2005).

In summary, we have demonstrated that acute changes in light intensity are associated with opposite, transient changes in WT. High values of light intensity plus morning, evening, and day-night contrast were associated with higher scores for rhythmic parameters related to good circadian WT and sleep rhythms, while nocturnal light exposure and fragmentation were associated with lower scores. However, the highest CFI scores for WT or sleep-wake rhythms were not necessarily associated with differences in light-dark exposure (data not shown). Accordingly, from our results we can conclude the combined use of a portable lux meter, along with skin WT and sleep recordings, allow the non-invasive ambulatory monitoring of the circadian system status and light-dark exposure in subjects under normal living conditions and with minimal discomfort. Naturalistic studies using 24-h ambulatory light monitoring can provide very relevant information on the characteristics of the LD cycle, the most important *zeitgeber* in humans, which is essential for maintaining healthy circadian functions. The corresponding modification of light-exposure patterns may help prevent and/or reverse some health problems generated by circadian disruption.

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REFERENCES

Atkinson G, Edwards B, Reilly T, Waterhouse J. (2007). Exercise as a synchronizer of human circadian rhythms: an update and discussion of the methodological problems. Eur J Appl Physiol. 99:331-41.

Brainard GC, Rollag MD, Hanifin JP. (1997). Photic regulation of melatonin in humans: Ocular and neural signal transduction. J Biol Rhythms. 12:537-46.

Buijs RM, Kalsbeek A. (2001). Hypothalamic integration of central and peripheral clocks. Nature Rev Neurosci. 2:521-6.

Buijs RM, la Fleur SE, Wortel J, Heyningen C, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol. 464:36-48.

Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, Orgül S, Wirz-Justice A. (2005). High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 90:1311-6.

Campbell SS, Kripke DF, Gillin JC, Hrubovcak JC. (1988). Exposure to light in healthy elderly subjects and alzheimer's patients. Physiol Behav. 42:141-4.

Cole RJ, Kripke DF, Wisbey J, Mason WJ, Gruen W, Hauri PJ, Juarez S. (1995). Seasonal variation in human illumination exposure at two different latitudes. J Biol Rhythms. 10:324-34.

Dijk DJ, Archer SN. (2009). Light, sleep, and circadian rhythms: Together again. PLoS Biol. 7:e1000145. doi:10.1371/journal.pbio.1000145.

Duffy JF, Wright KP. (2005). Entrainment of the human circadian system by light. J Biol Rhythms. 20:326-38.

Duguay D, Cermakian N. (2009) The crosstalk between physiology and circadian clock proteins. Chronobiol Int. 26(8):1479-513.

Dumont M, Beaulieu C. (2007). Light exposure in the natural environment: Relevance to mood and sleep disorders. Sleep Med. 8:557-65.

Emens JS, Yuhas K, Rough J, Kochar N, Peters D, Lewy AJ. (2009). Phase angle of entrainment in morning- and evening-types under naturalistic conditions. Chronobiol Int. 26:474-93.

Erren TC, Reiter RJ. (2009). Light hygiene: Time to make preventive use of insights-old and new-into the nexus of the drug light, melatonin, clocks, chronodisruption and public health. Med Hypotheses. 73:537-41.

Espiritu RC, Kripke DF, Ancoli-Israel S, Mowen MA, Mason WJ, Fell RL, Klauber MR, Kaplan OJ. (1994). Low illumination experienced by San Diego adults: Association with atypical depressive symptoms. Biol Psychiatry. 35:403-7.

Even C, Schroder CM, Friedman S, Rouillon F. (2008). Efficacy of light therapy in nonseasonal depression: A systematic review. J Affect Disord. 108:11-23.

Francis G, Bishop L, Luke C, Middleton B, Williams P, Arendt J. (2008). Sleep during the antarctic winter: preliminary observations on changing the spectral composition of artificial light. J Sleep Res. 17:354-60.

Garaulet M, Madrid JA. (2010). Chronobiology: Influences on metabolic syndrome and cardiovascular risk. Curr Cardio Risk Rep. 4:15-23.

Glickman G, Byrne B, Pineda C, Hauck WW, Brainard GC. (2006). Light therapy for seasonal affective disorder with blue narrow-band Light-Emitting Diodes (LEDs). Biol Psychiatry. 59:502-7.

Goulet G, Mongrain V, Desrosiers C, Paquet J, Dumont M. (2007). Daily light exposure in morning-type and evening-type individuals. J Biol Rhythms. 22:151-8.

Gronfier C, Wright KP Jr, Kronauer RE, Czeisler CA. (2007). Entrainment of the human circadian pacemaker to longer-than-24-h days. Proc Natl Acad Sci USA. 104:9081-6.

Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, Barnard AR, Cahill H, Badea TC, Zhao H, Hankins MW, Berson DM, Lucas RJ, Yau K-W Hattar S. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature Lett. 453:102-6.

Hebert M, Dumont M, Paquet J. (1998). Seasonal and diurnal patterns of human illumination under natural conditions. Chronobiol Int. 15:59-70.

Heil DP, Mathis SR. (2002). Characterizing free-living light exposure using a wrist-worn light monitor. Appl Ergon. 33:357-63.

Hood B, Bruck D, Kennedy G. (2004). Determinants of sleep quality in the healthy aged: the role of physical, psychological, circadian and naturalistic light variables. Age Ageing. 33:159-65.

Ishibashi K, Arikura S, Kozaki T, Higuchi S, Yasukouchi A. (2010). Thermoregulatory effect in humans of suppressed endogenous melatonin by pre-sleep bright-light exposure in a cold environment. Chronobiol Int. 27:782-806.

Kavanau JL. (1962). Twilight transitions and biological rhythmicity. Nature. 194:1293-5.

Khalsa SB, Jewett ME, Cajochen C, Czeisler CA. (2003). A phase response curve to single bright light pulses in human subjects. J Physiol. 549:945-52.

Kirisoglu C, Guilleminault C. (2004). Twenty minutes versus forty-five minutes morning bright light treatment on sleep onset insomnia in elderly subjects. J Psychosom Res. 56:537-42.

Krauchi K, Cajochen C, Werth E, Wirz-Justice A. (2000). Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol. 278:R741-8.

Mendoza J. Circadian clocks: Setting time by food. (2007). J Neuroendocrinol. 19:127-37.

Middleton B, Stone BM, Arendt J. (2002). Human circadian phase in 12:12 h, 200: <8 lux and 1000: <8 lux light-dark cycles, without scheduled sleep or activity. Neurosci Lett. 329:41-4.

Mishima K, Okawa M, Shimizu T, Hishikawa Y. (2001). Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. J Clin Endocrinol Metab. 86:129-34.

Mistlberger RE, Skene DJ. (2004). Social influences on mammalian circadian rhythms: Animal and human studies. Biol Rev. 79:533-56.

Moore RY, Speh JC, Leak RK. (2002). Suprachiasmatic nucleus organization. Cell Tissue Res. 309:89-98.

Mormont MC, Langouët AM, Claustrat B, Bogdan A, Marion S, Waterhouse J, Touitou Y, Lévi F. (2002). Marker rhythms of circadian system function: A study of patients with metastatic colorectal cancer and good performance status. Chronobiol Int. 19:141-55.

Mottram V, Middleton B, Williams P, Arendt J. (2011). The impact of bright artificial white and 'blue-enriched' light on sleep and circadian phase during polar winter. J Sleep Res. 20:154-61.

Myers BL, Badia P. (1995). Changes in circadian rhythms and sleep quality with aging: Mechanisms and interventions. Neurosci Biobehav Rev. 19:553-71.

Okudaira N, Kripke DF, Webster JB. (1983). Naturalistic studies of human light exposure. Am J Physiol Regul Integr Comp Physiol. 245:R613-5.

Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. (2010). A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comput Biol. 6:e1000996. doi:10.1371/journal.pcbi.1000996.

Pauley SM. (2004). Lighting for the human circadian clock: Recent research indicates that lighting has become a public health issue. Med Hypotheses. 63:588-96.

Phipps-Nelson J, Redman JR, Schlangen LJM, Rajaratnam SMW. (2009). Blue light exposure reduces objective measures of sleepiness during prolonged nighttime performance testing. Chronobiol Int. 26:891-912.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 25:1911-29.

Rahman SA, Kollara A, Brown TJ, Casper RF. (2008). Selectively filtering short wavelengths attenuates the disruptive effects of nocturnal light on endocrine and molecular circadian phase markers in rats. Endocrinol. 149:6125-35.

Reiter RJ, Tan D, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. (2007). Light at night, chronodisruption, melatonin suppression, and cancer risk: A review. Crit Rev Oncog. 13:303-28.

Sarabia JA, Rol MA, Mendiola P, Madrid JA. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate of new index of the circadian system. Physiol Behav. 95:570-80.

Savides TJ, Messin S, Senger C, Kripke D. (1986). Natural light exposure of young adults. Physiol Behav. 38:571-4.

Skene DJ, Lockley SW, Thapan K, Arendt J. (1999). Effects of light on human circadian rhythms. Reprod Nut Dev. 39:295-304.

Smith MR, Eastman CI. (2009). Phase delaying the human circadian clock with blue-enriched polychromatic light. Chronobiol Int. 26:709-25.

Stratmann M, Schibler U. (2006). Properties, entrainment, and physiological functions of mammalian peripheral oscillators. J Biol Rhythms. 21:494-506.

Tang IH, Murakami DM, Fuller CA. (1999). Effects of square-wave and simulated natural light-dark cycles on hamster circadian rhythms. Am J Physiol Regul Integr Comp Physiol. 276:R1195-202.

Turner PL, Mainster MA. (2008). Circadian photoreception: ageing and the eye's important role in systemic health. Br J Ophthalmol. 92:1439-44.

Turner PL, Van Someren EJW, Mainster MA. (2010). The role of environmental light in sleep and health: Effects of ocular aging and cataract surgery. Sleep Med Rev. 14:269-80.

Van Someren EJW. (2000). More than a marker: Interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. Chronobiol Int. 17:313-54.

Van Someren EJW, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. (1999). Bright light therapy: Improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int. 16:505-18.

Viola AU, James LM, Schlangen LJ, Dijk DJ. (2008). Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. Scand. J Work Environ Health. 34:297-306.

Weather station at the University of Murcia: https://estacion.um.es/.

Wirz-Justice A, Graw P, Krauchi K, Sarrafzadeh A, English J, Arendt J, Sand L. (1996). 'Natural' light treatment of seasonal affective disorder. J Affect Disord. 37:109-20.

3.4. EXPERIMENTAL CHAPTER 4

EFECTOS DE LA VARIACIÓN DIARIA EN LA TEMPERATURA AMBIENTAL SOBRE LA TEMPERATURA DE LA PIEL Y LA PRESIÓN ARTERIAL.

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3.4. EFECTOS DE LA VARIACIÓN DIARIA EN LA TEMPERATURA AMBIENTAL SOBRE LA TEMPERATURA DE LA PIEL Y LA PRESIÓN ARTERIAL.

Está ampliamente aceptado que la exposición al frío aumenta la presión arterial y, por tanto, el riesgo cardiovascular. El mecanismo subyacente parece deberse a los efectos adversos de los ajustes termorreguladores ya que el enfriamiento de la piel provoca vasoconstricción cutánea, centralización de la sangre y, a continuación, un incremento de la presión y viscosidad sanguínea. Sin embargo, existe una carencia de estudios longitudinales y de medidas de la temperatura ambiental a la que se ve expuesta la persona. Por tanto, el propósito de este trabajo fue analizar, en invierno y verano, la asociación temporal de la temperatura ambiental, la temperatura de la piel, la presión arterial y la frecuencia cardiaca en mujeres sanas durante el día en condiciones ambulatorias. Para ello se reclutaron 60 mujeres jóvenes en un diseño cruzado invierno/verano y se registraron ambulatoriamente durante 26 horas las variables termo- y cardiofisiológicas junto a la temperatura ambiental a nivel de la persona. Se analizó el periodo diurno (09:30-20:30) con el fin de que todos los sujetos estuvieran despiertos y favorecer una mayor variabilidad en la temperatura ambiental. Además, se seleccionó cada exposición a exteriores en la que hubiera al menos una medida de presión arterial, junto a los periodos previo y posterior con medidas de presión arterial para analizar los efectos a corto plazo de la temperatura ambiental sobre las variables termo- y cardiofisiológicas. Las variaciones a corto plazo de la temperatura ambiental que experimenta la persona modificaron la presión arterial mediante cambios en la temperatura de la piel distal y no de la proximal. Estos cambios son independientes de la temperatura media de la persona y de su aclimatación. Sin embargo, los cambios estacionales en la temperatura ambiental modificaron la temperatura de la piel sin alterar la presión arterial. En conclusión, la temperatura ambiental modifica la temperatura de la piel distal y ésta, a su vez, la presión arterial. Sin embargo, la aclimatación en mujeres jóvenes contrarresta los cambios estacionales esperados.

Key words: presión arterial, temperatura periférica distal, temperatura periférica proximal, temperature ambiental, path analysis, exposición a exteriores.

INTRODUCCION

Existe una mayor incidencia de enfermedades vasculares (infarto, ictus entre otras) y mortalidad en la población general durante el invierno (Alpérovitch et al., 2009; Brook et al., 2011; Goodwin et al., 2001; Keatinge, 2002; Modesti, 2013). Los mecanismos subyacentes no se comprenden completamente; sin embargo, los efectos adversos de los ajustes termoregulatorios parecen tener un papel importante. Por ejemplo, investigaciones observacionales, fisiológicas y epidemiológicas muestran que la exposición a frío promueve una vasoconstricción cutánea con la centralización de la sangre, aumento de la presión arterial (PA) y hemoconcentración (Brook et al., 2011; Keatinge et al., 1984; Keatinge, 2002).

El modelo termofisiológico core/shell desarrollado por Aschoff en 1956 proporciona una explicación posible a los descubrimientos mencionados anteriormente. En un medio frío el shell es grande y protege el core del enfriamiento (centralización de la sangre). En un medio cálido, y durante el sueño, el shell es pequeño y el cuerpo es propenso a enfriarse. (Kräuchi & Deboer, 2010). Las regiones distales están especialmente diseñadas para perder calor gracias a sus propiedades físicas (anastomosis arteriovenosas) y fisiológicas (alta relación superficie/volumen). La utilización de sensores inalámbricos de temperatura para medir la temperatura de la piel (TP) distal y proximal proporciona información sobre el estado termofisiológico del cuerpo humano. Por ejemplo, TP distal fria en relación a la TP proimal indica un shell grande (Kräuchi & Deboer, 2010).

La relación entre temperature ambiental y PA se ha estudiado principalmente en diseños transversales; sin embargo, para la obtención de información más fiable y precisa son necesarios estudios longitudinales (Modesti, 2013). A pesar de las evidencias de que la temperatura ambiental fría influye en la PA es sorprendente que la mayoría de estudios comparen la PA con los datos de temperatura ambiental obtenidos de institutos metereológicos. Esta limitación podría ser mejorada fácilmente midiendo la temperatura ambiental en el entorno de la persona (TAP) (Modesti, 2013). Además, la TAP afecta al cuerpo mediante la piel, por lo que parece lógico incluir también la medida de la temperatura de la piel con respecto a los cambios estacionales en PA. Al incluir estas medidas directas se puede mejorar el conocimiento sobre como los cambios en TAP se transforman en cambios en PA

En un estudio ambulatorio reciente se demostró que la PA media exhibe un patrón inverso a la TP sobretodo en las regiones distales (Blazquez et al., 2012; Kräuchi et al., 2012). Desafortunadamente, no se realizaron medidas de TAP impidiendo un análisis detallado y la interpretación de los cambios que produce TAP en TP y PA. El objetivo principal de este trabajo due analizar la asociación temporal entre las variables en individuos jóvenes sanos en invierno y verano. En un primer paso se estudió la variación durante 11-hr y posteriormente se realizó un análisis detallado de la exposición a exteriores.

MATERIAL Y MÉTODOS

Población de estudio y diseño

Se reclutaron mujeres jóvenes sanas mediante un anuncio en la plataforma de la universidad de Basel informando a las voluntarias potenciales de la oportunidad de participar en un proyecto de investigación. Para obtener una muestra con gran variabilidad en la respuesta vascular al frío se seleccionaron mujeres con incomodidad por frío en las extremidades (IFE) y sin ella. Se le preguntó por incomodidad térmica, que generalmente se percibe en invierno, a todos los sujetos de estudio potenciales con el cuestionario de *screening* de IFE (Kräuchi et al., 2012). Para la clasificación se hacían 3 preguntas: 1) ¿Sufre de manos y/o pies fríos con más intensidad que otras mujeres? 2) Durante el invierno, ¿Qué intensidad tiene su malestar en relación con las manos frías? 3) Durante el invierno, ¿Qué intensidad tiene su malestar en relación con pies fríos? Para ser clasificado como IFE debían responder con "sí" a la primera cuestión y "mucho" o "bastante" a las preguntas 2 y 3 mientras que un sujeto control debía responder con "no" a la primera cuestión y "poco" o "nada" a las dos preguntas restantes. Este cuestionario se envió a las 394 voluntarias, de las cuales 194 completaron el cuestionario. De entre ellas se seleccionaron 60 mujeres residentes en Basel (Suiza) y se monitorizaron durante 26 horas en un diseño cruzado invierno/verano. De estas, 39 mujeres comenzaron en invierno (02/12/11-09/03/12) y terminaron en verano (04/06/12-12/09/12), momento en el cual comenzaron 21 para terminar en el invierno siguiente (04/12/12-14/03/13). 52 mujeres completaron el estudio complete, 6 abandonaron en el primer invierno y dos durante el verano. Las características de los sujetos incluyendo el patron de sueño están en la Tabla 1

Es estudio, incluyendo todos los procedimientos experimentales y el consentimiento informado fueron aprobados por el comité de ética local para investigación en humanos (Ethikkommission beider Basel). Antes de la inclusión en el estudio, cada sujeto firmó el consentimiento informado y se le informó explícitamente de que podía abandonar el estudio en cualquier momento. Sin embargo, los únicos sujetos que abandonaron el estudio lo hicieron por razones de horario.

Para obtener un grupo de mujeres homogéneo respecto a las hormonas sexuales aquellas mujeres que no tomaban contraceptivos y con un ciclo menstrual estable fueron monitorizadas durante la fase luteínica, definida como el intervalo entre el día 14 y el final del ciclo menstrual. Se les solicitó a todas las voluntarias que mantuvieran su estilo y ritmo de vida habital evitando las actividades deportivas y la ducha o baño. Los criterios de exclusión se centraron en:

- IMC < 17 kg/m2 y > 28 kg/m2.
- Enfermedad física o mental ya fuese aguda o crónica.
- Alergías cutáneas, como por ejemplo al Níquel.
- Sin medicación alguna durante al menos un mes (exceptuando los anticonceptivos).
- Ciclo menstrual irregular (>±2 días).
- Trabajo a turnos en los últimos 3 meses o un viaje transmeridional en el último mes.
- Embarazo (se realizó un test de embarazo antes de comenzar el estudio).
- Fumador, abstinencia durante al menos 3 meses.
- Consumo de drogas.
- Consumo excesivo de cafeína (≤ 3 tazas al día) o alcohol (> 1 vaso al día).
- Hipertensión.
- Exceso de actividades deportivas (>3 días por semana).

Diarios de sueño

En el momento del despertar se apuntaba el momento estimado de irse a dormir y levantarse, de apagado y encendido de las luces, latencia de sueño, problemas de sueño (despertares durante la noche), calidad del sueño, sueño reparador y somnolencia en el momento de irse a dormir. Además, se pidió que anotaran las horas de actividad, el

tiempo que pasaban en exteriores y el momento de eventos especiales como la retirada de un sensor.

Medidas de presión arterial

La monitorización de la PA durante 24h con análisis integrado de la presión de pulso (APP) se realizó mediante un sistema oscilométrico ambulatorio (Mobil-O-Graph NG®, IEM GmbH, Stolberg, Germany). Se registraron presión arterial sistólica, diastólica y media, frecuencia cardiaca y las variables derivadas del APP (velocidad de la presión de pulso y PA sistólica y diastólica centrales) cada 15 minutos durante la fase activa y cada 30 minutos en la fase de descanso.

Medidas de la temperatura de la piel

El registro de la temperatura se realizó mediante *data loggers* (DS 1922L, Thermochron iButtons®; resolution 0.0625°C, accuracy 0.5°C; Maxim, Dallas, USA) en intervalos de un minuto, los cuales se fijaron a la piel con esparadrapo (Fixomull®; Beiersdorf, Hamburg, Germany) en ambos lados del cuerpo, izquierdo y derecho en las siguientes regiones: interior del talón, interior de la muñeca, muslo (en el centro del cuádriceps) y la región infraclavicular junto a un sensor de temperatura adicional en el estómago y otro en el esternón. El valor medio de tobillo, muñeca y muslo se consideró TP distal mientras que las regiones infraclaviculares, esternón y estómago promedidas se consideraron TP proximal.

Medidas de temperatura ambiental y humedad

La TAP y la humedad se monitorizaron mediante *data loggers* (DS 1923H, Hygrochron iButtons®; temperature resolution: 0.0625°C, accuracy: 0.5°C; relative humidity resolution: 0.04%, accuracy: 5%; Maxim, Dallas, USA) con una frecuencia de muestreo de un minuto, colocado en una malla por fuera de la ropa colgando del cinturón en la cadera derecha. Durante el sueño la bolsa se colocó en la mesilla al lado de la cama.

Análisis del periodo activo

Se excluyeron del análisis los datos de media hora antes de de la colocación y retirada de los sensores, reemplazando los datos faltantes por la media de los 30 minutos anteriores y posteriores (solamente se perdió el 0.2% de la información). Para analizar

la relación temporal entre la PA durante el día, la TP y TAP se promediaron los valores en intervalos de 1 hora entre las 09:30 y las 20:30.

Análisis de la exposición a exteriores

De acuerdo a los momentos anotados en el diario la última medida de PA antes de salir al exterior se nombró "pre-exposición" (PRE), durante la salida al exterior se nombró "exposición" (EXP, donde si había más de una medida se promediaba entre ellas) y la primera medida de vuelta al interior "post-exposición" (POST). Para TP se realizó el promedio de los valores desde 2 minutos antes hasta 2 minutos después de cada segmento temporal (PRE, EXP y POST). Si un sujeto tenía más de una salida al exterior se promediaba para todas las variables por segmento temporal y por estación.

Análisis estadístico

Para comprobar si cada variable presentaba un patrón durante el día (entre las 09:30 y las 20:30) o entre los segmentos temporales (PRE, EXP y POST) se realizó un análisis de la variaza para medidas repetidas (rANOVA) para todas las variables medidas con los factores hora del día (DAYTIME) o segmento temporal (EXPOSURE) y estación (SEASON) y los sujetos como factor aleatorio (modelo de efectos mixtos). Los análisis de regression individuals entre TAP, TP y PA se realizaron mediante un modelo de efectos mixtos con los factores estación (SEASON) y coeficiente, mientras que los sujetos se presentaron como factor aleatorio. El modelo de efectos mixtos se realizó mediante R 3.0.1.

Basado en descubrimientos previos (Kräuchi et al., 2012), se intentó explicar los cambios en PA mediante cambios en TAP y TP (Figura 2) usando modelo estructural multinivel (Preacher et al., 2010). Se realizó un modelo de dos niveles con las medidas de todos los participantes. En este modelo, la media de cada persona se utiliza como punto central y las medidas de cada persona se denotan como desviaciones de la media individual. Existe un interés particular en los cambios intra-individuales entre las 09:30 y las 20:30 para lo cual se sustrajo un polinomio de tercer grado para eliminar la tendencia temporal de las variables. La ecuación estructural multinivel fue analizada utilizando Mplus 5.2 (Muthén and Muthén, 2010).

3.4. Air temperature, skin temperatures and blood pressure

Se muestran los valores medios con sus EEM, DE, intervalos de confianza al 95% y los valores de p. Un valor de $p < 0.05$ se consideró estadísticamente significativo.

RESULTADOS

Los análisis estadísticos se realizaron en 4 pasos. Primero, se buscaron diferencias estacionales utilizando todos los datos tanto en los patrones como en los valores medios diarios. Para este propósito se promediaron los valores de verano e invierno en 11 intervalos de 1 hora entre las 09:30 y las 20:30 (todos los sujetos estaban despiertos en dicho intervalo). En un segundo paso, se realizó un análisis de correlación bivariado y multivariado entre las variaciones intraindividuales de TAP, TP y PA. Tercero, con el fin de descubrir una posible relación causal entre las variaciones intra-individuales de TAP, TP y PA se realizó un análisis de mediación. Finalmente, para examinar los efectos estacionales en detalle se realizó un análisis pormenorizado de los efectos de la exposición al exterior de las variables TAP, TP y PA durante el periodo del día.

Las características de los sujetos están detalladas en la Tabla 1, en la que los sujetos con y sin IFE no difirieron respecto a su asociación intraindividual en las variaciones de TAP, TP y PA. Por ello, y para una presentación de los datos más clara y concisa se mostrarán los datos sin el factor IFE. El análisis del perfil de 24h complete (incluyendo la fase de sueño) con respecto a TP y PA de ambos grupos se mostrará en otro trabajo en detalle, ya que una presentación completa de estos resultados estaría más allá del propósito de este trabajo.

Análisis de las variaciones diarias, niveles medios diarios y diferencias invierno-verano

La figura 1 muestra los patrones temporales de las variables durante la fase activa (09:30-20:30) de invierno y verano ajustados al valor medio de cada sujeto durante las once horas analizadas representado en el gráfico de barras (estadística en la Tabla 2). Ninguna de las variables presentó diferencias significativas entre los patrones temporales de invierno y verano.

Variable	SEASON (S)		DAYTIME (D)		S x D	
	F (df)	P	F (df)	P	F (df)	P
PET	253(1,1127)	**0.001**	4.0(10,1127)	**0.001**	0.61(10,1108)	0.81
Proximal ST	20.0(1,1161)	**0.001**	8.6(10,161)	**0.001**	1.20(10,1151)	0.27
Distal ST	64.4(1,1161)	**0.001**	5.3(10,1161)	**0.001**	0.83(10,1151)	0.60
MAP	0.1(1,1157)	0.76	1.3(10,1147)	0.25	0.26(10,1147)	0.98
DBP	1.0(1,1153)	0.31	1.90(10,1153)	**0.039**	0.54(10,1143)	0.86
SBP	0.36(1,1159)	0.55	0.80(10,1159)	0.63	0.41(10,1149)	0.94
PP	3.67(1,1153)	0.056	0.86(10,1153)	0.57	0.80(10,1143)	0.63
HR	1.42(1,1153)	0.23	2.0(10,1153)	**0.028**	0.44(10,1143)	0.93
PWV	0.02(1,1146)	0.90	1.10(10,1146)	0.36	0.71(10,1136)	0.72
PEH	211(1,1118)	**0.001**	3.5(120,1118)	**0.001**	0.76(10,1108)	0.66
OUTDOORS	0.01(1,1257)	0.98	4.80(10,1257)	**0.001**	0.54(10,1247)	0.86

Tabla 2. Tabla ANOVA basada en el modelo de efectos mixtos del patron entre 09:30 – 20:30h con factores SEASON y DAYTIME. SxD n.s.; Los efectos principales se chequearon sin interacción. La significación estadística se marca en negrita ($p<0.05$).

La TAP mostró un patrón temporal altamente significativo durante el periodo del día con valores más altos en la tarde que en la mañana (DAYTIME, $p<0.001$). Igualmente, la TP proximal fue mayoy por la tarde temprano y menor por la mañana (DAYTIME, $p<0.001$). La TP distal mostró un patron bimodal con el mínimo en la mañana (12:00) y el máximo por la tarde (16:00) (DAYTIME, $p<0.001$). La PA media no mostró un patrón termporal significativo durante el día (DAYTIME, n.s.), aunque mostró una tendencia a un patrón bimodal inverso al de TP distal. Los patrones diarios de PA sistolica (DAYTIME, n.s.) y diastólica (DAYTIME, $p<0.05$) mostraron un perfil similar a la PA media. La presión de pulso (PP), la velocidad de la onda de pulso (VOP y el índice de aumento no mostraron ningún efecto significativo. Un análisis posterior reveló que los sujetos pasaron más tiempo en exteriores por la mañana que por la tarde (DAYTIME, $p<0.001$), lo cual podría estar relacionado con el patrón de frecuencia cardiaca (DAYTIME, $p<0.03$). La humedad ambiental mostró un patron significativo (DAYTIME, $p<0.001$) e inverso al patrón diario de TAP, como ejemplifica la correlación de los 11 intervalos horarios intraindividual ($r=-0.617$ en invierno y $r=-0.606$ en verano, $p<0.001$, $n=52$ sujetos, 11 puntos horarios). Con el fin de evitar problemas de colinealidad y ya que todas las variables estaban más relacionadas con la TAP que con la humedad ambiental (datos no mostrados) se decidió excluir la humedad del análisis multivariante (ver a continuación).

El análisis de las diferencias estacionales en los valores medios (Figura 1 y Tabla 2) muestra una TAP 5ºC mayor en verano (SEASON, p<0.001). En contraste, la TP proximal fue menor en verano (0.25°C; SEASON, p<0.001), mientras que la TP distal fue mayor (0.75°C; SEASON, p<0.001). Además, la humedad fue mayor en verano (12.5%; SEASON, p<0.001), mientras que ninguna variable cardiofisiológica mostró cambios en los valores medios.

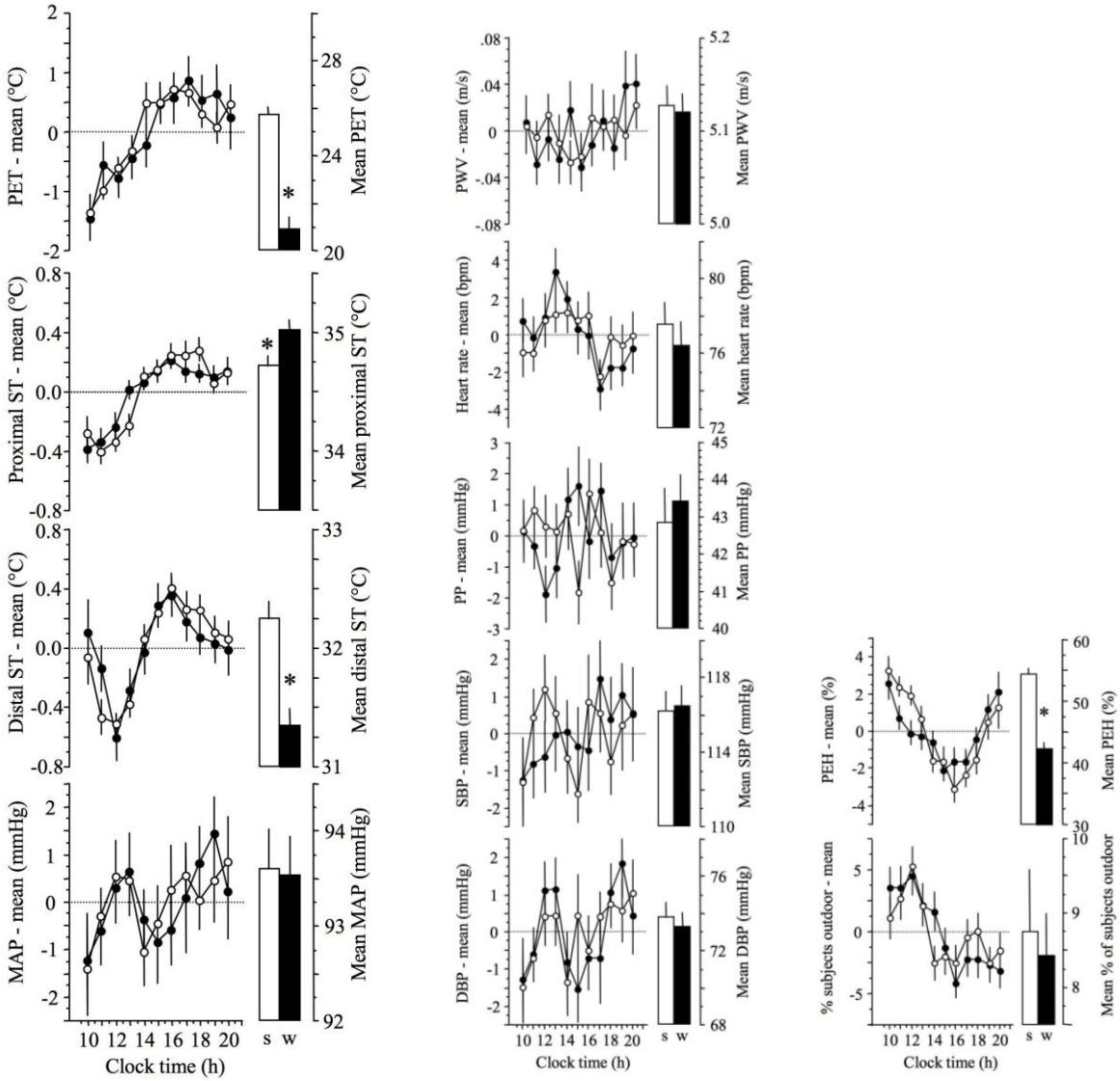

Figura 1. Patrón temporal de presión arterial y temperature de la piel y ambient. De arriba hacia abajo temperatura ambiental en el entorno de la persona (TAP), TP proximal, TP distal y PA media (MAP) en la columna izquierda; en la columna central la velocidad de la onda de presión (VOP), frecuencia cardiaca, presión de pulso, presión arterial sistólica y diastólica; humedad ambiental y porcentaje de sujetos en exteriores aparecen en la columna de la derecha. Cada variable se ajustó al valor medio del individuo y se muestra en intervalos horarios entre 09:30 y 20:30, a su lado el valor medio diario representado en barras. Media ± EEM de N=52 sujetos; los valores del verano se muestran con puntos y barras blancas y los de invierno en negro. Para ver la estadística mirar la Tabla 2.

Correlaciones bivariadas y multiples de las variaciones intraindividuales durante el día y por estación

Primero, los valores medios individuales de verano e invierno entre las 09:30 y las 20:30 se restaron de cada uno de los valores medios de las 11 horas (valores ajustados para los valores medios individuales. Figura 1). Además, para eliminar las fluctuaciones lentas en los residuos de las series temporales se ajustó a un polinomio cúbico antes de los siguientes análisis. El análisis de regresión vibariada entre TAP, TP y las variables cardiofisiológicas se realizó mediante un modelo de efectos mixtos. Los resultados incluyendo coeficientes de regresión (pendientes), intervalo de confianza al 95% y nivel de significación para las pendientes se muestra en la Tabla 3. TAP mostró una relación positiva significativa en ambas estaciones con TP proximal y distal aunque la relación con TP distal fue más fuerte que con proximal. La relación de TAP con TP proximal fue mayor en verano que en invierno (Tabla 3). Además, TAP se asoció negativa y significativamente con PA media, pero solo durante el invierno; con una asociaciónmás fuerte durante el invierno como era evidente. Lo que indicó que debían estudiarse separadamente invierno y verano.

La TP distal y proximal mostró una asociación similar con la PA media, siendo esta negativa y significativa en ambas estaciones. Las regresiones bivariadas entre TAP, TP y PA se muestran en la Figura 2 separadas para invierno y verano (estadística en tabla 3).

Variables	Season (S)	Coeff (C)	SEM	-95%CI	+95%CI	p(C)	p(SxC)
PET *vs* Distal ST	Summer	0.165	0.022	0.121	0.208	0.001	n.s.
	Winter	0.182	0.014	0.154	0.209	0.001	
PET *vs* Proximal ST	Summer	0.078	0.015	0.049	0.107	0.001	0.016
	Winter	0.042	0.010	0.021	0.062	0.001	
PET *vs* MAP	Summer	0.115	0.143	-0.169	0.402	n.s.	0.001
	Winter	-0.429	0.083	-0.589	-0.271	0.001	
Distal ST *vs* MAP	Summer	-1.94	0.361	-2.65	-1.23	0.001	n.s.
	Winter	-1.80	0.304	-2.40	-1.20	0.001	
Proximal ST *vs* MAP	Summer	-2.50	0.535	-3.55	-1.45	0.001	n.s.
	Winter	-2.02	0.547	-3.09	-0.949	0.001	

Tabla 3: Análisis de regresión lineal multiple. Regresión lineal multiple para TAP, TP y PA media en invierno y verano despues de eliminar la tendencia polinomial cúbica para el modelo de efectos mixtos.

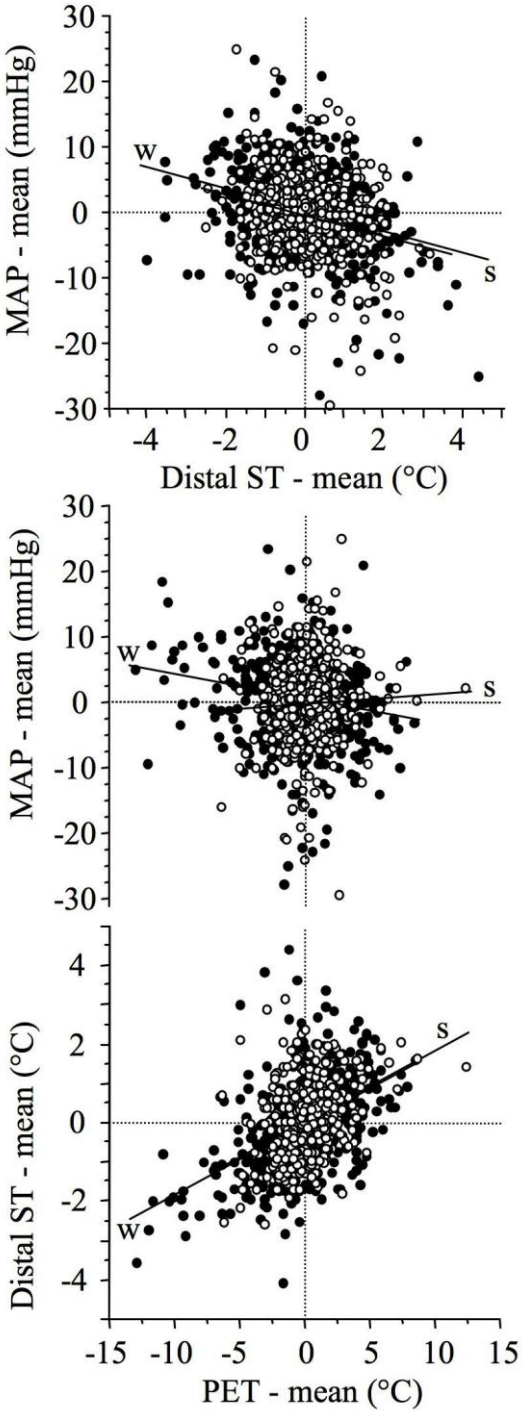

Figura 2: Análisis de regresión lineal bivariada para verano e invierno en intervalos de 1 hora entre 09:30-20:30h. Se muestra la TP distal (abajo), la PA media (medio) en relación con la temperatura ambiental (TAP). La asociación entre PA media y TP distal se muestra en el gráfico superior. Todos los datos (media horaria) fueron ajustados al nivel medio del individuo durante el día en verano e invierno (09:30-20:30). Los valores del verano se muestran como círculos blancos mientras que los del invierno aparecen como círculos negros, la estadística se muestra en la Tabla 3.

Path análisis de TAP, TP y variables cardiofisiológicas

Con el fin de comprobar la relación entre las variables desde un punto de vista más causal se realizaron modelos estructurales con PA media como variable puramente endógena, TP como parcialmente endógena (mediadores) y TAP como puramente exógena. Los posibles efectos de confusión de las fluctuaciones lentas de las series temporales se eliminaron mediante la sustracción del polinomio cúbico que mejor se ajustaba y los resultados del path análisis entre TAP, TP y PA se muestran en la Tabla 4. Todos los efectos 'entre sujetos' no alcanzaron significación estadística y no son presentados.

Para los datos de invierno hubo un efecto significativo total en los sujetos ($p<0.001$) con un incremento de 0.43 mmHg en PA media por cada grado de disminución de TAP (Tabla 4). La vía indirect correspondiente con TP distal fue significativa ($p<0.001$), mientras que no fue significativa con TP proximal ni la vía directa. Lo cual indica que el efecto intraindividuos de variación de TAP sobre PA media está casi completamente mediado por cambios en TP distal. Ya que una disminución de 1ºC en TAP durante el invierno reduce 0.19ºC la TP distal incrementando en 0.31mmHg la PA media (ver Figura 3).

Effects	Winter	Summer
Indirect Effect		
(PET → distal ST → MAP)	**-0.30±0.08, p<0.001**	**-0.25±0.07, p<0.001**
(PET → proximal ST → MAP)	0.01±0.03, n.s.	**-0.15±0.07, p<0.028**
Total Indirect	**-0.31±0.08, p<0.001**	**-0.39±0.09, p<0.001**
Direct Effect		
(PET → MAP)	-0.12±0.12, n.s.	**0.49±0.17, p<0.003**
Total within	**-0.43±0.10, p<0.001**	0.10±0.18, n.s.

Tabla 4. Efectos de la temperatura ambiental en el entorno de la persona (TAP) sobre la presión arterial media (PAM). Los modelos obtenidos se ajustaron perfectamente a los datos (CFI =1, TLI= 1, RMSEA=0). Todos los efectos entre sujetos no alcanzaron significación estadística ($p<0.05$). Nota: Existe un efecto indirecto significativo de TAP sobre TP distal y PA media en ambas estaciones.

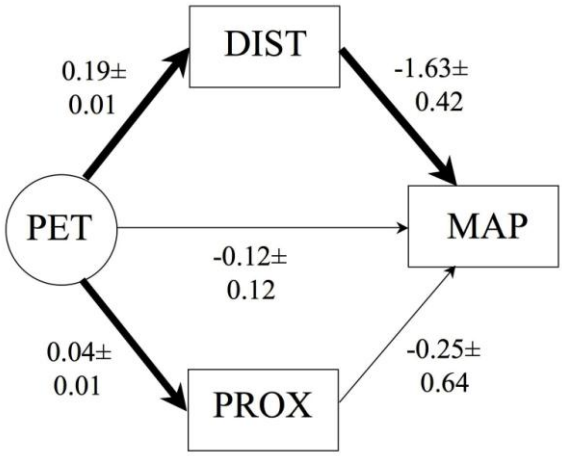

Figura 3. Path analisis. Diagrama de caminos mostrando la relación entre las variaciones intraindividuales en TAP, TP distal (DIST), proximal (PROX) y presión arterial media (MAP) durante invierno. Los caminos significativos ($p<0.001$) se indicant con fleches gruesas (ver Tabla 4), mientras que las flechas finas representan caminos no significativos ($p>0.3$). Los coeficientes de los caminos se muestran junto al camino correspondiente. Nota: Las variaciones individuals de TAP muestran una influencia en PA media vía cambios en TP distal.

Con similitude al análisis de caminos de los datos de invierno los datos del verano mostraron una vía de acción indirecta de TAP sobre PA media mediada por TP distal (Tabla 4). Sin embargo, también hubo un efecto indirect mediado por TP proximal y juntos influían en la misma medida sobre PA media que en el invierno. En contraste al análisis del invierno la TAP durante el verano mostró un efecto directo significativo sobre PA media cancelando los efectos negativos de los caminos indirectos, provocando la ausencia de un efecto total en lo que se llama mediación inconsistente

Análisis de la exposición a exteriores

La figura 4 y la tabla 5 muestran el análisis de exposición a exteriores de las variables principales. Los valores medios de TAP fueron más calidos en verano, por lo que la exposición a exteriores (EXP) aumentó la TAP. Cuando los individuos vuelven a interiors (POST) los valores no se recuperan del todo ($p<0.05$). Durante EXP la TP distal y proximal disminuyó en invierno y aumentó en verano ($p<0.05$) y casi se recuperaron en POST. En relación a los valores de verano de PA media (Figura 4), PA sistólica, diastólica, PP, frecuencia cardiaca y VOP (Figura 4) aumentaron durante EXP en invierno mientras que POST no difirió de PRE (Tabla 5).

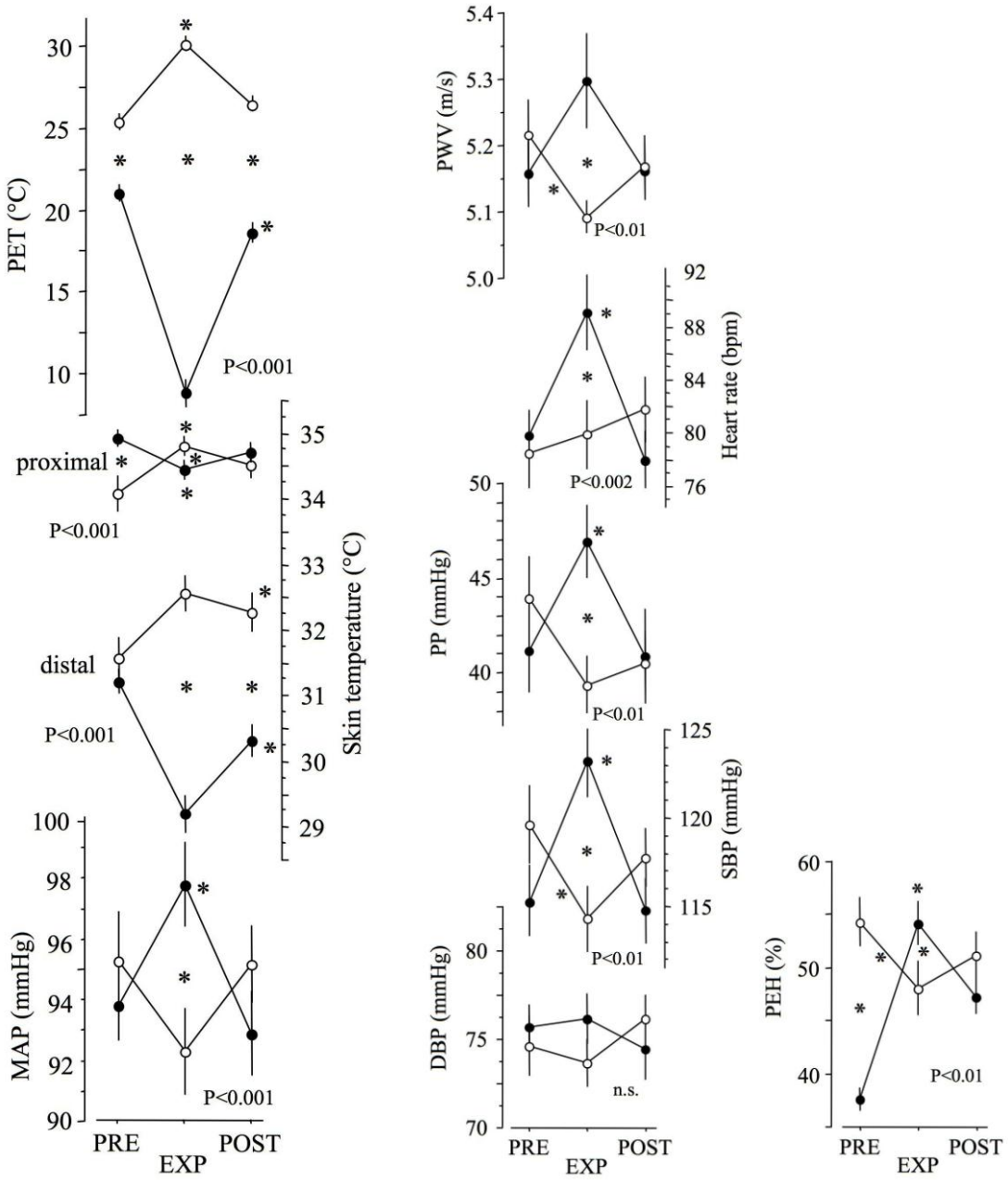

Figura 4. Efectos de la exposición a exteriores. Efectos de pre (PRE), durante (EXP) y post (POST)- exposición a exteriors en TAP, TP proximal y distal y PA media en el panel izquierdo; en velocidad de la presión de pulso (VOP), frecuencia cardiaca, presión de pulso (PP) y PA sistólica y diastólica en el panel central; y en humedad ambiental en el panel derecho durante el invierno (puntos negros) y el verano (puntos blancos). Se representa como media ±EEM, N=52 sujetos, * p<0.05.

Un análisis a posteriori detallado mostró que la PA sistólica, PP y frecuencia cardiaca fueron mayores en invierno durante EXP mientras que en verano solamente mostraron una reducción PA sistólica y VOP. La PA diastólica no reveló ningún efecto significativo. Sin embargo, la humedad ambiental mostró valores menores en invierno en PRE ($p<0.05$). Comparado con PRE, los valores de EXP disminuyeron en verano y aumentaron en invierno, pero no se recuperaron completamente durante POST.

Variable	SEASON (S)		EXPOSURE (D)		S x E	
	F (df)	P	F (df)	P	F (df)	P
PET	42.0(1,158)	**0.001**	27.0(2,158)	**0.001**	4.85(2,158)	**0.001**
Proximal ST	15.6(1,158)	**0.001**	6.3(2,158)	**0.003**	11.6(2,158)	**0.001**
Distal ST	5.9(1,158)	**0.017**	8.4(2,158)	**0.001**	48.3(2,158)	**0.001**
MAP	0.01(1,139)	0.92	11.10(2,139)	0.33	3.60(2,139)	**0.030**
DBP	0.45(1,139)	0.50	0.70(2,139)	0.50	1.44(2,139)	0.24
SBP	0.51(1,139)	0.21	1.59(2,139)	0.47	14.9(2,139)	**0.009**
PP	0.63(1,139)	0.43	1.37(2,139)	0.26	3.51(2,139)	**0.033**
Heart Rate	0.09(1,139)	0.76	0.70(2,139)	0.50	4.30(2,139)	**0.016**
PWV	1.70(1,116)	0.20	2.60(2,116)	0.077	4.85(2,116)	**0.034**
PEH	51.6(1,152)	**0.001**	4.1(1,152)	**0.019**	30.9(2,152)	**0.001**

Tabla 5: ANOVA del modelo de efectos mixtos para exposición a exteriores con los factores SEASON y EXPOSURE. Los análisis de PRE, EXP y POST exposición a exteriores en function de la estación para TAP, TP distal y proximal, PA media, sistólica y diastólica presión de pulso, frecuencia cardiaca, velocidad de la onda de pulso y humedad ambiental. Los valores estadísticamente significativos se muestran en negrita.

DISCUSSION

Este estudio ambulatorio en mujeres jóvenes sanas proporciona información de como las variaciones de TAP afectan la PA en la vida real. Los principales resultados muestran que en ambas estaciones las variaciones a corto plazo de TAP afectan a la PA media indirectamente mediante cambios en la TP, sobretodo distal. En contraste, y a pesar de la diferencia en los niveles medios estacionales no aparecieron diferencias significativas en TAP, TP o PA. Estos resultados confirman estudios previos, pero muestran por vez primera una interpretación de un punto de vista más causal con variables medidas a la vez en los mismos sujetos.

Los patrones temporales durante el día de TP distal y proximal fueron similares entre sí con una cierta relación de fase, mostrando un patrón inverso a la PA media. Esto indica que el gradiente de TP distal menos proximal y, por tanto, la redistribución de la sangre del *core* al *shell* también varía en función de la hora del día, confirmando nuestro estudio previo (Kräuchi et al., 2012). Se sabe que la TP y la PA están influidas por multitud de factores como la actividad, la ingesta de comida y bebida, los cambios posturales o los eventos emocionales (Brook et al., 2011; Goodwin et al., 2001; Jansen & Lipsitz, 1995; Kräuchi et al., 2012; Modesti, 2013), pero también por cambios ambientales como la temperatura o la humedad del aire y la presión atmosférica (Alpérovitch et al., 2009; Brook et al., 2011; Kräuchi et al., 2012). Los patrones diarios observados son una combinación de efectos enmascarantes junto a los patrones endógenos y, por tanto, de la muestra seleccionada para el estudio. En este trabajo nos centramos en un análisis principalmente relacional de las variaciones a lo largo del día entre TAP, TP y PA media.

Con la ayuda del *path* analisis de los datos longitudinales de entre las 09:30 y las 20:30 fue posible discernir que una disminución de 1ºC en TAP promovía un aumento de 0.43 mmHg, resultados similares a los encontrados en estudios previos (Modesti, 2013). La mayor parte del incremento en invierno (0.31±0.08mmHg) se alcanzó mediante la vía indirecta de la TP distal (0.30±0.08mmHg), pero no proximal (0.01±0.03mmHg), mientras que la vía directa de TAP sobre PA media no fue significativa. Estos resultados proporcionaron las primeras indicaciones de una reacción en cadena causal de que las variaciones en la vida real en TAP modifica la TP distal y, por tanto, en PA media. Muchos de los estudios previos epidemiológicos, de laboratorio y ambulatorios habían sugerido está relación, pero sin medir la TAP, TP y PA de forma simultanea (Modesti, 2013). Además, estos trabajos no utilizaron un análisis estadístico multivariante para separar efectos directos e indirectos de TAP sobre PA media.

El *path* análisis reveló un efecto intrasujetos significativo, pero el efecto intersujetos no alcanzo significación estadística. Esto indica que los diferentes niveles de TP de los sujetos no son predictivos del nivel de PA media del sujeto. Una conclusión similar se puede extraer del resultado de la falta de diferencias en PA media en función de los cambios en TAP y TP entre sujetos con y sin IFE (no mostrado). Se puede concluir que los cambios a corto plazo de TAP afectan a las personas eindependientemente de la aclimatación a largo plazo del sistema termorregulador de las mismas.

El estudio se llevo a cabo balanceadamente entre invierno y verano sin aparecer efecto significativo alguno por el orden respecto a las variaciones diarias o a la estación. La TAP, TP, PA media, frecuencia cardiaca (medida indirecta de actividad y gasto energético) y comportamiento en exteriores mostraron un patrón diario entre las 09:30 y las 20:30. Sin embargo, todos los patrones diarios fueron similares entre invierno y verano, indicando un comportamiento similar en los comportamientos diarios durante el invierno y durante el verano, lo que podría explicar la ausencia de diferencias estacionales en las variaciones intraindividuo de la PA media. La muestra de este estudio consta principalmente de estudiantes, intruidos para mantener una actividad similar en invierno y verano, por lo que podrían presentar resultados distintos en otros grupos de población.

El análisis pormenorizado de las exposiciones a exteriores reveló información adicional y similar a la del *path* análisis de entre 09:30 y 20:30. La exposición a exteriores durante invierno redujo TAP en 12.5ºC y TP distal en 2ºC aumentando PA media en 4mmHg, lo cual se ajusta a los cambios encontrados en el *path* análisis (TAP -1°C, TP distal -0.19°C, PA media +0.31mmHg, ver más arriba) indicando una asociación similar entre TAP y PA media mediante ambas aproximaciones. Además, el análisis de exposición a exteriores mostró que los incrementos y disminuciones agudas en TAP modificaban paralelamente TP y en dirección opuesta la PA con cambios mayores en invierno que en verano. Además, cambios mayores en TAP durante la exposición a exteriores reveló que la VOP, PP (ambas medidas de elasticidad arterial, Youn et al., 2007), PA sistólica y frecuencia cardiaca, pero no la PA diastólica, estaban más elevadas en invierno. Estos resultados confirman los resultados de studios previos que mostraban mayor sensibilidad de la PA sistólica a los cambios que la PA diastólica (Alpérovitch et al., 2009). El incremento de la vasoconstricción periférica con temperaturas ambientales más frías podrían incrementar la elasticidad arterial medida por VOP (Youn et al., 2007).

Los efectos sobre los niveles medios presentaron un comportamiento diferente. Como se esperaba, TAP y TP distal fueron menores en invierno que en verano. Pero la TP proximal fue mayor en invierno que en verano. La explicación más plausible para estos resultados es que los sujetos llevaban ropas más cálidas en invierno que en verano mientras que las regiones distales eran más sensibles y estaban expuestas más directamente a la TAP. Sin embargo, este efecto podría ser debido a un mecanismo indirecto, ya que se había visto que el enfriamiento de la cara, el área menos protegida

del cuerpo normalmente, activa el sistema nervioso simpático, reduce la TP distal y eleva la presión arterial por la activación del reflejo del trigémino (Li et al., 2009; O'Brien et al., 2011). Otro mecanismo adicional podría ser el incremento de la sudoración y el subsecuente enfriamiento de las regiones proximales en verano, la mayor tasa de sudoración se encontró en el torso (Taylor & Machado-Moreira, 2013). Sin embargo, todas estas conjeturas requieren verificación con datos reales.

De acuerdo con el comportamiento en exteriores auto-estimado los sujetos estuvieron aproximadamente dos terceras partes del tiempo en interiores y 3.5 horas en exteriores sin diferencias entre invierno y verano, lo que lleva a una disminución del nivel medio de TAP en invierno (4.7ºC) y de TP distal (0.9ºC). En contraste, la PA media no mostró cambios estacionales confirmando los resultados en estudios previos en sujetos jóvenes (Alpérovitch et al., 2009). Estos resultados abren la cuestión de porqué los cambios a largo plazo en TAP no se transforman en cambios en PA media al igual que hacen los cambios a corto plazo. Se sabe que tanto las respuestas adaptativas a corto plazo como las a largo plazo emergen para asegurar el mantenimiento de la PA y la distribución adeacuada del gasto cardiaco a los diferentes órganos. Las variaciones a corto plazo en PA parecen no estar tan estrictamente reguladas en el nivel como las a largo plazo indicando una interrelación entre los sistemas termoreguladores y cardiovasculares durante los cambios agudos en TAP. Esto indica que los cambios a corto plazo modifican la PA en función de la estación, pero no producen cambios a largo plazo en los valores medios diarios por lo que surge la pregunta de cómo interactúan ambos sistemas en la aclimatación a largo plazo. En cualquier caso no es possible explicar el mismo nivel de PA durante el día tanto en invierno como en verano por simples cambios en TP y circulación sanguínea. Esto implica, al menos en individuos jóvenes, que otros mecanismos adicionales deben contrarrestar los cambios en PA media, lo cual debería ocurrir en función de los cambios estacionales en TP. En cualquier caso, estos niveles similares de PA durante invierno y verano no pueden explicarse solamente por los cambios estacionales en el nivel de TP.

A pesar del hecho de que este es un estudio ambulatori sin ningún control posible sobre todas las influencias, los resultados pueden ser correctamente interpretados junto a los estudios previos en laboratorio. Se ha observado que en un medio frío los vasos periféricos se contraen y la sangre se retira del *shell* (más pronunciado en las extremidades) para centralizarse (Aschoff, 1956). Este movimiento de la sangre aumenta el estrés hemodinámico central y la PA media (Keatinge et al.,

1984). Además, junto al efecto directo del enfriamiento sobre la vasculatura local existen reflejos vasomotores simpáticos indirectos (Kenney et al., 2013). Justo lo contrario ocurre en un medio cálido, con una reducción de PA media atribuida a vasodilatación cutánea y a la perdida de agua y sales minerales por la sudoración (Kenney et al., 2013). Por tanto, el efecto indirect observado via TP distal en invierno y verano puede ser interpretado como parte del bucle del reflejo simpático vasomotor.

Además, durante el verano se encontró una vía positiva de acción de TAP sobre PA media, la cual podría ser responsable de la ausencia de un efecto global de TAP sobre la PA media. Un incremento de la presión arterial podría no estar relacionado únicamente con menores TAP sino también con incrementos de actividad (Goodwin et al., 2001). Sin embargo, las diferencias en el patron diario de actividad no parecen ser las responsables en nuestro estudio ya que el comportamiento en exteriores y la frecuencia cardiaca (medida indirecta del gasto de energía y la actividad) no difirieron entre ambas estaciones. No hay que descartar la posibilidad de una sensibilidad diferente del sistema cardiovascular a la misma cantidad de actividad entre ambas estaciones. Todo junto, el efecto positivo directo de la TAP sobre la PA media en verano puede ser tal efecto, la evidencia muestra que es bastante débil.

En conclusion, este studio ambulatorio proporciona evidencias de cambios intraindividuales en TAP que afectan a la TP distal de un modo directo y a la PA de un un modo inverso indicando un posible mecanismo causal para los cambios a corto plazo en PA media en la vida real. Dos aproximaciones distintas llegaron a la misma conclusión apoyándose la una en la otra: análisis de la exposición a exteriores con cambios grandes en las variables mostraron relaciones similares al *path* análisis utilizando el periodo completo de 11 horas del estudio con cambios relativamente pequeños. En contraste, a pesar de las distintas diferencias entre invierno y verano en TAP y TP no aparecieron diferencias estacionales en PA soportando el concepto de que cambios a largo plazo en la ditribución de la sangre no afectan a la PA, al menos en mujeres jóvenes sanas. Por tanto, se justifica la posibilidad de conseguir mejorar los tratamientos antihipertensivos personalizándolos a la medida del usuario final.

REFERENCES

Alpérovitch A, Lacombe JM, Hanon O, Dartigues JF, Ritchie K, Ducimetiere P, Tzourio C. (2009). Relationship between blood pressure and outdoor

temperature in a large sample of elderly individuals. The Three-City Study. Arch Intern Med. 169:75-80.

Aschoff J. (1971). Temperaturregulation. In Gauer OH, Kramer K, Jung R (Eds). *Energiehaushalt und Temperaturregulation. Physiologie des menschen*. München (Germany): Urban & Schwarzenberg, pp. 43-112.

Blazquez A, Martinez-Nicolas A, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity, and body position as determinants of the circadian pattern of blood pressure. Chronobiol Int. 29:747-56.

Brook RD, Weder AB, Rajagopalan S. (2011). "Environmental hypertensionology" the effects of environmental factors on blood pressure in clinical practice and research. J Clin Hypertens (Greenwich) 13:836-42.

Goodwin J, Pearce VR, Taylor RS, Read KLQ, Powers SJ. (2001). Seasonal cold and circadian changes in blood pressure and physical activity in young and elderly people. Age Ageing. 30:311-7.

Jansen RW, Lipsitz LA. (1995). Postprandial hypotension: epidemiology, pathophysiology, and clinical management. Ann Intern Med 122:286-95.

Keatinge WR. (2002). Winter mortality and its causes. Int J Circumpolar Health. 61:292-9.

Keatinge WR, Coleshaw SR, Cotter F, Mattock M, Murphy M, Chelliah R. (1984). Increases in platelet and red cell counts, blood viscosity, and arterial pressure during mild surface cooling: factors in mortality from coronary and cerebral thrombosis in winter. Br Med J. 289:1405-8.

Kenney WL, Stanhewicz AE, Bruning RS, Alexander LM. (2013). Blood pressure regulation III: What happens when one system must serve two masters: Temperature and pressure regulation? Eur J Appl Physiol. 114:467-79.

Kräuchi K, Deboer T. (2010). The interrelationship between sleep regulation and thermoregulation. Front Biosci. 15:604-25.

Kräuchi K, Gompper B, Hauenstein D, Flammer J, Pflüger M, Studerus E, Schötzau A, Orgül S. (2012). Diurnal blood pressure variations are associated with changes in distal-proximal skin temperature gradient. Chronobiol Int. 29:1273-83.

Li Y, Alshaer H, Fernie G. (2009). Blood pressure and thermal responses to repeated whole body cold exposure: effect of winter clothing. Eur J Appl Physiol 107:673-85.

Modesti PA. (2013). Season, temperature and blood pressure: A complex interaction. Eur J Intern Med. 24:604-7.

Muthén LK, Muthén BO. (2010). Mplus user's guide, 6th Edition, Los Angeles, CA

O'Brien C, Castellani JW, Sawka MN. (2011). Thermal face protection delays finger cooling and improves thermal comfort during cold air exposure. Eur J Appl Physiol 111:3097-105.

Preacher KJ, Zyphur MJ, Zhang Z. (2010). A general multilevel SEM framework for assessing multilevel mediation. Psychol Methods 15:209-33.

Taylor NA, Machado-Moreira CA. (2013). Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. Extrem Physiol Med 2:4.

Youn JC, Rim SJ, Park S, Ko YG, Kang SM, Choi D, Ha JW, Jang Y, Chung N. (2007). Arterial stiffness is related to augmented seasonal variation of blood pressure in hypertensive patients. Blood Press 16:375-80.

3.5. EXPERIMENTAL CHAPTER 5

ENVEJECIMIENTO DEL SISTEMA CIRCADIANO. CRONODISRUPCIÓN Y CRONOPOTENCIACIÓN

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3.5. ENVEJECIMIENTO DEL SISTEMA CIRCADIANO. CRONODISRUPCIÓN Y CRONOPOTENCIACIÓN

RESUMEN

El envejecimiento del sistema circadiano se caracteriza por aplanamiento, fragmentación y avance de fase. El amarilleamiento del cristalino con la edad deteriora la entrada de luz y, por tanto, provoca cronodisrupción en. Aunque la temperatura de la muñeca se ha propuesto como un índice del sistema circadiano apenas se conoce como envejece su patrón. Por ello, el propósito de este trabajo fue describir este ritmo en ancianos sanos junto a su estilo de vida comparado con jóvenes. También se evaluó la cronodisrupción y posibilidades de cronopotenciación. Para ello, se reclutaron 90 sujetos sanos, 46 ancianos (65-75 años) y 44 jóvenes (19-25 años) y se monitorizó su temperatura periférica, actividad, posición, luz y temperatura ambiental durante 5 días (entre semana). Para la luz se realizó un histograma, el índice de calidad de la luz (ICL) y de la oscuridad (ICO). La cronodisrupción se evaluó mediante la tasa de patrones anómalos de temperatura y las diferencias de fase entre luz, temperatura, y actividad. Con los características de cada variable se construyeron árboles de decisión para diferenciar grupos de edad. Los ancianos se exponían a menores intensidades luminosas disminuyendo su ICL, pero sin diferencias en ICO. La tasa de patrones anómalos de temperatura y la desincroninzación fue mayor en ancianos. Los árboles de decisión consiguieron una elevada tasa de acierto (superior al 80%) excepto para la luz, si bien, al combinarlos subió al 96,5% por lo que las diferencias en los ritmos permiten diferenciar jóvenes y ancianos sin considerar su edad. Además, mayores ICL e ICO en ancianos se relacionaron con mejores patrones de temperatura y una recuperación parcial de la zona de mantenimiento de la vigilia. En resumen, el envejecimiento se relacionó con un avance de fase, menor exposición a la luz y mayor cronodisrupción, aunque los ancianos con un mejor patrón luz mostraron un sistema circadiano más joven, resaltando la importancia de una adecuada exposición a este *zeitgeber*.

Palabras clave: temperatura de la muñeca, exposicion a la luz, temperatura ambiental, envejecimiento, actividad, cronodisrupción.

INTRODUCCIÓN

En humanos, la mayor parte de variables fisiologicas y comportamentales muestran cambios diarios con un periodo de aproximadamente 24 horas, que son regulados por n marcapasos central situado en el núcleo supraquiasmático del hipotálamo (SCN). El SCN es la parte más importante del sistema circadiano, y está estructurado jerárquicamente para sincronizar el organism al ambiente, y para generar y mantener los ritmos biológicos (Buijs & Kalsbeek, 2001; Duguay & Cermakian, 2009; Stratmann & Schibler, 2006).

Debido a la incapacidad para registrar de forma directa la actividad del marcapasos central, algunos ritmos abiertos han sido definidos como ritmos marcadores circadianos. Estos ritmos se derivan directamente de la actividad del marcapasos central y son estables, robustos, y fáciles de medir measure (Touitou & Haus, 1994). Los ritmos marcadores más ampliamente aceptados son variables fisiológicas como la temperatura central y el patron de secreción de melatonina, o comportamentales como el ritmo reposo-actividad o el ciclo sueño-vigilia.

Sin embargo, las medidas de melatonina y temperature central son incómodas para los voluntarios, mientras que los ritmos de actividad y el ciclo de sueño-vigilia son más subsidiarios de control voluntario (Hofstra & de Weerd, 2008). En este sentido, la temperature de la muñeca (WT) puede ser registrada de forma sencilla bajo condiciones ambulatorias gracias a dispositivos sin cable, con gran exactitud y reproductibilidad (Martinez Nicolas et al., 2013, Gompper et al., 2010, Kräuchi et al., 2012), y exhibe un patrón en inversión de fase y un pequeño avance de fase en relación con la temperatura central corporal (CBT), permitiendo la pérdida de calor interno necesaria para el sueño (Kräuchi et al., 2000; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). Este ritmo presenta también un fuerte componente endógeno (Bonmati-Carrion et al., 2014; Martinez-Nicolas et al., 2013). Por todo ello, el WT ha sido propuesto como un índice no invasive y robusto del funcionamiento del sistema circadiano (Ortiz-Tudela et al., 2010; Sarabia et al., 2008; Zornoza-Moreno et al., 2011), que determina de forma precisa la fase circadiana (Bonmati-Carrion et al., 2014), y madura a lo largo de los primeros 6 meses de vida (Zornoza-Moreno et al., 2011). Además, la WT se ve aplanada en la obesidad (Corbalan-Tutau et al., 2011) y en los pacientes con patrón no dipper de tensión arterial (Blazquez et al., 2012), sugiriendo que la WT es también un marcador de salud.

Como sucede en la mayor parte de funciones fisiológicas, el sistema circadiano pierde progresivamente su funcionalidad con el envejecimiento (Turek et al., 1995). El proceso de envejecimiento altera las entradas del sistema circadiano por predominancia de miosis pupilar y una reducción en la transmisión de luz azul por el cristalino (la principal longitud de onda para el encarrilamiento circadiano) (Turner & Mainster, 2008). El SCN es también susceptible al envejecimiento, ya que se ha descrito degeneración neuronal asociada a la senectud (Hofman & Swaab, 2006). Y, finalmente, las salidas también están alteradas con el envejecimiento, ya que los ritmos muestran una reducción de la amplitud, una mayor fragmentación y un avance de fase (Hardeland et al., 2011; Hofman & Swaab 2006; Van Someren et al., 1999; Weinert 2010).

Los ancianos muestran consistentes cambios de su estilo de vida con respect a los adultos, se despiertan antes, y muestran una mayor estabilidad interdiaria (Minors et al., 1998). También presentan una menor actividad global aunque con un mayor número de movimientos durante el period nocturno, lo cual se relaciona a una menor eficiencia de sueño (Huang et al., 2002). Con respect a la exposición a la luz, se exponen a mayors intensidades de luz a lo largo del día (Scheuermaier et al., 2010). Además, los sistemas de termorregulación también están alterados, por una reducción en la vasoconstricción periférica, del flujo sanguineo cutáneo, de la distribución de flujo sanguíneo durante el estrés, y de la producción metabólica de calor durante el estrés frío (Blatteis, 2012).

Estos cambios de los estilos de vida y el proceso del envejecimiento normal transforman a los ancianos en personas más sensibles a la cronodisrupción (CD), debido a la falta de contraste luz/oscuridad y a la disminución de la recepción de luz circadiana (Martínez-Nicolas et al., 2014; Turner & Mainster, 2008). La CD puede ser el resultado de luz insuficiente diurna y excesiva luz nocturna (Erren and Reiter, 2009), y se manifiesta como un aplanamiento, inestabilidad y fragmentación de los ritmos, y desplazamiento de fase entre los diferentes osciladores y/o entre el SCN y las claves externas. Sin embargo, otros factores capaces de desencadenar la CD son: temperatura ambiental elevada durante el periodo nocturno, que atenúa la CBT, o la actividad física, que induce un aumento en la CBT cuando se realiza por la noche, (Weinert & Waterhouse, 2007).

Además del envejecimiento prematuro, la CD se asocia a un riesgo aumentado de sufrir diversos tipos de cancer, syndrome metabolico, desórdenes afectivos y cognitivos, disregulación de la reproducción, eventos cardiovasculares, y alteraciones del sueño, entre otras patologías (Davis & Mirick, 2006; Garaulet & Madrid, 2010; Gronfier et al., 2007; Karlsson et al., 2001; Middleton et al., 2002; Pauley, 2004; Rodrigues Menezes et al., 2004; Schernhammer et al., 2003).

Por ello, el objetivo de este trabajo fue evaluar la influencia de la explosición a los sincronizadores circadianos en la temperatura de la muñeca en ancianos sanos en comparación con jóvenes, y además, la existencia de una desincronización interna entre las diferentes variables rítmicas, con la finalidad de proponer estrategias en los hábitos de vida que potencien el sistema circadiano.

MATERIAL Y MÉTODOS

Para el presente studio, 44 estudiantes pre-graduados voluntarios (20 hombres y 24 mujeres, de 19-25 años) residents en Murcia, España (latitud 38º 01’ N) y 46 voluntarios ancianos sanos (23 hombres y 23 mujeres, de 65-75 años) residentes en Toledo, España (latitud 39º 52’ N) fueron reclutados. Los participantes fueron instruidos a llevar todos los sensores durante 5 días consecutivos de la semana, y fueron animados también a mantener sus estilos de vida habituales. Todos los datos fueron registrados en condiciones de la vida real durante las dos primeras semanas de noviembre.

El studio cumplía los principios bioéticos fijados por la declaración de Helsinki. Los datos de los voluntaries fueron incluídos en una bse de datos y protegidos de acuerdo a la Ley Española 15/1999, del 13 de Septiembre. Todos los participantes recibieron información apropiada sobre las características del studio, y firmaron el consentimiento informado previo a su inclusión en este estudio (Portaluppi et al., 2010).

Todos los sujetos llevaban un Thermochron iButton DS1921H (Maxim Integrated Products, Sunnyvale, California, USA) para medir la temperatura de la piel de la muñeca (WT), con una precision de ±0.125 ºC. Este sensor de temperature se colocó en la muñeca de la mano no dominante sobre la arteria temporal, y fue aislado de la temperatura ambiental mediante una muñecera deportiva reversible de algodón, como

descrito previamente (Sarabia et al., 2008). El dispositivo fue programado para registrar cada 10 minutos a lo largo de 5 días.

Medición de la temperature de la piel de la muñeca

Todos los sujetos llevaban un Thermochron iButton DS1921H (Maxim Integrated Products, Sunnyvale, California, USA) para medir la temperatura de la piel de la muñeca (WT), con una precision de ±0.125 ºC. Este sensor de temperature se colocó en la muñeca de la mano no dominante sobre la arteria temporal, y fue aislado de la temperatura ambiental mediante una muñecera deportiva reversible de algodón, como descrito previamente (Sarabia et al., 2008). El dispositivo fue programado para registrar cada 10 minutos a lo largo de 5 días.

Posición corporal y medición del ritmo de actividad

La posición corporal y el ritmo de actividad fueron registrados cada 30 segundos, utilizando un HOBO Pendant G Acceleration Data Logger UA-004-64 (Onset Computer, Bourne, Massachusetts, USA) colocado en la mano no dominante con su eje X paralelo al húmero. Las especificaciones del dispositivo y el método para obtener estas variables han sido descritos previamente (Ortiz-Tudela et al., 2010). La actividad es medida como tasa de cambio en grados por minute, y la posición representa la inclinación del eje X del acelerómetro (paralelo al húmero) expresado como grados (90º para la posición vertical del brazo, 0º para la posición horizontal del brazo). Ambas variables fueron registradas cada 10 minutos para conseguir una frecuencia de registro que coincidiera con la utilizada para la WT.

Registro de temperatura ambiental y exposición a la luz

Se solicitó a todos los sujetos que llevaran un HOBO Pendant Temperature/Light Data Logger UA-002-64 (Onset Computer, Bourne, Massachusetts, USA) en una cadena en el cuello para registrar temperatura ambiental y exposición a la luz. Las especificaciones técnicas, de memoria, espectro y exactitud de la medición han sido descritas en un trabajo previo (Martinez-Nicolas et al., 2011). El dispositivo registró de forma regular con intervalos pre-programados de 30 segundos. Las intensidades de luz en luxes fueron convertidas en unidades logarítmicas y promediados cada 10 minutos para permitir comparaciones con los datos de temperatura.

Calculo del TAP

La actividad, posición corporal y temperatura de la muñeca fueron normalizados para obtener la variable TAP. Un valor de 0 en el TAP indica reposo, posición de decúbito y temperature de la muñeca elevada, lo cual es compatible con el sueño, mientras que un valor de 1 se alcanza cuando el sujeto está en bipedestación, activo, y con baja temperatura de la muñeca, lo cual indica una elevada activación (para más detalles véase Ortiz-Tudela et al., 2010).

Análisis de datos

Los artefactos producidos por la retirada de los sensores fueron eliminados por filtración de los datos. Además, para eliminar datos atípicos, se calculó la distancia intercuartílica (de Q1 a Q4) y entonces, cada punto cuyo cambio con respecto al previo fuese superior a la distancia intercuartílica era eliminado (van Marken Lichtenbelt et al., 2006). Se calculó el patrón medio por variable y por individuo y, posrteriormente, para el grupo completo.

Para analizar las posibles diferencias entre los patrones circadiano, realizamos un análisis no paramétrico (Van Someren et al., 1999). Se calcularon la Estabilidad Interdiaria (la constancia en el patron rítmico de 24-h, IS) y la Variabilidad Intradiaria (fragmentación, IV). La Amplitud Relativa (RA), para la temperatura de la muñeca fue calculada como la diferencia entre M5 (media para las 5 horas consecutivas con los máximos valores) y el L10 (media para las 10 horas consecutivas con los valores mínimos), divididos por su suma. Sin embargo, en el caso de variables con una acrofase que ocurre en el periodo de actividad (luz, temperatuae ambiental, actividad, posición y TAP), se utlizaron las cinco horas consecutivas de valores mínimos (L5) y las 10 horas consecutivas de valores máximos (M10). Por tanto, los valores máximos (M5 para la WT y M10 para el resto de las variables) fueron denotadas como MAX, mientras que los valores mínimos (L10 para la WT y L5 para el resto de las variables) como MIN, y sus tiempos como TMAX y TMIN (para MAX y MIN), respectivamente. Además, la zona de mantenimiento de la vigilia fue definida como el tiempo de las dos horas con los menores valores de temperatura de la muñeca (TL2).

La exposición a la luz fue analizada en detalle. En primer lugar, se realizó un histograma acumulado en intervalos de 10 minutos y expresado en log10lux, utilizando

el despertar como *zeitgeber time*. En segundo lugar, se realizó un histograma con 5 categorías: menos de 10 lux, entre 10 y 100 lux, entre 100 y 500 lux, entre 500 y 1000 lux, y más de 1000 lux), en función del día (08:00-23:50) y la noche estándar (00:00-07:50). En tercer lugar, se calculó el Indice de Calidad de la Luz (ICL) (Martinez-Nicolas et al., 2011) y de Calidad de la Oscuridad (ICO) calculado como: tiempo a <10 lux, menos el tiempo a >500 lux, dividido por la suma del tiempo a >500 lux y a <10 lux, oscilando entre +1 y -1 (exposición de <10 lux y de >500 lux respectivamente).

Para evaluar el grado de desincronización interna (ID) en ambos grupos de edad, se analizaron los sujetos con un ritmo de WT significativo y de periodo aproximado de 24 horas. Las diferencias de fase entre el *zeitgeber* principal (luz), temperatura de la muñeca, y actividad, fueron calculadas como sigue: tiempo L5 de la luz vs tiempo M5 para la temperatura, tiempo M5 de la temperatura vs tiempo L5 de la actividad, y tiempo L5 de la luz vs tiempo L5 de la actividad. Finalmente, se calculó la media las diferencias de fase con el fin de obtener una medida global del desequilibrio de fase de la ID. Entonces, se realizaron comparaciones entre jóvenes y ancianos.

Los índices no paramétricos de TAP, WT, actividad, posición, luz y temperatura ambiental fueron procesados en WEKA 3.0.0 (University of Waikato, New Zealand) utilizando el método J48 basado en el algoritmo C4.5 (Kotsiantis, 2007), para realizar un árbol de decisión por variable, de los cuales se combinaron los que tenían más del 66% de acierto. Esta combinación se realizó puntuando 1 cada vez que un patrón se definía como joven y 0 en caso contrario. Por tanto, cada sujeto tenía una puntuación entre 0 (todos los árboles de decisión lo definen como un anciano) y 5 (todos los árboles de decisión lo definen como joven).

Además, se realizó una clasificación de los individuos de acuerdo a su ICL y su ICO, para detectar si las diferencias en el patrón de WT en ancianos se asociaban a una exposición débil a zeitgeber. Se calculó entonces la onda media de 24-h por cuartil. El primer cuartil (Q1) representa los valores más bajos, mientras que el cuarto (Q4) los más altos. De nuevo, se realizaron análisis no paramétricos para comparar Q1 y Q4.

Todos los datos se expresaron como media ± SEM. Todos los análisis estadísticos (ANOVA de dos vías para medidas repetidas, t de Student, y test de chi-cuadrado) se realizaron con la versión 19.0 del SPSS (SPSS, Inc. Chicago, IL, USA).

RESULTADOS

Ondas medias

La onda media para todas las variables registradas se muestra en la Figura 1, y su correspondiente índice no paramétrico en la Tabla 1. Como puede observarse, el ritmo de temperature ambiental (Figura 1A) en la gente joven muestra un patron diario de baja amplitude alrededor de un valor medio de 23.19ºC, con los valores mínimos (menores de 22ºC) entre las 00:50 h y 08:30 h, mientras que los valores más altos (más de 24ºC) ocurrieron entre las 09:40 h y 23:00 h, mientras que en ancianos la temperatura oscila alrededor de 25.20 ºC ($p<0.001$ comparada con jóvenes, ver Tabla 1), valores inferiores a 24ºC entre 01:20 h y10:10 h, y superiores a 26 ºC entre 13:20 h y 23:40 h. Además, en ancianos, el ritmo de temperatura ambiental mostró un retraso durante el día (ver TMAX para ET en laTabla 1). La exposición media a la luz (Figura 1B) en jóvenes es inferior a 10 lux de 00:30 h a 07:50 h, y alrededor de 100 lux de 10:30 h a 16:10 h, con una lenta disminución de 17:00 h a 22:50 h, mientras que la exposición a la luz en ancianos es inferior a 10 lux de 22:20 h a 09:00 h y alrededor de 100 lux de 10:30 h a 15:00 h, con una disminución continua y progresiva a partir de esta hora.

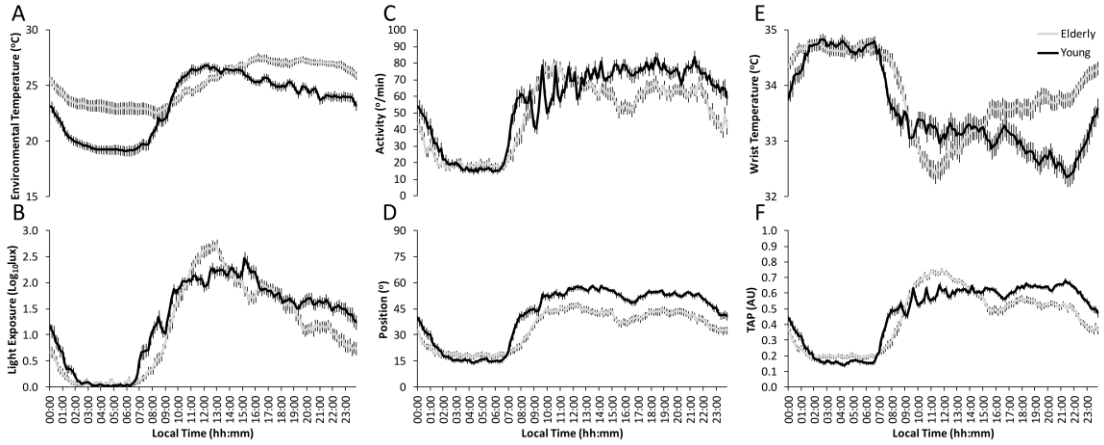


Figura 1. Estudio poblacional de ondas medias. Ondas medias para temperature ambiental (A), exposición a la luz (B), actividad (C), posición (D), temperature de la muñeca (E), y la variable integrada TAP (F) para jóvenes (línea negra) y ancianos (línea gris). Todas las variables son expresadas como media ± SEM.

Como se esperaba por resultados previos (Ortiz-Tudela et al. 2010), los patrones de actividad y posición de los jóvenes, (Figura 1C y D, respectivamente) mostraron valores bajos estables de 01:10 h a 07:10 h y altos y variables de 09:30 h a 23:00 h. El periodo de descanso para los ancianos se situó entre 00:20 h y 08:00 h y la actividad

entre 09:30 h y 21:20 h, con una disminución en el medio coincidiendo con la siestaen España (de 15:10 h a 17:20 h). Al comparer ambos grupos, los valores de actividad y posición eran más bajos, en general, en ancianos (Tabla 1), especialmente durante el día (Figura 1C y D, MAX en Tabla 1), mientras que los valores tendían a ser más altos durante la noche (MIN en Tabla 1). La amplitud y la estabilidad eran más bajos y con mayor fragmentación en ancianos, como se muestra en la Tabla 1, excepto para la fragmentación de la actividad.

		IS	IV	RA	MIN	MAX	TMIN	TMAX	CFI	MEAN
WT	Y	0.57±0.02	0.20±0.02	0.03±0.00	32.76±0.15	34.87±0.08	16:25±00:39	02:49±00:35	0.50±0.01	33.57±0.09
	E	0.69±0.02*	0.20±0.01	0.03±0.00	33.14±0.12*	34.86±0.07	13:41±00:19*	02:53±00:29	0.54±0.01*	33.93±0.09*
L	Y	0.70±0.02	0.25±0.01	0.98±0.01	0.03±0.01	2.08±0.06	04:24±00:06	15:22±00:21	0.85±0.01	1.27±0.04
	E	0.75±0.02	0.29±0.02*	0.97±0.01	0.03±0.01	2.03±0.06	03:54±00:12	14:44±00:14	0.86±0.01	1.08±0.04*
ET	Y	0.63±0.03	0.10±0.01	0.16±0.01	19.00±0.35	26.18±0.24	05:08±00:16	15:30±00:25	0.58±0.01	23.19±0.25
	E	0.71±0.02*	0.10±0.00	0.11±0.00*	22.16±0.38*	27.67±0.33*	06:08±00:32	17:21±00:38*	0.59±0.01	25.20±0.34*
ACT	Y	0.59±0.01	0.71±0.02	0.68±0.01	15.04±0.70	78.04±1.52	04:11±00:10	17:22±00:20	0.64±0.01	55.28±1.12
	E	0.53±0.01*	0.72±0.02	0.62±0.02*	16.18±0.10	68.52±1.72*	03:54±00:20	15:09±00:20*	0.60±0.01*	49.20±1.26*
POS	Y	0.70±0.02	0.26±0.01	0.59±0.02	14.33±0.72	55.40±0.83	04:14±00:12	15:22±00:17	0.72±0.01	41.04±0.62
	E	0.58±0.03*	0.34±0.02*	0.47±0.02*	16.49±1.11	44.83±1.35*	03:27±00:38	15:12±00:28	0.63±0.02*	34.25±1.00*
TAP	Y	0.74±0.02	0.25±0.01	0.62±0.02	0.15±0.01	0.64±0.01	04:15±00:10	17:07±00:18	0.74±0.01	0.47±0.00
	E	0.77±0.02	0.32±0.02*	0.55±0.02*	0.18±0.01*	0.63±0.01	03:36±00:21	14:17±00:08*	0.72±0.01	0.43±0.00*

Tabla 1. Análisis no paramétricos para WT y TAP y patrones de exposición a *zeitgebers* y otras variables rítmicas con efecto sincronizador, según la edad. Jóvenes (Y) y ancianos (E): características principales para temperatura de la muñeca (WT, exposición a la luz (L), temperatura ambiental (ET), actividad (ACT), posición (POS), y TAP. Indices no paramétricos: Estabilidad Interdiaria (IS), Variabilidad Intradiaria (IV), Amplitud Relativa (RA), media de las 10 horas consecutivas con los valores más bajos (MIN), y de las 5 horas consecutivas con los valores más altos (MAX) para la WT; media de las 10 horas consecutivas con los valores más altos (MAX) y las 5 horas consecutivas con los valores más bajos (MIN) para exposición a la luz, temperatura ambiental, actividad, posición y TAP. TMAX y TMIN indican el momento del punto medio para cada MAX o MIN, respectivamente. CFI: indice de funcionamiento circadiano. MEAN: media de los datos. TMIN y TMAX se expresan en hh:mm. MIN, MAX y MEAN se expresan en ºC para temperature ambiental y de muñeca, en $\log_{10}$lux para exposición a la luz, en º y º/min para posición y actividad respectivamente, y en unidades arbitrarias para TAP. Valores expresados en media ± SEM. * indica $p< 0.05$, according to a Student´s t test.

Análisis de la calidad de la luz y la oscuridad

El histograma de la luz (Figura 2) muestra las diferencias día/noche entre ambos grupos de edad en todas las categorías estudiadas. Además, hubieron diferencias significativas en las intensidades luminosas bajas entre ambos grupos de edad. (0-10 lux, 10-100 lux y 100-500 lux) pero no en las más altas (500-1000 lux y más de 1000 lux). Los ancianos se expusieron menos a las categorías de 10-100 y100-500 y más tiempo a menos de 10 lux durante el día y la noche. Además, ambos grupos de edad se

expusieron menos de dos horas a luz brillante. Por tanto, al considerar el histograma de exposición a la luz a lo largo del día, los ancianos se expusieron a menores intensidades que la gente joven cómo se muestra en la Figura 3 (161.13 ± 5.62 $\log_{10}$lux *vs* 186.19 ± 6.00 $\log_{10}$lux luz acumulada en 24 horas, $p<0.001$ e intensidad media como se ve en la Tabla 1). De hecho, los ancianos se expusieron a intensidades más altas que los jóvenes solo entre 2 y 5 horas después del despertar, pero esto cambia después. La exposición a la luz en gente jóven continúa aumentando hasta 16 horas después del despertar, alcanzando una meseta ambos grupos. Además, el ICL (ver Martinez-Nicolas et al., 2011) e ICO, aquí propuestos revelaron que ambos grupos de edad están expuestos a noches oscuras con un valor medio de ICO de 0.93 ± 0.02 para gente joven y 0.96 ± 0.01 para ancianos sin diferencias entre ellos, pero también a días oscuros (ICL, -0.16 ± 0.07 *vs* -0.34 ± 0.05 para jóvenes y ancianos respectivamente, $p<0.05$).

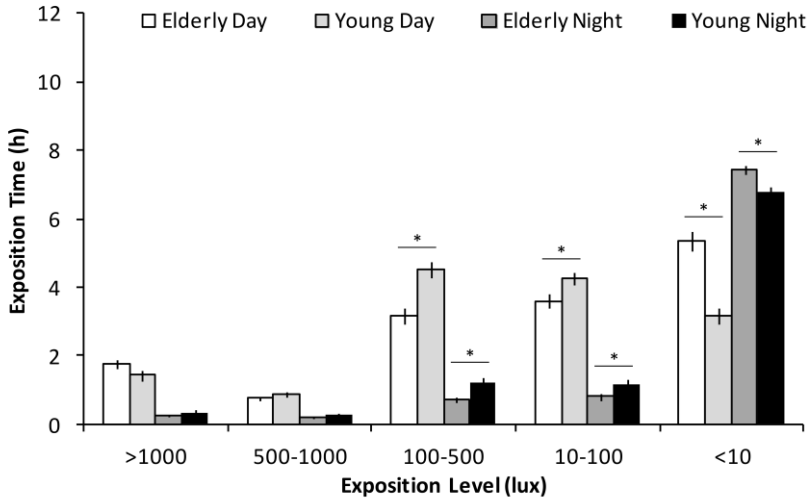


Figura 2. Histograma de exposición a la luz. Categorías de exposición a la luz (0-10, 10-100, 100-500, 500-1000 y más de 1000 lux) para jóvenes y ancianos durante el día (08:00-23:50) y la noche (00:00-07:50). Los ancianos durante el día se muestan en barras blancas, y por la noche en gris oscuro mientras que los jóvenes por el día se ven en gris claro mientras que por la noche se muestran en negro. Todos los valores se muestran como media ± EEM. * indica diferencias significativas $p<0.05$ mediante una ANOVA de dos factores y medidas repetidas.

Medida de la cronodisrupción

La tasa patrones anómalos de WT (no significativos o periodo distinto a 24 horas), una medida de cronodisrupción, fue mayor en ancianos que en jóvenes medido

por el test de la Chi cuadrado (42.6% *vs* 18.2%, p<0.05). Las diferencias de fase en los patrones de luz, TP y actividad fueron mayores en ancianos que en jóevenes (01:31 ± 00:14 h *vs* 00:56 ± 00:08 h, p<0.05), y entre luz y actividad (01:05 ± 00:11 h *vs* 00:25 ± 00:04 h, p<0.001, para ancianos y jóvenes, respectivamente). Sin embargo, no se encontraron diferencias significativas en el desfase entre actividad y TP (01:49 ± 00:19 h *vs* 01:09 ± 00:11 h, p=0.07) y entre luz y TP (01:37 ± 00:21 h *vs* 01:14 ± 00:11 h, p=0.33) entre ambos grupos de edad.

Predicción de la edad

En la figura 4 se muestran los árboles de decision para temperatura ambiental, posición, actividad, WT y TAP. El árbol de decision para la luz no está incluido puesto que no fue estadísticamente significativo. Un valor de M10 de posición mayor de 50º definió a la gente jóven con un 80% de acierto, mientras que si el M5 de temperatura ambiental aparecía después de las 15:00 apunta al grupo anciano con un acierto de un 80.9%. El árbol de decision para actividad era más complejo y necesitó tres ramas (los momentos de M5 y M10 y el valor de M10) alcanzando una tasa de acuerdo del 82.2%. En el caso de WT si aparecía el L2 antes de las 14:00 se trataba de un anciano con un 84.9% de acierto y en el caso del TAP si el momento del M10 era anterior a las 16:15 se alcanzaba una tasa de acierto del 86% para definir el patrón del anciano. Posteriormente, al conbinar todos los pronósticos (Figura 5), un sujeto que puntúase menos de 2 puntos o más de 3 puntos sería clasificado como anciano o joven, respectivamente, con una tasa de acierto del 100%.

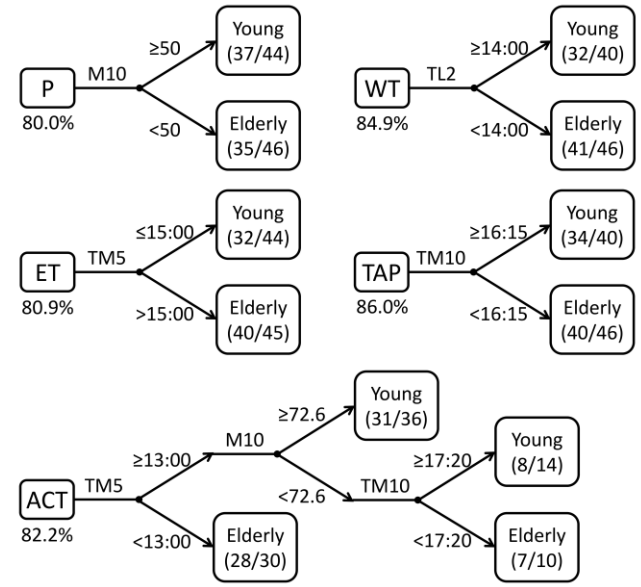
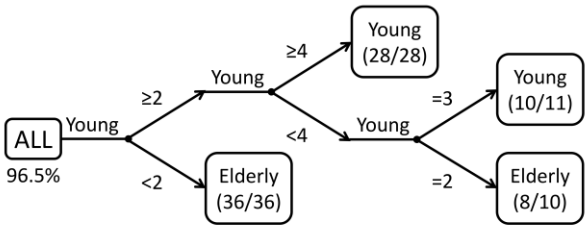


Figura 4. Arboles de decisión. Árboles de decision para temperatura ambiental (ET) define a los sujetos por la hora de M5; la posición (P) diferencia sujetos por el M10; la variable TAP por el momento de M10 (TM10); WT por la hora de la zona de mantenimiento de la vigilia (TL2), mientras que la actividad utiliza la hora de M5 y M10 y el valor de este último. Las tasas de acuerdo se muestran debajo del nombre de cada variable



Young Score	Number of subjects	Agreement Rate	Category
0	Elderly = 20 Young = 0	100	Elderly
1	Elderly = 16 Young = 0	100	Elderly
2	Elderly = 8 Young = 2	80	Elderly
3	Elderly = 1 Young = 10	90.9	Young
4	Elderly = 0 Young = 12	100	Young
5	Elderly = 0 Young = 16	100	Young

Figura 5. Arbol de decision que integra los previos. Cada sujeto puntúa 1 cada vez que es definido como jóven en los árboles de decisión individuales (temperatura ambiental, posicion, actividad, WT y TAP). La tasa de acuerdo global se muestra al principio del árbol de decisión (ALL). Cuando un sujeto se clasifica como jóven 3 o más veces se marca como jóven y en caso contrario como anciano.

Cronopotenciación

Cada grupo de edad, jóvenes (Figuras 6 y 8) y ancianos (Figuras 7 y 9), se clasificaron en función de alto y bajo ICL (Figuras 6 y 7) o ICO (Figuras 8 y 9), seleccionando los cuartiles cuarto y primero, respectivamente y se analizaron sus patrones circadianos de WT, actividad, posición y TAP.

La figura 6 muestra que un alto ICL en gente jóven se relaciona con mayor IS (0.75 ± 0.02 vs 0.65 ± 0.04, $p<0.01$), menor IV (0.21 ± 0.02 vs 0.33 ± 0.02, $p<0.001$), mayores valores máximos (2.37 ± 0.06 log10lux vs 1.63 ± 0.12 log10lux, $p<0.001$) y un

avance de fase de TMIN (03:53 ± 00:13 h vs 04:54 ± 00:25 h, p<0.05) para la luz, también se asoció con mayor RA (0.71 ± 0.01 vs 0.64 ± 0.02, p<0.01), menores valores MIN (6.37 ± 0.40 º/min vs 8.46 ± 0.50 º/min, p<0.01) y un avance de fase del TMIN (03:44 ± 00:11 h vs 04:43 ± 00:26 h, p<0.05) en actividad y mayor estabilidad en posición (0.79 ± 0.03 vs 0.68 ± 0.02, p<0.001) junto a un avance de fase (03:38 ± 00:16 h vs 04:51 ± 00:20 h, p<0.05) y mayor IS en TAP (0.79 ± 0.02 vs 0.73 ± 0.03, p<0.05).

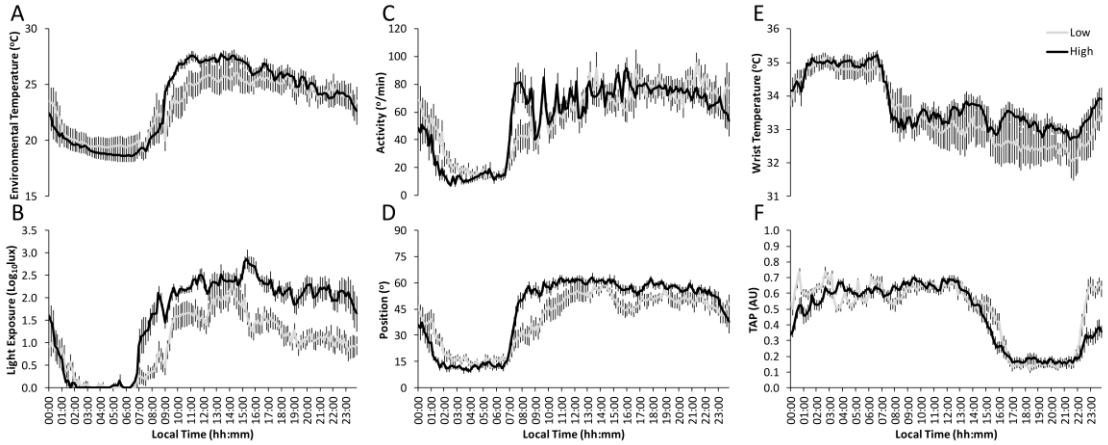


Figura 6. Ondas medias de jóvenes en function de alto y bajo contraste en ICL. Ondas medias de temperature ambiental (A), luz (B), actividad (C), posición (D), WT (E) y TAP (F) para gente joven con alto (black line) y bajo ICL (grey line). Todas las variables están expresadas como media ± SEM.

Los ancianos con alto ICL en comparación con un bajo ICL (Figura 7) mostraron mayor estabilidad (0.86 ± 0.03 *vs* 0.70 ± 0.03, p<0.001), menor fragmentación (0.24 ± 0.02 *vs* 0.39 ± 0.05, p<0.05), menores valores MIN (0.00 ± 0.00 log_{10}lux *vs* 0.04 ± 0.01 log_{10}lux, p<0.01) y mayores valores MAX (2.48 ± 0.08 log_{10}lux *vs* 1.51 ± 0.08 log_{10}lux, p<0.001) en luz que produjeron mayor RA (1.00 ± 0.01 *vs* 0.94 ± 0.02, p<0.01). Además, un alto ICL se asoció con menor fragmentación (0.68 ± 0.03 *vs* 0.83± 0.03, p<0.05) en el patron de actividad y un avance de fase en ambos marcadores, TMAX (13:55 ± 00:32 h *vs* 16:27 ± 00:51 h, p<0.05) and TMIN (03:45 ± 00:20 h *vs* 04:52 ± 00:18 h, p<0.05); mostraron también mayor estabilidad en posición (0.68 ± 0.03 *vs* 0.55 ± 0.05, p<0.05) y mayor estabilidad (0.83 ± 0.01 *vs* 0.72 ± 0.04, p<0.05) y menor fragmentación (0.27 ± 0.02 *vs* 0.36 ± 0.03, p<0.05) en TAP.

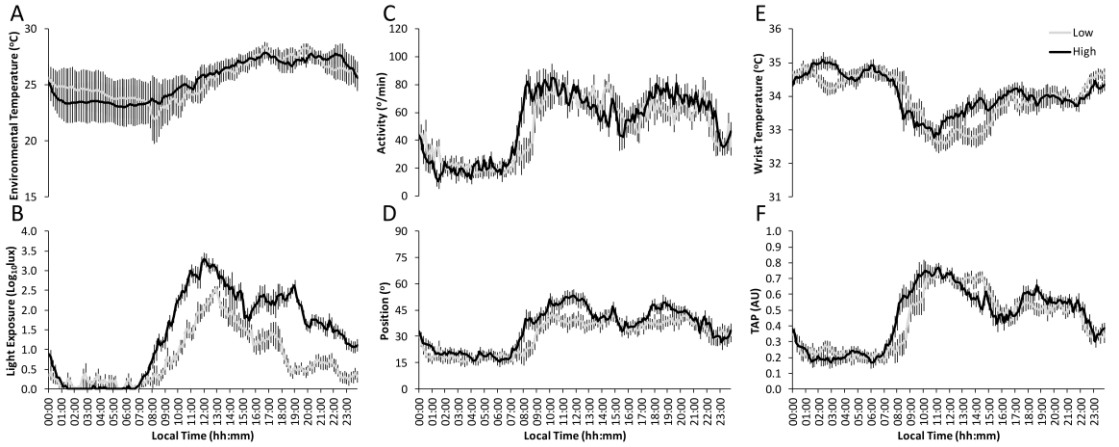


Figura 7. Ondas medias de ancianos en function de alto y bajo contraste en ICL. Ondas medias de temperature ambiental (A), luz (B), actividad (C), posición (D), WT (E) y TAP (F) para ancianos con alto (black line) y bajo ICL (grey line). Todas las variables están expresadas como media ± SEM.

Un mayor ICO en jóvenes (Figura 8) se asoció con mayor fragmentación (0.27 ± 0.02 *vs* 0.20 ± 0.02, p<0.01), menores valores MAX (1.89 ± 0.13 log_{10}lux *vs* 2.35 ± 0.081 log_{10}lux, p<0.01) y un avance de fase en TMAX (14:16 ± 00:26 h *vs* 17:55 ± 00:39 h, p<0.001) en exposición a la luz. Sin embargo, no mostraron cambios en ninguna otra variable excepto la RA de actividad, que fue menor con alto ICO (0.69 ± 0.01 *vs* 0.73 ± 0.01, p<0.05), probablemente debido a mayores valores mínimos (7.62 ± 0.37 º/min *vs* 6.21 ± 0.37 º/min, p<0.05).

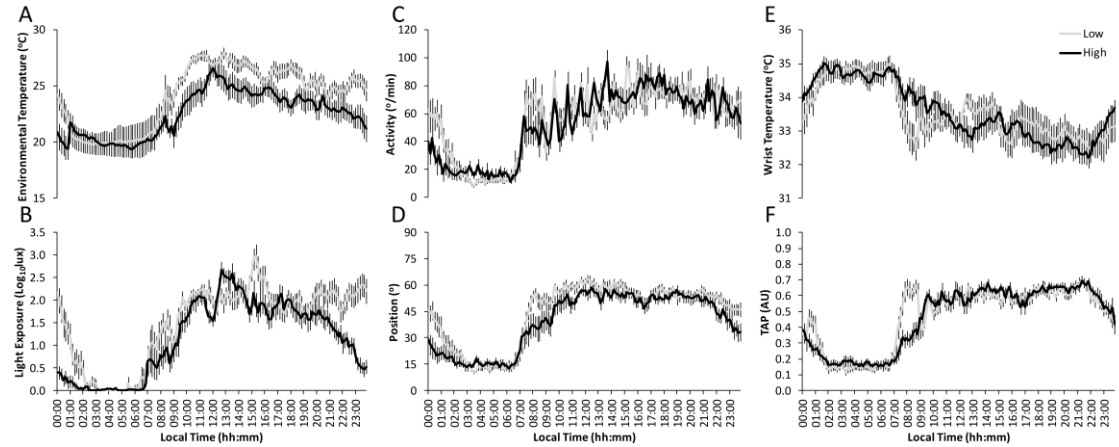


Figura 8. Ondas medias de jóvenes en function de alto y bajo contraste en ICO. Ondas medias de temperature ambiental (A), luz (B), actividad (C), posición (D), WT (E) y TAP (F) para gente joven con alto (black line) y bajo ICO (grey line). Todas las variables están expresadas como media ± SEM.

Los ancianos con alto ICO (Figura 9) presentaban valores más bajos de MAX durante la exposición a la luz (1.77 ± 0.10 log10lux vs 2.37 ± 0.10 log10lux, p<0.001). Además, se encontró una recuperación parcial de las caracteristicas de los jóvenes en el patron de WT, ya que la "zona de mantenimiento de la vigilia" de la tarde se recuperó parcialmente (33.84 ± 0.04 ºC vs 34.16 ± 0.04 ºC, p<0.001), junto con una mayor estabilidad (0.77 ± 0.02 vs 0.64 ± 0.04, p<0.01) y amplitud relativa (0.03 ± 0.00 vs 0.02 ± 0.00, p<0.001), and lower fragmentation (0.14 ± 0.01 vs 0.25 ± 0.02, p<0.01) and MIN values (32.67 ± 0.12 ºC vs 33.64 ± 0.20 ºC, p<0.001). Activity and position did not show significant changes according to the DQI score in old people, whereas TAP variable showed higher amplitude (0.57 ± 0.04 vs 0.46 ± 0.03, p<0.05).

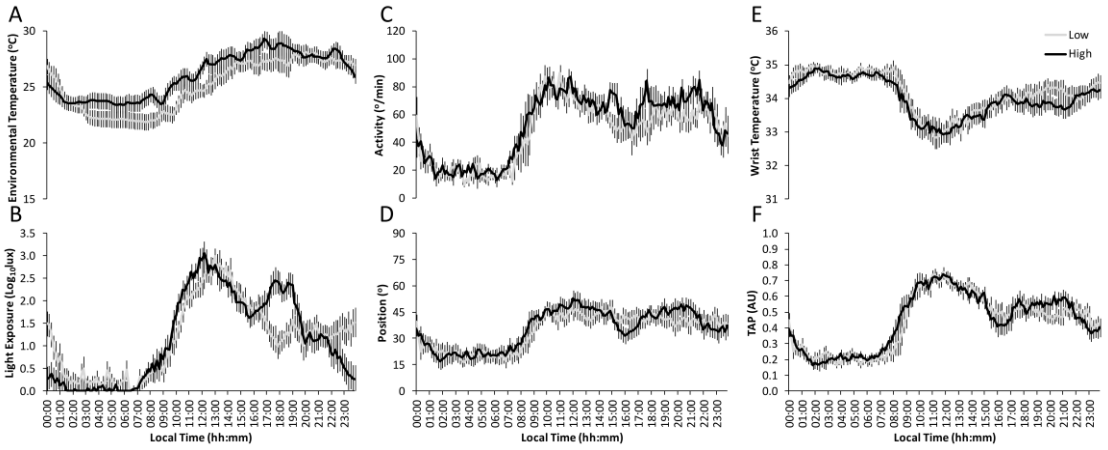


Figure 9. Onda media de ancianos en función de un ICO alto o bajo. Ondas medias de temperature ambiental (A), exposición a la luz (B), actividad (C), posición (D), temperature de la muñeca (E) y la variable integrada TAP (F) para ancianos con elevado (línea negra) y bajo ICO (línea gris). Todas las variables están expresadas en media ± SEM.

DISCUSION

Nuestros resultados revelaron que los ancianos están expuestos a zeitgebers más débiles, principalmente durante la tarde, cuando están inactivos y soñolientos. Además, experimentaron una desincronización de fase en los ritmos de temperatura de la muñeca, actividad, y exposición a la luz. Finalmente, una mejoría de la calidad de la exposición a la luz restaura, al menos parcialmente, un patrón circadiano de temperatura de a piel distal más joven.

Los ancianos se exponían a luz tenue o a oscuridad más de la mitad del día, mientras que la mayor parte de su exposición a la luz se concentra al mediodía. Desde el mediodía hasta la noche, la exposición a la luz muestra una disminución progresiva en gente anciana. Para los jóvenes, el pico de exposición a la luz occuría a mediodía, la oscuridad de la noche era más corta que en ancianos, y el atardecer y el inicio de la noche mostró un plateau de luz artificial, como se describió previamente (Martinez-Nicolas et al., 2011; 2013; 2014). Comparado con los jóvenes, el pico de exposición a la luz estaba adelantado en ancianos, tenían noches más largas con mayores interrupciones de luz y días más oscuros, principalmente durante la tarde y la noche, lo que implica que los ancianos pasan más tiempo en interiores y con niveles de intensidad de luz más bajos durante la fase activa, y exposición ocasional a la luz durante la fase de reposo. Aunque los ancianos se exponían a más de 1000 lux por un periodo de tiempo más prolongado que en jóvenes, este tiempo podría ser insuficiente para mantener de forma apropiada una sincronización circadiana. El amarilleamiento de la lente (Turner et al., 2010), que provoca que necesiten 5 veces más tiempo de más de 1000 lux ha sido propuesto como una de las razones de la disrupción circadiana en ancianos (Turner & Mainster, 2008).

En cuanto al ritmo de temperatura ambiental, se registraron temperaturas más bajas durante la noche y más elevadas durante el día en ambos grupos (ancianos y jóvenes), como se publicó anteriormente (Martinez-Nicolas et al., 2013). Sin embargo, la temperature ambiental media a lo largo del día era más cálida en ancianos y las máximas temperaturas estaban retrasadas a la tarde en lugar de a mediodía, como ocurría en jóvenes, por lo que el patrón ambiental estaba aplanado. Probablemente, la disminución en la percepción térmica, especialmente para percibir estímulos cálidos (Blatteis, 2012), justificaría por qué los ancianos prefieren exponserse a ambientes más cálidos. Los ancianos intentarían, de acuerdo con otros autores, compensar el deficit en la respuesta autonomica a los ambientes más fríos debida a una disminución en la tasa de descarga y funcionalidad de los nervios simpáticos (Holowatz et al., 2010; Holowatz & Kenney, 2010). Además, estos valores más elevados de temperature ambiental por la noche en ancianos podría relacionarse con las alteraciones del sueño que esta población experimenta, debido a su mayor dificultad para disipar calor (Kondo et al., 2007; Kräuchi, 2007; Wakamura & Tokura, 2002).

El ritmo de temperatura de la muñeca en ancianos mostró valores superiors a 34.5ºC durante la noche, que son comparables a valores descritos previamente para población joven (Sarabia et al., 2008). Además, la zona de mantenimiento de la vigilia para la temperature de la muñeca desapareció en ancianos, como descrito previamente para la vigilia (Münch et al., 2005) y los valores mínimos de WT estaban desplazados a la mañana, y su temperatura de la muñeca aumentaba de mediodía hasta la noche, con un plateau en la tarde. El ritmo de temperatura de la muñeca en jóvenes era similar al descrito previamente en otros estudios (Sarabia et al., 2008, Ortiz-Tudela et al., 2010, Martinez-Nicolas et al 2011, 2013, 2014, Blazquez et al., 2012). Además, los ancianos presentaron un period de noche más prolongado, valores más elevados durante el día, como si estuvieran soñolientos, que en jóvenes. Además de una somnolencia diurna real, una temperatura de la piel más elevada podría ser la consecuencia en la reducción de los mecanismos intrínsecos de termoregulación, con impacto en la capacidad del anciano para mantener la homeostasis térmica, particularmente de aquellos procesos asociados con la conservación del calor (Blatteis, 2012).

En cuanto a actividad y posición, jóvenes y ancianos mostraron una actividad más baja y una posición en decúbito durante la noche, como era esperable durante el sueño (Ortiz-Tudela et al., 2010). Sin embargo, los ancianos mostraron valores más altos de actividad y posición durante la noche, y valores más bajos durante el día, junto con un avance de fase de la actividad en comparación con jóvenes, hallazgos que se relacionan con el envejecimiento normal (Huang et al., 2002; Van Someren et al., 1999). Estos cambios en ancianos durante el día se relacionan con un estilo de vida sedentario, con posturas en posición horizontal, o semi-recostados, y baja actividad, mientras que los cambios nocturnos están relacionados con los despertares durante el sueño y un sueño menos profundo (Ortiz-Tudela et al., 2014).

La variable TAP integrada mostró valores más bajos durante la noche y más elevados durante el día, como descrito previamente (Ortiz-Tudela et al., 2010). Sin embargo, los ancianos mostraron un avance de fase y valores más altos en la mañana y más bajos en la tarde y el anochecer que los jóvenes. Además, los ancianos presentaron valores más altos durante la noche que, de nuevo, se asocian a un sueño más superficial (Ortiz-Tudela et al., 2014).

En el grupo de ancianos hay mayor número de sujetos con un ritmo de temperatura de la muñeca no significativo, lo que implica un estado de cronodisrupción. Además, un periodo mayor de 24 horas y una inestabilidad de fase también se asocian a los cambios relacionados con la edad del sistema circadiano (Turek et al., 1995). Estos efectos de la edad se deben probablemente a cambios en el núcleo supraquiasmático, como la reducción en la amplitud de la actividad neural (Nakamura et al., 2011). Asociado a ésto, los ancianos mostraron mayores diferencias de fase entre los ritmos manifiestos, y entre el reloj interno y el reloj externo evaluado como diferencias entre temperatura de la piel, actividad y exposición a la luz. Esto implica una elevada desincronización en el grupo de ancianos, que otros autores sugieren debido a discrepancias entre los ritmos manifiestos (Aschoff, 1965; Kohyama, 2009).

Las diferencias mencionadas en los ritmos circadianos entre jóvenes y adultos nos permiten, por primera vez, diferenciarlos sin tener en cuenta su edad biológica, sino únicamente las características de sus ritmos circadianos. Por ello, una acrofase precoz para exposición a temperature ambiental, valores bajos de posición diurna, ritmo de actividad con acrofase precoz y valores bajos, desplazamiento matutino de los mínomos de temperature en la muñeca (equivalente a la zona de mantenimiento de la vigilia), y acrofase precoz del TAP, se corresponden con los ancianos, y lo inverso con los jóvenes, con una tasa de acierto global del 96.5%, y solo 3 errores de 90.

El índice de calidad de la luz fue también útil para la caracterización de los ancianos, como descrito previamente para jóvenes (Martinez-Nicolas et al., 2011). Además, la selección de acuerdo a valores más altos del índice de calidad de la oscuridad se asoció a patrones robustos de WT en ancianos, como descrito previamente con el índice de calidad de la luz en jóvenes (Martinez-Nicolas et al., 2011), lo que sugiere que un alto contraste día/noche, y un aumento en la exposición a la luz por la tarde podría de algún modo minimizar, o al menos retrasar, algunas de las alteraciones relacionadas con la edad del sistema circadiano. Sin embargo, no se encontraron cambios en el ritmo de WT en jóvenes de acuerdo al ICO, lo que podría deberse a que los sujetos seleccionados por este índice también se exponían a niveles más bajos de luz durante el dia. Por ejemplo, un valor más bajo en el ICO se relacionó con una perdida de la zona de mantenimiento de la vigilia en el ritmo de temperatura de la muñeca, con una mayor fragmentación en el ritmo de actividad y amplitudes más bajas, todos ellos relacionados con el proceso de envejecimiento (Münch et al., 2005; Van Someren et al.,

1999). Además, había sujetos ancianos con una puntuación elevada en los indices de calidad luz/oscuridad cuyos patrones circadianos mostraban características de gente joven. Estos sujetos, desde un punto de vista cronobiológico, son candidatos a una mayor esperanza de vida y/o mejor calidad de vida; sin embargo, es necesario realizar otros experimentos para comprobar esta hipótesis.

En conclusion, el envejecimiento del sistema circadiano se asocia con una disminución del contraste día-noche, mayor fragmentación, avance de fase, y alteración del orden interno. Estos cambios son tan consistentes que los parámetros circadianos pueden ser implementados en árboles de decisión que permiten discriminar con precisión entre ancianos y jóvenes. El envejecimiento el sistema circadiano podría ser provocado por el envejecimiento del marcapasos circadiano, o cambios comportamentales obsrvados durante la tarde y el anochecer, caracterizados por una muy baja actividad, exposición a luz tenue durante el día, y alta somnolencia. Finalmente, el cambio del estilo de vida de los ancianos mejorando su patron de exposición a la luz debería ser tenida en cuenta como un tratamiento reforzador del sistema circadiano para mejorar su salud circadiana.

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REFERENCES

Aschoff J. (1965). Circadian rhythms in man. Science. 148:1427-32.

Blatteis CM. (2012). Age-dependent changes in temperature regulation – A mini review. Gerontology. 58:289-95.

Blazquez A, Martinez-Nicolas A, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity, and body position as determinants of the circadian pattern of blood pressure. Chronobiol Int. 29:747-56.

Bonmati-Carrion MA, Middleton B, Revell V, Skene DJ, Rol MA, Madrid JA. (2014). Circadian phase assessment by ambulatory monitoring in humans: Correlation with dim light melatonin onset. Chronobiol Int. 31:37-51.

Buijs RM, Kalsbeek A. (2001). Hypothalamic integration of central and peripheral clocks. Nat Rev Neurosci. 2:521-6.

Corbalan-Tutau MD, Madrid JA, Ordovás JM, Smith CE, Nicolas F, Garaulet M. (2011). Differences in daily rhythms of wrist temperature between obese and normal-weight women: Associations with metabolic syndrome features. Chronobiol Int. 28:425-33.

Davis S, Mirick DK. (2006). Circadian disruption, shift work and the risk of cancer: A summary of the evidence and studies in seattle. Cancer Causes Control. 17:539-45.

Duguay D, Cermakian N. (2009). The crosstalk between physiology and circadian clock proteins. Chronobiol Int. 26:1479-513.

Erren TC, Reiter RJ. (2009). Defining chronodisruption. J Pineal Res. 46:245-7.

Garaulet M, Madrid JA. (2010). Chronobiology: Influences on metabolic syndrome and cardiovascular risk. Curr Cardiovasc Risk Rep. 4:15-23.

Gompper B, Bromundt V, Orgül S, Flammer J, Kräuchi K. (2010). Phase relationship between skin temperature and sleep-wake rhythms in women with vascular dysregulation and controls under real-life conditions. Chronobiol Int. 27:1778-96.

Gronfier C, Wright KP, Kronauer RE, Czeisler CA. (2007). Entrainment of the human circadian pacemaker to longer-than-24-h days. Proc Natl Acad Sci USA. 104:9081-6.

Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. (2011). Melatonin-A pleiotropic, orchestrating regulator molecule. Prog Neurobiol. 93:350-84.

Hofman MA, Swaab DD. (2006). Living by the clock: The circadian pacemaker in older people. Ageing Res Rev. 5:33-51.

Hofstra WA, de Weerd AW. (2008). How to assess circadian rhythm in humans: A review of literature. Epilepsy Behav. 13:438-44.

Holowatz LA, Kenney WL. (2010). Peripheral mechanisms of thermoregulatoy control on skin blood flow in aged humans. J Appl Physiol. 109:1538-44.

Holowatz LA, Thompson-Torgerson C, Kenney WL. (2010). Aging and control of human skin blood flow. Front Biosci. 15:718-39.

Huang YL, Liu RY, Wang QS, Van Someren EJW, Xu H, Zhou JN. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. Physiol Behav. 76:597-603.

Karlsson B, Knutsson A, Lindahl B. (2001). Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 58:747-52.

Kohyama J. (2009). A newly proposed disease condition produced by light exposure during night: Asynchronization. Brain Dev. 31:255-73.

Kondo M, Tokura H, Wakamura T, Hyun KJ, Tamotsu S, Morita T, Oishi T. (2007). Physiological significance of cyclic changes in room temperature around dusk and dawn for circadian rhythms of core and skin temperature, urinary 6-hydroxymelatonin sulfate, and waking sensation just after rising. J Physiol Anthropol. 26:429-36.

Kotsiantis SB. (2007). Supervised machine learning: A review of classification techniques. Informatica. 31:249-68.

Kräuchi K. (2007). The thermophysiological cascade leading to sleep initiation in relation to phase of entrainment. Sleep Med Rev. 11:439-51.

Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. (2000). Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol. 278:R741-8.

Kräuchi H, Gompper B, Hauenstein D, Flammer J, Pflüger M, Studerus E, Schötzau A, Orgül S. (2012). Diurnal blood pressure variations are associated with changes in distal-proximal skin temperature gradient. Chronobiol Int. 29:1273-83.

Lavie P. (1968). Ultrashort sleep-waking schedule. III. ‘gates’ and ‘forbidden zones’ for sleep. Electroencephalogr Clin Neurophysiol. 63:414-25.

Martinez-Nicolas A, Madrid JA, Rol MA. (2014). Day-night contrast as source of health for the human circadian system. Chronobiol Int. 31:382-93.

Martinez-Nicolas A, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. Chronobiol Int. 28:617-29.

Martinez-Nicolas A, Ortiz-Tudela E, Rol MA, Madrid JA. (2013). Uncovering different masking factors on wrist skin temperature rhythm in free-living subjects. PLoS ONE. 8:e61142.

Middleton B, Stone BM, Arendt J. (2002). Human circadian phase in 12:12 h, 200: <8 lux and 1000: <8 lux light-dark cycles, without scheduled sleep or activity. Neurosci Lett. 329:41-4.

Minors D, Atkinson G, Bent N, Rabbitt P, Waterhouse J. (1998). The effects of age upon some aspects of lifestyle and implications for studies on circadian rhythmicity. Age Ageing. 27:67-72.

Münch M, Knoblauch V, Blatter K, Schröder C, Schnitzler C, Kräuchi K, Wirz-Justice A, Cajochen C. (2005). Age-related attenuation of the evening circadian arousal signals in humans. Neurobiol Aging. 26:1307-19.

Nakamura TJ, Nakamura W, Yamazaki S, Kudo T, Cutler T, Colwell CS, Block GD. (2011). Age-related decline in circadian output. J Neurosci. 31:10201-5.

Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. (2010). A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comput Biol. 6:e1000996.

Ortiz-Tudela E, Martinez-Nicolas A, Albares J, Segarra F, Campos M, Estivill E, Rol MA, Madrid JA. (2014). Ambulatory circadian monitoring (ACM) based on thermometry, motor activity and body position (TAP): A comparison with polysomnography. Physiol Behav. 126:30-8.

Pauley SM. (2004). Lighting for the human circadian clock: Recent research indicates that lighting has become a public health issue. Med Hypotheses. 63:588-96.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 27:1911-29.

Rodrigues Menezes MC, Nogueira Pires ML, Benedito-Silva AA, Tufik S. (2004). Sleep parameters among offshore workers: An initial assessment in the Campos Basin, Rio de Janeiro, Brazil. Chronobiol Int. 21:889-97.

Sarabia JA, Rol MA, Mendiola P, Madrid JA. (2008). Circadian rhythm of wrist temperature in normal-living subjects. A candidate of new index of the circadian system. Physiol Behav. 95:570-80.

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Fuchs CS, Colditz GA. (2003). Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst. 95:825-8.

Scheuermaier K, Laffan AM, Duffy JF. (2010). Light exposure patterns in healthy older and young adults. J Biol Rhythms. 25:113-22.

Stratmann M, Schibler U. (2006). Properties, entrainment, and physiological functions of mammalian peripheral oscillators. J Biol Rhythms. 21:494-506.

Touitou Y, Haus E. (1994). Aging of the human endocrine and neuroendocrine time structure. Ann N Y Acad Sci. 31:378-97.

Turek FW, Penev P, Zhang Y, van Reeth O, Zee P. (1995). Effects of age on the circadian system. Neurosci Biobehav Rev. 19:53-8.

Turner PL, Mainster MA. (2008). Circadian photoreception: ageing and the eye's important role in systemic health. Br J Ophthalmol. 92:1439-44.

Turner PL, Van Someren EJW, Mainster MA. (2010). The role of environmental light in sleep and health: Effects of ocular aging and cataract surgery. Sleep Med Rev. 14:269-80.

Van Marken Lichtenbelt WD, Daanen HAM, Wouters L, Fronczek R, Raymann RJEM, Severens NMW, Van Someren EJW. (2006). Evaluation of wireless determination of skin temperature using iButtons. Physiol Behav. 88:489-97.

Van Someren EJW, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. (1999). Bright light therapy: Improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int. 16:505-18.

Wakamura T, Tokura H. (2002). Circadian rhythm of rectal temperature in humans under different ambient temperature cycles. J Thermal Biol. 27:439-47.

Weinert D. (2010). Circadian temperature variation and ageing. Ageing Res Rev. 9:51-60.

Weinert D, Waterhouse J. (2007). The circadian rhythm of core temperature: Effects of physical activity and aging. Physiol Behav. 90:246-56.

Zornoza-Moreno M, Fuentes-Hernández S, Sánchez-Solis M, Rol MA, Larqué E, Madrid JA. (2011). Assessment of circadian rhythms of both skin temperature and motor activity in infants during the first 6 months of life. Chronobiol Int. 28:330-7.

3.6. EXPERIMENTAL CHAPTER 6

DAY-NIGHT CONTRAST AS SOURCE OF HEALTH FOR THE HUMAN CIRCADIAN SYSTEM

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3.6. DAY-NIGHT CONTRAST AS SOURCE OF HEALTH FOR THE HUMAN CIRCADIAN SYSTEM

ABSTRACT

Modern societies are characterized by a 24/7 lifestyle with no environmental differences between day and night, resulting in weak *zeitgebers* (dim daylight, absence of night darkness, constant environmental temperature, sedentary lifestyle and frequent snacking), and as a consequence, in an impaired circadian system through a process known as chronodisruption. Both weak *zeitgebers* and circadian system impairment are related to human pathologies, (certain cancers, metabolic syndrome, and affective and cognitive disorders), but little is known about circadian system chronoenhancement. The aim of this work is to propose practical strategies for chronoenhancement, based on increasing day/night contrast. For this, wrist temperature, activity, position, sleep and light and temperature exposure were recorded in 131 young subjects under free-living conditions for 1 week. Subjects with high contrast (HC) and low contrast (LC) for each variable were selected to analyze the high contrast effect in activity, position, sleep and light and temperature exposure would have on wrist temperature. We found that HC showed better rhythms than LC for every variable except sleep. Subjects with HC and LC for wrist temperature also demonstrated lifestyle differences, where HC subjects had an advanced night phase onset and a general increase in contrast. In addition, theoretical high day/night contrast calculated using mathematical models suggests an improvement by means of lifestyle contrast. Finally, some individuals classified as belonging to the HC group in terms of wrist temperature when they are exposed to the lifestyle characteristic of the LC group, while others exhibit wrist temperature arrhythmicity despite their good lifestyle habits, revealing two different wrist temperature components: an exogenous component modified by lifestyle and another endogenous component that is refractory to it. Therefore, intensifying day/night contrast in subject's lifestyle has proven to be a feasible measure to chronoenhance the circadian system.

Keywords: Chronodisruption, Chronoenhancement, Zeitgeber exposure, Contrast, Human circadian system, Ambulatory circadian monitoring, Wrist temperature.

INTRODUCTION

Nowadays, our society is characterized by a progressive trend towards a 24/7 lifestyle. Members of this society spend most of their time indoors, resulting in exposition to weak or contradictory environmental and behavioural synchronizing cues. Thus, their environment is characterized by poor light and warm temperature, while their behavior by irregularity in sleep time, low physical activity and frequent meals and/or snacks eaten at short intervals. Furthermore, the population is involved in more shift-work, takes more transmeridian flights and is exposed to increasing levels of social jet-lag (Rajaratnam & Arendt, 2001; Reiter et al., 2007; Wittmann et al., 2006). These features can lead to impairments of the circadian system or chronodisruption (significant disturbance of the internal temporal order of circadian rhythms) as the result of weak, absent or conflicting environmental cues (Erren & Reiter, 2009a).

In mammals, the circadian system is composed of a clock-like machinery consisting of the central pacemaker, the suprachiasmatic nucleus of hypothalamus (SCN), peripheral oscillators in most (if not all) of the tissues and organs, and the input and output pathways responsible for entrainment and generation of circadian rhythms, respectively. All these structures and pathways are hierarchically organized to generate biological rhythms and to synchronize the organism to the natural environment (Buijs & Kalsbeek, 2001; Duguay & Cermakian, 2009; Stratmann & Schibler, 2006).

The SCN is entrained everyday by periodical light input (Brainard et al., 1997; Skene et al., 1999) from the retina through the retinohypothalamic tract (Güler et al., 2008; Moore et al., 2002). Furthermore, there are other periodical cues, such as scheduled sleep, exercise and feeding can also contribute to entraining the circadian system (Atkinson et al., 2007; Danilenko et al., 2003; Mendoza, 2007; Mistlberger & Skene, 2004). It has also been found that environmental temperature influences core body temperature rhythm (Refinetti, 2010) and entrains cellular circadian rhythms in vitro and in vivo (Brown et al., 2002).

One of the major characteristics of an environmental variable in order to be effective as a *zeitgeber* (environmental cue able to entrain circadian clocks) is the contrast between its corresponding day and night levels. Thus, a high contrast in day/night exposure to light increases the amplitude of most rhythms, such as melatonin and core body temperature (Cajochen et al., 2005; Mishima et al., 2001; Park & Tokura,

1998). On the contrary, low contrast is associated with transient impaired circadian functionality over the short-term, and chronodisruption (CD) over the long-term (Erren and Reiter, 2009a). CD manifests by means of flattened, fragmented rhythms, the loss of rhythmicity and stability and phase shifts among the different oscillators and/or between the SCN and the external cues. CD can be present as the result of insufficient daytime light and excessive nighttime light intensities (Erren and Reiter, 2009b), producing phase instability or free running in the short-term, and an increased risk of suffering some types of cancer, metabolic syndrome, cognitive alterations, dysregulation of reproduction, cardiovascular events, and sleep disorders in the long-term among other health impairments (Davis & Mirick, 2006; Garaulet & Madrid, 2010; Gronfier et al., 2007; Karlsson et al., 2001; Middleton et al., 2002; Pauley, 2004; Rodrigues Menezes et al., 2004; Schernhammer et al., 2003). In addition, other factors are capable of triggering CD, such as high environmental temperature during the nighttime, which attenuates Core Body Temperature (CBT), or physical activity which induces an increase in CBT when performed at night, flattening the amplitude of CBT (Weinert & Waterhouse, 2007).

Several rhythms, called circadian marker rhythms, are currently used to evaluate the status of the circadian system. The marker rhythms most commonly used in human circadian assessment are melatonin, cortisol, CBT and actimetry (Mormont et al., 2002; Van Someren, 2000). Recently, distal skin temperature (DST) has been suggested as indicator of heat loss, a major regulator of CBT (Rubinstein & Sessler, 1990). DST is mainly measured in wrist and ankle (Gompper et al., 2010; Martinez-Nicolas et al., 2013; Sarabia et al., 2008; Zornoza-Moreno et al., 2011), and it has been demonstrated its utility to assay the circadian phase (Kolodyazhniy et al., 2011, 2012). DST exhibits an inverse phase relationship and a small phase advance with respect to CBT, allowing the internal heat loss necessary for sleep (Kräuchi et al., 2000; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). In addition, wrist temperature (WT) is flattened in obesity (Corbalan-Tutau et al., 2011) and in non-dipping blood pressure patients (Blazquez et al., 2012), suggesting WT as a health marker. Moreover, WT has been proposed as a non-invasive, robust, and easy-to-record index for circadian system functioning (Ortiz-Tudela et al., 2010; Sarabia et al., 2008; Zornoza-Moreno et al., 2011). However, all circadian marker rhythms are masked by external factors. Two procedures are capable of eliminating these interferences. One such procedure involves a constant routine

protocol that avoids masking effects by maintaining the subject awake in bed with low levels of activity, dim light and meals that are constant in terms of composition and timing. However, this protocol is quite different from natural home conditions and it introduces its own masking effects (Minors & Waterhouse, 1989; Rietveld et al., 1993; Weinert & Waterhouse, 2007). Fortunately, there are purification procedures (mathematical methods) capable of eliminating the masking effects on data collected under ambulatory conditions (Waterhouse et al., 2000; Weinert & Waterhouse, 1998).

To date, most of the research on the relationship between the circadian system and human health has focused on the impact of chronodisruption on human health and well-being (Garaulet & Madrid, 2010; Erren & Reiter, 2009a); however, little has been published about potentiation or chronoenhancement (potentiation of synchronizers and overt rhythms) of the circadian system. This is particularly relevant, considering the increasing incidence of chronodisruptive-related pathologies in Western societies. Thus, the aim of this work is to propose practical strategies for enhancing distal temperature pattern (as marker of the human circadian system) evaluated on the wrist. analyzing the influence of the day/night contrast in light and environmental temperature (two markers of external time with strong synchronizing effects) and motor activity, body position and sleep-wake patterns (three behavioural rhythms with synchronizing effects as the result of the feed-back they provide to circadian oscillators) on human circadian functionality, as assessed by an easy-to-measure and comfortable overt rhythm: wrist temperature.

MATERIAL AND METHODS

Subjects

For the present study, 131 undergraduate student volunteers (66 men and 65 women, 19-25 years old) residents in Murcia, Spain (latitude 38º 01’ N) were recruited. All data was recorded in real life during the last week in October and the first two weeks of November in 2011.

The study was abided by the bioethical principles set out by the Declaration of Helsinki. Data from the volunteers were included in a database and were protected according to Spanish Law 15/1999, of 13 September. All participants received

appropriate information about the study characteristics and signed an informed consent form prior to their inclusion in the study (Portaluppi et al., 2010).

Participants were instructed to wear all sensors for 7 consecutive days, and were also encouraged to maintain their habitual lifestyle.

Wrist skin temperature measurement

All subjects wore a Thermochron iButton DS1921H (Maxim Integrated Products, Sunnyvale, California, USA) to measure wrist skin temperature (WT), with a precision of ±0.125 ºC. This temperature sensor was placed on the wrist of the non-dominant hand over the radial artery and was isolated from the environmental temperature by a double-sided cotton sport wrist band, as previously described (Sarabia et al., 2008). The device was programmed to sample every 10 min over the course of the entire week.

Body position and activity rhythm measurement

The body position and activity rhythm were assessed every 30 seconds, using a HOBO Pendant G Acceleration Data Logger UA-004-64 (Onset Computer, Bourne, Massachusetts, USA) placed on the non-dominant arm by means of a sport band, with its X-axis parallel to the humerus bone. The manufacturing specifications and the method to obtain these variables have been described in a previous work (Ortiz-Tudela et al., 2010). Activity is measured as rate of change in degrees per minute, and position represents the position of the subject at each moment. Both variables are averaged every 10 minutes to obtain the same sampling frequency as that used for wrist temperature.

Environmental temperature and light exposure recording

In addition, all subjects were required to wear a HOBO Pendant Temperature/Light Data Logger UA-002-64 (Onset Computer, Bourne, Massachusetts, USA) on a neck chain to record environmental temperature and light exposure. Manufacturing specifications, memory, spectrum and accuracy have been described in a previous work (Martinez-Nicolas et al., 2011). The device recorded at regular pre-programmed intervals of 30 seconds. Light intensities in lux were converted into logarithmic units and averaged every 10 min to allow for comparison with temperature data.

Data analysis

Artifacts produced by temporarily removing the temperature, activity or light sensors were filtered from the raw data. In addition, to eliminate atypical data, the interquartile distance (from Q1 to Q4) was calculated and each time point for which the rate of change with respect to the previous value was higher than the interquartile distance was eliminated (Van Marken Lichtenbelt et al., 2006). If during the filtering process a subject lost more than 2 days of data from any one variable, the variable was discarded. The mean daily pattern for all variables was calculated per individual, and then averaged per group. Sleep probability was calculated from sleep logs. This parameter indicated the percentage of individuals asleep at any given time, as previously described (Sarabia et al., 2008).

To determine the effect of day/night contrast on circadian rhythmicity, we classified the subjects into deciles based on relative amplitude (RA, the parameter used as an index of contrast). In the case of variables with an acrophase that occurred during the rest period (WT and sleep), RA was calculated as the difference between M5 (average measured in 10-min intervals for the 5 consecutive hours with the maximum values) and L10 (average measured in 10-min intervals for the 10 consecutive hours with the minimum values), divided by the sum of M5 and L10, as previously published by Van Someren (Van Someren et al., 1999). However, in the case of variables with an acrophase that occurs during the activity period (light exposure, environmental temperature, activity and position), these calculations were modified by using the five consecutive hours of minimum values (L5) and the ten consecutive hours of maximum values (M10). Once the RA was calculated, we select the two extreme groups, the highest (HC) and lowest (LC) contrast groups (the 10th decile and 1st decile, respectively), and calculated their mean waveform for each variable. Moreover, to characterize these waveforms, the following non-parametrical indexes (previously reported by Van Someren (Van Someren et al., 1999)) were calculated: Interdaily Stability (IS), Intradaily Variability (IV), Relative Amplitude (RA), MAX (M5 for WT and sleep, and M10 for activity, position, light and environmental temperature) and MIN (L10 for WT and sleep, and L5 for activity, position, light and environmental temperature). Sleep, darkness, lying position and rest onset for both groups (HC and LC for WT) were calculated to describe the phase shift. The selected threshold nearest to sleep onset and maintained during at least 30 minutes was less than 40º/min for rest, less

than 10 lux for darkness, and less than 30º for lying position. To assess possible bias by including the same subjects when selecting HC or LC for the different variables, a coincidence matrix was performed. The maximum coincidence between 2 variables was less than a 40% (Table 1).

LC \ HC	ACTIVITY	POSITION	LIGHT	ET	SLEEP	WT
ACTIVITY	100	15	8	23	15	23
POSITION	8	100	27	36	0	18
LIGHT	15	9	100	27	9	18
ET	8	18	36	100	27	36
SLEEP	23	0	18	18	100	8
WT	31	27	0	9	23	100

Table 1. Coincidence matrix. The lower triangular matrix shows the percentage of coincident subjects between LC groups while the upper corresponds to HC groups for each variable. The diagonal matrix is coincident and maximum, since the coincidence of one variable with itself is 100%. The variables included are activity, position, light exposure (light), environmental temperature (ET), sleep and wrist temperature (WT).

To find out whether subjects exposed to general high contrast for physical (light and environmental temperature) and behavioural (activity, position and sleep) synchronizing variables also exhibited the highest contrast in WT rhythm, we calculated lifestyle (LS) contrast as the average for the RA decile of all variables with a synchronizing effect. The subjects were then classified by graphic matrix representations, according to two criteria:

a) Using LS contrast as the major classification criterion, and its corresponding WT pattern as a marker of circadian status. Individual physical and behavioural synchronizing variables were reordered by minimizing differences in mean deciles with respect to LS.

b) Using RA decile for WT as the main criterion, and then reordering the remaining variables in accordance with their mean minimal difference with WT deciles.

We applied demasking and mathematical modelling procedures to check whether subjects with the worst patterns were potentially sensitive to chronoenhancement by increasing the day/night contrast in the physical and behavioural synchronizing variables. The WT rhythm of subjects from the HC and LC deciles was

demasked by intercepts in one hour time bins, as described by Weinert and Waterhouse (Weinert & Waterhouse, 1998), using as intercepts complete darkness, no activity, lying position, and an environmental temperature of 20ºC, as proposed by Martinez-Nicolas (Martinez-Nicolas et al., 2013).

To simulate the theoretical pattern of WT in high contrast conditions, we used the previously demasked WT rhythms, superimposing a theoretical high contrast pattern for each masking variable defined as follows: a) dark (0 lux)/light (1000 lux); b) lying position (0º)/standing position (90º); c) no activity (0º/min)/high activity (50º/min) and d) cold temperatures (15ºC)/warm temperatures (25ºC), with low values during the rest phase (from 01:00 to 08:00 h) and high values during the activity phase (from 09:00 to midnight). The same procedure (superimposing a theoretical high contrast pattern on demasked data) was applied to deciles obtained according to day/night contrast in WT to determine if the robustness of the WT rhythm was associated with exposure to a particular *zeitgeber*.

Original, demasked and modeled WT patterns were analyzed with the cosinor method (see Tables 4 and 5), using the "El Temps" integrated package for temporal series analysis (A. Díez-Noguera, Universitat de Barcelona, 1999). All data were expressed as the mean ± SEM. The data were processed and non-parametrical indices calculated using Microsoft Office Excel 2010. Data processing flow (Figure 1) summarizes the methodological procedure followed and represents what is done in each methodological step and what pattern is obtained. All statistical analyses (One-way ANOVA for Tables 1 and 2 and repeated measures ANOVA with Bonferroni post hoc for Supplementary Tables 4 and 5) were performed with R 3.0.0. Values of $p < .05$ were considered to be statistically significant.

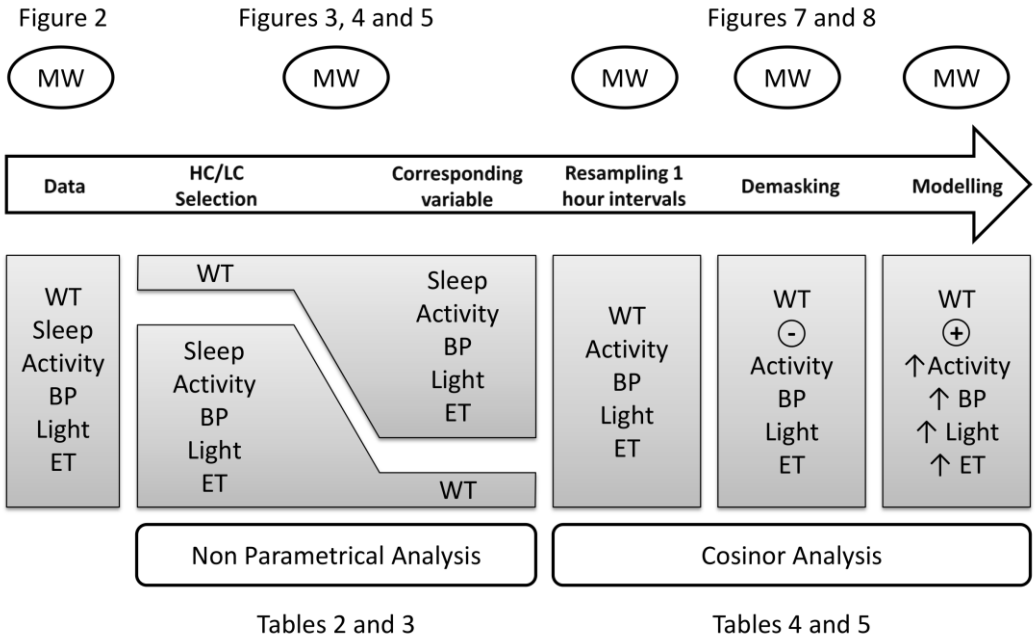

Figure 1. Diagram for data processing flow. Diagram for light, environmental temperature (ET), activity, body position (BP), sleep and wrist temperature (WT) processing flow represented by the horizontal arrow including data recording, high and low contrast selection (HC and LC, respectively), pattern obtained, resampling, demasking and modelling processes (in this order). The mean-waveforms (MW) shown in each figure are represented in the upper part, while data analyses shown in the tables are represented in the bottom part. Demasking processes are represented as WT minus each variable effect and modelling processes are represented as WT plus the enhanced effect (↑) by increasing contrast for each variable.

RESULTS

Mean patterns

The mean waveforms of all recorded variables are shown in Figure 2. As can be observed (Figure 2A), mean light exposure was less than 10 lux from 00:20 h (9.81 ± 1.15 lux) to 09:00 h (7.06 ± 1.10 lux) and greater than 100 lux from 12:30 h (122.43 ± 1.12 lux) to 15:50 h (119.30 ± 1.10 lux), with a plateau from 16:30 h (60.10 ± 1.11 lux) to 22:50 h (26.36 ± 1.12 lux). The environmental temperature rhythm (Figure 2A) showed a daily pattern of low amplitude, with the lowest values (decreasing to 22ºC) between 01:10 h (21.72 ± 0.32 ºC) and 09:20 h (21.96 ± 0.28 ºC), while the highest values (increasing to 23.5 ºC) occurred between 10:10 h (23.58 ± 0.29 ºC) and 00:10 h (23.56 ± 0.28 ºC). The minimal WTs (less than 32.6 ºC) were observed from 20:20 h to

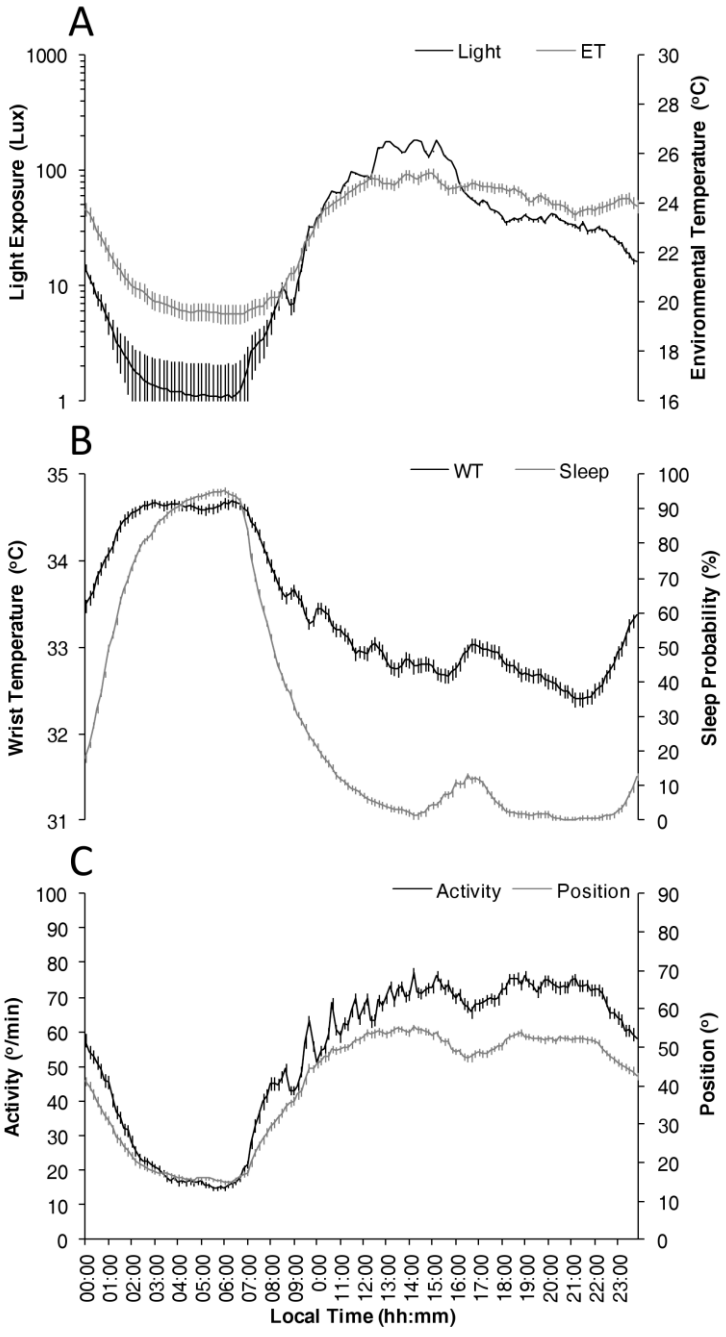

Figure 2. Mean-waveforms for the study population. Mean-waveforms for (**A**) light exposure (Light) and environmental temperature (ET), (**B**) wrist temperature (WT) and sleep probability and activity and position (**C**). All variables are expressed as the mean ± SEM (n=131).

22:20 h, coinciding with the minimal sleep probability (less than 1%), a period known as the wake maintenance zone (Figure 2B) (Lavie, 1968). WT reached maximum values (greater than 34.0 ºC) during the nighttime (from 01:00 h to 07:50 h), coinciding with higher sleep probability (greater than 50%). In addition, a second sleep probability peak (greater than 10%) was obtained from 16:10 h to 18:10 h as was described by Sarabia (Sarabia et al., 2008), associated with a slight increase of about .2 ºC in WT (32.94 ± 0.02 ºC during the second sleep probability peak, with respect to 32.71 ± 0.01 ºC during the hours before and after). As expected, activity and body position, (Figure 2C) exhibited stable, low values from 01:10 h to 07:50 h and higher values from 11:20 h to 22:10 h.

Synchronizing variables effect on wrist temperature

Figure 3 shows the WT pattern that is associated with high or low contrast in physical synchronizing variables, either light or environmental temperature. Subjects from the HC light group were exposed to nighttime light values of zero, while the LC group received some light during the nighttime (Figure 3A and Table 2A). The LC light group was associated with a significant reduction in IS, nocturnal values (MAX) and RA for WT (Table 2B), but not with phase changes. The LC environmental temperature group exhibited a slightly flattened WT pattern as the result of the reduction in the normal nighttime WT, together with a decrease in IS, as compared to the HC group (Figure 3B and Table 2B).

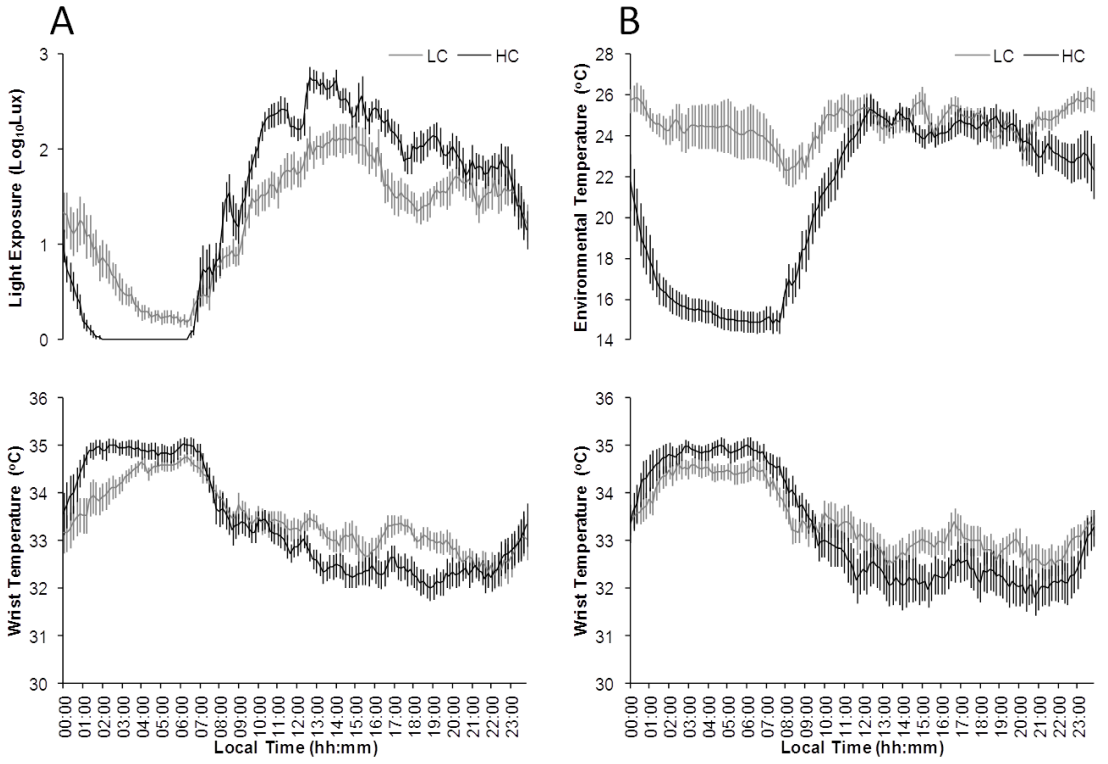

Figure 3. Wrist Temperature pattern for groups with high and low contrast in environmental *zeitgebers*. WT patterns (lower panels) selected by high and low contrast in environmental *zeitgebers* (upper panels), either for light exposure (A) or environmental temperature (B). LC indicates the first decile and HC the tenth decile, according to the corresponding RA. Each variable is expressed as the mean ± SEM (n=13 in each group).

Figure 4 shows the WT rhythm associated with high or low contrast in behavioural variables with a synchronizing effect (sleep, motor activity and body position). The sleep pattern in LC subjects was characterized by a delay in sleep onset (01:47 ± 00:12 vs. 01:01 ± 00:17 $p < 0.05$), reduced IS and RA, and an increase in MIN sleep probability, with respect to the HC sleep group (Figure 4A and Table 2A). In spite of these changes, no statistically significant alterations in the WT pattern were detected. The activity and position rhythms (Figure 4B and 4C, respectively and Table 2) in the LC group were more unstable, flattened and fragmented than in the HC group. The corresponding WT pattern showed reduced IS, RA and nighttime WT (MAX), together with increased IV and daytime WT, as shown in Table 2B. No statistically significant differences were observed in WT phasing between the low and high contrast groups when considered according to activity or position.

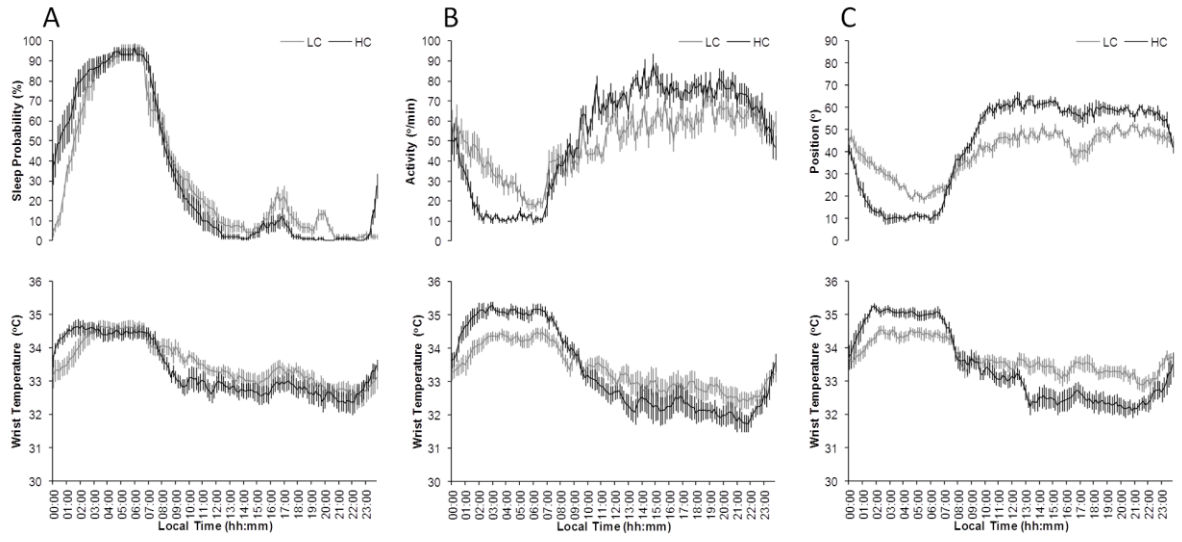

Figure 4. Wrist Temperature for groups with high and low contrast in rhythmic variables with a synchronizing effect. WT patterns selected by high and low contrast for sleep (A), activity (B) and position (C). LC indicates the first decile and HC the tenth decile, according to the corresponding RA. Each variable is expressed as the mean ± SEM (n=13 in each group).

A

	Group	Selected Variable: IS	IV	RA	MAX	MIN	PHASE
Light	HC	.67 ± .03#	.18 ± .01†	1.00 ± .00#	209.73 ± 1.22#	.00 ± .00#	04:02 ± 00:10*
	LC	.37 ± .02#	.27 ± .02†	.72 ± .04#	68.29 ± 1.21#	2.03 ± 1.13#	04:53 ± 00:20*
ET	HC	.61 ± .02#	.08 ± .01#	.25 ± .01#	24.73 ± .50	14.99 ± .54#	05:40 ± 00:17*
	LC	.23 ± .03#	.15 ± .01#	.07 ± .00#	25.90 ± .59	22.47 ± .58#	09:50 ± 01:47*
Act	HC	.46 ± .03#	.59 ± .02*	.76 ± .00#	77.81 ± 2.54†	10.69 ± .40#	04:09 ± 00:14†
	LC	.25 ± .01#	.76 ± .07*	.45 ± .02#	62.73 ± 4.13†	23.85 ± 1.68#	05:17 ± 00:16†
Pos	HC	.73 ± .02#	.17 ± .02#	.69 ± .02#	60.25 ± 1.29#	10.89 ± .71#	04:02 ± 00:13†
	LC	.34 ± .02#	.35 ± .02#	.36 ± .02#	48.00 ± 1.19#	22.42 ± .69#	05:14 ± 00:18†
Sleep	HC	.70 ± .04*	.08 ± .00†	1.00 ± .00#	93.12 ± 2.55	2.31 ± .60#	04:48 ± 00:16
	LC	.57 ± .04*	.10 ± .01†	.90 ± .01#	89.19 ± 2.80	7.28 ± .70#	04:53 ± 00:10

B

	Group	Wrist Temperature: IS	IV	RA	MAX	MIN	PHASE
Light	HC	.57 ± .03#	.13 ± .02	.04 ± .00†	34.98 ± .17*	32.30 ± .25	03:58 ± 00:18
	LC	.34 ± .03#	.16 ± .02	.03 ± .00†	34.56 ± .10*	32.78 ± .22	04:48 ± 00:20
ET	HC	.51 ± .05*	.14 ± .02	.04 ± .01	34.98 ± .16	32.14 ± .41	04:00 ± 00:21
	LC	.35 ± .04*	.18 ± .03	.03 ± .00	34.57 ± .16	32.71 ± .29	03:55 ± 00:26
Act	HC	.58 ± .03#	.11 ± .01†	.05 ± .00†	35.16 ± .10#	32.13 ± .26*	04:22 ± 00:14
	LC	.33 ± .04#	.20 ± .03†	.02 ± .00†	34.41 ± .13#	32.87 ± .19*	04:21 ± 00:43
Pos	HC	.57 ± .03#	.12 ± .02#	.04 ± .00#	35.11 ± .12#	32.40 ± .23†	03:36 ± 00:14
	LC	.30 ± .04#	.24 ± .03#	.02 ± .00#	34.50 ± .10#	33.20 ± .16†	04:05 ± 00:22
Sleep	HC	.39 ± .05	.19 ± .03	.03 ± .01	34.62 ± .17	32.59 ± .22	03:17 ± 00:30
	LC	.37 ± .05	.21 ± .04	.03 ± .01	34.61 ± .19	32.92 ± .24	04:11 ± 00:25

Table 2. Non-parametrical analysis of WT and corresponding exposure to *zeitgebers* or rhythmic variables with a synchronizing effect, according to their high or low contrast. HC and LC (High and low contrast, respectively) corresponds to the highest and lowest deciles, respectively, for the RA for each variable (n=13 in each group). Main characteristics for light exposure, environmental temperature (ET), activity, position and sleep 24-h high and low contrast patterns (Section A) and corresponding wrist temperature non-parametric indexes (Section B): Interdaily Stability (IS), Intradaily Variability (IV), Relative Amplitude (RA), mean of the 5 consecutive hours with the highest values (MAX), and the 10 consecutive hours with the lowest values (MIN) for wrist temperature and sleep and the mean of the 10 consecutive hours with the highest values (MAX), and the 5 consecutive hours with the lowest values (MIN) for light exposure, environmental temperature, activity, position. The PHASE indicates the timing for the midpoint of each MAX or MIN. Values are expressed as the mean ± SEM. # indicates $p < 0.001$, † $p < 0.01$, and * $p < 0.05$, according to a one-way ANOVA.

Wrist temperature contrast associations to life style

To determine how subjects belonging to low and high contrast WT groups exposed themselves to synchronizing variables, we selected only those subjects included in the upper and lower decile for WT contrast; their WT patterns are represented in Figure 5. Low contrast in WT was characterized by an arrhythmic pattern (Figure 5A) with lower stability, relative amplitude, and MAX values, together with higher fragmentation and MIN values than those observed for the HC group (Table 3). However, unexpectedly, this arrhythmicity was only associated with limited changes in their corresponding physical and behavioural synchronizing variables (Figures 5B, C, D, E and F). All these variables were characterized by a delay associated with the resting period time, i.e. a delay in sleep onset (02:08±00:14 vs. 00:54±00:10 $p < 0.001$, Figure 5B), darkness onset (00:05±00:26 vs. 01:09±00:16 $p < 0.05$, Figure 5C) and activity (02:15±00:19 vs. 00:56±00:15 $p < 0.01$, Figure 5E) and changes in body position towards proneness (02:14±00:22 vs. 00:34±00:15 $p < 0.001$, Figure 5F). In addition, a lower RA in environmental temperature, activity and body position, together with a significant increase in activity and sleep fragmentation were found in the LC WT group, with respect to the high contrast group (Table 3).

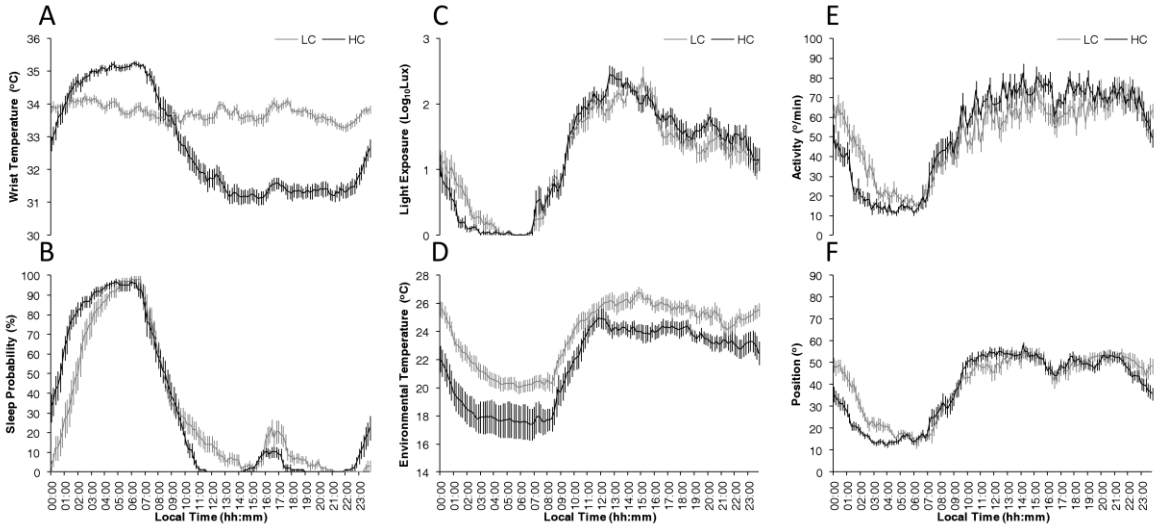

Figure 5. High and low contrast in WT pattern and corresponding exposure to *zeitgebers* or rhythmic variables with a synchronizing effect. High and low contrast in WT pattern (A) and the corresponding sleep (B), light exposure (C), environmental temperature (D), activity (E) and position (F) patterns. LC indicates the first decile and HC the tenth decile, according to the corresponding RA. Each variable is expressed as the mean ± SEM (n=14 in each group).

		Selected Variable					
	Group	IS	IV	RA	MAX	MIN	PHASE
WT	HC	.58 ± .02	.07 ± .00	.06 ± .00	35.12 ± .09	31.25 ± .15	04:57 ± 00:16
	LC	.20 ± .02#	.33 ± .03#	.01 ± .00#	34.15 ± .09#	33.55 ± .10#	00:50 ± 01:12†
Light	HC	.56 ± .02	.25 ± .02	.97 ± .01	103.6 ± 1.20	1.07 ± 1.03	04:47 ± 00:15
	LC	.50 ± .03	.24 ± .02	.94 ± .01	73.80 ± 1.16	1.14 ± 1.03	05:07 ± 00:11
ET	HC	.49 ± .03	.10 ± .01	.18 ± .02	24.45 ± .42	17.23 ± .91	05:55 ± 00:27
	LC	.41 ± .05	.11 ± .01	.13 ± .01*	26.03 ± .27†	20.16 ± .39†	05:48 ± 00:16
Act	HC	.44 ± .02	.68 ± .02	.70 ± .02	74.95 ± 1.77	13.32 ± .87	04:33 ± 00:16
	LC	.35 ± .02†	.79 ± .04*	.54 ± .03#	67.47 ± 3.45	20.29 ± 1.47#	05:03 ± 00:11
Pos	HC	.54 ± .03	.27 ± .02	.57 ± .02	51.64 ± 1.68	14.29 ± .68	04:30 ± 00:15
	LC	.47 ± .04	.30 ± .03	.48 ± .03*	51.50 ± 1.98	17.73 ± .86†	05:22 ± 00:12*
Sleep	HC	.72 ± .03	.07 ± .00	1.00 ± .00	93.55 ± 1.99	2.14 ± .54	04:40 ± 00:11
	LC	.63 ± .03	.09 ± .01†	.98 ± .01	91.28 ± 1.92	5.00 ± .91†	05:07 ± 00:12

Table 3. Non-parametrical analysis for high and low contrast in WT pattern and corresponding *zeitgebers* or rhythmic variables with a synchronizing effect pattern. HC and LC corresponds to the highest and lowest deciles for the relative amplitude of wrist temperature (WT) (n=14 in each group). Main characteristics for wrist temperature and corresponding light exposure, environmental temperature (ET), activity (Act), position (Pos) and sleep 24-h patterns, selected by high or low contrast in terms of WT: Interdaily Stability (IS), Intradaily Variability (IV), Relative Amplitude (RA), mean of the 5 consecutive hours with the highest values (MAX), and the 10 consecutive hours with the lowest values (MIN) for WT and sleep and the mean of the 10 consecutive hours with the highest values (MAX), and the 5 consecutive hours with the lowest values (MIN) for light, ET, Act and Pos. The PHASE indicates the timing for the midpoint of each MAX or MIN. Values are expressed as the mean ± SEM. # indicates $p < 0.001$, † $p < 0.01$, and * $p < 0.05$.

Subjects lifestyle and wrist temperature *Macroarrays*

Figure 6A and 6B show the graphic matrix (macroarrays) for our population ordered by LS and WT deciles, respectively. These figures show a color gradient ranging from the worst pattern (in red) in lifestyle or WT to the best pattern in the same variable (green). Most subjects showed a good (less than 3 deciles of difference) or moderate (between 3 and 6 deciles of difference, both included) coincidence between their LS and WT scores (89 and 61 out of 157, respectively), but a small number of subjects showed unexpected discordances among them (more than 6 deciles of difference), as some exhibited high LS contrast, but low WT rhythm contrast (3 out of 157), whereas others showed robust WT rhythms with low LS contrast patterns (4 out of 157).

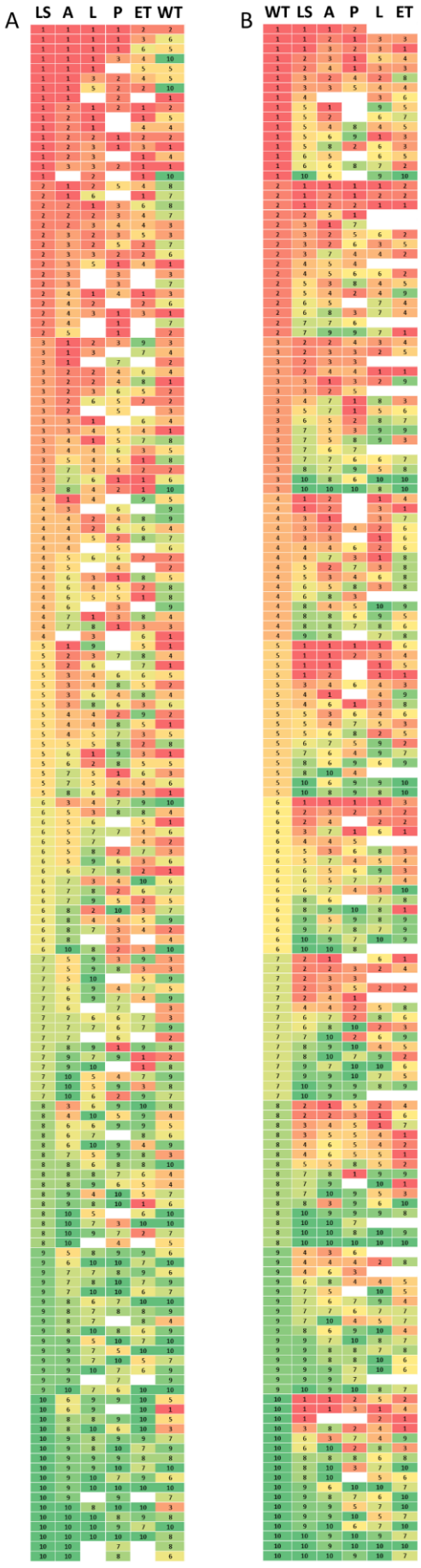

Figure 6. *Macroarrays* according to high and low contrast. Graphic matrix of the population studied, sorted by lifestyle (A) and Wrist Temperature (B) deciles (n=157). Color scale corresponds to the relative amplitude decile for each variable and subject: redder, shades indicate lower contrast, yellow indicates medium contrast, and greener shades indicate higher contrast. White cells indicate variables that have been discarded because more than 2 days of data were missing for that subject. For details on lifestyle, see the Materials and Methods section. The variables included in the *macroarrays* are activity (A), position (P), light exposure (L), environmental temperature (ET), wrist temperature (WT) and lifestyle (LS).

Simulation of chronoenhancement

Demasking and modelling procedures theoretically demonstrated that increasing the day/night contrast for environmental variables could improve the WT pattern (day/night contrast) according to high and low contrast either in zeitgebers or rhythmic variables with a synchronizing effect (Figure 7 and Table 4) or in WT pattern (Figure 8 and Table 5).

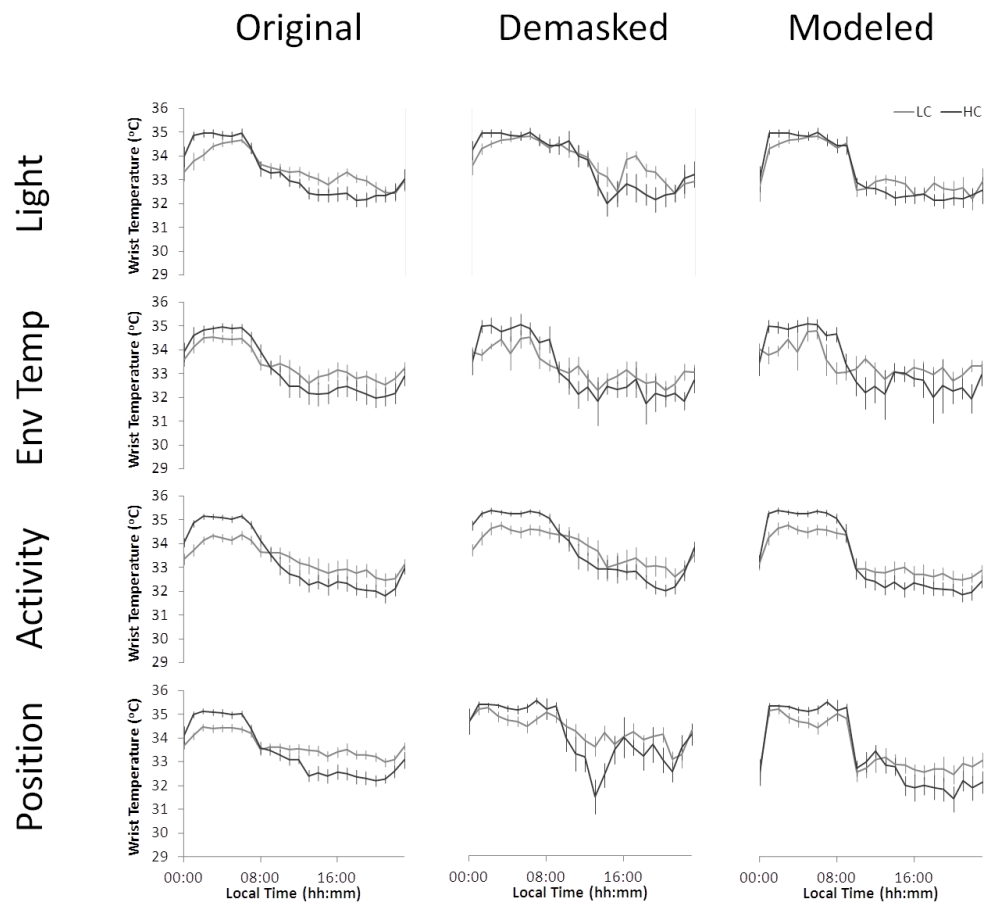

Figure 7. Original, demasked and modelled WT patterns according to high and low contrast in *zeitgebers* or rhythmic variables with a synchronizing effect. WT mean waveforms for HC and LC groups (black and grey, respectively) selected according to Light (First row), Environmental Temperature (Second row), Activity (Third row) and Position (Fourth row), without any processing (First column), demasked (Second column) and modelled (Third column), expressed as the mean ± SEM (n=13 in each group).

		Mesor			Amplitude			Acrophase		
	Group	Original	Demasked	Modeled	Original	Demasked	Modeled	Original	Demasked	Modeled
Light	HC	33.35 ± .19^{a}	33.68 ± .23^{b}	33.11 ± .19^{c}	1.43 ± .12	1.56 ± .16	1.34 ± .13	04:15 ± 00:17	05:09 ± 00:30	03:59 ± 00:11
	LC	33.46 ± .15^{a}	33.82 ± .14^{b}	33.19 ± .17^{c}	.91 ± .11*	.98 ± .10*	1.04 ± .12	05:21 ± 00:42^{a}	05:47 ± 00:35^{a}	03:44 ± 00:36^{b}
ET	HC	33.23 ± .24^{ab}	32.93 ± .27^{a}	33.09 ± .32^{b}	1.52 ± .27	1.78 ± .34	1.61 ± .37	04:11 ± 00:20	04:42 ± 00:17	04:33 ± 00:15
	LC	33.42 ± .19^{ab}	33.09 ± .24^{a}	33.32 ± .23^{b}	.95 ± .16	1.07 ± .31	1.00 ± .27	04:41 ± 00:35	04:08 ± 00:22	04:02 ± 00:23
Act	HC	33.37 ± .17^{a}	33.80 ± .14^{b}	33.21 ± .18^{c}	1.65 ± .19	1.68 ± .19	1.70 ± .19	04:41 ± 00:10^{a}	05:16 ± 00:19^{b}	04:27 ± 00:08^{a}
	LC	33.33 ± .16^{a}	33.76 ± .21^{b}	33.24 ± .15^{c}	.85 ± .21*^{a}	1.06 ± .23*^{b}	1.04 ± .18*^{b}	05:36 ± 00:30*^{a}	05:46 ± 00:32^{a}	04:28 ± 00:31^{b}
Pos	HC	33.44 ± .17^{a}	34.04 ± .13^{b}	33.07 ± .23^{c}	1.43 ± .12	1.41 ± .22	1.71 ± .17	04:22 ± 00:10	04:17 ± 00:25	04:29 ± 00:18
	LC	33.69 ± .11^{a}	34.29 ± .09^{b}	33.29 ± .15^{c}	.63 ± .11*^{a}	.77 ± .12*^{ab}	1.08 ± .20*^{b}	05:03 ± 00:42^{a}	04:39 ± 00:44^{ab}	03:38 ± 00:33^{b}

Table 4. Cosinor analysis for original, demasked and modelled WT patterns according to high and low contrast in *zeitgebers* or rhythmic variables with a synchronizing effect. Values for Mesor, Amplitude and Acrophase of each WT pattern, according to HC or LC and the mathematical procedure used (original, demasked or modelled), expressed as the mean ± 95% confidence interval (n=13 in each group). * indicates significant differences between HC and LC, and different letters indicate significant differences between original, demasked or modelled patterns, as determined by a repeated measures ANOVA.

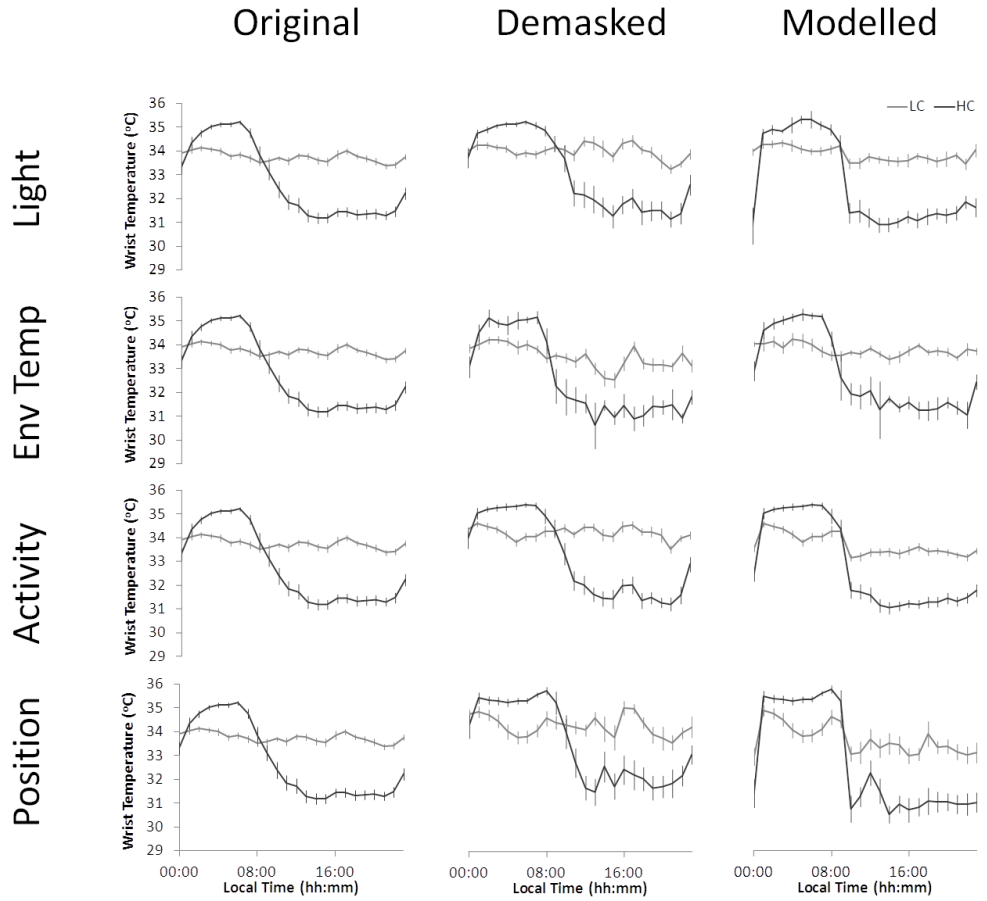

Figure 8. Original WT mean waveform, demasked and modelled by *zeitgebers* or rhythmic variables with a synchronizing effect, according to high and low contrast in WT pattern. WT mean waveforms according to HC or LC group (black and grey and red, respectively), selected by Light (First row), Environmental Temperature (Second row), Activity (Third row) and Position (Fourth row), without any processing (First column), demasked (Second column) and modelled (Third column), expressed as the mean ± SEM (n=14 in each group).

		Mesor			Amplitude			Acrophase		
	Group	Original	Demasked	Modeled	Original	Demasked	Modeled	Original	Demasked	Modeled
Light	HC	32.76 ± .12^{a}	33.12 ± .11^{b}	32.06 ± .21^{c}	2.13 ± .10^{a}	2.24 ± .14^{a}	1.59 ± .16^{b}	04:33 ± 00:16	04:48 ± 00:29	03:10 ± 00:25
	LC	33.75 ± .09^{*ab}	34.02 ± .11^{*a}	33.67 ± .11^{*b}	.21 ± .03*	.35 ± .05*	.42 ± .07*	03:40 ± 01:28	06:16 ± 03:20	00:02 ± 01:35*
ET	HC	32.76 ± .12^{a}	31.95 ± .34^{b}	32.59 ± .21^{ab}	2.13 ± .10^{a}	2.63 ± .22^{b}	2.42 ± .23^{b}	04:33 ± 00:16	03:58 ± 00:30	04:34 ± 00:18
	LC	33.75 ± .09^{*a}	32.44 ± .20^{*b}	33.77 ± .10^{*a}	.21 ± .03^{*a}	.51 ± .07^{*b}	.32 ± .04^{*a}	03:40 ± 01:28^{a}	04:28 ± 00:43^{a}	01:42 ± 02:08^{b}
Act	HC	32.76 ± .12^{a}	33.16 ± .13^{b}	32.64 ± .12^{c}	2.13 ± .10	2.30 ± .11	2.20 ± .11	04:33 ± 00:16^{a}	04:50 ± 00:19^{a}	04:10 ± 00:11^{b}
	LC	33.75 ± .09^{*a}	34.22 ± .11^{*b}	33.56 ± .10^{*c}	.21 ± .03^{*a}	.30 ± .05^{*ab}	.46 ± .05^{*b}	03:40 ± 01:28	05:40 ± 01:21	02:40 ± 00:33*
Pos	HC	32.76 ± .12^{a}	33.57 ± .17^{b}	32.23 ± .21^{c}	2.13 ± .10	2.20 ± .22	2.37 ± .17	04:33 ± 00:16	05:11 ± 00:27	03:44 ± 00:17
	LC	33.75 ± .09^{*a}	34.30 ± .10^{*b}	33.49 ± .17^{*a}	.21 ± .03*	.54 ± .10*	.59 ± .11*	03:40 ± 01:28	04:17 ± 03:24	02:00 ± 01:04

Table 5. Cosinor analysis for original, demasked and modelled WT mean waveform by *zeitgebers* or rhythmic variables with a synchronizing effect, according to high and low contrast in WT pattern. Values for Mesor, Amplitude and Acrophase of each WT pattern, according to HC or LC and the mathematical procedure used (original, demasked or modelled); expressed as the mean ± 95% confidence interval (n=14 in each group). * indicates significant differences between HC and LC, and different letters indicate significant differences between original, demasked or modelled patterns as determined by a repeated measures ANOVA.

DISCUSSION

Our results show that contrast reduction of the WT circadian rhythm is associated with a low contrast between day and night in both physical and behavioural variables with entraining capacity. In addition, we found a small population of subjects with dissociation between the contrast in WT and in LS variables. Thus, whereas some individuals show a high contrast in WT and a low contrast in LS variables, others exhibit arrhythmicity in WT despite their high contrast in LS variables, demonstrating that WT seems to consist of two components: one exogenous and susceptible to lifestyle, and another endogenous and more refractory to it.

People living in developed countries spend most of their time inside buildings, exposed to artificial environments. In most cases, these settings are characterized by low day-night contrast in light and temperature, which is quite different from the natural conditions for these physical *zeitgebers* (Pauley, 2004). In addition, people living and working in these artificially almost constant conditions are usually exposed to very low levels of physical exercise, delayed bed times and irregular habits, promoting the chronodisruption produced by the mismatch between internal and external time (Erren & Reiter, 2009b). However, there are certain habits that are associated with chronoenhancement, such as cold environmental temperatures during the nighttime, physical activity and bright light exposure during the daytime (Kondo et al., 2007;

Mishima et al., 2001). These habits improve circadian health and, therefore, could prevent CD and associated diseases.

The human circadian system is synchronized not only by physical environmental cues, such as sunlight or environmental temperature, but also by behavioural variables that are partially under voluntary control, such as motor activity, body position and sleep-wake rhythm (Cajochen et al., 2000; Kräuchi, 2007a; Kräuchi et al., 2005; Kräuchi & Wirz-Justice, 2001; Reilly & Waterhouse, 2009; Scheer et el., 1999; Wakamura & Tokura, 2002). In addition, the exposure to physical synchronizing variables is also conditioned by the circadian system, for example the synchronizing effect of light is partly dependent of the sleep-wake pattern. Thus, although light is considered a major input to the SCN, humans are exposed to both artificial and natural light, according to their own internal rhythms and social constraints. Similarly, the sleep-wake cycle and motor activity, two overt rhythms contingent upon SCN activity, are at the same time modulated by voluntary control. The sleep-wake rhythm, through changes in central and peripheral temperature, motor activity and light exposure, in itself contributes to SCN synchronization (Atkinson et al., 2007; Danilenko et al., 2003; Shibata et al., 2010). Therefore, because of the existence of such feedback loops, overlapping outputs and inputs from and to the internal clocks, and the excessive permanence in artificial environments, the human circadian system is particularly prone to chronodisruption. In order to simplify the terms used, we propose that all these variables (physical and behavioural) that exert an entraining activity on circadian clocks, but which are modulated by voluntary control, be grouped under the denomination of circadian lifestyle (LS) variables.

Circadian patterns of light exposure, motor activity, body position, sleep and WT are similar to those previously published for the Spanish population (Martinez-Nicolas et al., 2011; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). In addition, despite the fact that the daily pattern of these variables is similar to what has been described for people from other countries, a general phase delay was observed in this Spanish population. Differences in circadian phase among populations have already been reported (Adan & Almirall, 1990; Eastman et al., 2012). They could be due to different timing and intensity of light exposure and social customs that would provoke different phase angles of entrainment (Mongrain et al., 2004, 2006).

In general, it is true that in most individuals (95.5%), contrast in LS variables is closely associated with similar contrasts in WT rhythms, indicating that healthy circadian LS is a major determinant of WT rhythms robustness. The rhythms of individuals with a low contrast in circadian LS variables share several common characteristics, such as lower regularity, higher fragmentation and a phase delay with respect to the rhythms of the high contrast group. Considering the individual contribution of each circadian lifestyle variable, low contrast in light exposure is associated with two effects on WT: a decrease in stability and maximum WT values, probably promoted by reduced levels of daylight, together with excessive light at night, which is even able to induce arrhythmicity through circadian singularity behavior, i.e., a unique light stimulus produces a specific disruption that persists for a certain time, even in humans (Huang et al., 2006; Kohyama, 2011; Ukai et al., 2007). In the case of environmental temperature, warmer rooms during sleep are related to instability of the WT pattern and higher diurnal, but similar or even lower nocturnal WT values. Low contrast in activity or position reduces stability and amplitude, while increasing fragmentation of the WT pattern. Both variables (activity and position) are closely related to WT, through vasomotor changes produced by activity and the orthostatic reflex (Kario et al., 1999; Blazquez et al., 2012). Finally, despite the great homogeneity in the sleep habits of our population, low contrast in sleep patterns was associated with a delay in sleep onset and with higher diurnal WT values, which is in accordance with the strengthening of the relationship between sleep and WT (Ortiz-Tudela et al., 2010; Sarabia et al., 2008).

As discussed earlier, the contrast in LS variables is closely correlated with similar contrasts in WT rhythms; however, when individuals were classified based on their contrast in WT, the pattern of LS variables did not correlate accordingly. In general, LC in WT is associated with a phase delay in the values associated with sleep onset in all variables except sleep offset. These results highlight the risk of individuals with some degree of phase delay to be prone to CD, due to nighttime light exposure, as previously reported (Reiter et al., 2007). A delay in sleep onset, without a compensating delay in sleep offset, reduces sleep time as compared to that observed in high contrast WT subjects. This reduction in sleep time could induce daytime sleepiness, which would be related to high values of peripheral temperature during the day (Kräuchi & Wirz-Justice, 2001); however, this fact cannot explain the decrease in night WT values.

More surprising is the association between the low contrast WT group and the increased nocturnal and diurnal environmental temperature values that provided for a low contrast between them. One would expect that with higher nocturnal environmental temperatures, the nocturnal WT would also be higher. However, the opposite was observed. Warm nocturnal environments have been reported to induce an impairment of the CBT rhythm (Kondo et al., 2007; Wakamura & Tokura, 2002;). This effect could alter the nocturnal heat loss necessary to achieve deep sleep (Kräuchi & Wirz-Justice, 2001; Kräuchi & Deboer, 2010; Van Someren, 2000), which is associated with decreased skin temperature at night.

When subjects are classified by LS or WT, a gradation in the association between variables can be observed. Thus, when subjects were classified according to their contrast in LS, a progressive increase in mean deciles for activity, light, position, environmental temperature and WT was found, in that order. Just the opposite, when subjects were classified according to contrast in WT, the gradation was: lifestyle, activity, light, position, and environmental temperature; in other words, environmental temperature was the more distant from WT. These results confirm that the WT rhythm is not a passive response to physical and behavioural variables with synchronizing capacity, as previously proposed by our group (Blazquez et al., 2012; Martinez-Nicolas et al., 2011; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). Moreover, the fact that WT and environmental temperature showed the weakest association when subjects were classified according their WT contrast points to the relative independence of the contrast between the internal and external temperature.

Although most subjects were classified in the same category in terms of LS and WT, a small number of subjects exhibited unexpected associations. On the one hand, subjects with high WT contrast and low LS contrast were sometimes individuals with a robust circadian system (high day/night contrast and stability), despite presenting inadequate habits. However, and since our population was made up of young people, we cannot rule out the possibility that this robustness would be lost as the subjects age, following long-term exposure to these bad habits. On the other hand, subjects with low WT contrast and high LS contrast hypothetically could be individuals with some degree of impairment of the circadian system, thermoregulation or physiological blood vessel control (Kräuchi, 2002; Kräuchi et al., 2008), showing a flattened WT rhythm in spite

of their good habits. More experiments will be needed to characterize such subpopulations and assess whether they would be resistant to chronoenhancement.

Demasking and modelling theoretically demonstrated that the WT rhythm could be improved in subjects exhibiting flattened patterns by exposing them to a high contrast in LS variables. Although all variables studied had an influence on the WT rhythm, the most important seem to be activity, light and position, as they induce greater changes in the WT pattern. However, further experiments exposing subjects to both (high and low contrast) will confirm this hypothesis. Activity and position are variables that are closely associated with relaxation-induced effects on peripheral temperature, as previously described (Kräuchi, 2007b; Kräuchi et al., 1997). Bright light exposure has also been reported to induce acute changes in WT itself (Cajochen, 2007; Martinez-Nicolas et al., 2011). However, since this mathematical procedure does not allow for the analysis of all variables simultaneously, we cannot discount the existence of interactions between them, as previously described (Martinez-Nicolas et al., 2013).

Our results show that WT circadian rhythm impairment is associated with low contrast between day and night in physical (light, temperature) and behavioural (activity, body position and sleep) variables with entraining capacity. In addition, we found a small population with dissociation between day/night contrast in WT and LS variables, that is, suffering from circadian disruption. Thus, whereas some individuals show a high contrast in WT rhythm when they are exposed to a low contrast in lifestyle, others exhibit arrhythmicity in WT, despite their good habits in terms of a high contrast in LS; these individuals demonstrate two components for WT, one exogenous and susceptible to lifestyle, and another endogenous, and more refractory to it. Although our results are focused only in WT rhythm and alterations in other marker rhythms were not evaluated, they highlight the importance of strengthening the day-night contrast in lifestyle as a feasible measure to chronoenhance the circadian system and reduce circadian impairments associated with modern life.

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REFERENCES

Adan A, Almirall H. (1990). Estandarización de una escala reducidad de matutinidad en población española: Diferencias individuales. Psicothema. 2:137-49.

Atkinson G, Edwards B, Reilly T, Waterhouse J. (2007). Exercise as a synchroniser of human circadian rhythms: An update and discussion of the methodological problems. Eur J Appl Physiol. 99:331-41.

Blazquez A, Martinez-Nicolas A, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity, and body position as determinants of the circadian pattern of blood pressure. Chronobiol Int. 29:747-56.

Brainard GC, Rollag MD, Hanifin JP. (1997). Photic regulation of melatonin in humans: Ocular and neural signal transduction. J Biol Rhythms. 12:537-46.

Brown SA, Zumbrunn G, Fleury-Olela F, Preitner N, Schibler U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. Curr Biol. 12:1574-83.

Buijs RM, Kalsbeek A. (2001). Hypothalamic integration of central and peripheral clocks. Nat Rev Neurosci. 2:521-6.

Cajochen C. (2007). Alerting Effects of Light. Sleep Med Rev. 11:453-64.

Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, Orgül S, Wirz-Justice A. (2005). High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 90:1311-6.

Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. (2000). Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res. 115:75-83.

Corbalan-Tutau MD, Madrid JA, Ordovás JM, Smith CE, Nicolas F, Garaulet M. (2011). Differences in daily rhythms of wrist temperature between obese and normal-weight women: Associations with metabolic syndrome features. Chronobiol Int. 28:425-33.

Danilenko KV, Cajochen C, Wirz-Justice A. (2003). Is sleep per se a zeitgeber in humans? J Biol Rhythms. 18:170-8.

Davis S, Mirick DK. (2006). Circadian disruption, shift work and the risk of cancer: A summary of the evidence and studies in seattle. Cancer Causes Control. 17:539-45.

Duguay D, Cermakian N. (2009). The crosstalk between physiology and circadian clock proteins. Chronobiol Int. 26:1479-513.

Eastman CI, Molina TA, Dziepak ME, Smith MR. (2012). Blacks (african americans) have shorter free-running circadian periods than whites (caucasian americans). Chronobiol Int. 29:1072-7.

Erren TC, Reiter RJ. (2009a). Light hygiene: Time to make preventive use of insights-old and new-into the nexus of the drug light, melatonin, clocks, chronodisruption and public health. Med Hypotheses. 73:537-41.

Erren TC, Reiter RJ. (2009b). Defining chronodisruption. J Pineal Res. 46:245-7.

Garaulet M, Madrid JA. (2010). Chronobiology: Influences on metabolic syndrome and cardiovascular risk. Curr Cardiovasc Risk Rep. 4:15-23.

Gompper B, Bromundt V, Orgül S, Flammer J, Kräuchi K. (2010). Phase relationship between skin temperature and sleep-wake rhythms in women with vascular dysregulation and controls under real-life conditions. Chronobiol Int. 27:1778-96.

Gronfier C, Wright KP, Kronauer RE, Czeisler CA. (2007). Entrainment of the human circadian pacemaker to longer-than-24-h days. Proc Natl Acad Sci USA. 104:9081-6.

Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, Barnard AR, Cahill H, Badea TC, Zhao H, Hankins MW, Berson DM, Lucas RJ, Yau KW, Hattar S. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature. 453:102-5.

Huang G, Wang L, Liu Y. (2006). Molecular mechanism suppression of circadian rhythms by a critical stimulus. EMBO J. 25:5349-57.

Kario K, Schwartz JE, Pickering TG. (1999).Ambulatory physical activity as a determinant of diurnal blood pressure variation. Hypertension 34:685-91.

Karlsson B, Knutsson A, Lindahl B. (2001). Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 58:747-52.

Kohyama J. (2011). Neurochemical and neuropharmacological aspects of circadian disruptions: An introduction to asynchronization. Curr Neuropharmacol. 9:330-41.

Kolodyazhniy V, Späti J, Frey S, Götz T, Wirz-Justice A, Kräuchi K, Cajochen C, Wilhelm FH. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. J Biol Rhythms. 26:55-67.

Kolodyazhniy V, Späti J, Frey S, Götz T, Wirz-Justice A, Kräuchi K, Cajochen C, Wilhelm FH. (2012). An improved method for estimating human circadian phase derived from multichannel ambulatory monitoring and artificial neural networks. Chronobiol Int. 29:1078-97.

Kondo M, Tokura H, Wakamura T, Hyun KJ, Tamotsu S, Morita T, Oishi T. (2007). Physiological significance of cyclic changes in room temperature around dusk and dawn for circadian rhythms of core and skin temperature, urinary 6-hydroxymelatonin sulfate, and waking sensation just after rising. J Physiol Anthropol. 26:429-36.

Kräuchi K. (2002). How is the circadian rhythm of core body temperature regulated? Clin Auton Res. 12:147-9.

Kräuchi K. (2007a). The human sleep-wake cycle reconsidered from a thermoregulatory point of view. Physiol Behav. 90:236-45.

Kräuchi K. (2007b). The thermophysiological cascade leading to sleep initiation in relation to phase of entrainment. Sleep Med Rev. 11:439-51.

Kräuchi K, Cajochen C, Wirz-Justice A. (1997). A relationship between heat loss and sleepiness: Effects of postural change and melatonin administration. J Appl Physiol. 83:134-9.

Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. (2000). Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol. 278:R741-8.

Kräuchi K, Cajochen C, Wirz-Justice A. (2005). Thermophysiologic aspects of the three-process-model of sleepiness regulation. Clin Sports Med. 24:287-300.

Kräuchi K, Deboer T. (2010). The interrelationship between sleep regulation and thermoregulation. Front Biosci. 15:604-25.

Kräuchi K, Gasio PF, Vollenweider S, Von Arb M, Dubler B, Orgül S, Flammer J, Stutz EZ. (2008). Cold extremities and difficulties initiating sleep: Evidence of co-morbidity from a random sample of a swiss urban population. J Sleep Res. 17:420-6.

Kräuchi K, Wirz-Justice A. (2001). Circadian clues to sleep onset mechanisms. Neuropsychopharmacol. 25:S92-6.

Lavie P. (1968). Ultrashort sleep-waking schedule. III. ‘gates’ and ‘forbidden zones’ for sleep. Electroencephalogr Clin Neurophysiol. 63:414-25.

Martinez-Nicolas A, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. Chronobiol Int. 28:617-29.

Martinez-Nicolas A, Ortiz-Tudela E, Rol MA, Madrid JA. (2013). Uncovering different masking factors on wrist skin temperature rhythm in free-living subjects. PLoS ONE. 8:e61142

Mendoza J. (2007). Circadian clocks: Setting time by food. J Neuroendocrinol. 19:127-37.

Middleton B, Stone BM, Arendt J. (2002). Human circadian phase in 12:12 h, 200: <8 lux and 1000: <8 lux light-dark cycles, without scheduled sleep or activity. Neurosci Lett. 329:41-4.

Minors DS, Waterhouse J. (1989). Masking in humans: The problem and some attempts to solve it. Chronobiol Int. 6:29-53.

Mishima K, Okawa M, Shimizu T, Hishikawa Y. (2001). Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. J Clin Endocrinol Metab. 86:129-34.

Mistlberger RE, Skene DJ. (2004). Social influences on mammalian circadian rhythms: Animal and human studies. Biol Rev Camb Philos Soc. 79:533-56.

Mongrain V, Carrier J, Dumont M. (2006). Circadian and homeostatic sleep regulation in morningness-eveningness. J Sleep Res. 15:162-6.

Mongrain V, Lavoie S, Selmaoui B, Paquet J, Dumont M. (2004). Phase relationships between sleep-wake cycle and underlying circadian rhythms in morningness-eveningness. J Biol Rhythms. 19:248-57.

Moore RY, Speh JC, Leak RK. (2002). Suprachiasmatic nucleus organization. Cell Tissue Res. 309:89-98.

Mormont, MC, Langouët AM, Claustrat B, Bogdan A, Marion S, Waterhouse J, Touitou Y, Lévi F. (2002). Marker rhythms of circadian system function: A study of patients with metastatic colorectal cancer and good performance status. Chronobiol Int. 19:141-55.

Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. (2010). A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comput Biol. 6:e1000996.

Park SJ, Tokura H. (1998). Effects of different light intensities during the daytime on circadian rhythm of core temperature in humans. Appl Human Sci. 17:253-7.

Pauley SM. (2004). Lighting for the human circadian clock: Recent research indicates that lighting has become a public health issue. Med Hypotheses. 63:588-96.

Portaluppi F, Smolensky MH, Touitou Y. (2010). Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 27:1911-29.

Rajaratnam SMW, Arendt J. (2001). Health in a 24-h society. Lancet. 358:999-1005.

Refinetti R. (2010). Entrainment of circadian rhythm by ambient temperature cycles in mice. J Biol Rhythms. 25:247-56.

Reilly T, Waterhouse J. (2009). Circadian aspects of body temperature regulation in exercise. J Therm Biol. 34:161-70.

Reiter RJ, Tan DX, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. (2007). Light at night, chronodisruption, melatonin suppression, and cancer risk: A review. Crit Rev Oncog. 13:303-28.

Rietveld WJ, Minors DS, Waterhouse J. (1993). Circadian rhythms and masking: An overview. Chronobiol Int. 10:306-12.

Rodrigues Menezes MC, Nogueira Pires ML, Benedito-Silva AA, Tufik S. (2004). Sleep parameters among offshore workers: An initial assessment in the Campos Basin, Rio de Janeiro, Brazil. Chronobiol Int. 21:889-97.

Rubinstein EH, Sessler DI. (1990). Skin-surface temperature gradients correlate with fingertip blood flow in humans. Anesthesiol. 73:541-5.

Sarabia JA, Rol MA, Mendiola P, Madrid JA. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate of new index of the circadian system. Physiol Behav. 95:570-80.

Scheer F, Doornen L, Buijs R. (1999). Light and diurnal cycle affect human heart rate: Possible role for the circadian pacemaker. J Biol Rhythms. 14:202-12.

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Fuchs CS, Colditz GA. (2003). Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst. 95:825-8.

Shibata S, Tahara Y, Hirao A. (2010). The adjustment and manipulation of biological rhythms by light, nutrition, and abused drugs. Adv Drug Deliv Rev. 62:918-27.

Skene DJ, Lockley SW, Thapan K, Arendt J. (1999). Effects of light on human circadian rhythms. Reprod Nutr Dev. 39:295-304.

Stratmann M, Schibler U. (2006). Properties, entrainment, and physiological functions of mammalian peripheral oscillators. J Biol Rhythms. 21:494-506.

Ukai H, Kobayashi TJ, Nagano M, Masumoto KH, Sujino M, Kondo T, Yagita K, Shigeyoshi Y, Ueda HR. (2007). Melanopsin-dependent photo-perturbation reveals desynchronization underlying the singularity of mammalian circadian clocks. Nat Cell Biol. 9:1327-34.

Van Marken Lichtenbelt WD, Daanen HAM, Wouters L, Fronczek R, Raymann RJEM, Severens NMW, Van Someren EJW. (2006). Evaluation of wireless determination of skin temperature using iButtons. Physiol Behav. 88:489-97.

Van Someren EJW. (2000). More than a marker: Interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. Chronobiol Int. 17:313-54.

Van Someren EJW, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. (1999). Bright light therapy: Improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int. 16:505-18.

Wakamura T, Tokura H. (2002). Circadian rhythm of rectal temperature in humans under different ambient temperature cycles. J Therm Biol. 27:439-47.

Waterhouse J, Weinert D, Minors DS, Folkard S, Owens D, Atkinson G, Macdonald I, Sytnik N, Tucker P, Reilly T. (2000). A comparison of some different methods for purifying core temperature data from humans. Chronobiol Int. 17:539-66.

Weinert D, Waterhouse J. (2007). The circadian rhythm of core temperature: Effects of physical activity and aging. Physiol Behav. 90:246-56.

Weinert D, Waterhouse J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. Physiol Behav. 63:837-43.

Wittmann M, Dinich J, Merrow M, Roenneberg T. (2006). Social jetlag: Misalignment of biological and social time. Chronobiol Int. 23:497-509.

Zornoza-Moreno M, Fuentes-Hernández S, Sánchez-Solis M, Rol MA, Larqué E, Madrid JA. (2011). Assessment of circadian rhythms of both skin temperature and motor activity in infants during the first 6 months of life. Chronobiol Int. 28:330-7.

3.7. EXPERIMENTAL CHAPTER 7

DISPOSITIVO DE ILUMINACIÓN CIRCADIANO

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3.7. DISPOSITIVO DE ILUMINACIÓN CIRCADIANO

SUMMARY

Esta invención propone un dispositivo de iluminación circadiano preparado para funcionar en dos modos. El primer modo emite luz de espectro total y el Segundo bloquea una banda del espectro visible. Además, es capaz de cambiar entre ambos modos y modificar su intensidad en function de un segundo set de controladores. La banda bloqueada del espectro sera preferiblemente entre 450 y 480 nm.

CAMPO DE LA INVENCIÓN

La presente invención se engloba dentro del campo de los dispositivos para la iluminación.

ANTECEDENTES

El sistema circadiano está formado por un conjunto de estructuras encargadas de generar y sincronizar los diferentes ritmos circadianos entre sí y con los ciclos ambientales. Este sistema permite que las variables comportamentales, bioquímicas y fisiológicas del organismo muestren cambios rítmicos, con un periodo de 24 horas, y que se anticipen a los requerimientos periódicos generados por el medio ambiente. Para el correcto funcionamiento del sistema circadiano es necesario que una serie de factores ambientales cíclicos lo sincronicen a diario.

El ciclo de iluminación día-noche es la variable ambiental que marca las horas en los seres vivos poniendo en hora el reloj biológico de los seres humanos. La luz actúa sobre el reloj circadiano situado, en los núcleos supraquiasmáticos del hipotálamo, a través de la estimulación de un tipo especial de células de la retina, unos receptores visuales especiales denominados células ganglionares, que son especialmente sensibles a una banda azulada del espectro luminoso comprendida entre 450 y 480nm, y que transmiten la información a nuestro reloj a través del tracto retinohipotalámico.

En las sociedades desarrolladas, este ciclo está atenuado y distorsionado debido a que las actividades durante el día se desarrollan en el interior de edificios que están

poco y/o mal iluminados, mientras que a menudo los individuos se exponen a luz nocturna de intensidad y espectro inadecuados. De este modo, el ritmo circadiano de luz-oscuridad está atenuado, provocando o agravando una serie de patologías entre las que se encuentran ciertos tipos de cáncer, insomnio, síndrome metabólico, enfermedades cardiovasculares, deterioro cognitivo o síndromes afectivos.

Actualmente, existen filtros que permiten eliminar ciertas longitudes de onda, como los descritos en las solicitudes de patente US6141361A y US2008/0094566A1 y filtros que permiten eliminar toda emisión, a partir de una longitud de onda determinada, para evitar los problemas nocturnos que genera la luz sobre el sistema circadiano como describe la solicitud de patente US2008/0065177A1.

Era deseable por tanto un dispositivo de iluminación que eliminase la banda espectral de 450 a 480nm durante el periodo nocturno.

DESCRIPCIÓN DE LA INVENCIÓN

Los problemas generados o agravados por el mal funcionamiento del sistema circadiano pueden paliarse o incluso solventarse utilizando, durante el día, una fuente de iluminación similar a la luz solar, enriquecida en la longitud de onda azulada, y durante la noche, eliminando dicha banda azulada.

Los problemas generados se refieren a una mayor incidencia de cáncer de próstata y mama, los cuales han sido percibidos en regiones con mayor cantidad de luz nocturna o en trabajadores nocturnos, condiciones en las que se inhibe la síntesis de melatonina. Además, la supresión del ritmo de melatonina está directamente asociada a un incremento en la probabilidad de padecer tipos concretos de cáncer y al agravamiento de esta enfermedad en caso de que ya esté instaurada.

La luz que produce estos problemas se localiza en una banda entre 450 y 480nm que es la que inhibe la síntesis de melatonina.

Bajo este escenario, la presente invención presenta un dispositivo de iluminación capaz de funcionar en dos modos de funcionamiento. Un primer modo de funcionamiento que emite luz en todo el espectro visible y un segundo modo de funcionamiento que elimina una banda del espectro visible. Para ello, el dispositivo de

comprende unos medios de accionamiento accionados por unos primeros medios de interacción y unos segundos medios de interacción.

La invención permite la emisión de luz en todo el espectro visible durante el día y la eliminación de parte de la banda espectral del azul, concretamente, las longitudes de onda desde 450 a 480nm durante la noche.

El dispositivo de iluminación puede ser manual, automático o programable tanto en intensidad de luz emitida como en la banda del espectro eliminada.

Para ello, los primeros medios de interacción pueden comprender un interruptor que activará el usuario, o un temporizador o un espectrorradiómetro que enviará órdenes a los medios de accionamiento para que el dispositivo alterne entre el primer y el segundo modo de funcionamiento.

Los segundos medios de interacción pueden comprender un potenciómetro que activará el usuario, o un temporizador o un luxómetro que enviará órdenes a los medios de accionamiento para modificar la intensidad de luz emitida por el dispositivo de iluminación.

El dispositivo de iluminación puede comprender un filtro móvil que elimine la banda del espectro visible deseada si el dispositivo de iluminación funciona en el segundo modo de funcionamiento, durante el periodo nocturno, o que no elimine ninguna banda si el dispositivo de iluminación funciona en el primer modo de funcionamiento, durante el día.

El dispositivo puede comprender más de un filtro, según las necesidades, que se colocará delante del dispositivo de iluminación propiamente dicho o se retirará en función del modo de funcionamiento que esté activo.

En otro caso, el dispositivo de iluminación puede comprender una pluralidad de fuentes monocromáticas de forma que activará unas u otras según el modo de funcionamiento en el que se encuentre. En concreto, activará una fuente de iluminación roja, una fuente de iluminación verde y una fuente de iluminación azul si el dispositivo

de iluminación funciona en el primer modo de funcionamiento, durante el día, y activará una fuente de iluminación roja, una fuente de iluminación verde y una fuente de iluminación violeta (que no emite en la banda desde 450 a 480nm) si el dispositivo de iluminación funciona en el segundo modo de funcionamiento, durante el periodo nocturno.

El dispositivo puede comprender otras fuentes de iluminación monocromáticas o policromáticas que se agruparán en pequeños focos y estos a su vez en un único dispositivo.

Según lo descrito, se obtiene un dispositivo de iluminación que potencia las diferencias lumínicas entre el día y la noche. Además, permite la síntesis de melatonina nocturna aún en presencia de iluminación. Así, se potencia los efectos beneficiosos de la luz durante el día y se eliminan los efectos perniciosos de la luz nocturna.

Por otro lado, la luz brillante enriquecida en la longitud de onda azulada (450-480nm) durante el día produce la curación de enfermedades como el síndrome afectivo estacional o la mejoría en pacientes con enfermedades tipo alzheimer o demencia.

El empleo del dispositivo de iluminación permitirá iluminar interiores durante el día con un tipo de luz similar a la luz solar favoreciendo la sincronización del sistema circadiano de los individuos y en el caso de la noche suministrará un tipo de luz que no inhibirá la síntesis de la hormona melatonina (mediador químico de la oscuridad) y minimizará los efectos perjudiciales de la iluminación nocturna en la salud humana.

BREVE DESCRIPCIÓN DE LAS FIGURAS

A continuación se pasa a describir de manera muy breve una serie de dibujos que ayudan a comprender mejor la invención y que se relacionan expresamente con una realización de dicha invención que se presenta como un ejemplo no limitativo de ésta.

La Figura 1 muestra el espectro de radiación solar dentro del espectro visible en función de la longitud de onda en lumen/m2.

La Figura 2 muestra el espectro de absorción de los conos y los bastones de la retina en función de las diferentes longitudes de onda. El recuadro delimita la zona de absorción máxima de las células ganglionares de la retina.

La Figura 3 muestra el espectro de emisión de la luz de día y la luz de noche en función de la longitud de onda.

La Figura 4 muestra una realización del dispositivo de iluminación. El dispositivo está funcionando en el segundo modo de funcionamiento en el que el filtro móvil está situado delante del mismo.

La Figura 5 muestra la misma realización del dispositivo de iluminación que la figura 4 en la que el dispositivo está funcionando en el primer modo de funcionamiento.

La Figura 6 muestra otra posible realización del dispositivo de iluminación. El dispositivo comprende una fuente de iluminación roja, una fuente de iluminación verde, una fuente de iluminación azul y una fuente de iluminación violeta y un difusor que las cubre.

La Figura 7 muestra la combinación de varios de los dispositivos de iluminación mostrados en la figura 6.

DESCRIPCIÓN DETALLADA

La invención consiste en un dispositivo de iluminación que emite luz blanca de espectro total similar al espectro solar mostrado en la figura 1 con el fin de conservar una buena visión en color. Esto es debido a que los receptores visuales de la retina se dividen en dos tipos según el tipo celular, aparecen los bastones que no permiten distinguir colores que, como se muestra en la figura 2, tienen un espectro de absorción amplio 8 y los conos que tienen tres subtipos que se pueden distinguir según su espectro de absorción: Rojo 9, Verde 10 y Azul 11. Sin embargo, existe un fotorreceptor que no forma parte del sistema visual, un tipo especial de las células ganglionares de la retina, cuyo espectro de absorción se encuentra entre 450 y 480 nm 12 y cuando se estimula inhibe la producción de melatonina afectando a todo el organismo en general y al sistema circadiano en particular. Por ello se elimina esa banda del espectro en la luz

artificial nocturna 13 o se mantiene en la luz artificial diurna 14, tal y como se muestra en la figura 3.

Las figuras 4 y 5 muestran una posible realización del dispositivo de iluminación, el cual, emite luz en todo el espectro visible (luz artificial diurna) y tiene un filtro móvil 1 que se coloca automáticamente delante del dispositivo de iluminación para eliminar la banda del espectro entre 450 y 480nm durante el periodo considerado como nocturno.

La figura 6 muestra otra posible realización del dispositivo de iluminación el cual comprende cuatro fuentes de iluminación monocromáticas, la fuente de iluminación roja 2 y la verde 3 están funcionales tanto en el primer modo de funcionamiento, durante el día, como en el segundo modo de funcionamiento, durante la noche, pero la fuente de iluminación azul 4 solo está funcional en el primer modo de funcionamiento y la fuente de iluminación violeta 5 solo en el segundo modo de funcionamiento. El dispositivo de iluminación está cubierto por un difusor 6 para que las diferentes fuentes monocromáticas se combinen para dar la luz blanca.

La figura 7 muestra un dispositivo de iluminación que comprende una pluralidad de los dispositivos 7 mostrados en la figura 6.

El dispositivo de iluminación puede funcionar de forma manual, automática o programable para seleccionar el modo de funcionamiento.

Para ello, el dispositivo de iluminación tomará la información necesaria de un espectrorradiómetro, de la programación de un temporizador o mediante un interruptor, de la decisión del usuario. En el caso de que el dispositivo funcione en el primer modo de funcionamiento, en un modo de realización, se retirará el filtro móvil 1, y en otro modo de realización, el dispositivo de iluminación activará el rojo, el verde y el azul. En el caso de que el dispositivo funcione en el segundo modo de funcionamiento, en un modo de realización, se situará el filtro móvil 1 enfrente del dispositivo de iluminación,

y en otro modo de realización, el dispositivo de iluminación activará el rojo, el verde y el violeta.

El dispositivo de iluminación puede funcionar de forma manual, automática o programable para variar la intensidad de luz emitida.

Para ello, el dispositivo de iluminación tomará la información necesaria de un luxómetro que deberá ser igual que la determinada por el usuario, la preprogramada en un temporizador o la que exista en el medio ambiente mediante el empleo de otro luxómetro. En el caso de que la intensidad luminosa sea inferior o superior a un umbral preestablecido el dispositivo regulará la potencia para conseguir la intensidad luminosa descrita previamente.

Finalmente, el dispositivo de iluminación está configurado para funcionar en dos modos de funcionamiento:

- un primer modo de funcionamiento que emite luz en todo el espectro visible;
- un segundo modo de funcionamiento que elimina una banda del espectro visible;

que comprende unos medios de accionamiento configurados para alternar entre el primer modo y el segundo modo de funcionamiento en función de las órdenes recibidas de unos primeros medios de interacción y para modificar la intensidad de luz emitida en función de las órdenes recibidas de unos segundos medios de interacción.

Preferentemente, el segundo modo de funcionamiento eliminará la banda del espectro formada por las longitudes de onda desde 450 a 480nm.

Los primeros medios de interacción pueden comprender un interruptor a través del cual el usuario acciona los medios de accionamiento, o bien un temporizador que acciona los medios de accionamiento de forma programada, o bien un espectrorradiómetro que acciona los medios de accionamiento en función de la medida espectral obtenida.

Los segundos medios de interacción pueden comprender un potenciómetro a través del cual el usuario acciona los medios de accionamiento, o bien un temporizador que acciona los medios de accionamiento de forma programada, o bien un luxómetro que acciona los medios de accionamiento en función de la medida de iluminancia obtenida.

Preferentemente, el dispositivo de iluminación puede comprender al menos un filtro móvil 1 que elimina una banda del espectro visible y que es accionado por los medios de accionamiento, de forma que si el dispositivo de iluminación funciona en el primer modo de funcionamiento, los medios de accionamiento sitúan el filtro en la parte inferior del dispositivo de iluminación y si el dispositivo de iluminación funciona en el segundo modo de funcionamiento, los medios de accionamiento sitúan el filtro delante del dispositivo de iluminación.

Preferentemente, el dispositivo de iluminación puede comprender una pluralidad de fuentes monocromáticas, al menos una fuente de iluminación roja 2, una fuente de iluminación verde 3, una fuente de iluminación azul 4 y una fuente de iluminación violeta 5, todas las fuentes cubiertas por un difusor 6 que combina la iluminación de cada una de ellas, y donde los medios de accionamiento accionan la fuente de iluminación roja 2, la fuente de iluminación verde 3 y la fuente de iluminación azul 4 si el dispositivo de iluminación funciona en el primer modo de funcionamiento y accionan la fuente de iluminación roja 2, la fuente de iluminación verde 3 y la fuente de iluminación violeta 5 si el dispositivo de iluminación funciona en el segundo modo de funcionamiento.

Una vez descrita de forma clara la invención, se hace constar que las realizaciones particulares anteriormente descritas son susceptibles de modificaciones de detalle siempre que no alteren el principio fundamental y la esencia de la invención.

FIGURAS

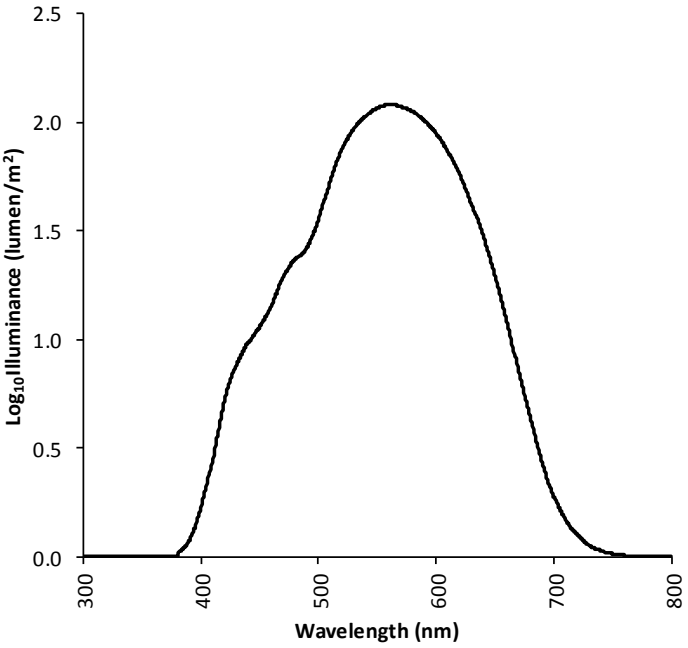

Figure 1. Visible solar light spectrum.

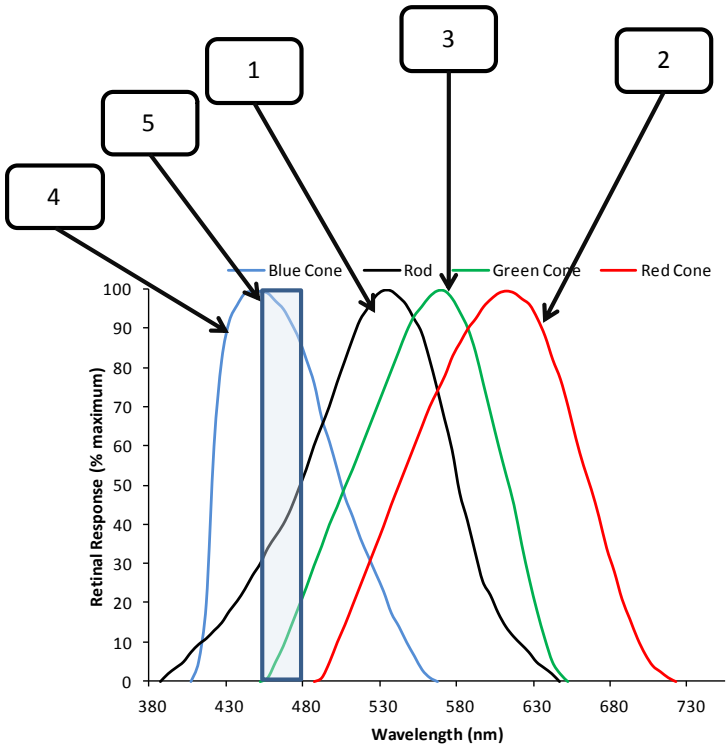

Figure 2. Activation spectra for cones and rods and the maximum response band for melanopsin ganglion cells.

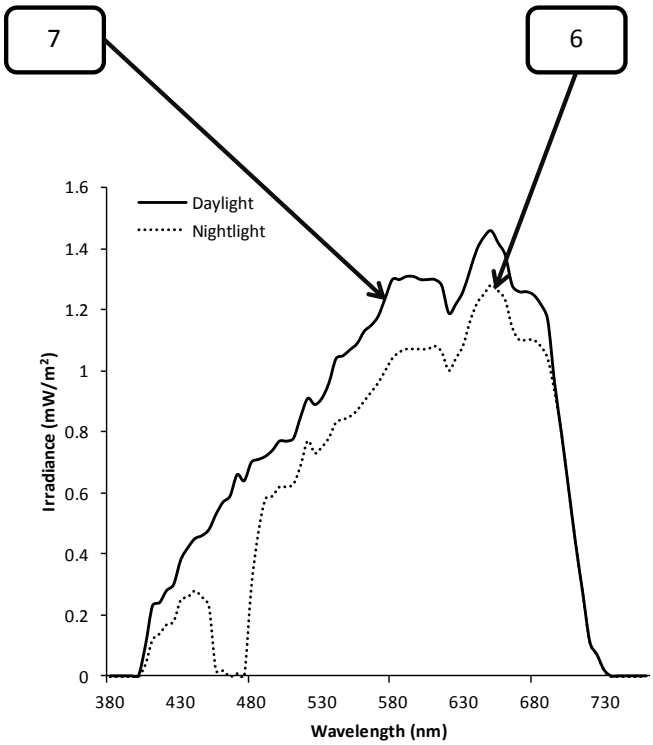

Figure 3. Emitted spectrum for daytime and nighttime light.

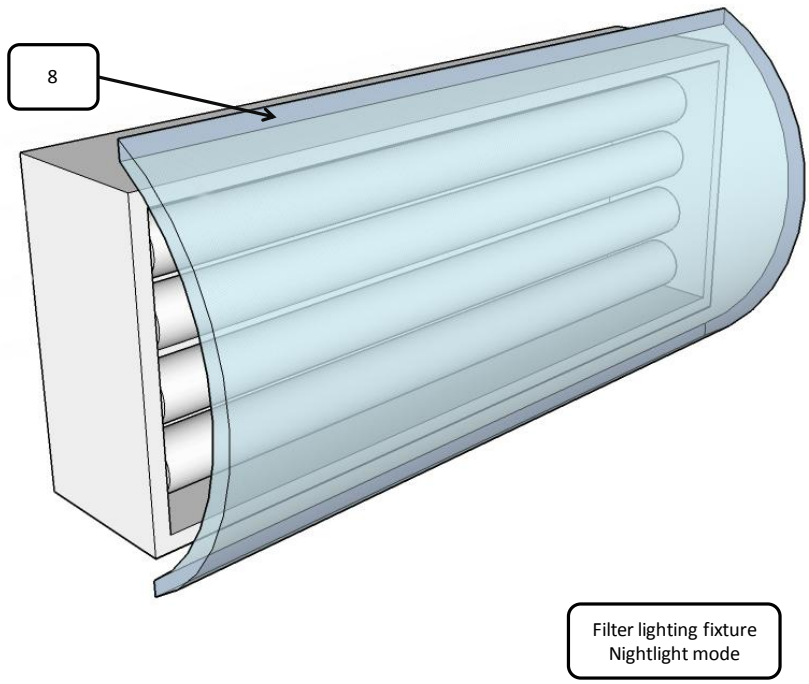

Figure 4. Lighting device working in night mode.

3.7. Circadian lighting device

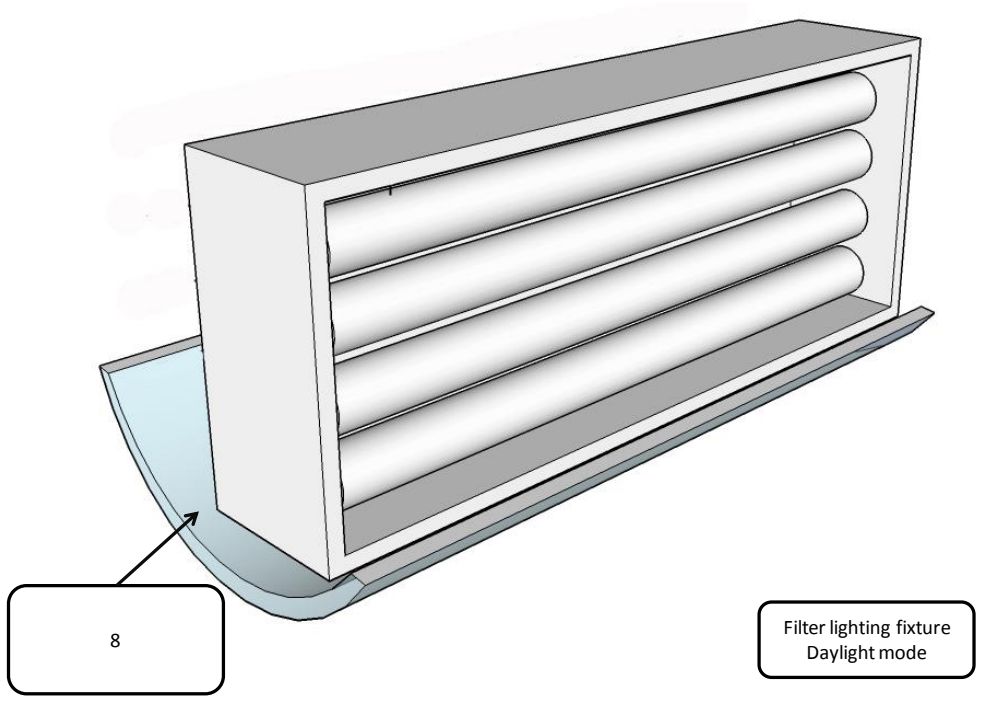

Figure 5. Lighting device working in day mode.

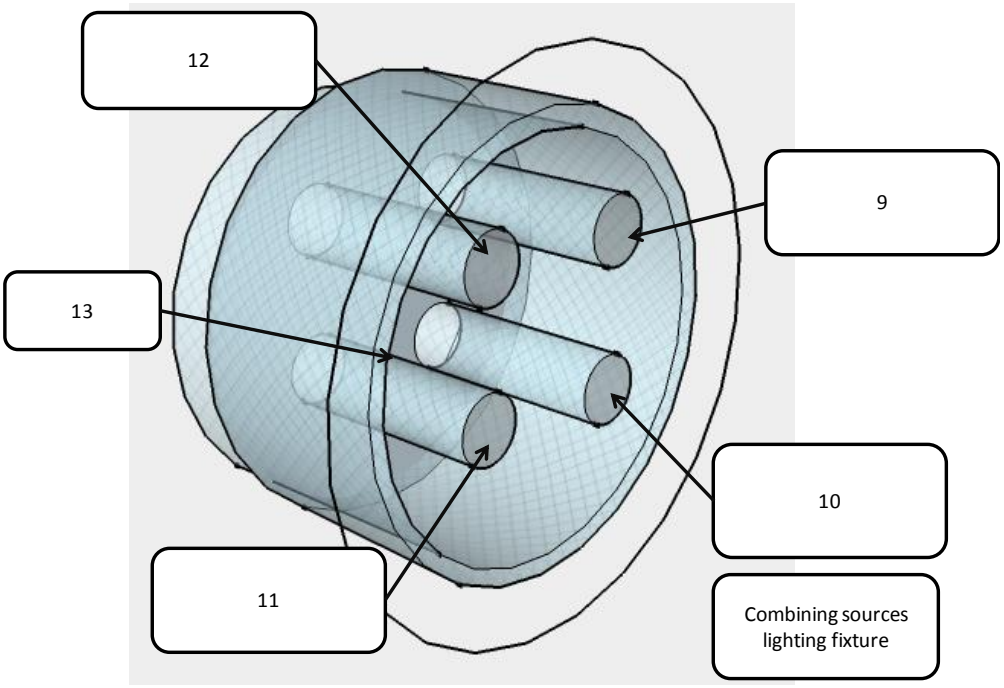

Figure 6. Lighting device composed by red, green, blue and violet monochromatic light sources.

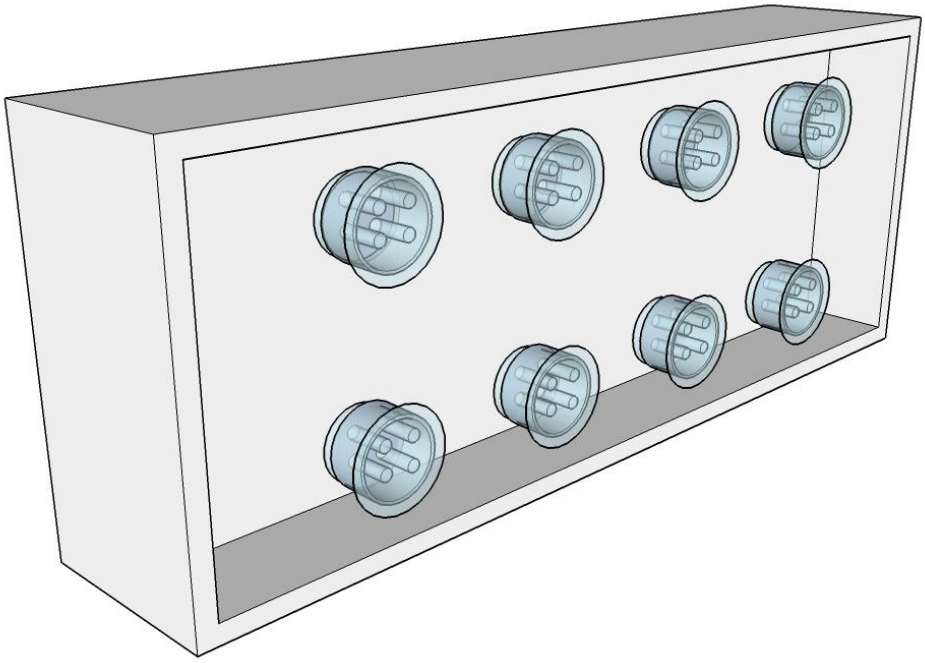

Figure 7. Lighting devices from Figure 6 combined in one lighting fixture.

4. Discussion

Western cities are functioning 24 hour a day and 365 days a year to provide services at any time. Thus, more and more members of these societies are involved in shift-work, and transmeridian flights increasing the exposure to jet-lag. In addition, the population lives mainly indoors with weak or contradictory exposure to synchronizers, which favors social jet-lag. These situations can impair the circadian system triggering chronodisruption.

Chronodisruption manifests as flattened, fragmented rhythms, loss of rhythmicity and stability and phase shifts among the different oscillators and/or between the SCN and the external cues. In the long term, chronodisruption increases the risk of suffering some types of cancer, metabolic syndrome, cognitive and affective impairments, dysregulation of reproduction, cardiovascular events, and sleep disorders among other health impairments (Davis & Mirick, 2006; Garaulet & Madrid, 2010; Gronfier et al., 2007; Karlsson et al., 2001; Middleton et al., 2002; Pauley, 2004; Rodrigues Menezes et al., 2004; Schernhammer et al., 2003).

Several rhythms, called circadian marker rhythms, are currently used to evaluate the circadian system. The marker rhythms most commonly used in human circadian assessment are melatonin and cortisol secretion patterns, core body temperature and actimetry (Mormont et al., 2002; Van Someren, 2000). In addition, distal skin temperature is increasingly considered as a marker rhythm because it has a stable phase relationship with other marker rhythms as core body temperature, which is slightly delayed and it is in antiphase, (Kräuchi et al., 2000; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). Distal skin temperature is maintained during constant routine protocols (Kräuchi et al., 2006) and persists after mathematical demasking procedures (Martinez-Nicolas et al., 2013) and it has been demonstrated its utility to assay the circadian phase (Kolodyazhniy et al., 2011; Kolodyazhniy et al., 2012; Bonmati-Carrion et al., 2014).

As previously demonstrated for other marker rhythms, the wrist temperature rhythm seems to be the result of two sets of influences: endogenous, such as autonomic balance directly controlled by the SCN (Buijs et al., 2003), and exogenous, attributable to variables such as light exposure (Cajochen, 2007; Cajochen et al., 2000; Rüger et al., 2006), environmental temperature (Kondo et al., 2007; Wakamura & Tokura, 2002), activity (Reilly & Waterhouse, 2009), body position (Blazquez et al., 2012) or sleep (Franken et al., 1992).

To obtain the endogenous pattern of a marker rhythm the stepwise multiple regression method is of interest, since it allows to simultaneously unmask several masking variables (Martinez-Nicolas et al., 2013). This method is useful to simulate wrist temperature changes in response to environmental variables and circadian patterns of sleep-wake or rest-activity rhythms (Martinez-Nicolas et al., 2013; Martinez-Nicolas et al., 2014). This model has revealed the existence of a phase-dependent masking effect for each variable, specifically that: a) high environmental temperatures affect WT during the wake period, but not during the sleep period, whereas high and low environmental temperatures respectively increase and decrease CBT during sleep (Kondo et al., 2007; Wakamura & Tokura, 2002); b) bright light reduces WT from the beginning of the sleep period until noon, as it is the case for distal temperature in accordance with the findings of Kräuchi et al., 2007; c) activity decreases WT throughout the day, except in the wake maintenance zone; d) body position modifies WT, but its effect is restricted to the usual times of the main changes in body position, such as awakening and sleep onset; and e) sleep increases WT, as previously described (Sarabia et al., 2008), but its effect is the lowest around 06:00 h, the time of maximum sleepiness. Additionally, it is worth noting that variables other than those considered here could contribute to masking and thus affect the unpurified wrist temperature pattern.

In addition, to be considered as a marker rhythm, it should be useful throughout the life and without intense gender differences. During ontogeny, the circadian rhythm of DST shows a progressive decrease of its values during rest period together with an increase in rhythm amplitude with no changes in phase markers. Although a 24 h rhythm is already present in newborns at 15 days and 1 month old, the predominance of ultradian rhythmicities (assessed by the circadianity index) and the very low amplitude, suggest that exogenous masking effects could be the major responsible of DST rhythm at these early ages. Environmental light and temperature together with behavioural influences of parents care could induce the small circadian rhythmicity observed in these newborns (Worobey, 2009). At six months of age, most infants show a robust daily pattern, which resembles to adults. It is characterized by higher circadianity index and amplitude than 15 days and 1 month old babies, reaching values similar to those observed in adults (Zornoza-Moreno et al., 2011).

In adults, gender differences were found, being women characterized by better circadian robustness than man, however this gender difference disappeared in older people.

Ageing is characterized by strong phase advances probably because older people go to bed early due to: a) their poor eyesight limits what they can do; b) they could be exposed to lower light intensities during the afternoon and evening; c) they became tired more easily in the second part of the day; d) or because their declining mental faculties promotes that they get bored more easily (Waterhouse et al., 2012). In addition, ageing of distal skin temperature pattern did not found significant changes in amplitude as expected by previous bibliography (Dijk et al., 2000; Harper et al., 2005). The absence of statistical signification in the reduction of amplitude according to our results can be explained by the compensatory effect of increase in lifestyle regularity associated to ageing.

Whatever the endogenous or exogenous causes of circadian system impairment in the older people, it is clear the convenience of including in their lifestyle procedures to increase the contrast between the active and resting phase. To this, activities such as bright light exposure, daytime physical activity outdoors, daytime mental activities, increasing artificial lighting levels, particularly during the afternoon and early evening should be scheduled. In addition, older people should be advised to sleep in complete darkness (light must be only available when necessary in relation to safety and care needs) and to keep under darkness or dim light when be awaken during the nocturnal sleep.

Considering the fact that contemporary humans spend most of their time indoors (Espiritu et al., 1994; Hebert et al., 1998; Mishima et al., 2001; Savides et al., 1986), with very low levels of natural light and with artificial light sources both during day and night (Martinez-Nicolas et al., 2011), the classical conception of light as a mere input to the circadian system should be questioned in humans. Light exposure is voluntarily and also unconsciously manipulated to match rest-activity rhythms, as well as working and leisure activities. The rhythm of light exposure should, therefore, be considered simultaneously as an input, and, in some aspects, a result of the circadian system function, which in turn provides feedback to the suprachiasmatic nuclei (SCN). Unlike our ancestors, who lived in natural environments, the last five generations of people

residing in developed countries have been able to self-select their light-dark cycle. The main differences between these two lifestyles with regard to light exposure are an overall decrease in light intensity and regularity; a modification in light timing with delayed and reduced exposure during the day and increased light at night and shift in the light spectrum towards artificial light sources. These changes in light input are hypothesized, as the reason why a large proportion of people suffer from some degree of or chronodisruption in modern society (Erren et al., 2009b; Francis et al., 2008; Mottram et al., 2011; Reiter et al., 2007). There is currently a growing body of scientific evidence that links chronodisruption to increased risk of developing certain diseases, and to worsening of pre-existing medical conditions, such as cancer, metabolic syndrome, insomnia, affective disorders, cognitive impairment, and cardiovascular diseases, as well as premature aging (Erren et al., 2009; Garaulet & Madrid, 2010; Reiter et al., 2007; Turner et al., 2010).

A common characteristic of natural light exposure is the high variability in intensity, which contrasts with the more constant values observed under laboratory conditions or artificial lighting. Acute changes in light exposure were associated with transient changes in WT in the opposite direction. Thus, positive changes in light intensity diminished WT, and consequently sleepiness, and vice versa. This inverse relationship could be explained by the alerting properties of light through sympathetic activation, inducing blood-vessel constriction and, in turn, reduction in skin temperature (Buijs et al., 2003), which is translated in a reduction of melatonin levels and the increasing of alertness, heart rate and core body temperature (Cajochen et al., 2005; Ishibashi et al., 2010; Phipps-Nelson et al., 2009).

In the long term, high regularity, amplitude and light quality index (a new index proposed by our group), in the light dark cycle are associated with the most robust circadian rhythms for WT and sleep, whereas high fragmentation and nocturnal light exposure are associated with the worst circadian sleep patterns. From these results, it seems that it may be possible to modify specific characteristics of the light-dark exposure in order to improve the organization of the circadian system. For example, improvement in the interdaily stability for WT can be achieved by an increase in light regularity. These results confirm previous observations indicating that natural bright-light exposure improves sleep quality and mood (Dumont & Beaulieu, 2007; Wirz-Justice et al., 1996), as previously demonstrated in light-controlled studies with blue-

enriched white light (Glickman et al., 2006; Viola et al., 2008) and bright-light therapy (Even et al., 2008; Kirisoglu & Guilleminault, 2004). However, our results show the circadian clock is influenced by the overall pattern of light-dark exposure, and not merely by isolated pulses of bright light, as it was thought from laboratory studies. Our results also confirm the importance of prior light history when analyzing subsequent light synchronizing effects, as already suggested (Smith & Eastman, 2009). However, some people seems refractory to chronoenhancement while others give the impression to be protected against chronodisruption since remain unaffected by damped rhythms in environmental and behavioural cues (Martinez-Nicolas et al., 2014), and the rational for both categories remains to be explored.

In addition to light, the second most important zeitgeber for mammals, environmental temperature, is also almost constant in modern societies thanks to heating and air conditioning in winter and summer, respectively. However, thermophysiological variables show global seasonal differences with lower proximal environmental temperature and distal skin temperature in winter, as it was expected from studies in lab conditions (Komulainen et al., 2004). A surprising fact was the inverse relationship between proximal skin temperature during winter regarding to summer, which could be due to a higher summer sweating rate or an increase of shelter clothes and the use of heating systems in winter. Although thermophysiological variables changed globally according to seasons, no seasonal changes were noticed in blood pressure (Goodwin et al., 2001).

The absence of a global influence from proximal environmental temperature on mean blood pressure levels is contrasted to the short-term inverse relationship found between them. This effect is mediated by distal, and not proximal, skin temperature indicating a causal chain of natural variations in proximal environmental temperature towards changes in distal skin temperature and hence to blood pressure. In addition, these changes are independent on person's temperature levels and also independent of the person's long-term acclimatization. However, age seems to be a critical factor because there is wide consensus that elderly subjects are prone to react more pronounced in blood pressure changes to seasonal and short-term environmental temperature variations than younger subjects (Goodwin, 2001).

It is said that older people is more prone to chronodisruption due to ageing produced impairments from the circadian system itself to every organ and system of the body. In these terms, the visual system is one of the most important because it is necessary to entrain the circadian system properly (Provencio et al., 2000; Ruby et al., 2002). Older people spent most of their time indoors resulting in a light-dark exposure pattern with dark days and light interrupted nights, which does not benefit circadian system health (Erren & Reiter, 2009b). In fact, when they were compared to young people, older people were phase advanced and they had darker days, mainly in the afternoon, and longer nights with more light interruptions. Although they were exposed longer to bright light than young people, probably it was not enough because the yellowing of the crystalline lens blocks the blue wavelength necessary for entraining (Turner and Mainster, 2008).

Elders also failed to expose themselves properly to cyclic environmental temperature, older people pattern was delayed and showed higher values throughout the whole day compared to young people, perhaps because they are less able to perceive the temperature of their surroundings and so, they are unable to forestall temperature changes by the suitable behaviour (Waterhouse et al. 2012). In addition, higher environmental temperature at night could be related to sleep disturbances due to the more difficulties for dissipating heat (Kondo et al., 2007; Kräuchi, 2007; Wakamura & Tokura, 2002).

Focussing on circadian system outputs, aging also affected WT pattern since the evening wake maintenance zone disappeared, as was previously described for arousal (Münch et al., 2005). Higher values during daytime, as if they were sleepier, were also observed. These impairments have been related with a reduction in the intrinsic mechanisms of thermoregulation, that could be attributed to changes in sensitivity to autonomic response to thermal changes (Blatteis, 2012; Holowatz et al., 2010; Holowatz & Kenney, 2010), but also customarily sedentary lifestyle activity can contribute to this changes. In this sense, older people showed higher night-time values for activity, position and TAP which are related to more frequent sleep awakenings and less sleep depth (Ortiz-Tudela et al., 2014); for daytime older people showed lower values and a phase advance for activity and TAP compared to young people, which are related to the normal ageing process (Huang et al., 2002; Van Someren et al., 1999).

Among older people there are more persons with a non-significant rhythm in wrist temperature, which means a higher chronodisrupted state itself. In addition, period higher than 24 hours and phase shifts are also related to age-related changes of the circadian system (Turek et al., 1995). These age-effects are probably due to changes in the suprachiasmatic nucleus (Nakamura et al., 2011). In addition to that, older people showed a higher misalignment between zeitgebers, internal clock and overt rhythms, which implies higher desynchronization in the older group as other authors suggest for internal desynchronization between overt rhythms (Aschoff, 1965; Kohyama, 2009).

The afore mentioned differences in the circadian rhythms between elderly and young people circadian rhythms allow us to differentiate them, for the first time, without taking into account their biological age but their circadian pattern characteristic. Thus, early acrophase of environmental temperature exposure, lower daytime position values, early acrophase and low values in activity, morning shift of minimum values for in wrist temperature and early acrophase for TAP corresponded with older people and the inverse for young people with a global agreement rate of 96.5% and only 3 mistakes out of 90.

Besides, two novelty indexes to summarize daytime and nighttime light exposure named light and dark quality indexes (LQI and DQI, respectively), were designed. It was found that both young and elderly people are exposed to dark nights but also, and surprisingly, considering the solar conditions in Spain, to dark days. In addition, those subjects with low scores in both indexes presented worse WT and activity patterns independently of their age.

Chronodisruption is becoming increasingly demonstrated as a social and health problem, and the importance of a correct light exposure, with high contrast between day and night is now unquestionable. However, artificially almost constant conditions in environmental temperature, delayed bed times, sedentary lifestyle and frequent snacking also contributes to the mismatch between internal and external time (Erren & Reiter, 2009b), and still, little has been published about how to counteract its potential harmful effects or chronoenhance the circadian system.

Lifestyle, grouping variables with synchronizing effects, allowed us to evidence that the robustness in wrist temperature rhythm was mainly determined by a healthy circadian lifestyle (Martinez-Nicolas et al., 2014). In addition, the relative independence

of internal and external temperature as previously proposed (Blazquez et al., 2012; Martinez-Nicolas et al., 2011; Martinez-Nicolas et al., 2013; Martinez-Nicolas et al., 2014; Ortiz-Tudela et al., 2010; Sarabia et al., 2008) was also evidenced. In fact, wrist temperature rhythm seems to present two components one exogenous susceptible to be impaired when contrast between day and night in both physical and behavioural variables with entraining capacity is reduced, and other endogenous more refractory to exogenous influences (Martinez-Ncolas et al., 2014).

In addition, demasking and modelling theoretical models demonstrated that wrist temperature rhythm could be improved in subjects exhibiting flattened patterns by exposing themselves to a high contrast in lifestyle variables providing, for the very first time, an approach to assess chonoenhance strategies and reduce circadian impairments associated with modern life characterized by low day-night contrast in light and temperature (Martinez-Nicolas et al., 2014). To this, there are certain habits that, in addition to adequate light exposure, can promote chronoenhancement, such as cold environmental temperatures during the nighttime or physical activity and bright light exposure during the daytime, especially during the afternoon and early evening (Kondo et al., 2007; Martinez-Nicolas et al., 2014; Mishima et al., 2001).

Considering most of the people here studied expend the majority of the time indoors under artificial lights that were design to allow sightseeing but not to specifically maintain circadian synchronization, a circadian lighting design was ideated for illuminating day but also the night protecting nocturnal melatonin secretion. This patented device illuminates indoors with sun-like of blue enriched light, stimulating circadian system entrainment and enhancing the healthy effects of light during day. The same device, shift to nightlight mode during nigh-time by eliminating blue wavelengths from light spectrum. The patent has been recently licensed to an international company for its commercial exploitation.

In summary, our results show that distal skin temperature rhythm could be used reliably and comfortably as circadian marker rhythm in healthy subjects because it shows a robust endogenous component in all studied age groups. However, as every rhythm driven by the central pacemaker, it is masked by zeitgebers and other variables with synchronizing effects as activity or sleep, that can be mathematically removed. The masking effect from light exposure and environmental temperature seems to be

mediated by the sympathetic tone. Subsequently, changes in distal skin temperature are related to other changes in alertness or blood pressure for example, pointing its potential usefulness to detect specific pathologies. Finally, distal skin temperature together with lifestyle simultaneous monitoring allow measuring chronodisruption and identification of those subjects prone to it, and thus, design strategies to prevent or empowering the circadian system that should be mainly based on the empowering day/night contrast.

5. Conclusions

The main conclusions of the present thesis are:

1. The stepwise multiple regression method allow us to reduce the masking influence on wrist temperature of light exposure, environmental temperature, sleep, activity and body position simultaneously. This rhythm has a strong endogenous component, in spite of the influence of the above mentioned variables, which affects in a phase-dependent manner. Also this demasking method could be extended to other marker rhythms.
2. Circadian maturation in distal skin temperature pattern is associated with a decrease in ultradian harmonics' power, an increase in amplitude and a reduction in skin temperature during sleep, whereas aging is related to a general phase advance. So, distal skin temperature allows assessing maturation and aging of the human circadian system.
3. Acute changes in light exposure are associated with transient changes in WT in the opposite direction, probably through sympathetic activation. In addition, higher day-night contrast and light quality index are related to more robust distal skin temperature and sleep patterns, whereas nighttime light exposure is associated with worst circadian patterns highlighting the importance of adequate life habits.
4. Variations in environmental temperature affect mean arterial blood pressure by changes produced in distal skin temperature.
5. Aged circadian system is characterized by less contrast in synchronizing variables, a generalized phase advance and internal order impairment; these differences allow discerning between a young and elderly circadian system.
6. Wrist temperature rhythm impairment is associated with low contrast between day and night in physical (light and temperature) and behavioral (activity, body position and sleep) variables with synchronizing capacity. Mathematical modelling also demonstrates that increasing contrast in lifestyle should improve wrist temperature rhythm.
7. A circadian lighting device with full-spectrum lighting during daytime and avoiding 450-480 nm wavelength band during night period to protect nocturnal melatonin secretion was patented.

GENERAL CONCLUSION

Wrist temperature has demonstrated to be a comfortable and reliable marker rhythm with a strong endogenous component that allows evaluating circadian system robustness and ageing in normal subjects under free-living conditions. Lifestyle assessment by multivariable recording constitutes a useful tool to screen for those subjects who should benefit from chronoenhancement therapies and reduce circadian impairment associated with modern life.

6. References

A

Abrahamson EE, Leak RK, Moore RY. (2001). The suprachiasmatic nucleus projects to posterior hypothalamic arousal systems. Neuroreport. 12:435-40.

Abrahamson EE, Moore RY. (2001). Suprachiasmatic nucleus in the mouse: retinal innervations, intrinsic organization and efferent projections. Brain Res. 916:172-91.

Acebo C, Sadeh A, Seifer R, Tzischinsky O, Wolfson AR, Hafer A, Carskadon MA. (1999). Estimating sleep patterns with activity monitoring in children and adolescents: How many nights are necessary for reliable measures? Sleep. 2:95-103.

Anders TF. (1982). Biological rhythms in development. Psychosom Med. 44:61-72.

Anisimov VN, Vinogradova IA, Panchenko AV, Popovich IG, Zabezhinski MA. (2012). Light-at-night-induced circadian disruption, cancer and aging. Curr Aging Sci. 5:170-7.

Antle MC, Foley DK, Foley NC, Silver R. (2003). Gates and oscillators: A network model of the brain clock. J Biol Rhythms. 18:339-50.

Aschoff J. (1965). Circadian rhythms in man. Science. 148:1427-32.

Aschoff J. (1969). Desynchronization and resynchronization of human circadian rhythms. Aerosp Med. 40:844-9.

Aschoff J. (1971). Temperaturregulation. In Gauer OH, Kramer K, Jung R (Eds). *Energiehaushalt und Temperaturregulation. Physiologie des menschen*. München (Germany): Urban & Schwarzenberg, pp. 43-112.

Aschoff J. (1979). Circadian rhythms: Influences of internal and external factors on the period measured in constant conditions. Z Tierpsychol. 49:225-49.

Aschoff J. (1983). Circadian control of body temperature. J Therm Biol. 8:143-7.

Aschoff J, Daan S, Figala J, Múller K. (1972). Precision of entrained circadian activity rhythms under natural photoperiodic conditions. Naturwissenschaften. 59:276-7.

Aschoff J, Fatranská M, Giedke H, Doerr P, Stamm D, Wisser H. (1971). Human circadian rhythms in continuous darkness: entrainment by social cues. Science. 171:213-5.

Aschoff J, Gerecke U, Wever R. (1967). Desynchronization of human circadian rhythms. Jpn J Physiol. 17:450-7.

Aschoff J, Pöppel E, Wever R. (1969). Circadian rhythms in men under the influence of light-dark cycles of various periods. Pflugers Arch. 306:58-70.

Atkinson G, Edwards B, Reilly T, Waterhouse J. (2007). Exercise as a synchroniser of human circadian rhythms: An update and discussion of the methodological problems. Eur J Appl Physiol. 99:331-41.

B

Bach V, Telliez F, Chardon K, Tourneux P, Cardot V, Libert JP. (2011). Thermoregulation in wakefulness and sleep in humans. In Montagna P, Chokroverty S (Eds). *Handbook of Clinical Neurology, Volume 98. Sleep Disorders, Part I*. Amsterdam (The Netherlands): Elsevier BV, pp. 215-27.

Benarroch EE. (2008). Suprachiasmatic nucleus and melatonin: Reciprocal interactions and clinical correlations. Neurology. 71:594-8.

Benloucif S, Guico MJ, Reid KJ, Wolfe LF, L'hermite-Balériaux M, Zee PC. (2005). Stability of melatonin and temperature as circadian phase markers and their relation to sleep times in humans. J Biol Rhythms. 20:178-88.

Berson DM. (2003). Strange vision: Ganglion cells as circadian photoreceptors. Trends Neurosci. 26: 314-20.

Berson DM, Dunn FA, Takao M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. Science. 295:1070-3.

Blatteis CM. (2012). Age-dependent changes in temperature regulation-A mini review. Gerontology. 58:289-95.

Blazquez A, Martinez-Nicolas A, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity, and body position as determinants of the circadian pattern of blood pressure. Chronobiol Int. 29:747-56.

Bonmati-Carrion MA, Middleton B, Revell V, Skene DJ, Rol MA, Madrid JA. (2014). Circadian phase assessment by ambulatory monitoring in humans: Correlation with dim light melatonin onset. Chronobiol Int. 31:37-51.

Brainard GC, Hanifin JP, Greeson JM, Byrne B, Glickman G, Gerner E, Rollag MD. (2001). Action spectrum for melatonin regulation in humans: Evidence for a novel circadian photoreceptor. J Neurosci. 21:6405-12.

Brown SA, Zumbrunn G, Fleury-Olela F, Preitner N, Schibler U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. Curr Biol. 12:1574-83.

Buijs RM, la Fleur SE, Wortel J, Heyningen C, Zuiddam L, Mettenleiter TC, Kalsbeek A, Nagai K, Niijima A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. J Comp Neurol. 464:36-48.

Buijs RM, Kalsbeek A. (2001). Hypothalamic integration of central and peripheral clocks. Nat Rev Neurosci. 2:521-6.

Bünning E. (1935). Zur Kenntnis der erblichen tagesperiodizitat bei den primarblattern von *Phaseolus multiflorus*. Jahrb wiss Bot. 81:411-8.

Bünning E, Stern K. (1930). Über die tagesperiodischen bewegungen der primärclätter von *Phaseolus multiflorus*. II. Die bewegungen bei thermo-konstanz. Ber Deutsche Bot Ges. 48:227-52.

C

Cajochen C. (2007). Alerting effects of light. Sleep Med Rev. 11: 453-64.

Cajochen C, Münch M, Kobialka S, Kräuchi K, Steiner R, Oelhafen P, Orgül S, Wirz-Justice A. (2005). High sensitivity of human melatonin, alertness,

thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 90:1311-6.

Cajochen C, Zeitzer JM, Czeisler CA, Dijk DJ. (2000). Dose-response relationship for light intensity and ocular and electroencephalographic correlates of human alertness. Behav Brain Res. 115:75-83.

Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, Belluzi J, Weaver DR, Leslie FM, Zhou QY. (2002). Prokineticin 2 transmits the behavioural rhythm of the suprachiasmatic nucleus. Nature. 417:405-10.

Clarke RJ, Ikeda H. (1985a). Luminance and darkness detectors in the olivary and posterior pretectal nuclei and their relationship to the pupillary light reflex in rat. I. Studies with steady luminance levels. Exp Brain Res. 57:224-32.

Clarke RJ, Ikeda H. (1985b). Luminance detectors in the olivary and their relationship to the pupillary light reflex in rat. I. Studies using sinusoidal light. Exp Brain Res. 59:83-90.

Czeisler CA. (1995). The effect of light on the human circadian pacemaker. Ciba Found Symp. 183:254-302.

D

Daan S, Pittendrigh CS. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: II. The variability of phase response curves. J Comp Physiol A. 106:253-66.

Danilenko KV, Cajochen C, Wirz-Justice A. (2003). Is sleep per se a zeitgeber in humans? J Biol Rhythms. 18:170-8.

Davis S, Mirick DK. (2006). Circadian disruption, shift work and the risk of cancer: A summary of the evidence and studies in seattle. Cancer Causes Control. 17:539-45.

Deboer T, Overeem S, Visser NAH, Duindam H, Frölich M, Lammers GJ, Meijer JH. (2004). Convergence of circadian and sleep regulatory mechanism on hypocretin-1. Neuroscience. 129:727-32.

DeCoursey PJ. (2003). Overview of biological timing from unicells to humans. In Dunlap JC, Loros JJ, & DeCoursey PJ (Eds). *Chronobiology: Biological Timekeeping*. Sunderland, MA (USA): Sinauer Associates, pp. 2-24.

Dijk DJ, Duffy JF, Czeisler CA. (2000). Contribution of circadian physiology and sleep homeostasis to age-related changes in human sleep. Chronobiol Int. 17:285-311.

Dijk DJ, Duffy JF, Riel E, Shanahan TL, Czeisler CA. (1999). Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol. 516:611-27.

Dowling GA, Graf C, Hubbard EM, Luxenberg JS. (2008). Light treatment for neuropsychiatric behaviors in Alzheimer's disease. West J Nurs Res. 29:961-75.

Duffy JF, Dijk DJ. (2002) Getting through to circadian oscillators: why use constant routines? J Biol Rhythms. 17:4-13.

Duffy JF, Wright KP. (2005). Entrainment of the human circadian system by light. J Biol Rhythms. 20:326-38.

Dumont M, Beaulieu C. (2007). Light exposure in the natural environment: Relevance to mood and sleep disorders. Sleep Med. 8:557-65.

E

Eide EJ, Kang H, Crapo S, Gallego M, Virshup DM. (2005). Casein kinase I in the mammalian circadian clock. Methods Enzymol. 393:408-18.

Erren TC, Reiter RJ. (2009a). Defining chronodisruption. J Pineal Res. 46:245-7.

Erren TC, Reiter RJ. (2009b). Light hygiene: Time to make preventive use of insights-old and new-into the nexus of the drug light, melatonin, clocks, chronodisruption and public health. Med Hypotheses. 73:537-41.

Erren TC, Reiter RJ, Piekarski C. (2003). Light, timing of biological rhythms, and chronodisruption in man. Naturwissenschaften. 90:485-94.

Escames G, Acuña-Castroviejo D, López LC, Tan DX, Maldonado MD, Sánchez-Hidalgo M, León J, Reiter RJ. (2006). Pharmacological utility of melatonin in

the treatment of septic shock: Experimental and clinical evidence. J Pharm Pharmacol. 58:1153-65

Espiritu RC, Kripke DF, Ancoli-Israel S, Mowen MA, Mason WJ, Fell RL, Klauber MR, Kaplan OJ. (1994). Low illumination experienced by San Diego adults: Association with atypical depressive symptoms. Biol Psychiatry. 35:403-7.

Even C, Schröder CM, Friedman S, Rouillon F. (2008). Efficacy of light therapy in nonseasonal depression: A systematic review. J Affect Disord. 108:11-23.

F

Francis G, Bishop L, Luke C, Middleton B, Williams P, Arendt J. (2008). Sleep during the antarctic winter: preliminary observations on changing the spectral composition of artificial light. J Sleep Res. 17:354-60.

Franken P, Tobler I, Borbély A. (1992). Sleep and waking have a major effect on the 24-hr rhythm of cortical temperature in the rat. J Biol Rhythms. 7:341-52.

Freedman MS, Lucas RJ, Soni B, von Schantz M, Munoz M, David-Gray Z, Foster R. (1999). Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. Science 284:502-4.

Furlan R, Guzzeti S, Crivellaro W, Dassi S, Tinelli M, Baselli G, Cerutti S, Lombardi F, Pagani M, Malliani A. (1990). Continuous 24-hour assessment of the neural regulation of systemic arterial pressure and RR variabilities in ambulant subjects. Circulation. 81:537-47.

G

Garaulet M, Madrid JA. (2010). Chronobiology: Influences on metabolic syndrome and cardiovascular risk. Curr Cardiovasc Risk Rep. 4:15-23.

Garcia J, Rosen G, Mahowald M. (2001). Circadian rhythms and circadian rhythm disorders in children and adolescents. Semin Pediatr Neurol. 8:229-40.

Gilbert SS, Burgess HJ, Kennaway DJ, Dawson D. (2000). Attenuation of sleep propensity, core hypothermia, and peripheral heat loss after temazepan tolerance. Am J Physiol Regul Integr Comp Physiol. 279:R1980-7.

Glickman G, Byrne B, Pineda C, Hauck WW, Brainard GC. (2006). Light therapy for seasonal affective disorder with blue narrow-band Light-Emitting Diodes (LEDs). Biol Psychiatry. 59:502-7.

Goodwin J, Pearce VR, Taylor RS, Read KLQ, Powers SJ. (2001). Seasonal cold and circadian changes in blood pressure and physical activity in young and elderly people. Age Ageing. 30:311-7.

Gooley JJ, Lu J, Fischer D, Saper CB. (2003). A broad role for melanopsin in nonvisual photoreception. J Neurosci. 23:7093-106.

Grone BP, Chang D, Bourgin P, Cao V, Fernald RD, Heller HC, Ruby NF. (2011). Acute light exposure suppresses circadian rhythms in clock gene expression. 26:78-81.

Gronfier C, Wright KP, Kronauer RE, Czeisler CA. (2007). Entrainment of the human circadian pacemaker to longer-than-24-h days. Proc Natl Acad Sci USA. 104:9081-6.

Guillaumond F, Dardente H, Giguere V, Cermakian N. (2005). Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. J Biol Rhythms. 20:391-403.

Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, Barnard AR, Cahill H, Badea TC, Chao H, Hankins MW, Berson DM, Lucas RJ, Yau KW, Hattar S. (2008). Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature. 453:102-6.

Guo YF, Stein PK. (2003). Circadian rhythm in the cardiovascular system: Chronocardiology. Am Heart J. 145:779-86.

H

Haldar C, Ahmad R. (2010). Photoimmunomodulation and melatonin. J Photochem Photobiol B. 98:107-17.

Hammel HT, Jackson DC, Stolwijk JAJ, Hardy JD, Stromme SB. (1963). Temperature regulation by hypothalamic proportional control with an adjustable set-point. J Appl Physiol. 18:1146-54.

Hankins MW, Peirson SN, Foster RG. (2008). Melanopsin: An exciting photopigment. Trends Neurosci. 31:27-36.

Hannibal J, Fahrenkrug J. (2004). Target areas innevated by PACAP-immunoreactive retinal ganglion cells. Cell Tissue Res. 316:99-113.

Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR. (2011). Melatonin-A pleiotropic, orchestrating regulator molecule. Prog Neurobiol. 93:350-84.

Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A. (2005). Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. Am J Geriatr Psychiatry.13:359-68.

Harrington ME. (1997). The ventral lateral geniculate nucleus and the intergeniculate leaflet: Interrelated structures in the visual and circadian systems. Neursoci Biobehav Rev. 21:705-27.

Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, Berson DM. (2006). Central projections of melanopsin-expressing retinal ganglion cells in the mouse. J Comp Neurol. 497:326-49.

Hattar S, Liao HW, Takao M, Berson DM, Yau KW. (2002). Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. Science. 295:1065-70.

Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M Hofmann F, Foster RG, Yau KW. (2003). Melanopsin and rod-cone

photoreceptive systems account for all major accessory visual functions in mice. Nature. 424:75-81.

Hebert M, Dumont M, Paquet J. (1998). Seasonal and diurnal patterns of human illumination under natural conditions. Chronobiol Int. 15:59-70.

Hirota T, Fukada Y. (2004). Resetting mechanism of central and peripheral circadian clocks in mammals. Zoolog Sci. 21:359-68.

Hofstra WA, de Werd AW. (2008). How to asses circadian rhythm in humans: A review of literature. Epilepsy Behav. 13:438-44.

Holowatz LA, Kenney WL. (2010). Peripheral mechanisms of thermoregulatoy control on skin blood flow in aged humans. J Appl Physiol. 109:1538-44.

Holowatz LA, Thompson-Torgerson C, Kenney WL. (2010). Aging and control of human skin blood flow. Front Biosci. 15:718-39.

Honma S, Katsuno Y, Tanahashi Y, Abe H, Honma KI. (1998). Circadian rhythms of arginine-vasopressin and vasoactive intestinal polypeptide do not depend on cytoarchitecture of dispersed cell culture rat suprachiasmatic nucleus. Neuroscience. 86:967-76.

Huang YL, Liu RY, Wang QS, Van Someren EJW, Xu H, Zhou JN. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. Physiol Behav. 76:597-603.

I

Inouye S, Kawamura H. (1979). Persistence of circadian rhythmicity in a mammalian hypothalamic “island” containing the suprachiasmatic nucleus. Proc Natl Acad Sci USA. 76:5962-6.

Ishibashi K, Arikura S, Kozaki T, Higuchi S, Yasukouchi A. (2010). Thermoregulatory effect in humans of suppressed endogenous melatonin by pre-sleep bright-light exposure in a cold environment. Chronobiol Int. 27:782-806.

K

Kalsbeek A, Rikkers M, Vivien-Roels B, Pevet P. (1993). Vasopressin and vasoactive intestinal peptide infused in the paraventricular nucleus of the hypothalamus elevate plasma melatonin levels. J Pineal Res. 15:46-52.

Karlsson B, Knutsson A, Lindahl B. (2001). Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. Occup Environ Med. 58:747-52.

Kirisoglu C, Guilleminault C. (2004). Twenty minutes versus forty-five minutes morning bright light treatment on sleep onset insomnia in elderly subjects. J Psychosom Res. 56:537-42.

Kohyama J. (2009). A newly proposed disease condition produced by light exposure during night: Asynchronization. Brain Dev. 31:255-73.

Kolodyazhniy V, Späti J, Frey S, Götz T, Wirz-Justice A, Kräuchi K, Cajochen C, Wilhelm FH. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. J Biol Rhythms. 26:55-67.

Kolodyazhniy V, Späti J, Frey S, Götz T, Wirz-Justice A, Kräuchi K, Cajochen C, Wilhelm FH. (2012). An improved method for estimating human circadian phase derived from multichannel ambulatory monitoring and artificial neural networks. Chronobiol Int. 29:1078-97.

Komulainen S, Oja T, Rintamäki H, Virokannas H, Keinänen-Kiukaanniemi S. (2004). Blood pressure and thermal responses to whole body cold exposure in mildly hypertensive subjects. J Therm Biol. 29:851-6.

Kondo M, Tokura H, Wakamura T, Hyun KJ, Tamotsu S, Morita T, Oishi T. (2007). Physiological significance of cyclic changes in room temperature around dusk and dawn for circadian rhythms of core and skin temperature, urinary 6-hydroxymelatonin sulfate, and waking sensation just after rising. J Physiol Anthropol. 26:429-36.

Kornmann B, Schaad O, Bujard H, Takahashi JS, Schibler U. (2007). System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. PLoS Biol. 5:e34.

Kräuchi K. (2007). The thermophysiological cascade leading to sleep initiation in relation to phase of entrainment. Sleep Med Rev. 11:439-51.

Kräuchi K, Cajochen C, Werth E, Wirz-Justice A. (2000). Functional link between distal vasodilation and sleep-onset latency? Am J Physiol Regul Integr Comp Physiol. 278:R741-8.

Kräuchi K, Deboer T. (2010). The interrelationship between sleep regulation and thermoregulation. Front Biosci. 15: 604-25.

Kräuchi H, Gompper B, Hauenstein D, Flammer J, Pflüger M, Studerus E, Schötzau A, Orgül S. (2012). Diurnal blood pressure variations are associated with changes in distal-proximal skin temperature gradient. Chronobiol Int. 29:1273-83.

Kräuchi K, Knoblauch V, Wirz-Justice A, Cajochen C. (2006). Challenging the sleep homeostat does not influence the thermoregulatory system in men: Evidence from a nap vs. sleep-deprivation study. Am J Physiol Regul Integr Comp Physiol. 290:R1052-61.

Kräuchi K, Wirz-Justice A. (2001). Circadian clues to sleep onset mechanisms. Neuropsychopharmacology. 25:S92-6.

Kraves S, Weitz CJ. (2006). A role for cardiotrophin-like cytokine in the circadian control of mammalian locomotor activity. Nat Neurosci. 9:212-9.

Kunz D, Schmitz S, Mahlberg R, Mohr A, Stoter C, Wolf KJ, Herrmann WM. (1999). A new concept for melatonin deficit: On pineal calcification and melatonin excretion. Neuropsychopharmacology. 21:765-72.

L

Leloup JC, Goldbeter A. (2001). A molecular explanation for the long-term suppression of circadian rhythms by a single light pulse. Am J Physiol Regul Integr Comp Physiol. 280:R1206-12.

Lewy AJ, Bauer VK, Ahmed S, Thomas KH, Cutler NL, Singer CM, Moffit MT, Sack RL. (1998). The human phase response curve (PRC) to melatonin is about 12 hours out of phase with the PRC to light. Chronobiol Int. 15:71-83.

M

Martinez-Nicolas A, Madrid JA, Rol MA. (2014). Day-night contrast as source of health for the human circadian system. Chronobiol Int. 31:382-93.

Martinez-Nicolas A, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. Chronobiol Int. 28:617-29.

Martinez-Nicolas A, Ortiz-Tudela E, Rol MA, Madrid JA. (2013). Uncovering different masking factors on wrist skin temperature rhythm in free-living subjects. PLoS ONE. 8:e61142.

McIntyre IM, Norman TR, Burrows GD, Armstrong SM. (1989). Human melatonin suppression by light is intensity dependent. J Pineal Res. 6:149-56.

Mendoza J. (2007). Circadian clocks: Setting time by food. J Neuroendocrinol. 19:127-37.

Middleton B, Stone BM, Arendt J. (2002). Human circadian phase in 12:12 h, 200: <8 lux and 1000: <8 lux light-dark cycles, without scheduled sleep or activity. Neurosci Lett. 329:41-4.

Mills JN, Minors D, Waterhouse J. (1978). Adaptation to abrupt time shifts of the oscillator[s] controlling human circadian rhythms. J Physiol. 285:455-70.

Minors DS, Waterhouse J. (1989). Masking in humans: The problem and some attempts to solve it. Chronobiol Int. 6:29-53.

Minors DS, Waterhouse J. (1992). Investigating the endogenous component of human circadian rhythms: A review of some simple alternatives to constant routines. Chronobiol Int. 9:55-78.

Mirmiran M, Kok JH. (1991). Circadian rhythms in early human development. Early Hum Dev. 26:121-8.

Mishima K, Okawa M, Shimizu T, Hishikawa Y. (2001). Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. J Clin Endocrinol Metab. 86:129-34.

Mistlberger RE, Skene DJ. (2004). Social influences on mammalian circadian rhythms: Animal and human studies. Biol Rev Camb Philos Soc. 79:533-56.

Mistlberger RE, Skene DJ. (2005). Nonphotic entrainment in humans? J Biol Rhythms. 20:339-52.

Moore RY. (2007). Suprachiasmatic nucleus in sleep-wake regulation. Sleep Med. 8:27-33.

Moore RY, Eichler VB. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in rat. Brain Res. 42:201-6.

Moore RY, Speh JC Leak RK. (2002). Suprachiasmatic nucleus organization. Cell Tissue Res. 309:89-98.

Morin LP. (2007). SCN organization reconsidered. J Biol Rhythms. 22:3-13.

Morin LP, Allen CN. (2006). The circadian visual system, 2005. Brain Res Rev. 51:1-60.

Morin LP, Blanchard JH, Provencio I. (2003). Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: bifurcation and melanopsin immunoreactivity. J Comp Physiol. 465:401-16.

Mormont MC, Langouët AM, Claustrat B, Bogdan A, Marion S, Waterhouse J, Touitou Y, Lévi F. (2002). Marker rhythms of circadian system function: A study of patients with metastatic colorectal cancer and good performance status. Chronobiol Int. 19:141-55.

Mottram V, Middleton B, Williams P, Arendt J. (2011). The impact of bright artificial white and 'blue-enriched' light on sleep and circadian phase during polar winter. J Sleep Res. 20:154-61.

Münch M, Knoblauch V, Blatter K, Schröder C, Schnitzler C, Kräuchi K, Wirz-Justice A, Cajochen C. (2005). Age-related attenuation of the evening circadian arousal signal in humans. 26:1307-19.

Myers BL, Badia P. (1995). Changes in circadian rhythms and sleep quality with aging: mechanism and interventions. Neurosci Biobehav Rev. 19:553-571.

N

Nakamura TJ, Nakamura W, Yamazaki S, Kudo T, Cutler T, Colwell CS, Block GD. (2011). Age-related decline in circadian output. J Neurosci. 31:10201-5.

O

Ohta H, Yamazaki S, McMahon DG. (2005). Constant light desynchronizes mammalian clock neurons. Nat Neurosci. 8:267-9.

Ortiz-Tudela E, Martinez-Nicolas A, Albares J, Segarra F, Campos M, Estivill E, Rol MA, Madrid JA. (2014). Ambulatory circadian monitoring (ACM) based on thermometry, motor activity and body position (TAP): A comparison with polysomnography. Physiol Behav. 126:30-38.

Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MA, Madrid JA. (2010). A new integrated variable based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. PLoS Comput Biol. 6:e1000996.

P

Pandi-Perumal SR, Trakht I, Srinivasan V, Spence DW, Maestroni GJ, Zisapel N, Cardinali DP. (2008). Physiological effects of melatonin: Role of melatonin receptors and signal transduction pathways. Prog Neurobiol. 85:335-53.

Pauley SM. (2004). Lighting for the human circadian clock: Recent research indicates that lighting has become a public health issue. Med Hypotheses. 63:588-96.

Peirson SN, Foster RG. (2010). Non-image-forming photoreceptors. In Albrecht U (Ed). *Protein reviews. Volume 12. The circadian clock*. Nueva York, NY (USA): Springer science, pp.105-13.

Peirson SN, Oster H, Jones SL, Leitges M, Hankins MW, Foster RG. (2007). Microarray analysis and functional genomics identify novel components of melanopsin signalling. Curr Biol. 17:1363-72.

Pevet P, Challet E. (2011). Melatonin: Both master clock output and internal time-giver in the circadian clocks network. J Physiol Paris. 105:170-82.

Phipps-Nelson J, Redman JR, Schlangen LJM, Rajaratnam SMW. (2009). Blue light exposure reduces objective measures of sleepiness during prolonged nighttime performance testing. Chronobiol Int. 26:891-912.

Pittendrigh CS. (1954). On temperature independence in the clock-system controlling emergence time in *Drosophila*. Proc Nat Acad Sci. 40:1018-29.

Pittendrigh CS. (1958). Perspectives in the study of biological clocks. In Buzati-Traverso AA (Ed). *Perspectives in Marine Biology*. Berkeley, CA (USA): University of California Press, pp.239-68.

Pittendrigh CS. (1960). Circadian rhythms and the circadian organization of living systems. Cold Spring Harb Symp Quant Biol. 25:159-84.

Pittendrigh CS. (1981). Circadian systems: Entrainment. In Aschoff J (Ed). *Handbook of Behavioral Neurobiology. Volume 4. Biological Rhythms*. New York, NY (USA): Plenum Press, pp. 95-124.

Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U. (2002). The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. Cell. 110:251-60.

Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. (2000). A novel human opsin in the inner retina. J Neurosci. 20:600-5.

R

Rajaratnam SM, Arendt J. (2001). Health in a 24-h society. Lancet. 358:999-1005.

Ralph MR, Foster RG, Davis FC, Menaker M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. Science. 247:975-8.

Reilly T, Waterhouse J. (2009). Circadian aspects of body temperature regulation in exercise. J Thermal Biol. 34:161-70.

Reiter RJ. (1995). Functional pleiotropy of the neurohormone melatonin: Antioxidant protection and neuroendocrine regulation. Front Neuroendocrinol. 16:383-415.

Reiter RJ, García JJ, Pie J. (1998) Oxidative toxicity in models of neurodegeneration: Responses to melatonin. Restor Neurol Neurosci. 12:135-42.

Reiter RJ, Tan D, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. (2007). Light at night, chronodisruption, melatonin suppression, and cancer risk: A review. Crit Rev Oncog. 13:303-28.

Refinetti R. (2010). Entrainment of circadian rhythm by ambient temperature cycles in mice. J Biol Rhythms. 25:247-56

Refinetti R, Cornélissen G, Halber F. (2007). Procedures for numerical analysis of circadian rhythms. Biol Rhythm Res. 38:275-325.

Reilly T, Waterhouse J. (2009). Circadian aspects of body temperature regulation in exercise. J Therm Biol. 34:161-70.

Reiter RJ, Tan D, Korkmaz A, Erren TC, Piekarski C, Tamura H, Manchester LC. (2007). Light at night, chronodisruption, melatonin suppression, and cancer risk: A review. Crit Rev Oncog. 13:303-28.

Reppert SM, Weaver DR. (2002). Coordination of circadian timing in mammals. Nature. 418:935-41.

Richter CP. (1967). Sleep and activity: Their relation to the 24-hour clock. Res Publ Assoc Ment Dis. 45:8-27.

Rietveld W, Minors D, Waterhouse J. (1993). Circadian rhythms and masking: An overview. Chronobiol Int. 10:306-12.

Ripperger JA, Brown SA. (2010). Transcriptional regulation of circadian clocks. In Albrecht U (Ed). *Protein reviews. Volume 12. The circadian clock*. Nueva York, NY (USA): Springer science, pp.37-78.

Rodrigues Menezes MC, Nogueira Pires ML, Benedito-Silva AA, Tufik S. (2004). Sleep parameters among offshore workers: An initial assessment in the Campos Basin, Rio de Janeiro, Brazil. Chronobiol Int. 21:889-97.

Roenneberg T, Merrow M. (1998). Molecular circadian oscillators: an alternative hypothesis. J Biol Rhythms. 13:167-79.

Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, O'Hara BF. (2002). Role of melanopsin in circadian responses to light. Science. 298:2211-3.

Rüger M, Gordijn MCM, Beersma DGM, de Vries B, Daan S. (2006). Time-of-day-dependent effects of bright light exposure on human psychophysiology: comparison of daytime and nighttime exposure. Am J Physiol Regul Integr Comp Physiol. 290:R1413-20.

S

Saad A, Dalla Man C, Nandy DK, Levine JA, Bharucha AE, Rizza RA, Basu R, Carter RE, Cobelli C, Kudva YC, Basu A. (2012). Diurnal pattern to insulin secretion and insulin action in healthy individuals. Diabetes. 61:2691:700.

Saper CB, Chou TC, Scammell TE. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. Trends Neurosci. 24:726-31.

Saper CB, Lu J, Chou TC, Gooley J. (2005). The hypothalamic integrator for circadian rhythms. Trends Neurosci. 28:152-7.

Sarabia JA, Rol MA, Mendiola P, Madrid JA. (2008). Circadian rhythm of wrist temperature in normal-living subjects. A candidate of new index of the circadian system. Physiol Behav. 95:570-80.

Sato TH, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, Hogenesch JB. (2004). A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. Neuron. 43:527-37.

Savides TJ, Messin S, Senger C, Kripke D. (1986). Natural light exposure of young adults. Physiol Behav. 38:571-4.

Schernhammer ES, Laden F, Speizer FE, Willett WC, Hunter DJ, Kawachi I, Fuchs CS, Colditz GA. (2003). Night-shift work and risk of colorectal cancer in the nurses' health study. J Natl Cancer Inst. 95:825-8.

Schmid HA, Requintina PJ, Oxenkrug GF, Sturner W. (1994). Calcium, calcification, and melatonin biosynthesis in the human pineal gland: A postmortem study into age-related factors. J Pineal Res. 16:178-83.

Sekaran S, Lall GS, Ralphs KL, Wolstenholme AJ, Lucas RJ, Foster RG, Hankins MW. (2007). 2-Aminoethoxydiphenylborane is an acute inhibitor of directly photosensitive retinal ganglion cell activity in vitro and in vivo. J Neurosci. 27:3981-6.

Seron-Ferre M, Ducsay CA, Valenzuela GJ. (1993). Circadian rhythms during pregnancy. Endocr Rev. 14:594-609.

Seron-Ferre M, Torres-Farfan C, Forcelledo ML, Valenzuela GJ. (2001). The development of circadian rhythms in the fetus and neonate. Semin Perinatol. 25:363-70.

Silver R, Lehman MN, Gibson M, Gladstone WR, Bittman EL. (1990). Dispersed cell suspensions of fetal SCN restore circadian rhythmicity in SCN-lesioned adult hamsters. Brain Res. 525:45-58.

Silver R, LeSauter J, Tresco PA, Lehman MN. (1996). A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. Nature. 382:810-3.

Simmoneaux V, Garidou ML, Ribelayga C, Pévet P. (2006). Mechanism underlying seasonal regulation of melatonin synthesis in rodents. In Pandi-Perumal SR,

Cardinali DP (Eds). *Melatonin: Biological basis of its function in health and disease*. Georgetown, TX (USA): Eurekah.com, pp 1-11.

Skene DJ. (2003). Optimization of light and melatonin to phase shift human circadian rhythms. J Neuroendocrinol. 15:438-41.

Smith MR, Eastman CI. (2009). Phase delaying the human circadian clock with blue-enriched polychromatic light. Chronobiol Int. 26:709-25.

Smith KA, Schoen MW, Czeisler CA. (2004). Adaptation of human pineal melatonin suppression by recent photic history. J Clin Endocrinol Metab. 89:3610-4.

Stephan FK, Zucker I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Nat Acad Sci USA. 69:1583-6.

Stevens RG. (2006). Artificial lighting in the industrialized world: Circadian disruption and breast cancer. Cancer Causes Control. 17:501-7.

T

Teclemariam-Mesbah R, Ter Host GJ, Postema F, Wortel J, Buijs RM. (1999). Anatomical demonstration of the suprachiasmatic nucleus-pineal pathway. J Comp Neurol. 406:171-82.

Thapan K, Arendt J, Skene DJ. (2001). An action spectrum for melatonin suppression: Evidence for a novel non-rod, non-cone photoreceptor system in humans. J Physiol. 535:261-7.

Touitou Y. (2001). Human aging and melatonin. Clinical relevance. Exp Geront. 36:1083-100.

Touitou Y, Selmaoui B. (2012). The effects of extremely low-frequency magnetic fields on melatonin and cortisol, two marker rhythms of the circadian system. Dialogues Clin Neurosci. 14:381-99.

Trejo LJ, Cicerone CM. (1984). Cells in the pretectal olivary nucleus are in the pathway for the direct light reflex of the pupil in the rat. Brain Res. 300:49-62.

Turek FW, Penev P, Zhang Y, van Reeth O, Zee P. (1995). Effects of age on the circadian system. Neurosci Biobehav Rev. 19:53-8.

Turner PL, Mainster MA. (2008). Circadian photoreception: ageing and the eye's important role in systemic health. Br J Ophthalmol. 92:1439-44.

Turner PL, Van Someren EJW, Mainster MA. (2010). The role of environmental light in sleep and health: Effects of ocular aging and cataract surgery. Sleep Med Rev. 14:269-80.

V

Vanselow JT, Kramer A. (2010). Posttranslational regulation of circadian clocks. In Albrecht U (Ed). *Protein reviews. Volume 12. The circadian clock.* Nueva York, NY (USA): Springer science, pp. 79-104.

Van Cauter E, Désir S, Decoster C, Féry F, Balasse EO. (1989). Nocturnal decrease in glucose tolerance during constant glucose infusion. J Clin Endocrinol Metab. 69:604-11.

Van Someren EJW. (2000). More than a marker: Interaction between the circadian regulation of temperature and sleep, age-related changes, and treatment possibilities. Chronobiol Int. 17:313-54.

Van Someren EJW, Nagtegaal E. (2007). Improving melatonin circadian phase estimates. Sleep Med. 8:590-601.

Van Someren EJW, Swaab DF, Colenda CC, Cohen W, McCall WV, Rosenquist PB. (1999). Bright light therapy: Improved sensitivity to its effects on rest-activity rhythms in Alzheimer patients by application of nonparametric methods. Chronobiol Int. 16:505-18.

Veerman DP, Imholz BP, Wieling W, Wesseling KH, van Montfrans GA. (1995). Circadian profile of systemic hemodynamics. Hypertension. 26:55-9.

Viola AU, James LM, Schlangen LJ, Dijk DJ. (2008). Blue-enriched white light in the workplace improves self-reported alertness, performance and sleep quality. Scand. J Work Environ Health. 34:297-306.

W

Wakamura T, Tokura H. (2002). Circadian rhythm of rectal temperature in humans under different ambient temperature cycles. J Thermal Biol. 27:439-47.

Waterhouse J, DeCoursey PJ. (2004). The relevance of circadian rhythms for human welfare. In Dunlap JC, Loros JJ, & DeCoursey PJ (Eds). *Chronobiology: Biological Timekeeping*. Sunderland, MA (USA): Sinauer Associates, pp. 325-56.

Waterhouse J, Fukuda Y, Morita T. (2012). Daily rhythms of the sleep-wake cycle. J Physiol Anthropol. 31:5. doi: 10.1186/1880-6805-31-5.

Waterhouse J, Kao S, Weinert D, Edwards B, Atkinson G, Reilly T. (2005). Measuring phase shifts in humans following a simulated time-zone transition: agreement between constant routine and purification methods. Chronobiol Int. 22:829-58.

Waterhouse J, Nevill A, Weinert D, Folkard S, Minors DS, Atkinson G, Reilly T, Macdonald I, Owens D, Sytnik N, Tucker P. (2001). Modeling the effect of spontaneous activity on core temperature in healthy human subjects. Biol Rhythm Res. 32:511-28.

Waterhouse J, Weinert D, Minors DS, Folkard S, Owens D, Atkinson G, Macdonald I, Sytnik N, Tucker P, Reilly T. (2000). A comparison of some different methods for purifying core temperature data from humans. Chronobiol Int. 17:539-66.

Webb AB, Angelo N, Huettner JE, Herzog ED. (2009). Intrinsic, nondeterministic circadian rhythm generation in identified mammalian neurons. Proc Natl Acad Sci USA. 106:16493-8.

Weinert D, Nevill A, Weinandy R, Waterhouse J. (2003). The development of new purification methods to assess the circadian rhythm of body temperature in Mongolian gerbils. Chronobiol Int. 20:249-70.

Weinert D, Waterhouse J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. Physiol Behav. 63:837-43.

Weinert D, Waterhouse J. (2007). The circadian rhythm of core temperature: Effects of physical activity and aging. Physiol Behav. 90:246-56.

Welsh DK, Logothetis DE, Meister M, Reppert SM. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phase circadian firing rhythms. Neuron. 14:697-706.

Wirz-Justice A, Graw P, Krauchi K, Sarrafzadeh A, English J, Arendt J, Sand L. (1996). 'Natural' light treatment of seasonal affective disorder. J Affect Disord. 37:109-20.

Y

Yamasaki Y, Kodama M, Matsuhisa M, Kishimoto M, Ozaki H, Tani A, Ueda N, Ishida Y, Kamada T. (1996). Diurnal heart rate variability in healthy subjects: effects of aging and sex difference. Am J Physiol. 271:H303-10.

Young MJ, Lund RD. (1994). The anatomical substrates subserving the papillary light reflex in rats: origin of the consensual pupillary response. Neuroscience. 62:481-96.

Z

Zornoza-Moreno M, Fuentes-Hernández S, Sánchez-Solis M, Rol MA, Larqué E, Madrid JA. (2011). Assessment of circadian rhythms of both skin temperature and motor activity in infants during the first 6 months of life. Chronobiol Int. 28:330-7.

7. Annex

ANNEX I. SCIENTIFIC PRODUCTION FROM THE EXPERIMENTS PERFORMED IN THE PRESENT PHD THESIS

1. PUBLICATIONS

1) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Crosstalk between environmental light and internal time in humans. Chronobiology International. 28:617-29. Impact Factor: 4.028, Q1 Category: Physiology.
2) Blazquez A, **Martinez-Nicolas A**, Salazar FJ, Rol MA, Madrid JA. (2012). Wrist skin temperature, motor activity and body position as determinants of the circadian pattern of blood pressure. Chronobiology International. 29:747-56. Impact Factor: 4.35, Q1 Category: Physiology.
3) Lucas Sánchez A, **Martinez-Nicolas A**, Escames G, de Costa J (2012). Aging of the circadian system. Revista Española de Geriatría y Gerontología. 47:76-80. (Spanish).
4) **Martinez-Nicolas A**, Ortiz-Tudela E, Rol MA, Madrid JA. (2013). Uncovering different masking factors on wrist skin temperature rhythm in free-living subjects. PLoS ONE. 8:e61142. Impact Factor: 4.09, Q1 Category: Multidisciplinary sciences.
5) **Martinez-Nicolas A**, Ortiz-Tudela E, Rol MA, Madrid JA. (2013). Influencia de la exposición a la luz sobre el sistema circadiano. Vigila y Sueño. 25-1:1-15. (Spanish).
6) **Martinez-Nicolas A**, Madrid JA, Rol MA. (2014). Day-night contrast as source of health for the human circadian system. Chronobiology International. 31:382-93. Impact Factor: 4.35, Q1 Category: Physiology.
7) Batinga H, **Martinez-Nicolas A**, Zornoza-Moreno M, Larqué E, Mondéjar MT, Moreno-Casbas M, García FJ, Rol MA, Madrid JA. Distal skin temperature rhythm as an index of circadian system ontogeny and ageing in humans. Physiology and Behaviour. *Submitted*.
8) **Martinez-Nicolas A**, Meyer M, Hunkler S, Madrid JA, Rol MA, Meyer AH, Schötzau A, Orgül S, Kräuchi K. Effects of daytime variation in ambient temperature on skin temperatures and blood pressure: An ambulatory winter vs. summer study in healthy young women. *In preparation*.

9) **Martinez-Nicolas A**, García FJ, Moreno-Casbas M, Madrid JA, Rol MA. Synchronizers exposure in healthy ageing: effect on wrist temperature rhythm. *In preparation*.

2. BOOK CHAPTERS

1) **Martinez-Nicolas A**, Ortiz-Tudela E, Lucas-Sanchez A, Madrid JA, Rol MA. (2011). Intercomunicación entre la exposición a la luz y el reloj biológico en humanos. In Libro de Resúmenes de las I Jornadas de Inicio a la Investigación de Estudiantes de la Facultad de Biología. 53. (Spanish).
2) **Martinez-Nicolas A**, Madrid JA, Rol MA. (in press). El Sistema Circadiano a lo largo de la vida. In Sociedad Española del Sueño (Eds). Tratado de medicina del sueño. 233-40. (Spanish).

3. COMMUNICATIONS TO NATIONAL AND INTERNATIONAL CONGRESSES, WORKSHOPS AND SEMINARS

1) Ortiz-Cullera, V., **Martinez-Nicolas, A.**, Ortiz, E, Madrid, J.A., Rol, M.A. (2007). A new synchronizer exposure test developed by chronobiology students. Second International School on Mind, Brain and Education. Basic and applied topics in biological rhythms and learning. Erice (Italy). Poster presentation.
2) **Martinez-Nicolas A**, Ortiz Tudela E, Madrid JA, Rol MA. (2008). Desarrollo de un nuevo método para evaluar la regularidad de un individuo: Chronozeit. V Congreso Internacional de Estudiantes de Ciencias Experimentales y de la Salud (CIE). Valencia (Spain). Oral communication.

   *** Prize for the best communication**
3) **Martinez-Nicolas A**, Ortiz E, Madrid JA, Rol MA. (2009). Influence of light exposure on skin wrist temperature rhythm in human under free-style living conditions. XI Congress of the European Biological Rhythms Society (EBRS). Strasbourg (France). Poster presentation.

   *** Prize for the best poster**
4) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2010). Efecto de la exposición voluntaria a la luz sobre el patrón de sueño y el estatus del sistema circadiano. XIX Reunión Anual de la Sociedad Española de Sueño (SES). Alcoy (Spain). Poster presentation. (Spanish).

5) **Martinez-Nicolas A**, Ortiz-Tudela E, Almaida-Pagan PF, Sarabia JA, Moreno M, Gonzalez-Maria E, Madrid JA, Rol MA. (2010). Effect of light exposure on wrist temperature rhythm. Effect of Aging. XXVI Conference of the International Society for Chronobiology (ISC). Vigo (Spain). Poster presentation.
6) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2010). Demasking wrist temperature rhythm under free-living conditions. XXVI Conference of the International Society for Chronobiology (ISC). Vigo (Spain). Poster presentation.
7) **Martinez-Nicolas A**. (2010). I Seminario de cronobiología aplicada: Controle el ritmo de su vida. "Seminarios Enfócate". Chronobiotech SL. Murcia (Spain). (Spanish). Conference.
8) **Martinez-Nicolas A**. (2011). II Seminario de Cronobiología Aplicada: Cronobiología y Trabajo a Turnos. "Seminarios Enfócate". Chronobiotech SL. Murcia (Spain). (Spanish). Conference.
9) **Martinez-Nicolas A**, Sarabia JA, Ortiz-Tudela E, Tortosa F, Madrid JA, Rol MA. (2011). Kronosensor, un nuevo dispositivo para el análisis de ritmos circadianos. XX Reunión anual de la Sociedad Española del Sueño (SES). Sevilla (Spain). Poster presentation. (Spanish).
10) **Martinez-Nicolas A**. (2011). Light pollution: Chronobiological effects. "Night sky protection against light pollution". Ministry of Environment. Malaga (Spain). (Spanish). Conference.
11) **Martinez-Nicolas A**. (2011). Espectros de diferentes Luminarias y de la Luz Solar. "Light pollution seminars". Delegación de Alumnos de la Facultad de Biología. Murcia (Spain). (Spanish). Conference.
12) **Martinez-Nicolas A**, Ortiz-Tudela E, Lucas-Sanchez A, Madrid JA, Rol MA. (2011). Intercomunicación entre la exposición a la luz y el reloj biológico en humanos. I Jornadas de Inicio a la Investigación de Estudiantes de la Facultad de Biología. Murcia (Spain). Oral communication. (Spanish).
13) **Martinez-Nicolas A**. (2011). Chronobiology: Light and light effects on human biology. "Sick Building Syndrome Seminars". Gea. Asociación de Estudios Geobiológicos. Barcelona (Spain). (Spanish). Conference.

14) **Martinez-Nicolas A**. (2011). Applied chronobiology: Natural and lighting effects on human health. "Sick Building Syndrome Seminars". Gea. Asociación de Estudios Geobiológicos. Barcelona (Spain). (Spanish). Conference.
15) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). A constant routine approach to analyse wrist temperature rhythm under free-living conditions. XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England). Poster presentation.
16) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2011). Light exposure pattern influences wrist temperature rhythm in humans. XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England). Poster presentation.
17) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2012). La exposición voluntaria a la luz influye en el ritmo de sueño. XXI Reunión anual de la Sociedad Española del Sueño (SES). Burgos (Spain). Poster presentation. (Spanish).

    *** Prize for the best poster**
18) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2012). La exposición voluntaria a la luz influye en el ritmo de sueño. XXI Reunión anual de la Sociedad Española del Sueño (SES). Burgos (Spain). Oral communication. (Spanish).
19) **Martinez-Nicolas A**, Madrid JA, Rol MA. (2013). Day-night contrast as source of health for the human circadian system. 5º World Congress on Sleep Medicine. Valencia (Spain). Poster presentation.
20) Guaita M, **Martinez-Nicolas A**, Madrid JA, Rol MA, Montserrat JM and Santamaria J. (2013). Daytime peripheral temperature changes during MWT and MSLT. 5º World Congress on Sleep Medicine. Valencia (Spain). Poster presentation.
21) **Martinez-Nicolas A**, Guaita M, Santamaría J, Montserrat JM, Madrid JA, Rol MA. (2013). Circadian impairment of the wrist temperature rhythm in patients with sleep disordered breathing. 5º World Congress on Sleep Medicine. Valencia (Spain). Poster presentation.

22) **Martinez-Nicolas A**, Madrid JA, Rol MA. (2013). Day-night contrast as source of health for the human circadian system. Jornada Sociedad Española de Sueño (SES). Valencia (Spain). Poster presentation.
23) **Martinez-Nicolas A**, Guaita M, Santamaría J, Montserrat JM, Madrid JA, Rol MA. (2013). Circadian impairment of the wrist temperature rhythm in patients with sleep disordered breathing. Jornada Sociedad Española de Sueño (SES). Valencia (Spain). Poster presentation.
24) **Martinez-Nicolas A**, Madrid JA, Rol MA. (2013). When the light is not properly switched on. Circadian impairment of Wrist Temperature pattern. 1st International Congress on Artificial Light At Night (ALAN). Berlin (Germany). Poster presentation.
25) **Martinez-Nicolas A**, Moreno-Casbas M, Madrid JA, Rol MA. (2014). Envejecimiento del sistema circadiano: Temperatura periférica y clasificación por edad. XXII Reunión anual de la Sociedad Española del Sueño (SES). San Sebastián (Spain). Oral Communication (Spanish).
26) **Martinez-Nicolas A,** Madrid JA, Rol MA. (2014). Envejecimiento del sistema circadiano y cronopotenciación a través del ritmo de temperatura periférica distal. 6ª Reunión Nacional de la Sociedad Española de Medicina Geriátrica (SEMEG). Pamplona (Spain). Poster presentation (Spanish).

4. ABSTRACTS PUBLISHED IN PEER-REVIEWED JOURNALS

1) **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid JA, Rol MA. (2010). Efecto de la exposición voluntaria a la luz sobre el patrón de sueño y el estatus del sistema circadiano. Vigilia y Sueño. 22-2:12. (Spanish).
2) **Martinez-Nicolas A**, Sarabia JA, Ortiz-Tudela E, Tortosa F, Madrid JA, Rol MA. (2011) Kronosensor, un nuevo dispositivo para el análisis de ritmos circadianos. Vigilia y Sueño. 23-1:12. (Spanish).
3) **Martinez-Nicolas A**, Ortiz-Tudela E, Rol MA, Madrid JA. (2012). La exposición voluntaria a la luz influye en el ritmo de Sueño. Vigilia y Sueño. 24-1:12. (Spanish).
4) **Martinez-Nicolas A**, Madrid JA, Rol MA. (2013). Day-night contrast as source of health for humans. Sleep Medicine. 14:e191. Impact Factor: 3.487, Q1 Category: Clinical Neurology.

5) **Martinez-Nicolas A**, Guaita M, Santamaría J, Montserrat J, Madrid JA, Rol MA. (2013). Circadian impairment of the wrist temperature rhythm in patients with sleep disordered breathing. Sleep Medicine. 14:e190-1. Impact Factor: 3.487, Q1 Category: Clinical Neurology.

5. PATENTS

1) Madrid JA, Rol MA, **Martinez-Nicolas A**, Sarabia JA. (2011). Dispositivo de Iluminación Circadiano. (2400590), University of Murcia. Murcia (Spain).

ANNEX II. ADDITIONAL SCIENTIFIC PRODUCTION RESULTING FROM COLLABORATIONS AND RESEARCH PROJECTS

1. PUBLICATIONS

1) Madrid JA, Ortiz-Tudela E, **Martinez-Nicolas A**, Rol de Lama MA. (2009). Sistema circadiano en el anciano: Valoración clínica e intervenciones terapéuticas. Informaciones Psiquiátricas. 195-196:51-9. (Spanish).
2) Ortiz-Tudela E, **Martinez-Nicolas A**, Campos M, Rol MA, Madrid JA. (2010). A New Integrated Variable Based on Thermometry, Actimetry and Body Position (TAP) to Evaluate Circadian System Status in Humans. PLoS Computational Biology. 6:e1000996. Impact Factor: 5.515, Q1 Category: Mathematical and Computational Biology.
3) Rol MA, Baño-Otalora B, **Martinez-Nicolas A**, Bonmatí Carrión MA, Ortiz-Tudela E, Madrid JA. (2011). El lado oscuro de la luz: Efectos de la contaminación lumínica sobre la salud humana. Física y Sociedad. 21:20-2. (Spanish).
4) Varela M, Ruiz-Esteban R, **Martinez-Nicolas A**, Cuervo-Arango JA, Barros C, Garcia Delgado E. (2011). "Catching the spike and tracking the flow": Holter-temperature monitoring in patients admitted in a general internal medicine Ward. International Journal of Clinical Practice. 65:1283-8. Impact Factor: 2.415, Q1 Category: Medicine, general and internal.
5) Bandín C, **Martinez-Nicolas A**, Ordovás JM, Ros Lucas JA, Castell P, Silvente T, Madrid JA, Garaulet M (2013). Differences in circadian rhythmicity in CLOCK 3111T/C genetic variants in moderate obese women as assessed by thermometry, actimetry and body position.

International Journal of Obesity. 37:1044-50. Impact Factor: 5.221, Q1 Category: Nutrition and Dietetics.

6) Lucas-Sanchez A, Almaida-Pagan PF, **Martinez-Nicolas A**, Madrid JA, de Costa J, Mendiola P. (2013). Rest-activity circadian rhythms in aged *Nothobranchius korthausae*. The effects of melatonin. Experimental Gerontology. 48:507-16. Impact Factor: 3.911, Q1 Category: Geriatrics and Gerontology.
7) Rubio-Sastre P, Gómez-Abellán P, **Martinez-Nicolas** A, Ordovás JM, Madrid JA, Garaulet M. (2014). Evening physical activity alters wrist temperature circadian rhythmicity. Chronobiology International. 31:276-82. Impact Factor: 4.35, Q1 Category: Physiology.
8) Bandín C, **Martinez-Nicolas A**, Ordovás JM, Madrid JA, Garaulet M. (2013). Circadian rhythmicity as a predictor of weight loss effectiveness. International Journal of Obesity (London). doi: 10.1038/ijo.2013.211. [Epub ahead of print]. Impact Factor: 5.221, Q1 Category: Nutrition and Dietetics.
9) Ortiz-Tudela E, Martinez-Nicolas A, Albares J, Segarra F, Campos M, Estivill E, Rol MA, Madrid JA. (2014). Ambulatory Circadian Monitoring (ACM) based on Thermometry, motor Activity and body Position (TAP): A comparison with Polysomnography. Physiology and Behaviour. 126:30-8.

2. BOOK CHAPTERS

1) Madrid JA, **Martinez-Nicolas A**, Sanchez Galvez RM. (2006). Cronotoxicología, Cronofarmacología y Cronoterapia. In Cronobiología básica y clínica. 597-629. (Spanish).
2) Rol MA, Baño Otalora B, Sarabia JA, Mondéjar-Abenza MT, **Martinez-Nicolas A**, Ortiz-Tudela E, Madrid Pérez JA. (2009). Enseñanza e investigación: las dos caras de una misma moneda. In Jornadas sobre el espacio europeo de educación superior: "El espacio europeo de educación superior en el horizonte 2010". (Spanish).
3) Lucas-Sanchez A, Almaida-Pagan PF, **Martinez-Nicolas A**, de Costa J, Mendiola P. (2011). Composición de ácidos grasos de dos especies de peces teleósteos y su relación con la longevidad. In Libro de Resúmenes

de las I Jornadas de Inicio a la Investigación de Estudiantes de la Facultad de Biología. 63. (Spanish).

4) Mendiola P, de Costa J, Ortiz-Tudela E, **Martinez-Nicolas A**, Bonmati-Carrion MA, Lucas-Sanchez A, Baño-Otalora B, Madrid JA, Rol MA. (2011). Teaching biological rhythms in endocrinology: cortisol and wrist temperature. In Congreso Internacional de Innovación Docente: Libro de Actas. 65.
5) Mendiola P, de Costa J, Ortiz-Tudela E, **Martinez-Nicolas A**, Bonmati-Carrion MA, Lucas-Sanchez A, Baño-Otalora B, Madrid JA, Rol MA. (2011). Teaching biological rhythms in endocrinology: cortisol and wrist temperature. In Congreso Internacional de Innovación Docente. 425.

3. COMMUNICATIONS TO NATIONAL AND INTERNATIONAL CONGRESSES, WORKSHOPS AND SEMINARS

1) Madariaga AM., Vivanco P, Ortiz V, **Martinez-Nicolas A**, Madrid JA, Rol MA. (2005). El Octodon degus, un modelo animal para el estudio del envejecimiento de los ritmos circadianos. SECAL-ESLAV 2005 International Congress. Elche (Spain). (Spanish)
2) Ortiz V, **Martinez-Nicolas A**, Ros F, Herrero MT, Madrid JA, Rol MA. (2007). Impaired circadian rhytmicity in a diurnal rodent (Octodon degus) with devascularizing cortical lesions. Effects of the NMDA antagonist memantine. The Second International Congress of Applied Chronobiology and Chronomedicine (ICACC). Tunis-Gammarth (Tunisia).
3) Ortiz-Tudela E, **Martinez-Nicolas A**, Madrid JA, Rol MA. (2008). Cortisol, Temperatura Periférica y el efecto de las salidas nocturnas durante el fin de semana. V Congreso Internacional de Estudiantes de Ciencias Experimentales y de la Salud (CIE). Valencia (Spain).
4) Ortiz-Tudela E, **Martinez-Nicolas A**, Rol MA, Campos M, Madrid JA. (2009). A new integrated index, based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in humans. XI Congress of the European Biological Rhythms Society (EBRS). Strasbourg (France).
5) Blázquez Manzanera AL, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2009). Correlation between wrist skin temperature circadian rhythm and

ambulatory blood pressure monitoring (ABPM). XI Congress of the European Biological Rhythms Society (EBRS). Strasbourg (France).

6) Rol MA, Baño-Otálora B, Sarabia JA, Mondéjar-Abenza MT, **Martinez Nicolas A**, Ortiz-Tudela E, Madrid JA. (2009). Enseñanza e Investigación: Las dos caras de una misma moneda. IV Jornadas Nacionales sobre el Espacio Europeo de Educación Superior (EEES). Murcia (Spain). (Spanish).
7) Cubero J, Sánchez CL, Sarabia JA, **Martinez-Nicolas A**, Ortiz-Tudela E, Rol MA, Rodríguez AB, Madrid JA, Barriga C. (2009). Comparación de diferentes parámetros de sueño entre las técnicas de actimetría y polisomnografía. I Congreso Ibérico de Biología y Sociedad - I Foro de Bioeconomía (CIBIOS). Badajoz (Spain).
8) Venero C, García S, Díaz C, Valencia A, Pereda Pérez I, Rol MA, Ortiz-Tudela E, **Martinez-Nicolas A**, Madrid JA, Perahita H. (2010). Marcadores neuropsicológicos y secreción circadiana de cortisol en la detección precoz del deterioro cognitivo ligero. IV Reunión Nacional de la Sociedad Española de Medicina Geriátrica (SEMEG). Salamanca (Spain). (Spanish).
9) Sosa M, Sosa J, **Martinez-Nicolas A**, Ortiz-Tudela E, Baño B, Madrid JA, Rol MA, Campos M. (2010). Circadianware: Una nueva herramienta informática para el análisis de los ritmos circadianos de temperatura, actividad y posición en humanos. XIX Reunión Anual de la Sociedad Española de Sueño (SES). Alcoy (Spain). (Spanish).
10) Ortiz-Tudela E, **Martinez-Nicolas A**, Sarabia JA, Almaida P, Campos M, Moreno M, Amillategui R, Madrid JA, Rol MA. (2010). Evaluación del grado de envejecimiento del sistema circadiano mediante el empleo combinado de actimetría, posición corporal y termometría. 52 Congreso de la Sociedad Española de Geriatría y Gerontología (SEGG). Valladolid (Spain). (Spanish).
11) Ortiz-Tudela E, **Martinez-Nicolas A**, Almaida-Pagan PF, Sarabia JA, Moreno M, Fuentelsaz C, Rol MA, Madrid JA. (2010). Biological rhythms characterization in elderly people: wrist temperature, locomotor activity, body position and TAP. XXVI Conference of the International Society for Chronobiology (ISC). Vigo (Spain).

12) Ortiz-Tudela E, **Martinez-Nicolas A**, Pagan G, Pozo P, Rol MA, Madrid JA. (2010). Assessment of circadian system status in patients with anorexia nervosa. XXVI Conference of the International Society for Chronobiology (ISC). Vigo (Spain).

13) Blazquez-Manzanera AL, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2010). Correlation between wrist skin temperature circadian rhythm, body position, activity and ambulatory blood pressure monitoring using Zeitgeber-Time. XXVI Conference of the International Society for Chronobiology (ISC). Vigo (Spain).

14) Blazquez-Manzanera AL, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2010). TAP: A new variable highly connected to blood pressure. XXVI Conference of the International Society for Chronobiology (ISC). Vigo (Spain).

15) Blazquez-Manzanera AL, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2011). Temperatura Periférica: Una nueva herramienta en el diagnóstico del patrón circadiano de presión arterial. XX Reunión anual de la Sociedad Española del Sueño (SES). Sevilla (Spain). (Spanish).

16) Bonmatí-Carrión MA, Rico F, **Martinez-Nicolas A**, Ortiz-Tudela E, Mondéjar MT, Otálora BB, Madrid JA, Rol MA. (2011). Evaluación del funcionamiento del sistema circadiano de ciegos mediante el registro de la temperatura de la piel de la muñeca. XX Reunión anual de la Sociedad Española del Sueño (SES). Sevilla (Spain). (Spanish).

17) Ortiz-Tudela E, **Martinez-Nicolas A**, Martinez C, Albares J, Segarra F, Rol MA, Estivill E, Madrid JA. (2011). Análisis de los ritmos de sueño-vigilia mediante el empleo combinado de Termometría, Actividad motora y Posición (TAP). Validación polisomnográfica. XX Reunión anual de la Sociedad Española del Sueño (SES). Sevilla (Spain). (Spanish).

18) Ortiz-Tudela E, **Martinez-Nicolas A**, Martinez C, Albares J, Segarra F, Rol MA, Estivill E, Madrid JA. (2011). Análisis de los ritmos de sueño-vigilia mediante el empleo combinado de Termometría, Actividad motora y Posición (TAP). Validación polisomnográfica. XX Reunión anual de la Sociedad Española del Sueño (SES). Sevilla (Spain). (Spanish).

19) Lucas-Sanchez A, Almaida-Pagan PF, **Martinez-Nicolas A**, de Costa J, Mendiola P. (2011). Composición de ácidos grasos de dos especies de peces teleósteos y su relación con la longevidad. I Jornadas de Inicio a la Investigación de Estudiantes de la Facultad de Biología. Murcia (Spain). (Spanish).

20) Mendiola P, de Costa J, Ortiz-Tudela E, **Martinez-Nicolas A**, Bonmati-Carrion MA, Lucas-Sanchez A, Baño-Otalora B, Madrid JA, Rol MA. (2011). Teaching biological rhythms in endocrinology: cortisol and wrist temperature. Congreso Internacional de Innovación Docente (CIID). Cartagena (Spain).

21) Blazquez-Manzanera AL, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2011). Wrist skin temperature and body position: new tools for the diagnostic of the blood pressuer circadian pattern. XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England).

22) Bonmatí-Carrión MA, **Martinez-Nicolas A**, Otálora BB, Madrid JA, Rol MA. (2011). Circadian system evaluation in blind people through wrist skin temperature rhythm. Should blind people be exposed to bright light? XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England).

23) Lucas-Sanchez A, **Martinez-Nicolas A**, Almaida-Pagan PF, Madrid JA, Mendiola P, de Costa J. (2011). Rest-activity rhythm in aged Nothobranchius korthausae: Effect of melatonin. XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England).

24) Lucas-Sanchez A, **Martinez-Nicolas A**, Madrid JA, Mendiola P, de Costa J. (2011). Rest-activity rhythms before death time in aged Nothobranchius rachovii. XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England).

25) Ortiz-Tudela E, **Martinez-Nicolas A**, Venero C, Madrid JA, Rol MA. (2011). Biological rhythms characterization in mild cognitive impairment. XII Congress of the European Biological Rhythms Society (EBRS). Oxford (England).

26) Nicolau C, Mouzo M, Fiol M, **Martinez-Nicolas A**, Sarabia JA, Sánchez-Campillo M. (2011). Bright light therapy improves the sleep and circadian rhythms in institutionalized elderly. Turkish Society of

Physiological Sciences and Federation of European Physiological Societies. Estambul (Turkey).

27) Blazquez A, **Martinez-Nicolas A**, Madrid JA, Rol MA. (2012). Influencia de la posición, la actividad motora y la temperatura periférica sobre el patrón circadiano de presión arterial. XXI Reunión anual de la Sociedad Española del Sueño (SES). Burgos (Spain). (Spanish).
*Prize for the best poster

28) Bandín Saura MC, **Martinez-Nicolas A**, Madrid JA, Ordovás JM, Garaulet M. (2012). Determinación del cronotipo en mujeres con sobrepeso en función del polimorfismo CLOCK 3111T/C. XIV Congreso de la Sociedad Española de Nutrición (SEN). Zaragoza (Spain). (Spanish).

29) Rubio-Sastre P, Gómez-Abellán P, **Martinez-Nicolas A**, Madrid JA, Garaulet M. (2013). Physical activity performed at evening time alters circadian rhythmcity towards a less healthy pattern. 20th International Congress of Nutrition (ICN). Granada (Spain).

30) Bandín MC; **Martinez-Nicolas A**; Madrid JA; Garaulet M. (2013). Circadian rhythmcity as a predictor of weight loss. 20th International Congress of Nutrition (ICN). Granada (Spain).

31) Argüelles-Prieto R, **Martinez-Nicolas A**, Bonmatí-Carrión MA, Madrid JA, Rol MA. (2013). Circadian healthy lights: Biological assessment. I Congreso Internacional de Jóvenes Investigadores en el Mediterráneo (MED-SOUK). Murcia (Spain).

32) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2013). Association between the impairment of skin temperature rhythm and hypertension. 5º World Congress on Sleep Medicine. Valencia (Spain).

33) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2013). Non dipping blood pressure pattern is related to an increase in daytime distal skin temperature. 5º World Congress on Sleep Medicine. Valencia (Spain).

34) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2013). Association between the impairment of skin temperature rhythm and hypertension. Jornada Sociedad Española de Sueño (SES). Valencia (Spain).

35) Blazquez A, **Martinez-Nicolas A,** Madrid JA, Rol MA. (2014). Sistema circadiano y presión arterial. Efecto del envejecimiento. 6ª Reunión Nacional de la Sociedad Española de Medicina Geriátrica (SEMEG). Pamplona (Spain). Poster presentation (Spanish).

4. ABSTRACTS PUBLISHED IN PEER-REVIEWED JOURNALS

1) Sosa, M, Sosa, J, **Martinez-Nicolas, A**, Ortiz-Tudela, E, Baño-Otalora, B,Madrid, JA, Rol, MA, Campos, M. (2010). Circadianware: Una nueva herramienta informática para el análisis de los ritmos circadianos de temperatura, actividad y posición en humanos. Vigilia y Sueño. 22-2:11. (Spanish).
2) Ortiz-Tudela E, **Martinez-Nicolas A**, Sarabia JA, Almaida P, Campos MT, Campos M, Moreno M, Amillategui R, Madrid JA, Rol de Lama MA. (2010). Evaluación del grado de envejecimiento del sistema circadiano mediante el empleo combinado de actimetría, posición corporal y termometría. Revista Española de Geriatría y Gerontología. 45:137-8. (Spanish).
3) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2011) Temperatura Periférica: Una nueva herramienta en el diagnóstico del patrón circadiano de presión arterial. Vigilia y Sueño. 23-1:18. (Spanish).
4) Ortiz-Tudela E, **Martinez-Nicolas A**, Martinez C, Albares J, Segarra F, Rol MA, Estivill E, Madrid JA. (2011) Análisis de los ritmos de sueño-vigilia mediante el empleo combinado de Termometría, Actividad motora y Posición (TAP). Validación polisomnográfica. Vigilia y Sueño. 23-1:26. (Spanish).
5) Bonmatí-Carrión MA, Rico F, **Martinez-Nicolas A**, Ortiz-Tudela E, Mondéjar MT, Baño-Otalora B, Madrid JA, Rol MA. (2011) Evaluación del funcionamiento del sistema circadiano de ciegos mediante el registro de la temperatura de la piel de la muñeca. Vigilia y Sueño. 23-1:21. (Spanish).
6) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2012). Influencia de la Posición, la Actividad Motora y la Temperatura Periférica sobre el patrón circadiano de Presión Arterial. Vigilia y Sueño. 24-1:47. (Spanish).

7) Bandín MC; **Martinez-Nicolas A**; Madrid JA; Garaulet M. (2013). Circadian rhythmycity as a predictor of weight loss. Annals of Nutrition and Metabolism. 63(S1):368. Impact Factor: 1.661, Q3 Category: Nutrition and Dietetics; Q4 Category: Endocrinology and Metabolism.
8) Rubio-Sastre P, Gómez-Abellán P, **Martinez-Nicolas A**, Madrid JA, Garaulet M. (2013). Physical activity performed at evening time alters circadian rhythmicity towards a less healthy pattern. Annals of Nutrition and Metabolism. 63(S1):790. Impact Factor: 1.661, Q3 Category: Nutrition and Dietetics; Q4 Category: Endocrinology and Metabolism.
9) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2013). Association between the impairment of skin temperature rhythm and hypertension. Sleep Medicine. 14:e191-2. Impact Factor: 3.487, Q1 Category: Clinical Neurology.
10) Blazquez A, **Martinez-Nicolas A**, Rol MA, Madrid JA. (2013). Non dipping blood pressure pattern is related to an increase in daytime distal skin temperature. Sleep Medicine. 14:e191. Impact Factor: 3.487, Q1 Category: Clinical Neurology.
11) Guaita M, **Martinez-Nicolas A**, Madrid JA, Rol MA, Montserrat JM and Santamaria J. (2013). Daytime peripheral temperature changes during MWT and MSLT. Sleep Medicine. 14:e144. Impact Factor: 3.487, Q1 Category: Clinical Neurology.

5. PATENTS

1) Sosa M, Mondejar MT, **Martinez-Nicolas A**, Ortiz-Tudela E, Sarabia JA, Sosa J, Otalora BB, Rol MA, Madrid JA, Campos M, Marín R. (2010). Circadianware. (MU/171/2010), University of Murcia. Murcia (Spain)
2) Sarabia JA, Martinez-Nicolas A, Madrid JA, Rol MA, Ortiz-Tudela E. (2010). Dispositivo que comprende un sensor de posición y actividad corporal, un sensor de temperatura periférica y un sensor de luz para ofrecer información del estado del sistema circadiano. (2398866), University of Murcia. Murcia (Spain).

ANNEX III. STAYS IN LABORATORIES OUT OF THE UNIVERSITY OF MURCIA DURING THIS PHD

1) Chronobiology group, University of Barcelona, Barcelona, Spain.

Responsible researcher: Antoni Diez Noguera
Duration: 2 weeks (01/05/09- 16/05/09).

2) Estivill's Sleep Clinic, Dexeus Institute, Barcelona, Spain.
Responsible researcher: Eduard Estivill Sancho
Duration: 3 weeks (26/04/10- 17/05/10).
3) Thermophysiological chronobiolgy laboratory, Universitäre Psychiatrische Kliniken Basel, Basel, Switzerland.
Responsible researcher: Kurt Kräuchi
Duration: 13 weeks (01/07/12- 30/09/12).

ANNEX IV. RESEARCH PROJECTS SUPPORTING THE EXPERIMENTS PERFORMED IN THE PRESENT PHD

1) Project Title: Chronodisruption and aging: animal models
Funding: Fundación Séneca (05700/PI/07)
Duration: 2007-2009
2) Project Title: Research network on aging and fragility-RETICEF
Funding: Instituto de Salud Carlos III (RD06/0013/0019)
Duration: 2007-2013
3) Project Title: The development of clinical applications for ambulatory thermography and actigraphy.
Funding: Centro Médico Virgen de la Caridad CDTII (DI-2010.001)
Duration: 2010-2011
4) Project Title: Exercise as a modulating factor of aging. Effect on oxidative stress
Funding: Fundación Séneca (12005/PI/09)
Duration: 2010-2013
5) Project Title: Electroestimulador Muscular Submental para el Tratamiento del Síndrome de Apnea Obstructiva del Sueño (SAOS)
Funding: Chronobiotech SL
Duration: 2010-2012
6) Project Title: Preventing chronodisruption by circadian-healthy lighting. Relevance in aging and cancer
Funding: CICYT (BFU2010-21945-C02-01)
Duration: 2011-2013

7) Project Title: Contrato de Licencia de Explotación de Patente Nº201031894
   Funding: Chronobiotech SL
   Duration: 2011-2012
8) Project Title: Contrato de Licencia de Explotación de Patente Nº201130509
   Funding: Chronobiotech SL
   Duration: 2011-2012
9) Project Title: Circadian system functionality, work environment and the organization of nursing care in hospitals of the Spanish National Health System.
   Funding: Instituto de Salud Carlos III
   Duration: 2012-2014
10) Project Title: Servicio de estudios e informes del sueño para el análisis de los datos recogidos en el proyecto "Circadian system functionality, work environment and the organization of nursing care in hospitals of the Spanish National Health System".
   Funding: Instituto de Salud Carlos III
   Duration: 2012-2014
11) Project Title: Asesoría Circadiana.
   Funding: Confidential
   Duration: 2012-2015
12) Project Title: Research network on aging and fragility-RETICEF
   Funding: Instituto de Salud Carlos III (RD12/0043/0011)
   Duration: 2013-2014

ANNEX V.

During his PhD and the writing of the Doctoral Thesis, Martinez-Nicolas A has been granted with a University of Murcia Scholarship to Lecturers training, from January 2009 to December 2012 and a Researcher's contract by Aging and Fragility Network and Cooperative Research Centers – RETICEF. Instituto de Salud Carlos III, from April 2013 to December 2013.

8. Resumen en Español

OBJETIVOS

El objetivo general de la presente tesis fue:

Establecer el patrón de temperatura de la piel de la muñeca como ritmo marcador para evaluar de forma fiable y confortable la robustez del sistema circadiano humano. Para ello se plantearon los siguientes objetivos específicos:

1. Obtener el ritmo endógeno de temperatura periférica distal por medio de procedimientos matemáticos para eliminar simultáneamente el efecto enmascarante de la luz y la temperatura ambiental, la actividad, la posición y el sueño y determinar su efecto sobre el ritmo de temperatura en la muñeca.
2. Describir el patrón de temperatura periférica distal considerando su ontogenia y el proceso de envejecimiento e identificar aquellos parámetros rítmicos más fiables para diferenciar a los sujetos de acuerdo con su edad.
3. Caracterizar el régimen de exposición a luz natural y su influencia sobre el sistema circadiano evaluado mediante el registro del ritmo de temperatura en la muñeca.
4. Analizar el efecto de la estacionalidad en la temperatura ambiental sobre las variables termofisiológicas y cardiofisiológicas en mujeres jóvenes.
5. Evaluar el estilo de vida, la exposición a sincronizadores, sus efectos en el sistema circadiano de sujetos sanos tanto ancianos como jóvenes y el posible grado de cronodisrupción en ambos grupos de edad.
6. Estudiar la influencia del contraste día/noche en la exposición a sincronizadores y en los hábitos de vida con efecto sincronizador sobre el sistema circadiano y proponer un método para evaluar la edad del sistema circadiano a partir de las características del patrón circadiano y no en función de la edad biológica.
7. Idear una luminaria saludable para el sistema circadiano que mejore la exposición a la luz durante el día y minimice los efectos nocivos de la luz durante la noche.

CAPÍTULO EXPERIMENTAL 1

DESCUBRIMIENTO DE DIFERENTES FACTORES ENMASCARANTES SOBRE EL RITMO DE TEMPERATURA DE LA PIEL DE LA MUÑECA EN SUJETOS EN CONDICIONES DE VIDA LIBRE.

La mayoría de los ritmos circadianos están controlados por el marcapasos principal localizado en el núcleo supraquiasmático del hipotálamo. Algunos de estos ritmos, denominados ritmos marcadores, sirven para caracterizar la fase del orden temporal interno. Sin embargo, estas variables son susceptibles de enmascaramiento por la actividad, la posición corporal, la exposición a la luz, la temperatura ambiental y el sueño. Recientemente, se ha propuesto el ritmo de temperatura periférica en la muñeca como un nuevo índice para evaluar el estado del sistema circadiano. A la luz de las evidencias previas que sugieren una relación estrecha entre el ritmo temperatura periférica en la muñeca y la regulación del ritmo de temperatura corporal central, el objetivo de este trabajo fue purificar el patrón de temperatura periférica de la muñeca con el fin de obtener su ritmo endógeno mediante la aplicación de técnicas de desenmascaramiento múltiple. Con este fin se reclutaron 103 sujetos (18-24 años) en los que se registró durante una semana sus ritmos de temperatura periférica de la muñeca, actividad, posición corporal, exposición a la luz, temperatura ambiental y sueño en condiciones ambulatorias. Se aplicó el desenmascaramiento por categorías o intercerptos del ritmo de temperatura periférica de la muñeca para simular un protocolo de rutina constante (despierto, luz tenue, tumbado, baja actividad y temperatura ambiental templada). Aunque el patrón circadiano general de temperatura periférica de la muñeca fue similar independientemente de los efectos enmascarantes, la amplitud fue el parámetro que resultó más afectado por las condiciones ambientales. Acrofase y mesor fueron los parámetros más robustos para caracterizar este ritmo. Además, el efecto enmascarante de cada variable individual presentaba modulación circadiana. El rimo de temperatura periférica en la muñeca presenta un fuerte componente endógeno a pesar de la existencia de múltiples influencias externas, lo que se pudo evidenciar al eliminar simultáneamente el efecto de actividad, posición corporal, exposición a la luz, temperatura ambiental y sueño. Por tanto, proponemos que se podría considerar este ritmo como un instrumento valioso y mínimamente invasivo para la monitorización de la fisiología circadiana en condiciones ambulatorias.

CAPÍTULO EXPERIMENTAL 2

EL RITMO DE TEMPERATURA PERIFÉRICA DISTAL COMO UN ÍNDICE PARA EVALUAR LA ONTOGENIA Y EL ENVEJECIMIENTO DEL SISTEMA CIRCADIANO HUMANO.

En términos circadianos, la ontogenia humana se caracteriza por la emergencia del patrón diario, a partir del patrón ultradiano previo, en los seis primeros meses de vida para la mayoría de las variables. El envejecimiento circadiano en humanos se acompaña de un avance de fase, y fragmentación y aplanamiento del ritmo. A pesar de la creciente bibliografía centrada en la temperatura periférica distal, existe muy poca información disponible acerca de su ontogenia y prácticamente nada sobre los cambios que experimenta esta variable con el avance de la edad. Por tanto, el propósito de este trabajo fue evaluar el grado de maduración y envejecimiento del sistema circadiano humano, utilizando el patrón de temperatura periférica distal para identificar aquellos parámetros que se modifican a lo largo de la vida, permitiendo diferenciar a los sujetos de acuerdo a su edad. Para ello se monitorizó el ritmo de temperatura periférica distal en 197 voluntarios (55% mujeres), incluyendo bebes de 15 días (30 sujetos), un mes (28 sujetos), 3 meses (31 sujetos) y 6 meses (10 sujetos) y adultos jóvenes de 19 años (37 sujetos), de mediana edad con 46 años (27 sujetos) y ancianos de 72 años (34 sujetos). La maduración del sistema circadiano se asoció con un incremento en la amplitud del ritmo circadiano y una reducción de la temperatura periférica distal durante el sueño. En la etapa adulta, las mujeres mostraron un patrón más robusto (menor fragmentación, mayor temperatura periférica distal nocturna, amplitud, índice de funcionamiento circadiano y potencia del primer armónico); sin embargo, estas diferencias se pierden con la edad, un periodo de la vida que está consecuentemente asociado consistentemente a un avance de fase del ritmo. En resumen, el patrón de temperatura periférica distal puede utilizarse como una variable robusta para evaluar el grado de maduración y envejecimiento del sistema circadiano humano.

CAPÍTULO EXPERIMENTAL 3

INTERCOMUNICACIÓN ENTRE LA LUZ AMBIENTAL Y EL TIEMPO INTERNO EN HUMANOS.

La exposición diaria a la luz ambiental es el *zeitgeber* más importante en humanos y todas las características del patrón de luz (horario, intensidad, tasa de cambio, duración y espectro) afectan al sistema circadiano. Sin embargo, y debido a la falta de estudios actuales sobre la exposición a la luz ambiental, el propósito de este trabajo fue determinar las características del régimen natural de exposición a la luz y su relación con el funcionamiento del sistema circadiano humano. Para ello, se reclutaron 88 estudiantes universitarios (18-23 años) en Murcia, España (latitud 38º 01' N) en los que se registró la temperatura de la muñeca, la exposición a la luz y el sueño durante una semana en condiciones ambulatorias y mientras mantenían su estilo de vida habitual. Posteriormente, se calculó el horario de exposición a la luz, tasa de cambio, regularidad, intensidad y se analizaron sus efectos sobre el patrón de sueño y el de temperatura de la muñeca. En general, valores más altos de estabilidad interdiaria, amplitud relativa, exposición media a la luz durante la mañana e índice de calidad de la luz (ICL) se correlacionaron con una mayor estabilidad interdiaria y amplitud relativa junto a un avance de fase en sueño, además de mayor estabilidad interdiaria y un avance de fase en el ritmo de temperatura de la muñeca. Por otro lado, una mayor fragmentación del ritmo de exposición a la luz se asociaba con un sueño más fragmentado. Los estudios naturalísticos utilizando monitorización ambulatoria de la luz durante 24 horas proporcionan información esencial sobre la principal entrada al sistema circadiano, necesaria para mantener una sincronización circadiana saludable. Corregir en consecuencia, los patrones de exposición a la luz puede ayudar a prevenir o incuso revertir problemas de salud asociados a la disrupción circadiana.

CAPÍTULO EXPERIMENTAL 4

EFECTOS DE LA VARIACIÓN DIARIA EN LA TEMPERATURA AMBIENTAL SOBRE LA TEMPERATURA DE LA PIEL Y LA PRESIÓN ARTERIAL.

Está ampliamente aceptado que la exposición al frío aumenta la presión arterial y, por tanto, el riesgo cardiovascular. El mecanismo subyacente parece deberse a los efectos adversos de los ajustes termorreguladores ya que el enfriamiento de la piel provoca vasoconstricción cutánea, centralización de la sangre y, a continuación, un incremento de la presión y viscosidad sanguínea. Sin embargo, existe una carencia de estudios longitudinales y de medidas de la temperatura ambiental a la que se ve expuesta la persona. Por tanto, el propósito de este trabajo fue analizar, en invierno y verano, la asociación temporal de la temperatura ambiental, la temperatura de la piel, la presión arterial y la frecuencia cardiaca en mujeres sanas durante el día en condiciones ambulatorias. Para ello se reclutaron 60 mujeres jóvenes en un diseño cruzado invierno/verano y se registraron ambulatoriamente durante 26 horas las variables termo- y cardiofisiológicas junto a la temperatura ambiental a nivel de la persona. Se analizó el periodo diurno (09:30-20:30) con el fin de que todos los sujetos estuvieran despiertos y favorecer una mayor variabilidad en la temperatura ambiental. Además, se seleccionó cada exposición a exteriores en la que hubiera al menos una medida de presión arterial, junto a los periodos previo y posterior con medidas de presión arterial para analizar los efectos a corto plazo de la temperatura ambiental sobre las variables termo- y cardiofisiológicas. Las variaciones a corto plazo de la temperatura ambiental que experimenta la persona modificaron la presión arterial mediante cambios en la temperatura de la piel distal y no de la proximal. Estos cambios son independientes de la temperatura media de la persona y de su aclimatación. Sin embargo, los cambios estacionales en la temperatura ambiental modificaron la temperatura de la piel sin alterar la presión arterial. En conclusión, la temperatura ambiental modifica la temperatura de la piel distal y ésta, a su vez, la presión arterial. Sin embargo, la aclimatación en mujeres jóvenes contrarresta los cambios estacionales esperados.

CAPÍTULO EXPERIMENTAL 5

ENVEJECIMIENTO DEL SISTEMA CIRCADIANO. CRONODISRUPCIÓN Y CRONOPOTENCIACIÓN.

El envejecimiento del sistema circadiano se caracteriza por aplanamiento de los ritmos, fragmentación y avance de fase. El amarilleamiento del cristalino con la edad deteriora la entrada de luz (principal *zeitgeber*) y, por tanto, contribuye a generar cronodisrupción en ancianos al dificultar el encarrilamiento. Como la temperatura periférica de la muñeca se ha propuesto recientemente como un índice para evaluar el sistema circadiano apenas se conoce como se modifica su patrón con el envejecimiento. Por ello, el propósito de este trabajo es describir este ritmo en ancianos sanos junto con sus hábitos de vida comparando con jóvenes. También se evaluó la posible existencia de cronodisrupción y las posibilidades de cronopotenciación del sistema circadiano. Para ello, se reclutaron 90 sujetos sanos, 46 ancianos (65-75 años) y 44 jóvenes (19-25 años) y se monitorizó su temperatura periférica, actividad, posición, exposición a luz y temperatura ambiental durante 5 días (entre semana) en condiciones ambulatorias. Para la luz se realizó un histograma acumulado, el índice de calidad de la luz y de la oscuridad (LQI y DQI, respectivamente). La cronodisrupción se evaluó mediante la tasa de patrones anómalos de temperatura periférica y las diferencias de fase entre luz, temperatura periférica, y actividad. Con los principales índices de cada variable se construyeron árboles de decisión para diferenciar ancianos y jóvenes. Los ancianos se expusieron a menores intensidades luminosas disminuyendo su LQI, pero sin diferencias en DQI. La tasa de patrones anómalos de temperatura periférica y la desincroninzación entre los diferentes ritmos fue mayor en ancianos. Los árboles de decisión consiguieron una elevada tasa de acierto (mínimo de 80%) excepto para la luz, si bien, al combinar los árboles de decisión se obtuvo una tasa de acierto del 96,5% por lo que las diferencias en las características de los ritmos monitorizados permiten diferenciar sujetos jóvenes y ancianos sin considerar su edad biológica. Además, valores elevados de LQI y DQI en ancianos se relacionaron con mejores patrones de temperatura periférica y una recuperación parcial de la zona de mantenimiento de la vigilia. En resumen, el envejecimiento se relacionó con un avance de fase, menor exposición a la luz y mayor grado de cronodisrupción, aunque los ancianos con un mejor patrón de exposición a la luz mostraron un sistema circadiano más joven, resaltando la importancia de una adecuada exposición a este *zeitgeber*.

CAPÍTULO EXPERIMENTAL 6

CONTRASTE DÍA-NOCHE COMO FUENTE DE SALUD PARA EL SISTEMA CIRCADIANO HUMANO.

Las sociedades modernas se caracterizan por un estilo de vida 24/7 sin diferencias ambientales entre el día y la noche, lo que resulta en una débil exposición a *zeitgebers* (luz poco intensa durante el día, ausencia de oscuridad por la noche, temperatura ambiental constante, un estilo de vida sedentario y picoteo frecuente), y como consecuencia, en un sistema circadiano alterado, proceso conocido como cronodisrupción. Tanto la debilidad en la exposición a *zeitgebers* como un sistema circadiano dañado se relacionan con determinadas patologías (ciertos tipos de cáncer, síndrome metabólico y desordenes afectivos y enfermedades cognitivas), pero la cronopotenciación del sistema circadiano apenas se ha explorado. El objetivo de este trabajo fue proponer estrategias prácticas de cronopotenciación basadas en aumentar el contraste día/noche. Para ello, se reclutaron 131 sujetos jóvenes para monitorizar su temperatura periférica de la muñeca, actividad, posición corporal, exposición a la luz, temperatura ambiental y sueño durante una semana en condiciones ambulatorias. Se seleccionaron los sujetos con alto y bajo contraste en actividad, posición corporal, temperatura ambiental, exposición a la luz y sueño para analizar su efecto sobre la temperatura periférica de la muñeca. Los sujetos con alto contraste mostraron mejores ritmos que los que tenían bajo contraste en todas las variables excepto el sueño. Los sujetos con alto y bajo contraste en temperatura periférica de la muñeca también presentaron diferencias en su estilo de vida, así sujetos con alto contraste presentaban un ligero adelanto en el inicio de la fase nocturna y un aumento de la amplitud en todas las variables analizadas. Además, un alto contraste teórico calculado mediante modelos matemáticos sugiere una mejoría en el ritmo de temperatura periférica de la muñeca al aumentar el contraste en el estilo de vida. Finalmente, algunos individuos mostraron un ritmo de temperatura periférica de la muñeca con elevado contraste a pesar de su bajo contraste en cuanto al estilo de vida, mientras que otros sujetos resultaron arrítmicos para el patrón de temperatura de la muñeca a pesar de sus buenos hábitos, lo que pone de manifiesto la existencia de dos componentes diferentes en la temperatura periférica: uno exógeno modificado por el estilo de vida y otro endógeno refractario a dicha modificación. Por tanto, un aumento del contraste día/noche en el estilo de vida de un sujeto es una medida factible para cronopotenciar el sistema circadiano.

CAPÍTULO EXPERIMENTAL 7

DISPOSITIVO DE ILUMINACIÓN CIRCADIANO.

Las enfermedades provocadas o empeoradas por la cronodisrupción pueden ser revertidas o paliadas mediante iluminación de espectro solar o enriquecida en azul durante el día y eliminando la banda entre 450 y 480 nm durante la noche. De entre las enfermedades comentadas anteriormente destacan el mayor riesgo de cáncer de próstata y de mama relacionados con contaminación lumínica, trabajo nocturno o en turnos rotatorios y jet-lag frecuente debido a la inhibición de la secreción de melatonina por la luz emitida en la banda 450-480 nm.

A la luz de estos hechos, esta invención presenta un dispositivo de iluminación que funciona en dos modos distintos emitiendo luz de espectro total o suprimiedo una banda azul (450-480 nm). Para ello, el dispositivo está compuesto por una serie de ajustes manuales (interruptor y/o potenciómetro), automáticos (espectrorradiómetro o luxómetro) y o programables (temporizador) para eliminar la banda del espectro y/o modificar la intensidad. Estos ajustes intercambiarán entre ambos modos.

El dispositivo puede constar de un filtro móvil para eliminar la banda deseada para el modo nocturno o permitir su paso durante el diurno. Además puede utilizarse más de un filtro en caso de necesidad, los cuales se colocaran delante del dispositivo o no en función del modo activo. En otro caso, la luminaria puede estar compuesta por múltiples fuentes monocromáticas, cuya activación depende del modo activo. Específicamente se activará rojo, verde y azul durante el día y rojo, verde y violeta (evitando la banda 450-480 nm) durante la noche. El dispositivo puede estar compuesto por otras fuentes de iluminación monocromáticas o policromáticas agrupadas en pequeños focos y estos a su vez en un único dispositivo.

Cómo se ha mencionado, el dispositivo maximiza el contraste día-noche. Además, permite la secreción de melatonina minimizando los efectos nocivos de la luz por la noche y potenciando los efectos saludables de la luz durante el día mediante luz enriquecida en azules, que mejora síntomas de demencias y sana desordenes afectivos. La utilización de este dispositivo permitirá iluminar interiores con luz de espectro solar estimulando el encarrilamiento del sistema circadiano mientras que durante la noche evitará los daños sobre la salud humana preservando la secreción de melatonina.

CONCLUSIONES

Las principales conclusiones de la presente tesis fueron:

1. El método de regresión múltiple permite deducir simultáneamente la influencia del enmascaramiento por la exposición a la luz, la temperatura ambiental, el sueño, la actividad y la posición corporal sobre el ritmo de temperatura periférica distal. Este ritmo presenta un fuerte componente endógeno a pesar de la influencia de las variables anteriormente mencionadas, que afectan a la temperatura periférica distal de un modo diferente en función de la fase. Esta técnica de purificación puede también aplicarse a otros ritmos marcadores.
2. La maduración circadiana del patrón de temperatura periférica de la muñeca se asocia con una disminución de la potencia de los armónicos ultradianos, un aumento en amplitud y una reducción de la temperatura de la piel durante el sueño, mientras que el envejecimiento se relaciona con un adelanto de fase general. Así la temperatura periférica de la muñeca permite evaluar la maduración y el envejecimiento del sistema circadiano en humanos.
3. Cambios agudos en la exposición a la luz se asocian con cambios transitorios en temperatura periférica en la dirección opuesta, probablemente por activación simpática. Además, un mayor contraste entre el día y la noche en la exposición a la luz y un mayor valor en el índice de calidad de la luz se relaciona con mejores patrones de temperatura de la muñeca y de sueño, mientras que la exposición a luz nocturna se asocia a los peores patrones, lo que subraya la importancia de unos hábitos adecuados.
4. Las variaciones en la temperatura ambiental afectan la presión arterial media a través de cambios en la temperatura periférica distal.
5. El sistema circadiano del anciano se caracteriza por un menor contraste en las variables sincronizadoras, un avance de fase generalizado y una mayor cronodisrupción; estas características permiten diferenciar entre patrones circadianos jóvenes y ancianos.
6. Las alteraciones en el ritmo de temperatura de la muñeca se asocian con un bajo contraste entre el día y la noche en las variables físicas (luz y temperatura) y las variables comportamentales con capacidad sincronizadora (actividad, posición corporal y sueño), aunque algunos sujetos muestran dos componentes para este ritmo, uno exógeno y susceptible a las influencias externas y otro endógeno, más

refractario a ellas. El modelado matemático también apunta a que el aumento del contraste en el estilo de vida podría mejorar el ritmo de temperatura en la muñeca

7. Se diseño y patentó un dispositivo de iluminación circadiano con luz de espectro total durante el día y que evita la emisión en la banda de 450 a 480 nm durante la noche permitiendo la secreción nocturna de melatonina.

CONCLUSIÓN GENERAL

La temperatura periférica de la piel de la muñeca ha demostrado ser un ritmo marcador fiable y cómodo con un fuerte componente endógeno que permite valuar la robustez del sistema circadiano y el envejecimiento en sujetos normales en condiciones ambulatorias. La evaluación del estilo de vida mediante registros multivariable constituye una herramienta útil para seleccionar aquellos sujetos susceptibles de beneficiarse de las terapias de cronopotenciación y reducir las alteraciones circadianas asociadas a la vida moderna.

Porque todavía no nos conocemos

nos conocemos de vista

estamos locos el uno por el otro

no nos vemos desde hace tiempo

estamos de algún modo emparentados

nunca llegaremos a conocernos, pero a pesar de ello, espero, pensaremos siempre con cariño el uno en el otro…

Esto es para ti

Con lo que tú ya sabes y por lo que probablemente ya sabes.

Neil Gaiman

Because we haven't yet met

have only a glancing acquaintance

are just crazy about each other

haven't seen each other in much too long

are in some way related

will never meet, but will, I trust, despite that, always think fondly of each other…

This is for you

With you know what and you probably know why.

Neil Gaiman