Approaches to Aging Control

Journal of Spanish Society of Anti-Aging Medicine and Longevity

Nº 22
October 2018

SEMAL

www.semal.org

Journal of Spanish Society of Anti-Aging Medicine and Longevity and Latin-American Federation of Anti-Aging Medicine Societies

Information and Subscription
Secretaría Técnica SEMAL
Colegio Oficial de Médicos de Sevilla
Avda. de la Borbolla, 47 - 41013 Sevilla
Tel.: 954 08 47 00
Web: www.semal.org E-mail: info@semal.org

Publisher
Evento XXI
Depósito Legal: SE-3645-05
ISSN: 1885-4028

Ageing of the circadian system. From monitoring to chronoenhancement.

Martinez-Nicolas A[1,2], Almaida-Pagán PF[1,2], Martinez-Madrid MJ[1,2], Argüelles R[1,2], Ortega-Sabater C[1,2], Fernández-Ortiz M[2,3], de Costa J[1,2], Madrid JA[1,2], Rol MA[1,2]

[1]*Chronobiology Lab, Department of Physiology, College of Biology, University of Murcia, Mare Nostrum Campus. IUIE, IMIB-Arrixaca, Spain.*

[2]*Ciber Fragilidad y Enwejecimiento Saludable (CIBERFES), Madrid, Spain.*

[3]*Department of Physiology, College of Medicine, University of Granada, Granada, Spain*

Corresponding author: Rol MA, angerol@um.es.

Keywords: Circadian rhythm, ageing, circadian ambulatory monitoring, chronoenhancement.

ABSTRACT

The circadian system (CS) organizes the temporal order of all living beings. Its general structure is very similar among species and consists of receptors of the temporal information (inputs), a central pacemaker along with several peripheral clocks that depend on it (machinery), and a set of overt rhythms driven by the central clock (outputs). The CS ages like any other structure of the organism, this process being characterized by a poorer reception of the temporal information, a general impairment of the central pacemaker and a phase advance, fragmentation and dampening of the overt rhythms. In order to assess the functioning of the CS, some overt rhythms have been selected as markers since they mirror the activity of the central pacemaker. Some of the most used marker rhythms are those of melatonin and cortisol secretion, rest-activity and sleep-wake patterns, and core body and distal skin temperature. Nevertheless, these rhythms can be masked by external variables and thus, to simultaneously record several marker rhythms is recommended. As the CS ages, the ability of an organism to adjust the internal temporal order of physiological, biochemical and behavioural circadian rhythms to the environmental cycles is compromised and chronodisruption can appear, which is related with several diseases. Fortunately, there are some strategies that one person can follow in order to enhance the functioning of the CS: to increase the contrast between day and night (i.e. to exposure to bright days and dark nights), to have melatonin (if needed), to do regular exercise, to improve sleep and meal schedules or to increase social contacts.

INTRODUCTION

From the origins of life, Earth has been rotating around its own axis and revolving all round the Sun. Thus, first biological clocks developed as an adaptative benefit for all living creatures since they were capable to anticipate and prevent a cyclic environmental change. The biological clock is integrated in the circadian system (CS) and, although it displays an endogenous activity, it needs to be winded up everyday to entrain to 24-h environmental changes (the term circadian comes from the Latin *circa*, that means "approximately", and *diem*, meaning "day"). The CS,

as the remaining systems of the organism, ages being affected each and every of its components [1].

1. The circadian system

The CS is responsible for organising the internal temporal order of every process in an organism, in accordance with the environment, producing the circadian rhythms. The CS structure consists of: 1) inputs, i.e. receptors gathering information from the main zeitgebers (from the German "time giver"), such as light, environmental temperature or food availability [2-4]; 2) a central pacemaker, which receives, integrates and transmits the information to the CS outputs [5-7]; 3) outputs, which transmit the temporal signal from the central pacemaker to every cell of the organism [8]. In addition to the central pacemaker, there are many oscillators operating in the brain and in peripheral organs, such as kidney, liver, intestine or adipose tissue [9].

1.1. *Inputs*

Although the light-dark cycle is the most important environmental cue and the main input of the CS for most of the organisms, there are non-photic synchronisers that also send information to the central pacemaker in order to entrain the CS [10]. Circadian photoreception occurs through a subgroup of intrinsically photosensitive ganglion cells in the retina (ipRGCs) and more specifically, through the melanopsin, a photopigment that belongs to the opsin family of light-sensitive retinal proteins [11]. Melanopsin shows a maximum sensitivity in vivo to wavelengths from 440 to 480 nanometres [12]. These ipRGCs receive also light information from rods and cones [13], integrate it together and transmit it to the central pacemaker [14,15]. Environmental cyclic temperature is also an important zeitgeber to the CS, which is capable to entrain cellular cultures in vitro, core body temperature of ectotherm organisms and mice in vivo [8]. Besides, some overt rhythms also present a synchroniser effect, such as feeding time, scheduled sleep and activity [16].

1.2. *Central pacemaker*

The mammalian master clock is located in the suprachiasmatic nucleus of the hypothalamus (SCN), which receives the light information from the retina via the retinohypothalamic tract. The SCN is organized in two differentiated regions: 1) the ventrolateral region, which receives the light information and 2) the dorsomedial region or the pacemaker itself [6]. Every neuron in the SCN is an independent oscillator, which is ensembled and synchronized to all other neurons to produce a common periodicity [17]. These neurons show endogenous circadian rhythms in mRNA expression and synthesis of key clock components that are working even in absence of rhythmic inputs [18]. The transcription factors BMAL1 and CLOCK (NPAS in the SCN) constitute a heterodimer, which favours the expression of the transcription factors PER and CRY, as well as many other clock-controlled genes (CCG) [19]. PER and CRY dimerize and inhibit their own expression, and also repress CLOCK:BMAL1 [20]. As this molecular clock has been demonstrated to be ubiquitous in every studied cell-type [21], a question emerged regarding the existing molecular differences between the master and the peripheral clocks. In this sense, it is proposed that the SCN, which autonomously generates the circadian oscillations, is the one that synchronizes peripheral clocks [9].

1.3. **Outputs**

The SCN drives the temporal organization of the organism by means of neural or humoral mediators. The SCN connects with several brain areas, regulates the release of a variety of hormones, such as corticoids, gonadotropin or melatonin, and controls overt rhythms like the sleep-wake cycle and feeding [8]. In order to study the human circadian clock

functioning, marker rhythms are used; plasma melatonin has been considered as the gold standard since its synthesis and release is directly dependent of the SCN [22]. Melatonin concentration in plasma shows a peak at night and a minimum during daytime since it is produced during subjective night in absence of light [8]. Cortisol secretion is also considered as a marker rhythm that shows a stable phase with melatonin secretion and a peak linked to the usual awakening. Core body temperature (CBT) is another marker rhythm with high values during daytime and low values at night, while its nadir coincides with the peak of melatonin [23]. However, distal skin temperature (DST) is beginning to be considered as a marker rhythm since it is more easily measured, presents a stable phase relationship with melatonin secretion and core body temperature [24,25] and its pattern is maintained under constant routine protocols [26] and after demasking procedures [27].

2. Ageing of the CS

Ageing process affects all the physiological functions in a way that is in part, genetically determined. The CS, as the remaining organs and systems, is affected by the ageing at all levels [8].

2. 1. *Inputs*

Ageing causes pupillary myosis and crystalline lens yellowing, which impairs light (especially blue light) perception [28]. Thus, aged people should be longer exposed to bright light (intensities higher than 1000 lux) to counteract this age-related impairment. In addition, aged people show an alteration in thermoreception and a decrease in their ability to perceive warm stimuli [29]. This could result also in altered exposure to environmental temperature cycles.

2. 2. *Central pacemaker*

The ageing of the SCN is characterized by a reduction in the number of neurons, a lower functionality (measured as electrical activity), an alteration and/or reduction of synapses, an attenuation of the firing rate pattern that results in reduced day-night contrast, and an uncoupling among neurons [8]. In addition to these alterations, biochemical and morphological alterations of the SCN occur, and the molecular clock rhythmicity experiences a general dampening [8].

2. 3. *Outputs*

As the outputs are the last step in the chain of events taking place in the CS functioning, it is worthy to note that the age-related changes described for the outputs could be due to an indirect effect of the inputs and/or central pacemaker ageing. The main changes observed in the overt rhythms include phase advance, fragmentation increase and an amplitude decrease [30], including melatonin secretion [31], CBT and sleep-wake cycle [30,32,33]. Activity pattern also reduces its amplitude [32,34] while DST advances its phase [96]. In contrast to the general behaviour, a group of genes (known as late life cyclers) increase their rhythmicity with ageing [8].

3. Ambulatory circadian monitoring (ACM)

Due to its location, the direct study of the SCN results impossible and therefore marker rhythms are used, as already commented. Marker rhythms must be driven by the SCN, be easy to measure and show large amplitude and a specific phase relationship with the SCN [8]. In spite of the high reliability of the most used marker rhythms, there are external variables that can mask their pattern [27]. To diminish the inherent variability of the different lifestyles, it is recommendable to record them for several complete cycles (days).

3. 1. *Environmental light and temperature*

As the main synchronizer of the CS, environmental light exposure gives information about how the clock

is entrained every day. Environmental light monitoring systems are programmable data logger provided with photosensitive cells placed in the subject wrist, glasses frame or a necklace (depending on the model) [35]. Some sensors register light on the visible spectrum while others only record specific wavelenghts (lights of a specific colour). Especially interesting are those that register blue light exposure as the CS is particularly sensitive to it (see for example those in Figure 1A and B) [28]. To measure environmental temperature exposure, a thermometer in a sealed chamber placed on a necklace, a belt or a jacket lapel is commonly used [36].

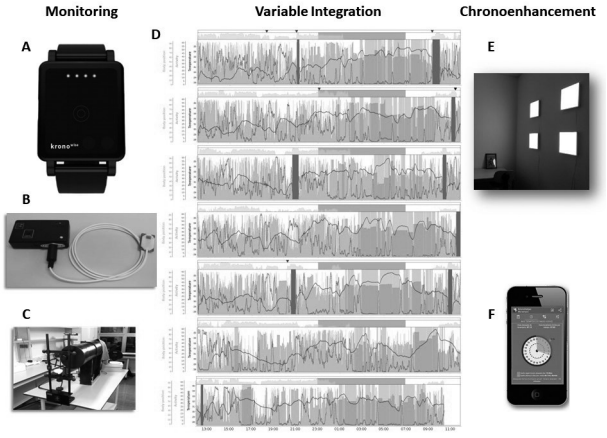

Figure 1. Ambulatory Circadian Monitoring. Circadian monitoring devices are shown in the left part including Kronowise® for ambulatory monitoring (A), Kronobed for inpatients (B) and Pupilabware© to assess pupil light reflex (C). Circadian processing by Circadianware© and/or Kronowizard (https://kronowizard.um.es) is shown in the middle part with an example of sleep estimation (D). Chronoenhancement devices are shown in the right part including Kronolight for circadian lighting (E) and Kronohelper app© for sleep hygiene and behavioural intervention (F).

3. 2. *Pupil-light reflex*

A typical pupillary light response consists of two components: When light is turned ON, there is a transient phase characterized by a short-latency, high-velocity maximal change in pupil size. Thereafter, the pupil partly redilates to a state of partial constriction that represents the sustained phase of the pupil light reflex. When a light stimulus ends, the pupil starts to recover its original size after a period (which does not always occur) in which some degree of contraction persists after light stimulus [37]. It is known that ipRGCs are necessary to reach the maximal pupil constriction and to sustain constriction under prolonged light stimulus in pupil light reflex (see Figure 1C) [38]. Since ipRGCs also transmit light-dark cycle information to the central pacemaker, pupil light reflex is considered a good index to indirectly assess the state of the CS. Measurement of pupil reflex is faster and more comfortable for patients than other assays, i.e. melatonin inhibition [39].

3. 3. *Core Body and Skin Temperature*

CBT is the consequence of the heat produced, stored and lost by the body and thus, reflects the heat gain/loss balance. To measure CBT, rectal probes or temperature pills are used. However, this rhythm requires several days of recording, making the rectal probes uncomfortables while temperature pills are not useful due to their short stay in the organism [40]. In this sense, DST rhythm arises as an alternative to CBT. Some of its strengths are robustness, stability across many different situations, and its stable phase relationship with the melatonin secretion pattern. DST rhythm shows an inversed pattern and a 100-minute phase-advance when compared to CBT rhythm [24,41]. The increase of DST promotes sleep onset as it activates specific hypothalamic areas involved in thermoregulation. In fact, some studies suggest that heat loss from the extremities may drive circadian

CBT rhythm [40]. Finally, DST rhythm can be easily and comfortably recorded by using iButtons and watch-like devices (see Figure 1A and B) [1,24].

3. 4. *Melatonin*

The pineal gland releases melatonin at night and in absence of light, what makes it the "chemical darkness" for the organism [42]. Its pattern is influenced mainly by light, but also by activity level, caffeine and some drugs (antiinflamatories and beta-blockers) [43]. Melatonin levels can be measured in blood, saliva and urine (in this case, 6-sulfatoxymelatonin is assessed) during night-time. Nevertheless, dim-light melatonin onset (DLMO) method is considered as the best to determine phase. It assesses the onset of melatonin secretion under dim light conditions based on the rapid increase of melatonin levels at dusk [25]. Sampling starts in the early evening until one hour after usual sleep time [25]. Once samples have been collected, melatonin concentration can be quantified, mainly by RIA or ELISA, although other methods can also be used [44].

3. 5. *Cortisol*

Cortisol is secreted by the adrenal gland and presents a marked and robust pattern. Cortisol secretion peaks at the beginning of the activity phase to prepare awakening by rising blood pressure, cardiac output and glucose concentration in blood. Cortisol pattern could be affected by light, hyperprotein intake, stress, and sleep-wake cycle among others [45]. The cortisol levels can be measured from blood or saliva. Cortisol assessment begins at awakening and continues until 9-15 hours later with different sampling frequency, which allows to quantify the usual decrease of this hormone secretion until dusk or early night. Measurement methods are similar (applying the appropriate modifications) to those used for melatonin.

3. 6. *Activity*

The motor activity pattern acts both as a synchronizer for the CS [16] and as an output [46]. The rest-activity pattern is the most usual ambulatory long recording method to assess sleep-wake cycle, due to the obvious similarities between both patterns. Actigraphy requires an accelerometer or actimeter placed on the wrist of the non-dominant hand, arm or on the hip (see Figure 1A and B). In fact, some smartphone apps convert the smartphone into an actimeter using their own accelerometer, although its reliability could be argued. Most actimeters register counts, some add information about actimeter movement between counts, a few report time in movement between measurements and only very few give information about the current position of the actimeter. However, actigraphy has a major concern with commercial devices since they usually preprocess information making raw data access impossible. In addition, activity pattern is subjected to the influence of daily situations that alter the record such as car's vibration, bed partner movements or the voluntary withdrawal of the sensor. However, despite these disadvantages, its low cost and comfort have made it the method of choice to assess sleep and circadian disorders [47].

3. 7. *Sleep detection and variable integration*

One of the most apparent circadian rhythms is the sleep-wake rhythm, which almost parallels the rest-activity rhythm [48]. The gold standard technique for sleep studies is polysomnography, which includes electroencephalography, electrooculography, electrocardiography, electromyography and respiratory variables monitoring (with a finger pulse oximeter, cannula, thermistor, abdominal and thoracic strain gauges), that must be performed in synchronized way with an audiovisual recording [40]. This technique allows the description of the sleep's architecture with three or four 90-120 minutes cycles. Each cycle is divided into rapid eye movement sleep (REM) and non-REM sleep (subdivided into light and slow wave sleep) [49,50]. Nevertheless, the high cost of

the equipment, the need for trained specialists and the limitations patients are subjected to during the measurement, make alternative procedures a necessity. As a partial solution to these methodological issues, the use of other ambulatory devices, such as actimeters, has been proposed [47]. Actigraphy has proven to be very sensitive to sleep but, since it is based on the detection of periods of immobility, its capacity to correctly evaluate all sleep phases or awakenings is reduced [51]. In this sense, incorporation of DST to sleep detection by actigraphy allows more accurate predictions (see Figure 1D) [52].

Ambulatory multivariable recordings are currently used to counteract the inaccuracy associated with the use of a single variable (due to masking or artefacts) [51,52]. The ambulatory circadian monitoring (ACM) combines endogenous variables, such as skin temperature, with other more dependent of willingness like motor activity and body position; and exogenous, such as light exposure and environmental temperature, providing information about lifestyle and the bidirectional crosstalk between internal time and external synchronizers. ACM has been validated for sleep-wake detection (it shows higher sensitivity and specificity than actigraphy alone) by comparison with polysomnography [52] and it can be used instead of dim light melatonin onset (DLMO) to predict the internal phase [25]. Its usefulness has been proven in very different populations, such as shift workers [53], babies [1], hypertensive subjects and patients with metabolic syndrome [54,55], aged [1], mild cognitive impairment subjects [56], cancer patients [57] or more recently in people with sleep-disordered breathing [40].

3. 8. *Blood pressure and heart rate*

Blood pressure and heart rate show a similar circadian pattern with high values during daytime and lower ones at night-time, and they are tightly related to the rest-activity rhythm and the sympathetic-parasympathetic balance. Decline of blood pressure at night defines four different patterns according to the percentage of nocturnal dip: 1) dipper: when physiological dip involves a nocturnal decline between 10% and 20%; 2) extreme dipper: a night decline higher than 20%; 3) non-dipper: with a nocturnal decline between 0% and 10% and 4) riser: a night increase of blood pressure [54]. The most effective and accurate method for continuous blood pressure registering is ambulatory blood pressure monitoring. This is a non-invasive technique in which blood pressure is automatically measured every 15 to 20 minutes during the daytime and every 30 to 40 minutes at night [58]. Its use prevents blood pressure measurements from most of masking effects. However, patients often find the device uncomfortable and it may even alter their sleep-wake cycle. For that reason, it is recommended to monitoring at least 48h since patients have the chance to get used to the device, avoiding false diagnosis.

3. 9. *Clock genes*

The central pacemaker neurons and peripheral oscillator cells show autonomous rhythmicity at gene expression level. The implication of clock and CCG genes in numerous physiological processes and their possible desynchronization in some pathologies highlight the importance to measure and characterise their activity. The most used techniques are based in the polymerase chain reaction (PCR). Since it is impossible to evaluate clock genes expression from the SCN in vivo, peripheral tissues are normally used, mainly leukocytes and cells from the oral mucosa. For leukocytes, blood samples with a concrete sample rate are needed; then, leukocytes are isolated from the rest of blood cells [59]. Regarding oral mucosa samples, the usual technique involves a biopsy with local anaesthesia or by means of an oral mucosa scrape [60], thus expression in hair follicle cells seems to be a futures practical solution [61].

4. Chronoenhancement

Developed societies are characterized by a 24/7 lifestyle. Thus, members of these societies are exposed to contradictory synchronizing cues that lead to the appearance of chronodisruption (CD) as a new health concern [62]. This concern generates the necessity of preventive or therapeutical measures to counteract it (see Figure 1 E and F). The main strategy to prevent CD consists in empowering circadian inputs and increasing day-night contrast. Because of that, the number of possible strategies is as large as the number of CS inputs:

• Light/dark cycle: as main zeitgeber, bright light is able to produce an increase of amplitude and stability when applied at appropriate timing (see Figure 1 E) [35]. In addition to light, darkness is also necessary to entrainment since its absence contributes to CD [36].

• Melatonin: it has similar effects than darkness when administrated at the proper time in order to avoid contradictory information to the CS [63]

• Regular exercise: at the right moment, it can also synchronize the human CS while improving physical health [2].

• Meal schedule: especially important as synchronizer for most peripheral clocks, it increases synchronization between physiological and behavioural rhythms in animal models [3].

• Environmental temperature: it is able to entrain animal models [64], but its influence in humans is restricted to enhance temperature rhythm in a warm-cold day-night cycle [65,66].

• Sleep habits: in spite of its weak synchronizing power, sleep is able to determine light exposure and drift some marker rhythms by a fixed schedule [67].

• Social interaction: although not considered a zeitgeber [4], to keep social interactions could also be helpful for the CS.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Economy and Competitiveness, through CIBERFES grant (CB16/10/00239, CB16/10/00238), and grant 19899/GERM/15 awarded to JAM (co-financed by FEDER).

References

1. Batinga H, Martinez-Nicolas A, Zornoza-Moreno M, Sánchez-Solis M, Larqué E, Mondejar MT, et al. Ontogeny and aging of the distal skin temperature rhythm in humans. Age. 2015; 37:29.

2. Atkinson G, Edwards B, Reilly T, Waterhouse J. Exercise as a synchroniser of human circadian rhythms: an update and discussion of the methodological problems. Eur J Appl Physiol. 2007; 9:331-41.

3. Mendoza J. Circadian clocks: setting time by food. J Neuroendocrinol. 2007; 19:127-37.

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

5. Antle MC, Foley DK, Silver R. Gates and oscillators: a network model of the brain clock. J Biol Rhythms. 2003; 18:339-50.

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

7. Morin LP. SCN organization reconsidered. J Biol Rhythms. 2007; 22:3-13.

8. Terzibasi-Tozini E, Martinez-Nicolas A, Lucas-Sánchez A. The clock is ticking. Ageing of the circadian system: From physiology to cell cycle. Semin Cell Dev Biol. 2017; 70:164-76.

9. Ko CH, Yamada YR, Welsh DK, Burh ED, Liu AC, Zhang EE, et al. Emergence of noise-induced oscillations in the central circadian pacemaker. PLoS Biol. 2010; 8:e1000513.

10. Morin LP, Allen CN. The circadian visual system. Brain Res Rev. 2006; 51:1-60.

11. Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, et al. Role of melanopsin in circadian responses to light. Science. 2002; 298:2211-3.

12. Peirson SN, Foster RG. Non-image-forming photoreceptors. In: Albrecht U, editor. Protein Reviews. Volume 12. The Circadian Clock. Springer Science: New York; 2010. pp. 105-113.

13. Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW, et al. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature. 2003; 424:75-81.

14. Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, et al. Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature. 2008; 453:102-6.

15. Berson DM. Strange vision: ganglion cells as circadian photoreceptors. Trends Neurosci. 2003; 26:314-20.

16. Roenneberg T, Merrow M. Molecular circadian oscillators: an alternative hypothesis. J Biol Rhtyhms. 1998; 13:167-79.

17. Webb AB, Angelo N, Huettner JE, Herzog ED. Intrinsic, nondeterministic circadian rhythm generation in identified mammalian neurons. Proc Natl Acad Sci USA. 2009; 106:16493-8.

18. Lowrey PL, Takahashi JS. Mammalian circadian biology: elucidating genome-wide levels of temporal organization. Annu Rev Genomics Hum Genet. 2004; 5:407-41.

19. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. Nature. 2002; 418:935-41.

20. Vanselow JT, Kramer A. Posttranslational regulation of circadian clocks. In Albrecht U, editors. Protein Reviews. Volume 12. The Circadian Clock. Springer Science: New York; 2010. pp. 79-104.

21. Yagita K, Tamanini F, van Der Horst GT, Okamura H. Molecular mechanisms of the biological clock in cultured fibroblasts. Science. 2001; 292:278-81.

22. Van Someren EJW, Nagtegaal E. Improving melatonin circadian phase estimates. Sleep Med. 2007; 8:590-601.

23 Hofstra WA, de Werd AW. How to assess circadian rhythms in humans: A review of literature. Epilepsy Behav. 2008; 13:438-44.

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

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

26. Kräuchi K, Knoblauch V, Wirz-Justice A, Cajochen C. Challenging the sleephomeostat does not influence the thermoregulatory system in men: Evidence from a nap vs. sleep-deprivation study. Am J Physiol Regul Integr Comp Physiol. 2006; 290:R1052-61.

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

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

29. Blatteis CM. Age-dependent changes in temperature regulation-A mini review. Gerontology. 2012; 58:289-95.

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

31. Srinivasan V, Maestroni GJM, Cardinali DP, Esquifino AI, Pandi-Perumal SR, Miller SC. Melatonin, immune function and aging. Immun Ageing. 2005; 2:17.

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

33. Mander BA, Winer JR, Walker MP. Sleep and human aging. Neuron. 2017; 94:19-36.

34. Duffy JF, Czeisler CA. Age-related change in the relationship between circadian period, circadian phase, and diurnal preference in humans. Neurosci. Lett. 2002; 318:117-20.

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

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

37. Bonmati-Carrion MA, Hild K, Isherwood C, Sweeney SJ, Revell VL, Skene DJ, et al. Relationship between human pupillary light reflex and circadian system status. PloS ONE. 2016; 11:e0162476.

38. Zhu Y, Tu DC, Denner D, Shane T, Fitzgerald CM, Van Gelder RN. Melanopsin-dependent persistence and photopotentiation of murine pupillary light responses. Invest Ophthalmol Vis Sci. 2007; 48:1268-75.

39. Park JC, Moura AL, Raza AS, Rhee DW, Kardon RH, Hood DC. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. Invest Ophthalmol Vis Sci. 2011; 52:6624-35.

40. Martinez-Nicolas A, Guaita M, Santamaría J, Montserrat JM, Rol MA, Madrid JA. Circadian impairment of distal skin temperature rhythm in patients with sleep-disordered breathing: The effect of CPAP. Sleep. 2017; doi: 10.1093/sleep/zsx067.

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

42. Bonmati-Carrion MA, Arguelles-Prieto R, Martinez-Madrid MJ, Reiter R, Hardeland R, Rol MA, et al. Protecting the melatonin rhythm through circadian healthy light exposure. Int J Mol Sci. 2014; 15:23448-500.

43. Stehle JH, von Gall C, Korf HW. Melatonin: a clock-output, a clock-input. J Neuroendocrinol. 2003; 15:383-9.

44. De Almeida EA, Di Mascio P, Harumi T, Spence DW, Moscovitch A, Hardeland R, et al. Measurement of melatonin in body fluids: Standards, protocols and procedures. Childs Nerv Syst. 2011; 27:879-91.

45. Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): Facts and future directions. Int J Psychophysiol. 2009; 72:67-73.

46. Mormont MC, Waterhouse J, Bleuzen P, Giacchetti S, Jami A, Bogdan A, et al. Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. Clin Cancer Res. 2000; 6:3038-45.

47. Morgenthaler TI, Lee-Chiong T, Alessi C, Friedman L, Aurora RN, Boehlecke B, et al. Practice parameters for the clinical evaluation and treatment of circadian rhythm sleep disorders. An American Academy of Sleep Medicine report. Sleep. 2007; 30:1445-59.

48. Pollak CP, Tryon WW, Nagaraja H, Dzwonczyk R. How accurately does wrist actigraphy identify the states of sleep and wakefulness? Sleep. 2001; 24:957-65.

49. Morris CJ, Aeschbach D, Scheer FAJL. Circadian system, sleep and endocrinology. Mol Cell Endocrinol. 2012; 349:91-104.

50. Silber MH, Ancoli-Israel S, Bonnet MH, Chokroverty S, Grigg-Damberger MM, Hirshkowitz M, et al. The visual scoring of sleep in adults. J Clin Sleep Med. 2007; 3:121-31.

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

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

53. Moreno-Casbas MT, Ruzafa-Martinez M, Rol MA, Madrid JA, Serrano Pinto A, González-María E, et al. Sleepiness in Spanish nursing staff - Influence of chronotype and care unit in circadian rhythm impairment: research protocol. J Adv Nurs. 2014; 70:211-9.

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

55. Corbalán-Tutau MD, Madrid JA, Ordovás JM, Smith CE, Nicolás F, Garaulet M. Differences in daily rhythms of wrist temperature between obese and normal-weight women: associations with metabolic syndrome features. Chronobiol Int. 2011; 28:425-33.

56. Ortiz-Tudela E, Martinez-Nicolas A, Díaz-Mardomingo C, García-Herranz S, Pereda-Pérez I, Valencia A, et al. The characterization of biological rhythms in mild cognitive impairment. Biomed Res Int. 2014; 2014:524971.

57. Ortiz-Tudela E, Iurisci I, Beau J, Karaboue A, Moreau T, Rol MA, et al. The circadian rest-activity rhythm, a potential safety pharmacology endpoint of cancer chemotherapy. Int J Cancer. 2014; 134:2717-25.

58. Lemmer B, Scholtze J, Schimitt J. Circadian rhythms in blood pressure, heart rate, hormones and on polysomnographic parameters in severe obstructive sleep apnea syndrome patients: effects of continuous positive airway pressure. Blood Press Monit. 2016; 21:136-43.

59. Gaspar L, Brown SA. Measuring circadian clock function in human cells. Methods Enzymol. 2015; 552:231-56.

60. Bjarnason GA, Jordan RCK, Wood PA, Lincoln DW, Sothem RB, Hrushesky WJM, et al. Circadian expression of clock genes in human oral mucosa and skin. Am J Pathol. 2001; 158:1793-801.

61. Takahashi M, Haraguchi A, Tahara Y, Aoki N, Fukazawa M, Tanisawa K, et al. Positive association between physical activity and PER3 expression in older adults. Sci Rep. 2017; 7:39771.

62. Erren TC, Reiter RJ, Piekarski C. Light, timing of biological rhythms, and chronodisruption in man. Naturwissenschaften. 2003; 90:485-94.

63. Stevens RG. Artificial lighting in the industrialized world: Circadian disruption and breast cancer. Cancer Causes Control. 2006; 17:501-7.

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

65. Kondo M, Tokura H, Wakamura T, Hyun KJ, Tamotsu S, Morita T, et al. 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. 2007; 26:429-36.

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

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