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Ambulatory Assessment of the Functional Status of the Human Circadian System

Evaluación Ambulatoria del Estatus Funcional del Sistema Circadiano Humano

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"Count your age by friends, not years.
Count your life by smiles, not tears."
— John Lennon

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“The only reason for time is so that everything doesn’t happen at once”

— Albert Einstein

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1. GENERAL INTRODUCTION

1.1. Biological rhythms and its properties.

Faced with cycled environmental cues, all living beings have developed biological clocks that facilitate the anticipation to these repetitive phenomena. These cyclic variations like the light-dark cycle or seasonality are caused by the Earth rotation over itself and the Earth's revolution around the Sun, and they are one of the reasons for the existence of the so-called biological rhythms (Figure 1).

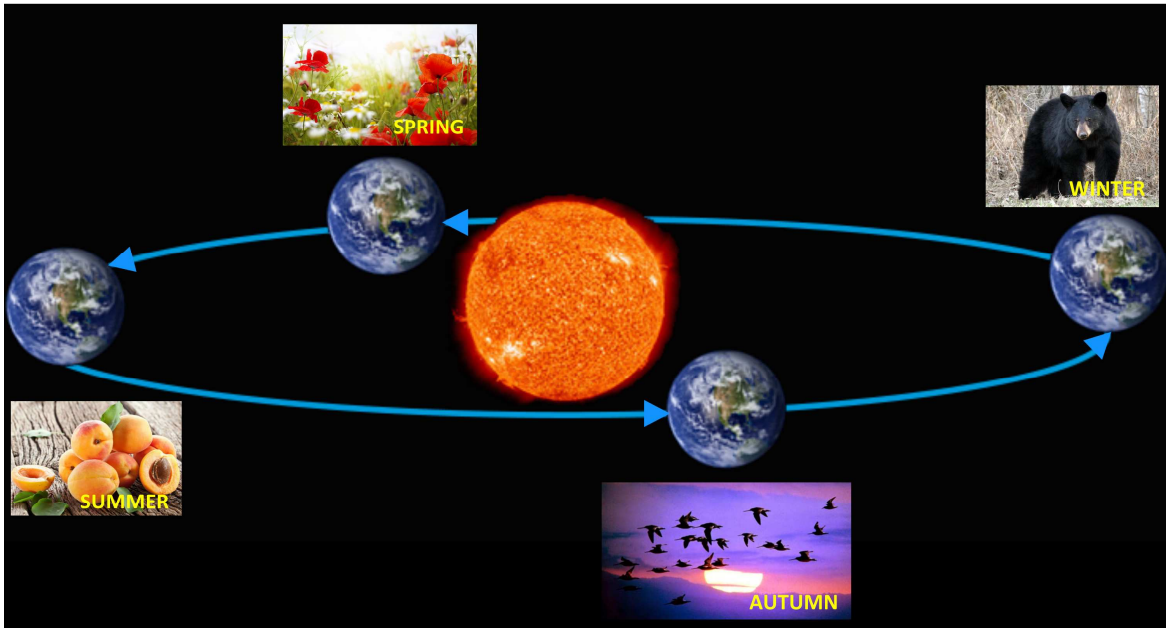

Figure 1. Schematic representation of the Earth's revolution around the Sun that generates seasons.

These cyclic conditions have allowed all living beings to develop biological clocks that facilitate the anticipation to these changes, i.e. the accumulation of fat in the black bear before the arrival of winter, the spring bloom of certain flowers, fruit's maturation on summer or autumn migration of some birds.

Despite being the reason why natural selection facilitated the existence of biological clocks, biological rhythms are not generated by environmental cyclic signals. In fact, the most important feature of biological rhythms is that they persist even in constant environments, without any external signal providing timing information. That's the definite demonstration of the endogenous generation of biological rhythms by an internal clock (Pittendrigh, 1960). Thus, biological rhythms are not driven by environmental signals, but synchronized to them.

Each species has a specific intrinsic endogenous period (τ = tau). In humans, this period is slightly over 24 hours. Thus, in order to generate rhythms synchronized with

the environment, biological clocks have the ability to get entrained by several of these cyclic external cues, called *zeitgeber* (from German: "time giver").

The most important *zeitgeber* for most living organisms is the light-dark cycle (LD) and it directly entrains the central pacemaker. However, in humans, exposure to other *zeitgebers* like, physical exercise, social contacts, or feeding schedule are also relevant to synchronize the biological clock (Mendoza, 2007; Roenneberg et al., 2013).

Each generated biological rhythm repeats itself with a characteristic period that allow the classification of rhythms in three categories (Refinetti, 2010):

- Circadian rhythms, from Latin *circa* = about and *die* = day, with a period ranging between 20 and 28 hours. Examples of these rhythms are the body temperature rhythm, the sleep-wake pattern, some hormonal rhythms like melatonin or cortisol secretion, blood pressure pattern… In humans, circadian rhythms are the most deeply studied rhythms because of their implications on health and disease.

- Ultradian rhythms. These are the rhythms with a frequency of oscillation of less than a day (period < 20 hours). In humans, there are several examples of these rhythms, such as the luteinizing hormone (LH) secretion or the brain wave electrical activity.

- Infradian rhythms. For these rhythms the frequency of oscillation is lower than one cycle per day and thus, they count on periods >28 hours. A good example in this category is the menstrual cycle in women (with a period of approximately 28 days).

Another important feature of biological rhythms is that each one possesses a specific phase angle with respect to a reference ZT (Pittendrigh and Daan, 1976). In the body, each rhythm acts like an instrument of an orchestra. Each instrument plays at the right moment and not everyone at the same time. The maintenance of stable phase relationships among instruments/rhythms leads to a desired situation of "internal temporal order", essential for keeping the melody of the orchestra and the well being in the organism. The opposite situation arises when instruments play at the wrong time, i.e. rhythms uncouple leading to circadian disruption or "chronodisruption" (Erren & Reiter, 2009).

1.2. Mammalian circadian timing system

1.2.1. An open system: SCN, inputs and outputs

Mammalian circadian timing system is formed by a set of hierarchically organized structures, in which the core piece lies in the suprachiasmatic nuclei of the hypothalamus (SCN), the so-called "circadian pacemaker". These SCN consists of two sets of 10.000-15.000 neurons located ventrally on the hypothalamus (Cassone et al., 1988; Slat et al., 2013; Swaab et al., 1985), bordering the optic chiasm (Figure 2). Each SCN can be differentiated in two main divisions, originally defined by different neuropeptides expression: the dorsomedial region or "*shell*" expressing arginine-vasopressin peptide (AVP) and the ventrolateral or "*core*" region with high expression of vasoactive intestinal peptide (VIP) (Dierickx and Vandesande, 1977; Samson et al., 1979). Although the specific functions of each region haven't been completely deciphered, it seems that the core region is responsible for the synchronizing of SCN timekeeping processes with the light-dark cycle (as it receives inputs from the retina via the retinohipotalamic tract, RHT). However, the shell region receives mainly inputs from the core and is responsible for the communication with the rest of the brain and body through neuropeptide signaling and thus, its role should be that of modulation of clock outputs. Although the SCN is designated as the central pacemaker, it is more correctly described as a chief synchronizer able to unify cell phases throughout the body (Yoo et al., 2004). That is so, since neurons isolated from the SCN are able to display circadian rhythms on their own (Balsalobre et al., 1998; Brown and Azzi, 2013; Tosini and Menaker, 1996; Yamazaki et al., 2000). To this purpose, the synchrony between SCN neurons has to be maximal and seems to be mediated by VIP, since mice lacking VIP expression or its receptor (VPAC2) display errant rest-activity cycles and synchrony is not maintained among SCN neurons (Aton and Herzog, 2005; Aton et al., 2005; Colwell et al., 2003; Harmar et al., 2002).

The mammalian SCN is entrained to the light cycle thanks to specific photoreceptors found in the eye's retinal ganglion cells (RGCs) that contains the photopigment melanopsin, which is maximally sensitive in the blue part of the spectrum (around 480 nm) (Berson, 2007; Hankins et al., 2008). The light information travels through the retinohypothalamic tract (RHT) until reaching the SCN (Berson et al., 2002; Hattar et al., 2002; Moore and Lenn, 1972).

The SCN then communicates time to the rest of the brain and body through a set of neural, humoral and systemic signals (Brown and Azzi, 2013). One of the most important signals is the hormone melatonin (for more information, see section 3.1).

The circadian system's abilities to 1) receive information from the environment, 2) self-elaborate de timing signal and 3) set the pace for the rest of the body, make this three-way system a perfect machine that permits the anticipation to cyclic changes.

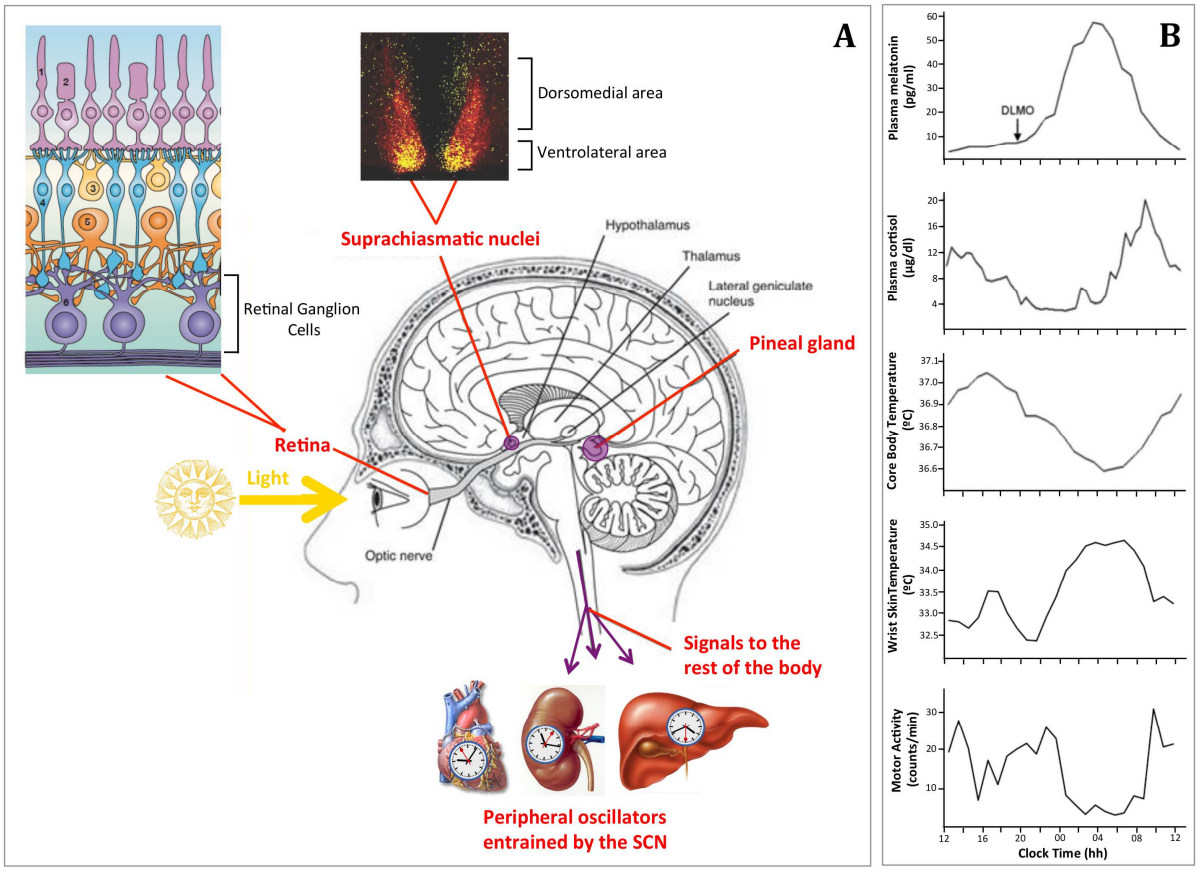

Figure 2. Mammalian circadian timing system organization (A) and several overt rhythms (B).

(A) The central pacemaker lies in the SCN of the hypothalamus. Light impacts RGCs that send light information through the retinohypothalamic tract until reaching the SCN. The SCN elaborate and convey the timing signal to the rest of the organism. For more information, see the text. The retina diagram has been modified from Wässle, 2004.

(B) A typical 24-hour period of several circadian rhythms considered to be markers of the status of circadian system: plasma melatonin and cortisol secretions, core body temperature, wrist skin temperature and motor activity. For more information, please see the text.

1.2.2. Peripheral oscillators

The circadian timing system is not only formed by the SCN but also counts on peripheral oscillators on almost every cell in the body that rhythmically express clock

genes (Cuninkova and Brown, 2008; Kornmann et al., 2007; Schibler, 2009; Stratmann and Schibler, 2006; Vansteensel et al., 2008). These oscillators, contrarily to SCN clocks, are not self sustainable as they depend on SCN signals and other environmental cues to display consistent and synchronized circadian rhythms (Brown and Azzi, 2013). These SCN signals entraining peripheral clocks could be direct nervous stimuli mediated by both, the sympathetic and the parasympathetic systems, hormonal signals (specially glucocorticoids and melatonin), and indirect signals like feeding time and body temperature (Brown and Azzi, 2013). These "slave" oscillators are able to regulate gene expression specifically for every tissue and besides maintain circadian rhythms.

1.2.3. Molecular clock

Virtually every cell of the organism has a circadian molecular clock, consisting of a set of feedback loops that create oscillations in gene expression at mRNA and protein levels with a period of about 24 hours (Huang et al., 2011; Ko and Takahashi, 2006).

These clock genes control the rhythmic expression of up to 10% of the transcriptome (Panda et al., 2002; Storch et al., 2002). Besides, some post-translational rhythms appear to be independent from the transcriptional rhythms (O'Neill et al., 2011). Thus, the antioxidant proteins peroxiredoxins undergo approximately 24 hours rhythms in human red blood cells, where there are no nuclei or DNA and then, transcriptional capability is inexistent (O'Neill and Reddy, 2011).

In these feedback loops, there are positive and negative elements, which are the heterodimers formed by the proteins CLOCK and BMAL1 and PER and CRY, respectively (Reppert and Weaver, 2002).

The transcriptional factors CLOCK and BMAL1, heterodimerize in the cytoplasm and when translocate back to the nucleus, bind to an Ebox in the DNA activating the expression of *Per*, *Cry*, *Rev-erbα*, *Rorα*, several clock controlled genes (*Ccg*) and a number of genes implicated in cell cycle (reviewed in chapter 4). The heterodimer PER:CRY translocates to the nucleus and inhibits the activity of CLOCK:BMAL1, restraining therefore their own expression. Similarly, REV-ERB*α* binds and deactivates the transcription of *Bmal1*. On the contrary, ROR*α* activates the expression of this gene (Figure 3) (Buhr and Takahashi, 2013).

The 24h rhythmicity of this molecular clock is mainly maintained by post-translational modifications such as phosphorylation and ubiquitination that are implicated in the translocation of the clock proteins into the nucleus (Gallego and Virshup, 2007; Lee et al., 2001). These modifications involve casein-kinase epsilon and delta (CKIε/δ).

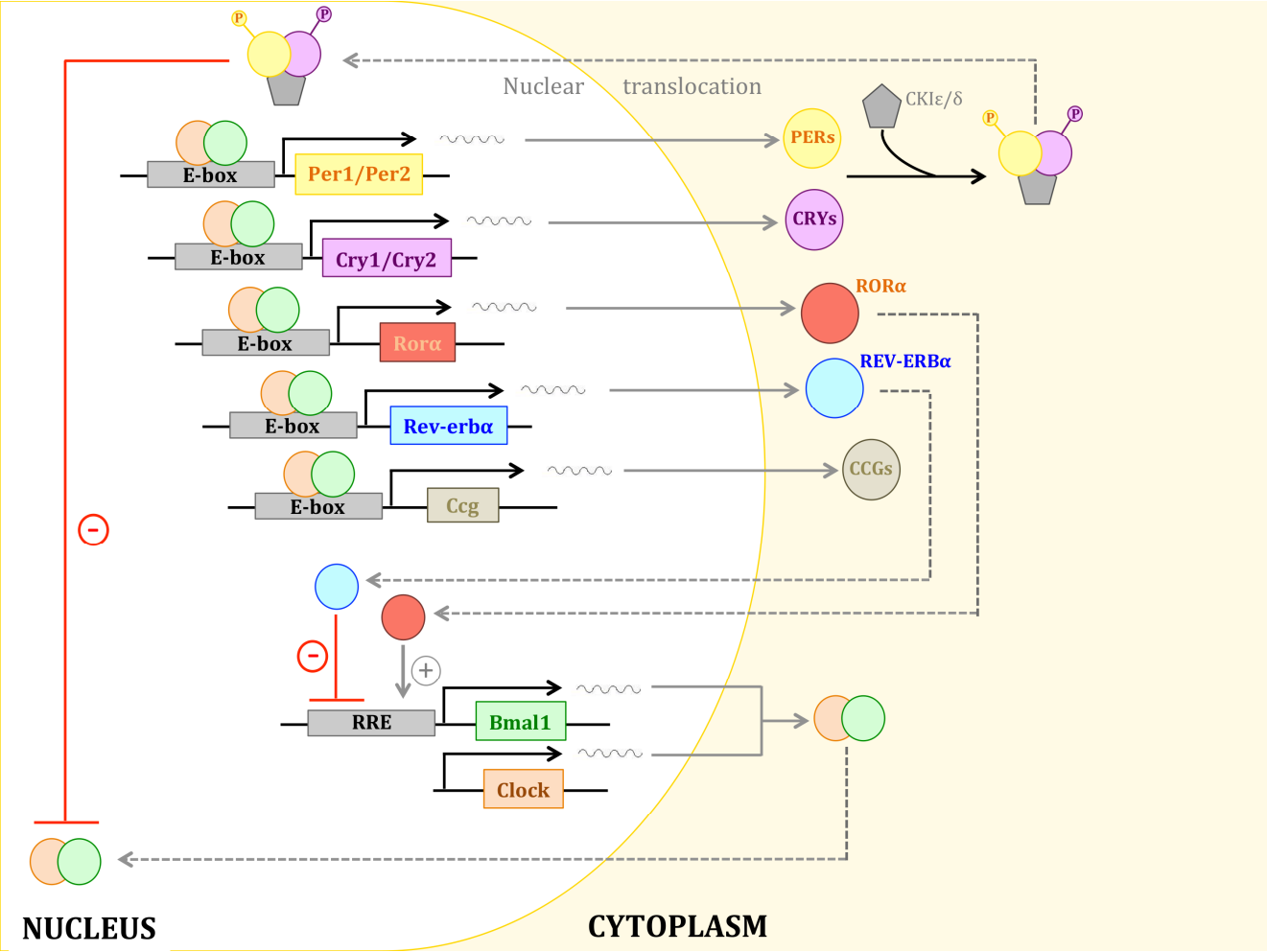

Figure 3. *The molecular clock counts on two feedback loops: one positive that includes the heterodimer CLOCK:BMAL1 that activates the transcription of Per, Cry, Rorα, Rev-erbα and several Clock controlled genes and one negative that includes PER:CRY and inhibits the CLOCK:BMAL1 heterodimer. For more details, see the text.*

1.3. Circadian disruption

The terms chronodisruption and circadian disruption make reference to the prolonged perturbation of physiological, behavioral and biochemical rhythms within the organism (Erren and Reiter, 2009). This alteration can be exemplified by ablation of rhythmicity, phase instability among days, extreme phase advances or delays,

desynchronization among rhythms within a subject, or with the environmental cues, and/or even by the phase inversion of circadian rhythms, all of them causing internal temporal impairment (Garaulet and Madrid, 2010; Ortiz-Tudela et al., 2012). Besides, the loss of coordination among peripheral oscillators can also lead to circadian disruption (Dibner et al., 2010).

The best example of chronodisruption is found among rotating shift workers, like nurses or flight attendants, living in chronic jetlag situations. Already in 2007, the International Agency for Research on Cancer (IARC) classified shift work with chronodisruption as a probable human carcinogen (group 2A) (World Health Organization and the International Agency for Research on Cancer Monographs on the Evaluation of Carcinogenic Risks to Humans (1997)). Besides the higher cancer incidence (Innominato et al., 2010), chronic uncoupling of rhythms is associated with a higher risk of developing metabolic syndrome, obesity (Evans and Davidson, 2013; Garaulet and Madrid, 2010), cardiovascular dysfunction (Evans and Davidson, 2013), immune dysregulation (Evans and Davidson, 2013), reproductive problems (Evans and Davidson, 2013), mood disorders, affective and cognitive impairments (Cochrane et al., 2012; Evans and Davidson, 2013), and accelerated aging (Kondratova and Kondratov, 2012; Ortiz-Tudela et al., 2012).

1.4. Marker rhythms for the study of circadian timing system's status.

Human chronobiological studies encounter the difficulty of reliably measuring the biological clock functioning as the central pacemaker lies in the deep brain. However, and to this end, circadian outputs can be evaluated. These circadian outputs are overt rhythms that can be easily measured for long periods, and with minimal subject discomfort and thus, they can be considered marker rhythms to assess circadian system function (Hofstra and De Weerd, 2008a; Ortiz-Tudela et al., 2014).

Since the purpose of this thesis was to study non-invasive marker rhythms, only those who meet this characteristic will be summarized here (for a more thorough review, please see Elisabet Ortiz-Tudela, Madrid, & Rol, 2014):

1.4.1. Melatonin

Melatonin is a neurohormone produced mainly, but not only, by the pineal gland under the control of the SCN. It is known as the "chemical expression of darkness"

(Reiter, 1991), since the duration of the night-time elevation is proportional to the duration of the dark phase. In fact, night-time lighting produces the blunt inhibition of melatonin production, specially blue (wavelengths around 480 nm) or white light (Lockley et al., 2003). Apart from the pineal gland, local production of melatonin has been described in other organs as retina and the gastrointestinal tract (Bubenik, 2008; Hardeland et al., 2011). However, although it has been suggested that gastrointestinal melatonin could be secreted into the blood in a circadian manner (Lee PPN et al., 1991), for the moment its effects are considered to be mainly local (Reiter et al., 2003).

The precursor for melatonin synthesis is the aminoacid L-tryptophan that is transformed to 5-hydroxytryptophan and then to serotonin. The key enzyme of this process is the aryalkylamine N-acetyltransferase (AA-NAT) that transform serotonin in N-acetyl serotonin. This enzyme presents a circadian rhythm in its activity and level that is indirectly controlled by the SCN (Pandi-Perumal et al., 2006). Finally, the enzyme hydroxyindole-O-methyltransferase (HI-OMT) transforms N-acetyl serotonin in melatonin.

The control of SCN over melatonin synthesis goes through the paraventricular nucleus of the hypothalamus that sends projections to the intermediolateral column of the spinal cord, to the superior cervical ganglion and finally, reaching the pineal gland that produces melatonin (Figure 2) (Benarroch, 2008).

Melatonin has many properties, being the most important one its chronobiotic effects on the body. Melatonin is able to convey the temporal signal from the SCN to the rest of the brain and body, acting through its MT1 and MT2 receptors. However, the discovery of its antioxidants, anti-inflammatory, immunomodulatory, neuroprotective, antitumoral and sleep promoting properties have made it object of extensive studies (for reviews on melatonin see Hardeland et al., 2011; Pandi-Perumal et al., 2006).

Besides accounting with the properties summarized above, the melatonin rhythm is considered to be one of the most reliable marker rhythms. However, its measurement is time consuming, and plasma or saliva sampling requires an intravenous catheter or the subject's active collaboration, respectively. Moreover, the melatonin rhythm in subjects under normal living conditions can be masked by a number of factors including posture, exercise, sleep, caffeine, certain drugs, such as beta-blockers and NSAIDS, and, in particular, nocturnal light exposure (Chang et al., 2011; Deacon and

Arendt, 1994; Hardeland et al., 2012; Mayeda et al., 1998; Stoschitzky et al., 1999). The most common procedure to evaluate melatonin is the so-called DLMO method (from Dim Light Melatonin Onset), in which saliva or plasma is collected in 30 minute intervals in conditions of dim light (<50 lux), during 2 to 3 hours before the habitual bedtime if the individual's phase is approximately normal (Hofstra and De Weerd, 2008a; Pandi-Perumal et al., 2007) (Figure 2).

1.4.2. Cortisol

Cortisol is a glucocorticoid produced by the zona fasciculata of the adrenal cortex subjected to circadian regulation. In humans, the highest values are found in the early morning coinciding with wake-up time. These values decline during daytime until the "quiet period" when cortisol presents its lowest levels corresponding to early night hours (Haus, 2007).

Given its circadian rhythmicity and easy measurement, cortisol rhythm has been commonly used as a marker rhythm. The nadir or acrophase, the onset of the evening rise, and the start or end of the quiet period can be evaluated as markers of circadian phase (Hofstra and De Weerd, 2008). However, this rhythm is masked by many factors, such as physical exercise, stress, lighting conditions, the sleep-wake cycle and high-protein intake (Bairagi et al., 2008; Haus, 2007; Scheer and Buijs, 1999).

1.4.3. Temperature

The core body temperature (CBT) rhythm results from a balance between heat production and loss. Thus, CBT decreases when heat loss is higher than heat production. The rhythm presents low values during nighttime corresponding to a high distal vasodilation and reaches the higher values during daytime (Kräuchi and Wirz-Justice, 1994). Conversely, the peripheral skin temperature rhythm presents an almost inverse pattern: high nighttime values and low daytime values with the point of inflexion coinciding with the awakening moment (Sarabia et al., 2008). This rhythm is in part the result of an alternating balance between parasympathetic (vasodilation) and sympathetic (vasoconstriction) actions on peripheral skin vessels, driven by the SCN (Kräuchi et al., 2005; Van Someren, 2004). Wrist skin temperature increases during rest periods associated with sleep and decreases during activity periods (Sarabia et al., 2008).

CBT has traditionally been recorded with a rectal probe during several days, which is not readily accepted by the subjects volunteering for these tests and entrains a risk of rectal perforation, especially in children (Friedrichs et al., 2013). However, in 2008, Sarabia et al., 2008 showed that the peripheral temperature rhythm measured on the wrist was a very robust rhythm whose phase estimation coincides with that obtained from the DLMO (Bonmati-Carrion et al., 2013).

Furthermore, evidence suggests that sleepiness may be more closely linked to increased peripheral skin temperature than to a decrease in core temperature (Kräuchi et al., 2005; Van Someren, 2004). To date, wrist temperature has been used to evaluate circadian rhythms under several physiological and pathological conditions, such as newborn circadian maturation (Zornoza-Moreno et al., 2011), metabolic syndrome (Corbalán-Tutau et al., 2011), and obesity (Corbalán-Tutau et al., 2011); and a correlation has even been found with clock gene polymorphism (Bandín et al., 2013). However, like core body temperature, peripheral temperature is subjected to environmental and physiological influences, including physical activity, body position, high environmental temperatures and sleep itself (Kräuchi, 2007; Reilly and Waterhouse, 2009; Wakamura and Tokura, 2002).

1.4.4. Rest-Activity

Rest-activity (R-A) rhythm measurement by actimetry is a simple, non-invasive and long-time recording method for indirectly evaluating the sleep-wake cycle. Therefore, it can be considered a marker rhythm. But, as occurs with other methods, actimetry is subjected to masking and artifacts, such as difficulties related to differentiate between the onset of nocturnal rest and sensor removal for bathing before going to bed, bed partner movements, sleeping when travelling in a car or train, etc (Acebo and LeBourgeois, 2006; Sadeh and Acebo, 2002).

The R-A rhythm has been extensively evaluated in multitude pathological conditions such as cancer (Barsevick et al., 2010; Berger et al., 2010a, 2010b; Grutsch et al., 2011; Innominato et al., 2012; Levin et al., 2005; Martinez-Nicolas et al., 2011), dementia (Carvalho-Bos et al., 2007; Oosterman et al., 2009; Tranah et al., 2011), circadian rhythm sleep disorders (Morgenthaler et al., 2007; Sack et al., 2007a, 2007b), autism (Hare et al., 2006), bipolar disorder (Kaplan et al., 2012)… Besides, the rest-activity rhythm has further served to monitor circadian maturation in children (Zornoza-

Moreno et al., 2011) and normal evolution of circadian timing system in the elderly (Buchman et al., 2014).

1.4.5. Body Position

Body position has rarely been considered for this purpose, because most actimeters are placed on the wrist, and thus they provide no information about the horizontal/vertical position of the subject. However, when the sensor is appropriately positioned, as has already been suggested (Blazquez et al., 2012; Kolodyazhniy et al., 2011; Ortiz-Tudela et al., 2010), this signal helps depict daily habits, and permits distinguishing when the subject is lying down or sitting (for example, at a computer), in spite of low activity levels in both cases (Ortiz-Tudela et al., 2010).

1.4.6. Sleep-Wake rhythm

The alternation between sleep and wake represents perhaps the most obvious 24-hour oscillation. It runs almost in parallel with rest-activity rhythm, since rest periods are very often associated with sleep (Pollak et al., 2001).

In healthy adults, sleep is concentrated on the nocturnal period. However, sleep timing is affected by age and gender. Thus, children present a higher tendency to morningness than adults, meaning an earlier hour to go to bed. This morningness character goes on until the age of 20, when young adults reach their highest eveningness. Finally, with increasing age this rhythm shows a progressive phase advance and become fragmented and distributed during the rest of the day (Dijk et al., 2000), for more details see section 5.1). Taking into consideration gender, during adulthood, eveningness is more common in men than in women, since they go later to bed (Roenneberg et al., 2007).

Sleep regulation was described as early as 1982 as a two-process model (Borbély, 1982). On the one hand, there is a homeostatic process in which there is an increasing pressure for sleeping from the awakening moment until the next sleep episode and this "need" declines from the moment of beginning to sleep. On the second hand, there is a circadian process, which independently of the homeostatic load, determines the propensity of falling sleep depending on the hour of the day (for a more updated review, see Morris, Aeschbach, & Scheer, 2012). However, sleep is a very complex

process also influenced by the light-dark cycle, melatonin and social timing (Fisher et al., 2013).

Sleep architecture during one typical night consists of 3 or 4 cycles of 90 to 120 minutes of duration. Each cycle counts on several steps of different depth, divided in non-rapid eye movement (nREM) and REM sleep. Non-REM sleep is further subdivided into light sleep (stages 1 and 2) and deep or slow wave sleep, SWS (stage 3). REM sleep is a very active period for the brain as dreaming occurs at this moment, although muscles are paralyzed (Morris et al., 2012; Silber et al., 2007). SWS is more predominant during the first half of the night and contrarily, during the second half of the night REM sleep is more frequent (Morris et al., 2012). This architecture changes during both healthy and pathological ageing (Pace-Schott and Spencer, 2011; Zeitzer, 2013).

Sleep function is still an unresolved matter. However, a role on memory consolidation has been previously described (Rasch and Born, 2013). Furthermore, very recently, a role on brain clearance from potentially neurotoxic waste products have been reported (Xie et al., 2013).

PSG is the gold standard for sleep studies and it is the preferred tool to diagnose sleep-related pathologies (Ancoli-Israel et al., 2003). Nevertheless, the high cost of the equipment, the need of trained specialists and the constraints for the subjects of this technique, force the introduction of alternative procedures to detect accurately sleep-wake cycles (see chapters 1 and 2). The employment of small devices, actimeters, placed generally on the wrist, recording the activity of the subject all along the 24h-period and during weeks or even months, the so-called actigraphy, has been proposed as a partial solution to these methodological problems. Besides, it represents a cheaper technique with higher acceptance among the volunteers. Already in 1997, the American Academy of Sleep Medicine (AASM) considered actigraphy as a useful tool for sleep studies (Thorpy et al., 1995). Later in 2007, the AASM declared actigraphy as clinically appropriate for the study of several sleep-related pathologies and circadian rhythm disorders (Morgenthaler et al., 2007). Numerous studies have employed actigraphy to evaluate sleep quality and therefore, it has been proposed as reliable method to discern wake from sleep periods (Ancoli-Israel et al., 2003). Actigraphy has shown to be very sensitive for detecting sleep but since it considers motionless moments as sleep, its ability to detect wake is smaller (Pollak et al., 2001).

In relation to the sleep field, the involvement of temperature on sleep initiation is being increasingly studied. In this sense, the distal-proximal skin temperature gradient has been proposed as a good predictor of sleep onset latency (Kräuchi and Wirz-Justice, 2001). Physiologically, sleep onset is associated to a core body temperature drop. For this to happen, peripheral temperature has to increase by the activation of peripheral vasodilation (Kräuchi et al., 2005; Van Someren, 2004). As a result, the peripheral rhythm of temperature precedes the central temperature rhythm (Van Someren, 2006). Thus those techniques combining both temperature and actimetry would presumable increase sleep detection accuracy (see chapter 2).

1.4.7. Multivariable recordings

To counteract the inaccuracy associated with the use of a single variable, multivariable recordings under ambulatory conditions have recently been proposed (Kolodyazhniy et al., 2012, 2011; Ortiz-Tudela et al., 2010). These ambulatory circadian monitoring procedures integrate a combination of variables, such as temperature, activity and body position, which provide complementary information about circadian system functionality.

Since masking, that is, the influence of external signals that affect overt rhythms (Martinez-Nicolas et al., 2013; Ortiz-Tudela et al., 2010), not only occurs with actigraphy, as already mentioned, but also when studying any single variable (such as peripheral temperature), new approaches to overcome potential artifacts during ambulatory measurements have been developed. These include multivariable recordings (Kolodyazhniy et al., 2011) and integrated variables, such as TAP, as described by Ortiz-Tudela *et al* (2010) (for more information, see chapters 1, 2 and 3).

1.5. THE CIRCADIAN TIMING SYSTEM STATUS IN HEALTHY AND PATHOLOGICAL CONDITIONS: IMPLICATIONS FOR EARLY DETECTION AND SURVIVAL

1.5.1. Healthy ageing

Normal aging manifests with a progressive deterioration of circadian rhythms (Myers and Badia, 1995). Usually this impairment is presented as a decrease in amplitude, an increase in fragmentation and a phase advance of rhythms (Dijk et al., 2000;

Oosterman et al., 2009; Van Cauter et al., 1996; Weinert and Waterhouse, 2007). There are several hypotheses that could account for this decline (Ortiz-Tudela et al., 2012). Firstly, if the input of information from environmental cues to the central pacemaker is weak, the entrainment of SCN to *zeitgebers* could be affected. In this sense, a reduction of retinal sensitivity to light has been reported (Hardeland et al., 2011; Myers and Badia, 1995) as well as a specific reduced responsiveness to the effects of blue light in elders. Besides, there could be changes in the afferent and efferent neural and chemical connections with the SCN (Myers and Badia, 1995). Furthermore, the conductual changes associated with age on conjunction with a reduction in bright light exposure in the elders could also have an impact on circadian synchronization (Myers and Badia, 1995). Secondly, even if the input of information remains adequate, the circadian timing system status could also be influenced by changes in the morphology and neurochemistry of the SCN and the pineal gland, affecting melatonin production (Cardinali et al., 2010; Hardeland et al., 2011; Myers and Badia, 1995).

These changes are more than evident with respect to the sleep-wake rhythm, since an increase parallel to aging in the complaints related to disturbed sleep has been widely reported (Raymann et al., 2008). These disturbances are translated in a rise in nocturnal awakenings and an earlier final awakening (Dijk et al., 2000; Raymann et al., 2008). Besides, quiet sleep (slow wave sleep, SWS) is also reduced in elders and REM sleep become more abundant in the first half of sleep (Dijk et al., 2000; Raymann et al., 2008), confirming the changes in the sleep-wake architecture (Dijk et al., 2000; Pace-Schott and Spencer, 2011).

Sleep onset is related to temperature rhythms, since an increase in peripheral temperature followed by a decrease in central temperature is associated with sleep onset (Murphy and Campbell, 1997; Raymann et al., 2008). Thus, an artificially induced increase in peripheral temperature by only 0.4ºC is able to suppress nocturnal wakefulness and shift sleep into deeper stages in old persons. Also, this little increase was able to double SWS proportion and diminish the probability of an early morning awakening (Raymann et al., 2008).

Actually, thermoregulation in the elders is often compromised as peripheral vasoconstriction is reduced (Weinert and Waterhouse, 2007). This difficulty in regulation of body temperature could be responsible for the observed central temperature amplitude's decrease in the elders (Dijk et al., 2000; Touitou et al., 1986;

Weinert and Waterhouse, 2007). Furthermore, a phase advance of temperature rhythm has been reported in elderly people compared to young (Dijk et al., 2000; Weinert and Waterhouse, 2007).

The rest-activity rhythm is also affected by ageing as higher fragmentation (Oosterman et al., 2009) and reduced general activity (Weinert and Waterhouse, 2007) is found. Cortisol rhythm presents also reduced amplitude in the elders by means of higher levels during nighttime (Haus, 2007; Van Cauter et al., 1996) in addition to a phase advance in its secretion (Van Cauter et al., 1996).

1.5.2. Pathological ageing: mild cognitive impairment (MCI) and Alzheimer's Disease (AD)

The modifications experienced in the aged circadian system reviewed above have also been related to a progressive impairment in cognitive performance (Pace-Schott and Spencer, 2011). Furthermore, an exacerbation of circadian system impairment is found in Alzheimer's Disease (AD) (Weldemichael and Grossberg, 2010). However, brain degeneration in AD begins 20 to 30 years before clinical symptoms' appearance (Cardinali et al., 2010) and thus, the relevance of an early detection results evident. Thus, mild cognitive impairment (MCI), which is the recognized previous stage to dementia that progresses to AD at a 12% rate every year (Cardinali et al., 2010), is being increasingly studied.

MCI patients already experience sleep problems, cognitive impairment and sundowning, characterized by nocturnal agitation and emotional disturbances. Besides, melatonin concentration on the cerebrospinal fluid (CSF) is decreased years before the clinical onset of AD (Cardinali et al., 2010) and phase advanced in MCI with respect to a clinically healthy age-matched control group (Naismith et al., 2013). However, a phase delay of the rest activity rhythm has been reported as a risk hazard for developing MCI (Schlosser Covell et al., 2012; Tranah et al., 2011). Cortisol rhythm disturbances have also been described (Venero et al., 2013). Thus, melatonin treatment has been proposed and proved to be a useful add-on drug to correct these symptoms (Furio et al., 2007; Lin et al., 2013).

Interestingly, decreased nocturnal melatonin levels correlate with the severity of mental impairment of demented patients (Magri et al., 1997). So, in AD, low melatonin levels and acrophase variability have been described (Lin et al., 2013; Mishima et al.,

1999). In addition, sleep-wake disturbances increase as the disease progresses (Cardinali et al., 2010) and this seems to have a negative impact on memory and cognitive impairment (McCurry et al., 2000). Besides, cortisol disturbances (Giubilei et al., 2001) and phase delays in core body temperature and rest-activity rhythms (Harper et al., 2001; Figure 4) have also been described. For more details, please see chapter 7.

The circadian perturbations in these AD's patients could be caused by a decreased expression of AVP in the SCN of these patients (Liu et al., 2000). Furthermore, a decrease in VIP expressing neurons in the SCN has also been described for female presenile AD patients (Zhou et al., 1995) indicating that the clock itself seems to be affected in a higher degree than in age-matched elders.

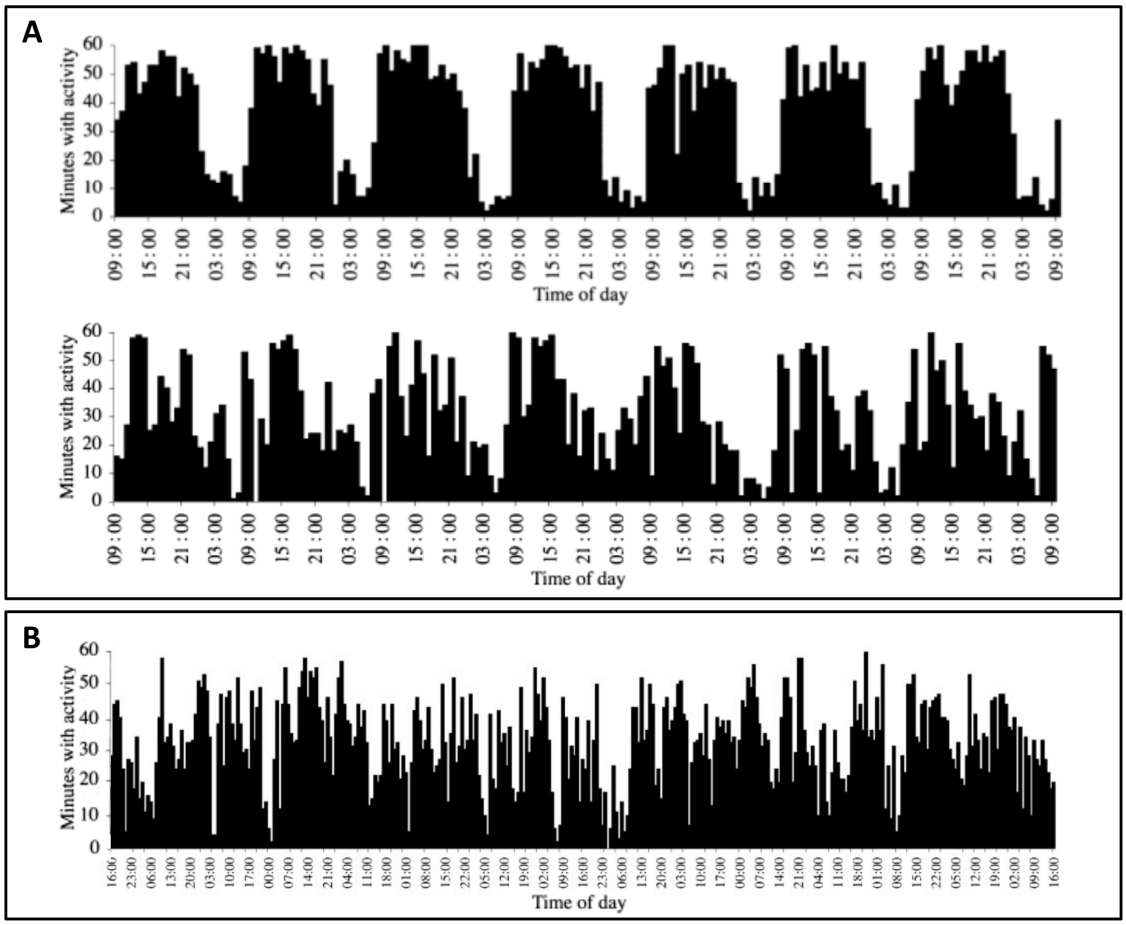

Figure 4. *Rest-activity individual profiles from an aged subject scoring above average in cognitive performance (panel A, top), an aged subject scoring below average in cognitive performance (panel A, bottom, modified from Oosterman et al., 2009) and an elderly demented woman (panel B, modified from Carvalho-Bos S et al., 2007).*

1.5.3. Cancer

Cancer is a systemic disease that profoundly affects daily activities, such as sleep and feeding (Barsevick et al., 2010; Mormont and Lévi, 1997), and several other functions, including immune system (Tohyama et al., 2013) and cognitive performance (Gibson and Monje, 2012). Cancer patients on chemotherapy further experience treatment-related adverse events such as nausea, vomiting, or diarrhea, which also impair their quality of life (Van Ryckeghem and Van Belle, 2010).

A hitherto overlooked possible consequence of chemotherapy drugs administration is the disruption of the circadian system (Innominato et al., 2009, 2012). In experimental models, circadian disruption can arise as a consequence of the administration of anticancer drugs, including antimetabolites, mitotic spindle poisons, alkylators or cytokines as a function of dose and timing (Ahowesso et al., 2011; Li et al., 2006, 2002; Li and Lévi, 2007; Ohdo et al., 2001). In humans, cancer drugs could enhance the release of pro-inflammatory cytokine from healthy and/or cancerous cells and these high levels of serum cytokines (TGFα, VEGF, IL-1 and IL-6) have been correlated to chemotherapy-induced fatigue (Mills et al., 2005; Rich et al., 2005; Schubert et al., 2007). Moreover, cytokines may influence sleep regulatory functions (Opp, 2005; Palesh et al., 2013) and thus, the reported increase of the level of cytokines in cancer patients could be partially responsible for the high prevalence of sleep disturbances (Liu et al., 2012). Furthermore, cancer-related fatigue and sleep problems are two of the most common and distressing symptoms reported by cancer patients (Berger et al., 2012; Palesh et al., 2013) and they are both very related to the rest-activity rhythm and the sleep-wake rhythm. Besides, it has also been shown how cancer chemotherapy is able to disturb the rest-activity rhythm, proving its potentially harmful effect on the circadian system (Ancoli-Israel et al., 2012; Berger et al., 2010a; Elisabet Ortiz-Tudela et al., 2013; Savard et al., 2009).

On the other hand, circadian robustness has been used as a prognostic index for cancer survival. Thus, values of rest-activity's I<O lower than 97.5%, has been associated with shortened survival and poorer quality of life of colorectal patients (Innominato et al., 2012) while flattening or aberrant diurnal cortisol rhythm predicts for early breast and lung cancer death (Kim, Kim, Oh, Kim, Choi, 2012; Sephton, Sapolsky, Kraemer, & Spiegel, 2000; Sephton et al., 2013; Figure 5). These data contribute to growing evidence that chronodisruption, or temporal order impairment is associated with an

increased incidence certain types of cancers (such as breast, prostate and colorectal) (Haus and Smolensky, 2013).

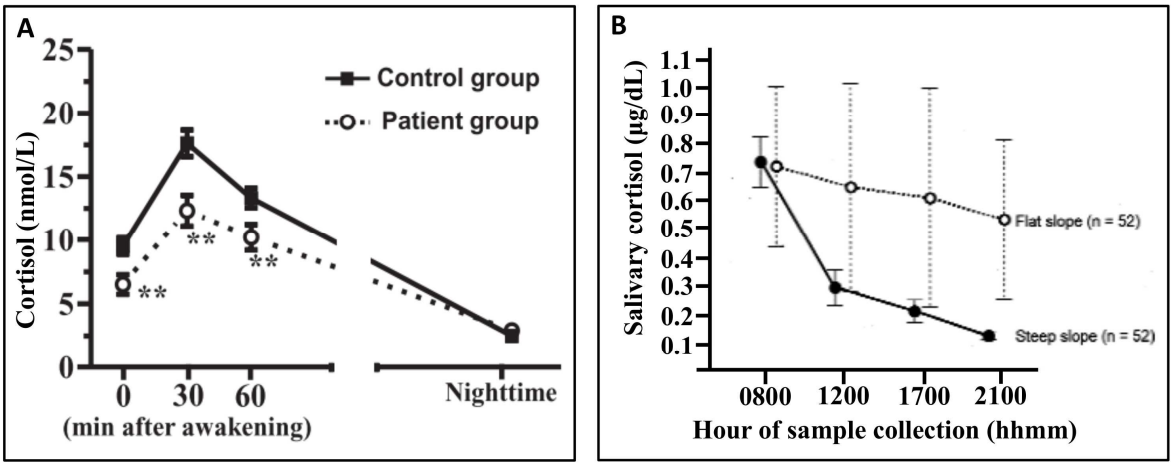

Figure 5. Profiles of cortisol secretion in cancer patients.

Panel A shows the increased secretion of morning cortisol in advanced lung cancer patients compared to a control healthy group (modified from Kim KS et al., 2012). Panel B shows mean profiles of cortisol of two groups of breast cancer patients (modified from Sephton S et al., 2000). Those patients presenting a flat slope presented also an earlier mortality.

Thus, the relevance of evaluating circadian system status and circadian disruption on these patients seems evident, that is precisely the main focus studied in chapters 5 and 6. The degree of synchrony or desynchrony among rhythms in individuals could have an impact on survival prognosis and cancer risk. Thus, the development of non-invasive and reliable methods to measure these aspects is becoming an increasing area of interest.

This PhD will then, focus on the evaluation of circadian system status, sleep disturbances and circadian rhythm coordination both in health and in different pathological conditions such as cancer and unhealthy aging.

2. OBJECTIVES

This thesis aims to develop a reliable, consistent, non-invasive and easily applied tool based on multivariable recordings, able to depict circadian system status and circadian rhythmic synchrony in humans.

In order to pursue this final goal, the following specific objectives were determined:

1. To create a multivariable tool able to reliably and non-invasively ambulatory assess the circadian system status by integrating wrist temperature, motor activity and body position rhythms into the composite TAP variable.
2. To validate this integrated variable for sleep and wake detection against the *gold standard* for this purpose, the polysomnography and test if TAP improves the detection from that obtained with actigraphy alone.
3. To test the validity of TAP variable in specific pathological conditions with known circadian impairments:
    3.1. To review, the previous knowledge on cancer and circadian rhythms, as well as circadian-based treatments for this disease.
    3.2. To study the inter-individual differences in rest-activity rhythm, that could potentially affect treatment outcome, in colorectal cancer patients.
    3.3. To assess how chronomodulated treatment affects internal synchronization on cancer patients by multivariable recordings.
    3.4. To evaluate circadian disturbances in mild cognitively impaired subjects, a previous condition to Alzheimer's Disease, in order to establish its potential usefulness to objectively assess the disease progression.
4. To transfer the usefulness of TAP implemented in an ambulatory monitoring device for its clinical application and its potential commercialization by an international patent.

3. EXPERIMENTAL CHAPTERS

3.1. Experimental Chapter 1

A NEW INTEGRATED VARIABLE BASED ON THERMOMETRY, ACTIMETRY AND BODY POSITION (TAP) TO EVALUATE CIRCADIAN SYSTEM STATUS IN HUMANS.

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3.1. A NEW INTEGRATED VARIABLE BASED ON THERMOMETRY, ACTIMETRY AND BODY POSITION (TAP) TO EVALUATE CIRCADIAN SYSTEM STATUS IN HUMANS

ABSTRACT

The disruption of the circadian system in humans has been associated with the development of chronic illnesses and the worsening of pre-existing pathologies. Therefore, the assessment of human circadian system function under free living conditions using non-invasive techniques needs further research. Traditionally, overt rhythms such as activity and body temperature have been analyzed separately; however, a comprehensive index could reduce individual recording artifacts. Thus, a new variable (TAP), based on the integrated analysis of three simultaneous recordings: skin wrist temperature (T), motor activity (A) and body position (P) has been developed. Furthermore, we also tested the reliability of a single numerical index, the Circadian Function Index (CFI), to determine the circadian robustness.

An actimeter and a temperature sensor were placed on the arm and wrist of the non-dominant hand, respectively, of 49 healthy young volunteers for a period of one week. T, A and P values were normalized for each subject. A non-parametric analysis was applied to both TAP and the separate variables to calculate their interdaily stability, intradaily variability and relative amplitude, and these values were then used for the CFI calculation. Modeling analyses were performed in order to determine TAP and CFI reliability.

Each variable (T, A, P or TAP) was independently correlated with rest-activity logs kept by the volunteers. The highest correlation ($r=-0.993$, $p<0.0001$), along with highest specificity (0.870), sensitivity (0.740) and accuracy (0.904), were obtained when rest-activity records were compared to TAP. Furthermore, the CFI proved to be very sensitive to changes in circadian robustness.

Our results demonstrate that the integrated TAP variable and the CFI calculation are powerful methods to assess circadian system status, improving sensitivity, specificity and accuracy in differentiating activity from rest over the analysis of wrist temperature, body position or activity alone.

3.2. Experimental Chapter 2

AMBULATORY CIRCADIAN MONITORING (ACM) BASED ON THERMOMETRY, MOTOR ACTIVITY AND BODY POSITION (TAP): A COMPARISON WITH POLYSOMNOGRAPHY.

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3.2. AMBULATORY CIRCADIAN MONITORING (ACM) BASED ON THERMOMETRY, MOTOR ACTIVITY AND BODY POSITION (TAP): A COMPARISON WITH POLYSOMNOGRAPHY

ABSTRACT

An integrated variable based on the combination of wrist Temperature, motor Activity and body Position (TAP) was previously developed at our laboratory to evaluate the functioning of the circadian system and sleep-wake rhythm under ambulatory conditions. However, the reliability of TAP needed to be validated with polysomnography (PSG). 22 subjects suffering from sleep disorders were monitored for one night with a temperature sensor (iButton®), an actimeter (Hobo®) and exploratory PSG. Mean waveforms, sensitivity (SE), specificity (SP), agreement rates (AR) and comparisons between TAP and sleep stages were studied. The TAP variable was optimized for SE, SP and AR with respect to each individual variable (SE: 92%; SP: 78%; AR: 86%). These results improved upon estimates previously published for actigraphy. Furthermore, TAP values tended to decrease as sleep depth increased, reaching the lowest point at phase 3. Finally, TAP estimates for sleep latency (SL: 37 ± 9 min), total sleep time (TST: 367 ± 13 min), sleep efficiency (SE: 86.8 ± 1.9%) and number of awakenings (NA>5 min: 3.3 ± .4) were not significantly different from those obtained with PSG (SL: 29 ± 4 min; SE: 89.9 ± 1.8%; NA>5 min: 2.3 ± .4), despite the heterogeneity of the sleep pathologies monitored. The TAP variable is a novel measurement for evaluating circadian system status and sleep-wake rhythms with a level of reliability better to that of actigraphy. Furthermore, it allows the evaluation of a patient's sleep-wake rhythm in his/her normal home environment, and at a much lower cost than PSG. Future studies in specific pathologies would verify the relevance of TAP in those conditions.

3.3. Experimental Chapter 3

CANCER CHRONOTHERAPEUTICS: EXPERIMENTAL, THEORETICAL, AND CLINICAL ASPECTS.

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3.3. CANCER CHRONOTHERAPEUTICS: EXPERIMENTAL, THEORETICAL, AND CLINICAL ASPECTS.

ABSTRACT

The circadian timing system controls cell cycle, apoptosis and drug bioactivation, transport and detoxification mechanisms in healthy tissues. As a consequence, the tolerability of cancer chemotherapy varies up to several-fold as a function of circadian timing of drug administration in experimental models. Best antitumor efficacy of single agent or combination chemotherapy usually corresponds to the delivery of anticancer drugs near their respective times of best tolerability. Mathematical models reveal that such coincidence be-tween chronotolerance and chronoefficacy is best explained by differences in the circadian and cell cycle dynamics of host and cancer cells, especially with regard circadian entrainment and cell cycle variability. In the clinic, a large improvement in tolerability was shown in international randomized trials where cancer patients received the same sinusoidal chronotherapy schedule over 24 h as compared to constant rate infusion or wrongly timed chronotherapy. However, sex, genetic background and lifestyle were found to influence optimal chronotherapy scheduling. These findings support Systems Biology approaches to cancer chronotherapeutics. They involve the systematic experimental mapping and modeling of chronopharmacology pathways in synchronized cell cultures, and their adjustment to mouse models of both sexes and distinct genetic background, as recently shown for irinotecan. Model-based personalized circadian drug delivery aims at jointly improving tolerability and efficacy of anticancer drugs based on the circadian timing system of individual patients, using dedicated circadian biomarker and drug delivery technologies.

3.4. Experimental Chapter 4

THE CIRCADIAN REST-ACTIVITY RHYTHM, A POTENTIAL SAFETY PHARMACOLOGY ENDPOINT OF CANCER CHEMOTHERAPY

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Published in *International Journal of Cancer* (2013). In press.

3.4. THE CIRCADIAN REST-ACTIVITY RHYTHM, A POTENTIAL SAFETY PHARMACOLOGY ENDPOINT OF CANCER CHEMOTHERAPY

ABSTRACT

The robustness of the Circadian Timing System (CTS) was correlated to quality of life and predicted for improved survival in cancer patients. However, chemotherapy disrupted the CTS according to dose and circadian timing in mice. A continuous and repeated measures longitudinal design was implemented here in order to characterize CTS dynamics in patients receiving a fixed circadian-based chemotherapy protocol. The rest-activity rhythm of 49 patients with advanced cancer was monitored using a wrist actigraph for 13 days split into 4 consecutive spans of 3-4 days each i.e. before, during, right after and late after a fixed chronotherapy course. The relative amount of activity in bed vs out of bed (I<O, main endpoint), the autocorrelation coefficient r24, the relative 24-h amplitude (Amp), interdaily stability (IS) and intradaily variability (IV) were compared according to study span. Circadian disruption (I<O < 97.5%) resulted from the administration of the fixed chronotherapy protocols, with all five rest-activity rhythm parameters being worsened in the whole group of patients ($p<0.05$). Mean parameter values subsequently recovered to near baseline values. The occurrence of circadian disruption on chemotherapy was associated with a higher risk of clinically relevant fatigue ($p=0.028$) or body weight loss ($p=0.05$). Four CTS dynamic patterns characterized treatment response including no change (9.5% of the patients); improvement (14.3%); alteration and complete recovery (31%) or sustained deterioration (45%), possibly due to inadequate chronotherapy dosing and/or timing. Improved clinical tolerability could result from the minimization of circadian disruption through the personalization of chronotherapy delivery.

3.5. Experimental Chapter 5

ASSESSING CIRCADIAN PHASE BY MEANS OF AMBULATORY CIRCADIAN MONITORING IN CANCER PATIENTS

Ortiz-Tudela E, Innominato PF, Rol MA, Lévi F, Madrid JA

Close to submission

3.5. ASSESSING CIRCADIAN PHASE BY MEANS OF AMBULATORY CIRCADIAN MONITORING IN CANCER PATIENTS

ABSTRACT

PURPOSE. Circadian disruption based on rest-activity monitoring predicts for poor survival outcome in cancer patients. However, other rhythmic variables can also assess the circadian system status along a chemotherapy course, like the integrated variable TAP (from distal Temperature, Activity and body Position rhythms) that tempts to allow personalizing cancer chemotherapy based on the individual circadian characteristics of patients.

EXPERIMENTAL DESIGN. TAP was monitored every 10 min for 4-day spans before, during and after a course of a fixed chronotherapy schedule for colorectal (n=21), pancreatic (n=2) or esophagus cancer (n=1) patients (11 males and 13 females, 60.6±2.4 y.o). Non-parametrical analysis, I<O and a new biomarker of the degree to temporal internal order maintenance, DI, were computed for each patient and period.

RESULTS. The three circadian rhythms studied and TAP rhythm presented lower stability and higher fragmentation related to treatment administration. Besides, large inter- and intra-individual changes were found for T, A, P and TAP patterns, with phase differences up to 12 hours among patients. A moderate perturbation of temporal internal order was found but the administration of fixed chronomodulated chemotherapy was able to partly resynchronize temperature and activity rhythms by the end of the study. Gender differences at baseline existed and women presented more robust rhythms than men but also they were also the most affected by chemotherapy.

CONCLUSION. The integrated variable TAP, as well as the asynchrony among rhythms thanks to the new biomarker, DI, in cancer patients will allow personalization of cancer chronotherapy considering individual circadian phase markers.

3.6. Experimental Chapter 6

BIOLOGICAL RHYTHMS CHARACTERIZATION IN MILD COGNITIVE IMPAIRMENT

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Subtmitted

3.6. BIOLOGICAL RHYTHMS CHARACTERIZATION IN MILD COGNITIVE IMPAIRMENT

ABSTRACT

BACKGROUND**.** Patients with dementia, especially those suffering from Alzheimer's disease (AD), present abnormal architecture in sleep-wake rhythm and altered circadian patterns of other rhythmic variables. This finding may be related to an accelerated perturbation of their biological clock caused by the illness itself, and not only related to age. However, detailed information regarding circadian organization in patients on an earlier stage of dementia is scarce. Thus, the objective of this work was to elucidate if there were already alterations on the circadian system of patients of mild cognitive impairment (MCI) compared to matched healthy aged subjects.

METHODS**.** 40 subjects (21 patients diagnosed with MCI, 74.1 ± 1.5 years old, and 19 healthy subjects, 71.7 ± 1.4 years old) were monitored ambulatorily for wrist's skin temperature (iButton, ThermoChron®, Data loggers I-button, IDC S.A., Spain), motor activity and body position (Hobo® Pendant G Acceleration Data Logger) during one week. For rhythmic characterization of each variable, non-parametrical analyses were applied. Finally, a new integrated index designed by our lab named TAP (from Temperature, Activity and Position), able to describe rest-activity rhythm accurately and assess circadian system status, was calculated, both individually and per group.

RESULTS**.** MCI patients exhibited a significant phase advance in two circadian phase markers of temperature, M5 (mean ± SEM: 04:20 ± 00:21 *vs* 02:52± 00:21, for healthy *vs* MCI, respectively) and L10 (mean ± SEM: 14:35 ± 00:27 *vs* 13:24 ± 00:16, for healthy *vs* MCI, respectively) and in two phase markers of TAP, L5 (mean ± SEM: 04:18 ± 00:14 *vs* 02:55 ± 00:30, for healthy *vs* MCI, respectively) and M10 (mean ± SEM: 14:30 ± 00:18 *vs* 13:28 ± 00:23, for healthy *vs* MCI, respectively). Furthermore, wake maintenance zone for temperature's rhythm (characterized by minimum temperature values between 19:00 to 21.00 h) tends to disappear in healthy elderly subjects, a phenomenon that is more pronounced in subjects with MCI.

CONCLUSIONS**.** These results prove that in an early stage of dementia, significant advances in the biological clock begin to occur, showing an accelerated decline of its function compared to a healthy population of the same age.

3.7. Experimental Chapter 7

DEVICE CONSISTING OF A BODY POSITION AND ACTIVITY SENSOR, A PERIPHERAL TEMPERATURE SENSOR AND A LIGHT SENSOR TO PROVIDE INFORMACIONT ON THE STATUS OF THE CIRCADIAN SYSTEM

Madrid Pérez JA, Sarabia Carrazo JA, Martinez-Nicolás A, Rol MA, **Ortiz-Tudela E.**

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3.7. DEVICE CONSISTING OF A BODY POSITION AND ACTIVITY SENSOR, A PERIPHERAL TEMPERATURE SENSOR AND A LIGHT SENSOR TO PROVIDE INFORMACIONT ON THE STATUS OF THE CIRCADIAN SYSTEM

4. GENERAL DISCUSSION

The study of circadian system has been attracting more and more interest given its already proven importance for well-being and disease prognosis (Innominato et al., 2012, 2009; Rajaratnam et al., 2013; Sephton et al., 2013, 2000). However, the reliable evaluation of the clock machinery functioning is quite a complex problem. On the one hand, the localization of the central pacemaker, in the suprachiasmatic nuclei of the hypothalamus (SCN), prevents the direct study in humans. Consequently, the indirect assessment of circadian system status through the evaluation of clock outputs, so-called marker rhythms, represents the more plausible choice. On the other hand, and given the masking processes influencing every isolated variable, this option presents also disadvantages. Thus, the purpose of this PhD was to develop a reliable, easily applicable, consistent and non-invasive tool, based on multivariable recordings, to evaluate circadian system status and its internal synchrony in large populations. To that purpose, we chose 3 circadian rhythms: wrist skin temperature, rest-activity and body position.

In **Chapter 1 and 2** we propose to integrate skin temperature, along with actimetry and body position data into a single variable to evaluate the status of the human circadian system under normal living conditions and demonstrate its validity for sleep studies.

Wrist temperature is a strong circadian rhythm resulting from internal and external influences that provides integrated information about the master pacemaker function and internal and external *zeitgebers* (Martinez-Nicolas et al., 2013; Ortiz-Tudela et al., 2010; Sarabia et al., 2008). Besides, high wrist temperature is closely linked to sleepiness, probably through parasympathetic activation and skin blood vessels vasodilation, while it drops during arousal periods, associated with sympathetic activation and vasoconstriction (Van Someren, 2006). The temperature rhythm minimum, occurring between 20:00-22:00h, a period previously known as “wake maintenance zone”, coincides with the start of the nocturnal melatonin surge: the dim light melatonin onset (DLMO) (Bonmati-Carrion et al., 2013; Lewy et al., 1999), thus, it could be used as a reliable phase marker for circadian system timing.

The rest-activity rhythm for circadian and sleep analysis has been widely studied through wrist actigraphy (Ancoli-Israel et al., 2003; Sadeh and Acebo, 2002) in several pathological conditions like cancer, dementia (Carvalho-Bos et al., 2007; Oosterman et al., 2009; Tranah et al., 2011), circadian rhythm sleep disorders (Morgenthaler et al.,

2007; Sack et al., 2007a, 2007b), and even in human trypanosomiasis (Njamnshi et al., 2012). Commercial actigraphs have proven its usefulness for the ambulatory assessment of rest-activity rhythm and, for at least, some characteristics of sleep, especially in healthy individuals (Ancoli-Israel et al., 2003). Commercial actigraphs count on different methods for transforming the analogic signals registered into digitized data. The most common are "time above threshold", "zero crossing method" and "digital integration". Lately, working with activity counts, with almost unprocessed raw data, is also becoming more frequent to avoid the disadvantages of each one of these methods (Ancoli-Israel et al., 2003). In this PhD, a new methodology based on expressing activity as the cumulative change of position in a specific time period has been developed (Ortiz-Tudela et al., 2010). This new method allows evaluating the amplitude and movements, unlike "time above threshold" and "zero-crossing method". However, it fails to take into account, as occurs with the "digital integration" method, the duration of each movement as it considers movements as a whole during the time epoch studied.

The third rhythm included in our studies is body position. Classically this rhythm hasn't been considered in circadian studies, mainly because the placement of the activity sensor, usually in the wrist, prevents position detection. Nevertheless, recent studies show its importance for improving the diagnosis of non-dipping blood pressure patterns (Blazquez et al., 2012; Morris et al., 2013).

However, each rhythmic variable is masked, at least to a certain degree, by environmental signals. Even that considered as the most robust marker rhythm, melatonin secretion, is influenced by several factors like prior photic history (Chang et al., 2011), posture (Deacon and Arendt, 1994), caffeine (Peuhkuri et al., 2012), certain drugs, such as beta-blockers (Mayeda et al., 1998; Stoschitzky et al., 1999) and, in particular, nocturnal light exposure (Hardeland et al., 2012). Likewise, core body temperature is influenced by sleep, posture, high caloric meals… (Hofstra & De Weerd, 2008); rest-activity rhythm can be influenced by a bed partner's movement, sensor removal… (Acebo and LeBourgeois, 2006; Sadeh and Acebo, 2002); body position can be confounded between standing and siting... Thus, the rationale under which the integration of rhythmic variables could be beneficial for circadian timing system evaluation lies in the minimization of artifacts affecting one isolated measured variable and the strengthening of meaningful rhythmic events. Thus, we developed

TAP algorithm consists on the integration of these 3 variables (wrist Temperature, motor Activity and body Position), as explained in **Chapter 1**, to obtain a time series values scoring from 0 when the subject is resting (with high temperature values, low activity levels and body position values near to 0 degrees) up to 1 when the subject is active (with low temperature values, high activity levels and body position values near 90 degrees).

According to our results TAP recording was able to capture individual rhythmic singularities and population rhythmic patterns, overcoming single variables' artifacts in healthy young subjects. Besides, circadian robustness at a specific period of time could be reliable assessed thanks to the development of the Circadian Function Index (CFI), based on non-parametric indexes. This parameter calculated on TAP, integrates the information concerning rhythm fragmentation within days, rhythm stability among different days and rhythm amplitude, thus providing important information to understand globally the status of circadian system. Unlike other frequently employed indexes like I<O (Mormont et al., 2000), CFI considers several rhythmic aspects of one variable and thus, provides a more complete insight of the rhythmic status of one subject.

Our second goal was the validation of TAP for sleep screening studies, tackled in **Chapters 1 and 2**. The relevance of this possibility lies in the non-invasiveness, in-home, cheap and comfortable way to evaluate the sleep-wake cycle and sleep quality in big populations. In this sense, actigraphy alone has already been proven useful and good correlations have been reported for this method against the gold standard for this purpose, the polysomnography (PSG) (Ancoli-Israel et al., 2003). However, actigraphy tends to overestimate sleep, as it considers motionless periods as sleep (Carrier et al., 2002; Pollak et al., 2001). Thus, in the literature to date, actigraphy has proven to be quite accurate in detecting sleep periods during rest spans, as compared to PSG. However, the specificity of wakefulness detection is weaker (Jean-Louis et al., 2001; Meltzer et al., 2012). This can be explained by the fact that sleep episodes of healthy subjects usually comprise more than 90% of sleep, and thus, total agreement rates would be high despite the large discrepancies in wake detection. This can lead to the misinterpretation of data from, for example, insomniac patients who experience trouble falling asleep, but who lay quietly in bed (Chambers, 1994; Sadeh and Acebo, 2002).

This fact led us to pursue validation studies comparing TAP against, on the one hand, sleep logs filled by healthy subjects (**Chapter 1**) and, on the other hand, an overnight standard PSG performed in a sample of patients experiencing sleep problems (**Chapter 2**).

Wrist temperature scored the highest value in specificity, proving its high ability to detect wakes states. This variable on its own, was able to further detect differences among sleep stages 1+2 and 3, probably because sleep onset is related to peripheral vasodilation and therefore, to increased values of wrist temperature (Van Someren, 2004). This is an important finding considering that such differentiation can only be reached by PSG, and highlights temperature's contribution to sleep screening studies (Ancoli-Israel et al., 2003).

With respect to actigraphy, our study also supports the finding of higher sensitivity, but low specificity. Furthermore, activity underwent no changes between sleep phases in our patients, so it cannot discern sleep depth by itself. Although previous studies seem to have found differences between NREM and REM sleep in subjects who experience sleep pathologies such as periodic limb movements (Pollmächer and Schulz, 1993), this capacity seems to be limited to a condition that specifically affects patient movements.

Overall, TAP proved to be more sensitive, specific and agree more with sleep logs and PSG than actigraphy on its own. Besides, TAP had a tendency to diminish with sleep depth, showing lower values in slow wave sleep (phase 3) than in phase 1+2. Finally, subjects readily accepted to wear the 2 sensors TAP requires as much as 1 entire week for **Chapter 1**. Bearing this in mind, we can conclude that TAP constitutes a reliable tool to screen sleep pathologies in big populations, without being a substitute for PSG. Furthermore, a recent study from our group showed that TAP's M5 (a phase marker signaling the central timing of the five consecutive hours with the highest values) is highly correlated to DLMO (Bonmati-Carrion et al., 2013), supporting also its reliability for circadian phase assessments.

Once we could validate TAP for healthy subjects we tried to assess circadian system status in 2 populations with known circadian disturbances: cancer patients (**Chapters 4** and **5**) and mild cognitively impaired subjects (**Chapter 6**).

Circadian alterations have been described in cancer patients (reviewed in **Chapter 3**), especially with respect to the rest-activity rhythm (Innominato et al., 2009; Savard et al., 2009; Berger et al., 2010). Importantly, disruption of the rest-activity rhythm or cortisol production have been shown to be related with a worse prognosis for the illness and a shorter survival (Mormont et al., 2000; Innominato et al., 2009, 2012; Sephton et al., 2000, 2013; Kim et al., 2012). Thus, in **Chapter 4,** we focused on quantifying treatment-induced circadian system alterations, characterizing rhythm recovery dynamics and establish a possible relation between circadian impairment and adverse events in individual patients receiving chronomodulated treatments. Firstly, our results confirmed those that have previously stated that chemotherapy administration produced deterioration on the rest-activity rhythm of cancer patients (Berger et al., 2010b; Innominato et al., 2009; Savard et al., 2009). However, and in spite of the global perturbation observed, important inter-individual differences aroused among patients. Forty-five percent of them weren't able to overcome the disruption caused by chemotherapy administration by the end of the study, a 10% remained with robust rest-activity rhythm through all the study, 31% were able to recover from the disruption, and a 14% of patients consistently improved their rhythms from baseline until the end of the study, in spite of receiving chemotherapy. These marked inter-individual dissimilarities force the study of the mechanisms underlying these differences and pave the path into personalized chemotherapy (Ortiz-Tudela et al., 2013).

Once we had verified the perturbation of the rest-activity rhythm in cancer patients and discovered the differences in response to chemotherapy, in **Chapter 5**, we evaluated the circadian system robustness not for an isolated variable but using multivariable recordings including wrist temperature, motor activity, body position and TAP variables. In addition, for the very first time an index to evaluate internal circadian misalignment was proposed. This index for health status could potentially be the base for personalized treatments. Thus, we demonstrated that in addition to the general effect of chemotherapy-induced disruption of rest-activity rhythm, wrist temperature, body position and TAP were also impaired. Again, huge differences among patients at baseline (before treatment administration) existed. In this sense, there were patients whose rhythms were robust and in phase with respect to each other while other patients presented rhythms out of tune and flat. More than that,

there was around a 12-hour difference among phase markers of different patients at baseline. These results call into question the meaning of chronomodulated chemotherapy when the time of day and each drug's mechanism of action for its administration (reviewed in **Chapter 3**) are decided according to pre-established internal timings, and not individual circadian phase, that is, in a personalized fashion. The huge interindividual timing differences could have an impact on these treatments' efficacy, since the same scheduled protocol could be applied in two patients with quite different internal times (as much as 12-h apart) and thus, hamper the known benefits of this therapy: the higher efficacy and the lower toxicity associated to the treatment (Ortiz-Tudela et al., 2013).

To date and to our knowledge, no one has before tried to quantify temporal order synchrony or impairment in humans, undoubtedly because recordings of circadian rhythms have been traditionally carried on one single variable (rest-activity, melatonin, temperature or cortisol, mostly). Thus, only multivariable recordings such as TAP allowed the further assessment of this aspect and the creation of a new parameter, DI from Desynchronization Index, which evaluates the degree of coupling between the rest-activity and the wrist temperature rhythm. In this study, the huge shock provoked by chronomodulated chemotherapy administration caused a moderate re-synchronization of, at least, temperature and activity rhythms at population level by the end of the study. Finally, **Chapter 5** contributed to track the changes in the circadian timing system of cancer patients undergoing chemotherapy treatments.

Another advantage of TAP is that our methodology allows the evaluation of the follow-up on patients' quotidian habits. Evidences exist on literature regarding the benefits of chronoenhancement in cancer patients either by melatonin administration (Seely et al., 2012), or by rightly-timed bright light exposure (Ancoli-Israel et al., 2012), although other circadian therapeutic approaches including sleep hygiene, social contacts, regular feeding schedules could be implemented. For example, in mice, the circadian amplification of the core body temperature rhythm through meal timing was associated with halving experimental cancer growth (Li et al., 2010). With TAP recording, we can reliably track the sleep-wake rhythm and evaluate the regularity in bed timing and the tendency to morningness or eveningness, an important issue when suggesting behavioral guidelines to improve the input to the biological clock, since

accomplish can be followed.

Elderly's circadian timing system shares with cancer some aspects of circadian deterioration. Rhythms tend to be more fragmented (Dijk et al., 2000; Oosterman et al., 2009), with smaller amplitude (Van Cauter et al., 1996; Weinert and Waterhouse, 2007) and sleep-wake cycle is altered (Raymann et al., 2008). However, unlike cancer, in older persons the problem lies in the entraining mechanisms via a retina desensitization to light (Hardeland et al., 2011; Myers and Badia, 1995) or a decreased exposure to light (Myers and Badia, 1995). Besides, changes in brain morphology, especially in SCN (such as a decrease in subpopulations of SCN neurons expressing VIP and AVP, SCN network modifications and photic information processing; Farajnia, Deboer, Rohling, Meijer, & Michel, 2013) and pineal gland (due to its progressive calcification; Schmid, Requintina, Oxenkrug, & Sturner, 1994), could also account for these modifications (Cardinali et al., 2010; Hardeland et al., 2011; Myers and Badia, 1995). These symptoms of deterioration accompanied with a progressive impairment of cognitive performance are found in AD (Weldemichael and Grossberg, 2010). Thus, the possibility of early detection of circadian disturbances other than those considered normal in the healthy age-matched, could result in the implementation of therapies that could delay the clinical onset of AD and/or helping to prevent some of the symptoms (Cardinali et al., 2010).

In this sense, some work has already been done in the precedent stage to AD, that is, mild cognitive impairment (MCI). Several studies have reported disturbances in the rest-activity and melatonin secretion rhythms in MCI patients. However, there are conflicting results. On the one hand, a phase delay of the rest-activity rhythm in MCI subjects (Cochrane et al., 2012) related to a risk factor for developing AD (Schlosser Covell et al., 2012; Tranah et al., 2011) has been described. On the other hand, a phase-advance in melatonin rhythm has also been reported (Naismith et al., 2013). This last result is coherent with the hypothesis of an accelerated ageing process in AD patients as the elderly advance their internal circadian phase of awakening (Dijk et al., 2000). In **Chapter 6** we evaluated wrist temperature, motor activity and body position rhythms in MCI patients and in a healthy age-matched population, in order to try to improve early AD detection by multivariable recordings. In our study, we found a consistent phase advance of all studied variables, although significant differences were only found for wrist temperature and TAP. A possible explanation for this incoherence

between our study and those from Cochrane (2012), Schlosser (2012) and Tranah (2011) could lie on how phases are calculated. In their case, they calculate the acrophase of the rest-activity rhythm with the cosinor method. This method tries to fit a sine wave to a time series (Minors and Waterhouse, 1988). However, rest-activity rhythm resembles more a squared wave than a sine wave and given the nocturnal agitation called "sundowning" that appears in AD (Cardinali et al., 2010; Weldemichael and Grossberg, 2010), cosinor may delay the moment of maximal activity (acrophase). On the contrary, in our study a non-parametrical rhythm characterization that doesn't assume *a priori* any waveform (Van Someren et al., 1999) was employed. Anyway, what seems evident is that subtle changes in the circadian timing system of MCI subjects begin to appear and they could potentially be used to screen the illness progression and, at least, start earlier available therapies in order to delay the onset of AD as much as possible.

After validating the use of TAP for circadian and sleep studies in healthy and circadian impaired subjects, we implemented TAP's algorithm in a new ambulatory monitoring device that has been registered in an international patent (**Chapter 7**), which is currently being exploited.

To sum up, this thesis has proven that an ambulatory, integrated and new methodology based on multivariable recordings represents a reliable option for the assessment of circadian system status, sleep architecture and circadian internal synchronization in humans. Furthermore and based on our results, TAP widens the horizon of cancer chemotherapy by suggesting the need of personalized treatments that take into account the internal timing of patients. Finally, we postulate that it could work as a prognosis tool helping to early diagnose AD.

5. CONCLUSIONS

SPECIFIC CONCLUSIONS

1. The integration of the wrist Temperature, motor Activity and body Position rhythms into TAP variable, guarantees the reliable assessment of circadian system status in humans.

2. The integrated variable TAP is powerful enough to discern sleep from wake states, as compared with the gold standard for sleep studies, polysomnography. Furthermore, TAP scores consistently better than actigraphy alone for sleep-wake detection.

3. Circadian-based cancer treatments substantiated on the high efficacy and low side effects associated to the drugs administrated in a chronomodulated fashion represent an interesting option to traditional therapies.

4. Cancer chemotherapy administration produces an overall disturbance of rest-activity rhythm. However, several patterns of evolution arise when chemotherapy is administered, suggesting again the need for personalized treatments.

5. Pre-chemotherapy differences among cancer patients are such that 12-hour spread phase markers are found. Besides, a high degree of internal desynchronization was discovered among these patients. These interindividual differences together with the existing circadian order impairment have an effect on treatment efficacy and survival, so personalized treatments suggested on conclusion 4 could benefit from TAP tool in order to guide and adjust these treatments.

6. Mild cognitively impaired subjects present a consistent phase-advance of their rhythms, proving that small but significant changes occur before the onset of Alzheimer's Disease and could be used for screening purposes. TAP was able to capture these, still subtle, differences.

7. The usefulness of integrated variable TAP for circadian status evaluation, circadian synchronization and sleep-wake detection have proven to be so relevant that an international patent implementing TAP algorithm in a new ambulatory monitoring device has been registered.

GENERAL CONCLUSION

The reliable ambulatory monitoring of circadian system in humans has proven very relevant for disease prognosis in such conditions as cancer, mild cognitive impairments and sleep disturbances. The joint recording and following integration of several clock outputs increase results' consistency as isolated variable artifacts are minimized by the integration of several variables and enables the calculation of internal desynchronization among different rhythmic variables.

TAP, integration of wrist Temperature, motor Activity and body Position rhythms, has supported the premises of high reliability, acceptance and versatility for the long-period ambulatory study of human circadian system status under several conditions of health and disease. Thus, these positive results have facilitated the implementation of TAP's algorithm in a new device for ambulatory monitoring and registered in an international patent, already in exploitation.

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7. ANNEX

7.1. SCIENTIFIC PRODUCTION RESULTING FROM THE PRESENT PhD THESIS

7.1.1. Publications

1. Ortiz-Tudela E, Martinez-Nicolas A, Campos M, Rol MÁ, 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(11):e1000996. Impact factor: 5.76, 1st Quartile, category: Mathematical & Computational Biology (1/37).

2. Ortiz-Tudela E, Martinez-Nicolas A, Albares J, Segarra F, Campos M, Estivill E, Rol MÁ, Madrid JA (2013) Ambulatory Circadian Monitoring (ACM) based on Thermometry, motor Activity and body Position (TAP): A comparison with Polysomnography. *Physiology & Behavior*. In press. Impact factor: 3.16, 2nd Quartile, category: Behavioral Sience (19/49).

3. Ortiz-Tudela E, Iurisci I, Beau J, Karaboue A, Moreau T, Rol MÁ, Madrid JA, Lévi F, Innominato PF (2013) The circadian rest-activity rhythm, a potential safety pharmacology endpoint of cancer chemotherapy. *Int J Cancer*. In press. Impact factor: 6.20, 1st Quartile, category: Oncology (23/149).

4. Ortiz-Tudela E, Innominato PF, Rol MÁ, Lévi F, Madrid JA (2014) Assessing circadian phase by means of Ambulatory Circadian Monitoring in cancer patients. Close to submission.

5. Ortiz-Tudela E, Martinez-Nicolas A, Venero C, Madrid JA, Rol MÁ (2014) Biological rhythms characterization in mild cognitive impairment. Submitted.

6. Ortiz-Tudela E, Bonmatí-Carrión Mde L, De la Fuente M, Mendiola P (2012) Chronodisruption and ageing [article in Spanish]. *Rev Esp Geriatr Gerontol*. 47(4):168-73. doi: 10.1016/j.regg.2011.09.013.

7. Palesh O, Aldridge-Gerry A, Ulusakary A, **Ortiz-Tudela E**, Capuron L, Innominato PF (2013) Sleep disruption in breast cancer patients and survivors. *J Natl Compr Cancer Netw (JNCCN)*. 11:1523-1530. Impact factor: 5.11, 1st Quartile, category: Oncology (34/197).

8. Madrid JA, **Ortiz-Tudela E**, Martínez-Nicolás A, Rol, MÁ (2009) Sistema circadiano en el anciano: Valoración clínica e intervenciones terapéuticas. *Informaciones Psiquiátricas*. (195-196): 51-9.

7.1.2. Book chapters

1. Ortiz-Tudela E, Mteyrek A, Ballesta A, Innominato PF, Lévi F (2013) Cancer chronotherapeutics: experimental, theoretical, and clinical aspects. *Handb Exp Pharmacol*. (217):261-88. doi: 10.1007/978-3-642-25950-0_11.

2. Ortiz-Tudela E, Madrid JA, Rol MÁ (2014) Técnicas de estudio del sistema circadiano. *Tratado de medicina de sueño*. Section XVIII: methodology, chapter 116.

7.1.3. Communications to national and international congresses, workshops and seminars

1. Ortiz-Tudela E, Martínez-Nicolás A, Rol MÁ, Campos M, Madrid JA (2009) A new integrated index, based on thermometry, actimetry and body position (TAP) to evaluate circadian system status in human. *XI International Congress of the European Biological Rhythms Society*. Strasbourg (France). Oral communication.

2. Ortiz-Tudela E, Martinez-Nicolas A, Almaida-Pagan PF, Sarabia JA, Moreno M, Fuentelsaz C, Rol MÁ, Madrid JA (2010) Biological rhythms characterization in elderly people: wrist temperature, motor activity, body position and TAP. *International Conference of the International Society of Chronobiology*. Vigo (Spain). Oral communication.

3. Venero C, García S, Díaz C, Valencia A, Pereda-Pérez I, Rol MÁ, **Ortiz-Tudela E**, Martínez-Nicolás A, Madrid JA, Peraita H (2010) Neuropsychological markers and circadian secretion of cortisol for early detection of mild cognitive impairment. *IV National meeting of the Spanish Society of Geriatric Medicine (SEMEG)*. Salamanca (Spain). Poster

4. Ortiz-Tudela E, Martinez-Nicolas A, Martinez C, Albares J, Segarra F, Rol MÁ, Estivill E, Madrid JA (2011) Sleep-wake rhythms analysis by means of the integrated study of

Thermometry, motor Activity and body Position (TAP): polysomnographic validation. *XX National Annual meeting of the Spanish Sleep Society (SES)*. Sevilla (Spain). Poster.

4. Ortiz-Tudela E, Martinez-Nicolas A, Venero C, Madrid JA, Rol MÁ (2011) Biological rhythms characterization in mild cognitive impairment. *XII International Congress of the European Biological Rhythms Society (EBRS)*. Oxford (United Kingdom). Oral communication

5. Ortiz-Tudela E, Innominato PF, Rol MÁ, Madrid JA, Lévi F (2012) Circadian patterns in integrated wrist Temperature, rest-Activity, and Position (TAP) as a biomarker for personalized cancer chronotherapeutics. *International Congress of the Society for Research and Biological Rhythms (SRBR)*. Destin, Florida (United States of America). Poster**.**

7.1.4. International patents

1. Madrid Pérez JA, Sarabia Carrazo JA, Martinez-Nicolás A, Rol MA, **Ortiz-Tudela E**. Device consisting of a body position and activity sensor, a peripheral temperature sensor and a light sensor to provide information on the status of the circadian system. Publication date: March, the 22th of 2013. International Classification of Patents: A61B5/00.

7.2. ANNEX II. ADDITIONAL SCIENTIFIC PRODUCTION RESULTING FROM DIVERSE COLLABORATIONS AND RESEARCH PROJECTS

7.2.1. Publications

1. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MA (2011) Crosstalk between environmental light and internal time in humans. *Chronobiol Int*. 28(7):617-29.

2. 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*. 2013;8(4):e61142.

7.2.3. Communications to national and international congresses

1. Rol MÁ, **Ortiz-Tudela E**, Mendiola P, de Costa J (2007) Teaching biological rhythms in endocrinology: cortisol, wrist temperature and impact of weekend night's fever. *Second International School on Mind, Brain and Education. Basic and applied topics in biological rhythms and learning.* Erice (Italy). Oral communication.

2. Ortiz-Cullera V, Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (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.* Eirce (Italy). Oral communication.

3. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (2008) Development of a new method for the assessment of regularity: Chronozeit. *V International of Experimental and Health Science Students Congress.* Valencia (Spain) 2008. Oral communication.

4. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (2009) Influence of light exposure on skin wrist temperature rhythm in human under free-style living conditions. *XII International Congress of the European Biological Rhythms Society (EBRS)*. Strasbourg (France). Poster.

5. Cubero J, Sánchez CL, Sarabia JA, Martínez A, **Ortiz-Tudela E**, Rol MÁ, Rodríguez AB, Madrid JA, Barriga C (2009) Comparison of different parameters of sleep between

actimetry and polisomnography. *I Iberic congress of Biology and Society (CIBIOS)*. Badajoz (Spain). Poster

6. Ortiz-Tudela E, Martinez-Nicolas A, Pagan G, Pozo P, Madrid JA, Rol MÁ (2010) Assessment of circadian system status in patients with anorexia nervosa. *26th International Conference of the International Society of Chronobiology*. Vigo (Spain). Poster

7. Ortiz-Tudela E, Martinez-Nicolas A, Sarabia JA, Almaida P, Campos M, Moreno M, Amillategui R, Madrid JA, Rol MÁ (2010) Ageing of the circadian system by means of the integration of actimetry, body position and thermometry. *52 National congress of the Spanish Society of Geriatrics and Gerontology (SEGG)*. Valladolid (Spain). Poster

8. Martinez-Nicolas A, **Ortiz-Tudela E**, Almaida-Pagan PF, Sarabia JA, Moreno M, Gonzalez-Maria E, Madrid JA, Rol MÁ (2010) Effect of light exposure on wrist temperature rhythm. Effect of ageing. *26th International Conference of the International Society of Chronobiology*. Vigo (Spain). Poster

9. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (2010) Demasking wrist temperature rhythm under free-living conditions. *26th International Conference of the International Society of Chronobiology*. Vigo (Spain). Poster

10. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (2010) Effect of the voluntary light exposure on the sleep pattern and circadian system status. *XIX National annual meeting of the Spanish Sleep Society (SES)*. Alcoy (Spain). Poster.

11. Sosa M, Sosa J, Martinez-Nicolas A, **Ortiz-Tudela E**, Baño B, Madrid JA, Rol MÁ, Campos M (2010) Circadianware: a new informatic tool for the analysis of temperature, activity and body position circadian rhythms in humans. *XIX National annual meeting of the Spanish Sleep Society (SES)*. Alcoy (Spain). Poster.

12. Ortiz-Tudela E. Effects of light pollution on biodiversity and human health. Workshop on Dark Sky Protection. Sevilla (Spain) 2011. Invited talk.

13. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (2011) A constant routine approach to analyse wrist temperature rhythm under free-living conditions. *XII International Congress of the European Biological Rhythms Society (EBRS)*. Oxford (United Kingdom). Poster.

14. Martinez-Nicolas A, **Ortiz-Tudela E**, Madrid JA, Rol MÁ (2011) Light exposure pattern influences wrist temperature rhythm in humans. *XII International Congress of the European Biological Rhythms Society (EBRS)*. Oxford (United Kingdom). Poster.

15. Mendiola P, de Costa J, **Ortiz-Tudela E**, Martinez-Nicolas A, Bonmati-Carrion MA, Lucas-Sanchez A, Baño-Otalora B, Madrid JA, Rol MÁ (2011) Teaching biological rhythms in endocrinology: cortisol and wrist temperature. International Congress on Teaching Innovation (CIID). Cartagena (Spain)

16. Bonmatí-Carrión MA, Rico F, Martínez-Nicolás A, **Ortiz-Tudela E**, Mondéjar MT, Otálora BB, Madrid JA, Rol MA (2011) Circadian timing system assessment in blind people by means of wrist skin temperature. *XX National annual meeting of the Spanish Sleep Society (SES)*. Sevilla (Spain). Poster.

17. Martinez-Nicolas A, Sarabia JA, **Ortiz-Tudela E**, Tortosa F, Madrid JA, Rol MÁ (2011) Kronosensor, a new device for circadian rhythm analysis. *XX National annual meeting of the Spanish Sleep Society (SES)*. Sevilla (Spain). Poster.

18. Ortiz-Tudela E, Martinez-Nicolas A, Madrid JA, Rol MÁ (2013) Cortisol, Perifpheral temperature and the effect of nocturnal exits in the week end. *V International of Experimental and Health Science Students Congress*. Valencia (Spain) 2008. Oral communication.

7.2.4. 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. Circadianware (MU/171/2010), University of Murcia. Murcia (Spain) 2010.

7.3. ANNEX III. STAYS IN LABORATORIES OUT OF THE UNIVERSITY OF MURCIA DURING THE REALIZATION OF THE PhD

1. Estivill's Sleep Clinic (Instituto Dexeus from Barcelona, Spain).
Responsible researchers: MD Eduard Estivill
Duration: 3 weeks (04/26/2010 – 05/16/2010)

2. INSERM U776 "Biological Rhythms and Cancer". Hôpital Paul Brousse (Villejuif, France)
Responsible researchers: MD PhD Francis Lévi
Duration: 47 months (09/01/2010 – 12/22/2010 & 09/01/2011 – 06/16/2012)

7.4. ANNEX IV. RESEARCH PROJECTS SUPPORTING THE EXPERIMENTS PERFORMED IN THE PRESENT PhD

1. Project Title: Chronodisruption and aging: animal models
Funding Body: Fundación Séneca (05700/PI/07)
Duration: 2007-2009

2. Project Title: Premature aging and disruption of the circadian system. Role of melatonin
Funding Body: CICYT (BFU2007-60658)
Duration: 2007-2010

3. Project Title: Research network on aging and fragility-RETICEF
Funding Body: Instituto de Salud Carlos III (RD06/0013/0019)
Duration: 2007-2012

4. Project Title: The development of clinical applications for ambulatory thermography and actigraphy.
Funding Body: Centro Médico Virgen de la Caridad
Duration: 2010-2011

5. Project Title: Exercise as a modulating factor of aging. Effect on oxidative stress
Funding Body: Fundación Séneca (12005/PI/09)
Duration: 2010-2011

6. Project Title: Preventing chronodisruption by circadian-healthy lighting. Relevance in aging and cancer
Funding Body: CICYT (BFU2010-21945-C02-01)
Duration: 2011-2013

7. Project Title: Circadian system functionality, work environment and the organization of nursing care in hospitals of the Spanish National Health System.
Funding Body: Instituto de Salud Carlos III
Duration: 2012-2014

7.5. ANNEX V.

During her PhD and the writing of the Doctoral Thesis, Elisabet Ortiz-Tudela has been granted with a Spanish Ministry of Science PhD Scholarship AP2008-02850 (FPU: Formación de Profesorado Universitario), from July 2009 to July 2013.

8. RESUMEN EN ESPAÑOL

OBJETIVOS

Esta tesis pretende desarrollar una herramienta de fácil aplicación, fiable, consistente y no invasiva que sea capaz de evaluar el estatus del sistema circadiano humano a partir de la información suministrada por sensores de temperatura, actividad y posición.

Para conseguir este objetivo, se plantearon los siguientes objetivos específicos:

1. Crear un algoritmo que facilite la evaluación ambulatoria y no invasiva del estado funcional del sistema circadiano mediante la integración de los ritmos de temperatura periférica, actividad motora y posición corporal en la variable compuesta TAP.
2. Validar la variable integrada TAP para la detección de sueño y vigilia en comparación con el *gold standard* para este propósito, la polisomnografía. Además, se pretende comprobar si TAP mejora la detección de sueño con respecto a las estimaciones obtenidas mediante la actigrafía convencional
3. Transferir la utilidad de TAP, implementada en un dispositivo de registro ambulatorio, para su aplicación clínica y su potencial comercialización en una patente internacional.
4. Evaluar la validez de la variable TAP para la detección de alteraciones circadianas en condiciones patológicas:

   4.1. Revisar los conocimientos previos sobre cáncer y ritmos circadianos, prestando especial atención a los tratamientos que tienen en cuenta el sistema circadiano para esta enfermedad.

   4.2. Estudiar las diferencias interindividuales del ritmo de actividad-reposo que podrían potencialmente afectar el resultado del tratamiento en pacientes de cáncer colorrectal.

   4.3. Evaluar como los tratamientos cronomodulados afectan la sincronización interna de diferentes ritmos circadianos en pacientes de cáncer.

   4.4. Evaluar las perturbaciones circadianas de sujetos con deterioro cognitivo leve, una condición previa a la enfermedad de Alzheimer, con el fin de establecer la utilidad de la variable integrada TAP para evaluar objetivamente la progresión de la enfermedad.

CAPÍTULO 1: Una nueva variable integrada basada en Termometría, Actimetría y Posición corporal (TAP) para evaluar el estatus del sistema circadiano en humanos.

La disrupción del sistema circadiano en humanos se ha asociado con el desarrollo de enfermedades crónicas y con el empeoramiento de patologías pre-existentes. Por lo tanto, la evaluación de la función del sistema circadiano en condiciones ambulatorias mediante el uso de técnicas no invasivas requiere más investigaciones. Tradicionalmente, los ritmos manifiestos tales como actividad y temperatura corporal se han analizado por separado. Sin embargo, un análisis integrado podría reducir los artefactos individuales. De este modo, se ha desarrollado una nueva variable, denominada TAP, basada en el análisis integrado de tres medidas simultáneas: el ritmo de temperatura de la piel de la muñeca (T), el ritmo de actividad motora (A) y el ritmo de posición corporal (P). Igualmente validamos la fiabilidad de un índice numérico individual, el Índice de Funcionamiento Circadiano (CFI), para determinar la robustez circadiana en un periodo de tiempo determinado.

Para ello, se colocaron un actímetro y un sensor de temperatura en el brazo y la muñeca de la mano no dominante, respectivamente, de 49 sujetos jóvenes y sanos durante una semana. Los valores de T, A y P se normalizaron para cada sujeto. Se llevaron a cabo análisis cronobiológicos no paramétricos para el cálculo de la estabilidad interdiaria, variabilidad intradiaria y amplitud relativa de TAP y cada una de las variables individuales. Estos valores se utilizaron para la determinación del CFI de cada sujeto. Además, con el fin de determinar la fiabilidad de TAP y CFI a la hora de detectar alteraciones circadianas, se llevaron a cabo simulaciones matemáticas en las que se generaron diferentes patrones circadianos. Cada variable (T, A, P and TAP) se correlacionó con los diarios de actividad-reposo cumplimentados por los voluntarios. La correlación más alta con dichos diarios ($r=0,993$; $p=0,0001$), además de la mayor especificidad (0,870), sensibilidad (0,740) y grado de concordancia (0,904) fue la obtenida para el TAP. Además, CFI mostró ser muy sensible a los cambios de robustez circadiana. Nuestros resultados muestran que la variable integrada TAP y el cálculo de CFI son métodos muy potentes para evaluar el estatus del sistema circadiano, mejorando la sensibilidad, especificidad y tasa de acuerdo en la detección de reposo y actividad por encima del análisis de la temperatura periférica, posición corporal o actividad motora consideradas individualmente.

CAPÍTULO 2: Monitorización circadiana ambulatoria (ACM) basada en Termometría, Actividad motora y Posición corporal (TAP): comparación con polisomnografía.

Con el fin de determinar la capacidad de la variable integrada TAP para la detección de patrón de sueño-vigilia, se validó frente al *gold standard* para los estudios de sueño, la polisomnografía (PSG).

Para ello, se estudiaron durante una noche 22 sujetos que padecían algún desorden del sueño mediante un sensor de temperatura (iButton®), un actímetro (Hobo®) y una PSG exploratoria. Se analizaron las ondas medias de cada variable, comparando la variable TAP con las etapas de sueño y se calcularon los valores de sensibilidad (SE), especificidad (SP) y tasa de concordancia (AR) para todas las variables, individuales y compuestas.

La variable integrada TAP optimizó los valores de SE, SP y AR con respecto a las variables individuales (SE: 92%; SP: 78%; AR: 86%). Además, estos resultados mejoraron las estimaciones previamente publicadas para la actimetría. Asimismo, los valores de TAP tendían a disminuir conforme la profundidad de sueño se incrementaba, alcanzando el valor más bajo en la fase 3. Finalmente, las estimaciones de TAP para la latencia de sueño (SL: 37 ± 9 min), tiempo total de sueño (TST: 367 ± 13 min), eficiencia de sueño (SE: 86.8 ± 1.9%) y número de despertares (NA>5 min: 3.3 ± .4) no fueron significativamente distintos a las estimaciones obtenidas de la PSG (SL: 29 ± 4 min; SE: 89.9 ± 1.8%; NA>5 min: 2.3 ± .4) a pesar de la heterogeneidad en las patologías estudiadas. Por lo tanto, la variable integrada TAP representa un nuevo sistema de medida para la evaluación del estatus del sistema circadiano y del ritmo de sueño-vigilia con un nivel de fiabilidad mayor que el de la actigrafía. Además, este sistema permite la evaluación del ritmo de sueño-vigilia de un paciente en su propio ambiente a un coste mucho menor que el de una PSG. Estudios futuros en patologías concretas podrán verificar la fiabilidad de TAP en esas condiciones.

CAPÍTULO 3: Cronoterapia del cáncer: aspectos experimentales, teóricos y clínicos.

El sistema circadiano controla el ciclo celular, la apoptosis, la bioactivación de medicamentos y los mecanismos de transporte y detoxificación en tejidos sanos. Consecuentemente, la tolerancia de la quimioterapia del cáncer varía hasta varias veces en función de la hora circadiana de administración en modelos experimentales. La mayor eficacia antitumoral de un agente quimioterápico, o de la combinación de varios, normalmente se consigue cuando su administración se acerca a las correspondientes horas de mejor tolerancia. Los modelos matemáticos revelan que esa coincidencia entre cronotolerancia y cronoeficacia se explica a través de diferencias en la dinámica circadiana y en el ciclo celular del huésped y del tumor, especialmente con respecto al encarrilamiento circadiano y a la variabilidad del ciclo celular.

En la clínica, se ha demostrado una gran mejora de la tolerancia en ensayos internacionales aleatorizados donde los pacientes de cáncer recibieron la misma cronoterapia, modulada de modo sinusoidal, en periodos de 24 horas en comparación con infusiones a tasa constante o cronoterapias desplazadas en el tiempo. Sin embargo, las características genéticas, el sexo y el estilo de vida influyen a la hora de optimizar la cronoterapia. Estos resultados apoyan las aproximaciones de biología de sistemas que implican el mapeo sistemático experimental, el modelado de vías cronofarmacológicas en cultivos celulares sincronizados y su ajuste en modelos de ratón de ambos sexos y distinta carga genética, tal y como se ha demostrado recientemente para el irinotecan. La administración de agentes quimioterápicos antitumorales en base a las características circadianas individuales, usando biomarcadores circadianos y basada en modelos matemáticos apunta a que las tecnologías de administración de medicamentos de modo circadiano permiten una mejora conjunta de la tolerabilidad y la eficacia de estos agentes.

CAPÍTULO 4: El ritmo circadiano de actividad-reposo, un objetivo farmacológico potencial para la quimioterapia del cáncer.

La robustez del sistema circadiano se ha correlacionado con la calidad de vida y con la predicción de una supervivencia más prolongada en pacientes de cáncer. Sin embargo, la administración de quimioterapia es capaz de perturbar el sistema circadiano en función de la dosis y hora circadiana en ratones.

Así, con el fin de caracterizar la dinámica del sistema circadiano en pacientes de cáncer sometidos a un protocolo estándar de cronoterapia, se aplicó un diseño continuo, longitudinal y de medidas repetidas. El ritmo actividad-reposo de 49 pacientes con cáncer avanzado se monitorizó empleando actigrafía convencional durante 13 días consecutivos que se correspondían con 4 periodos consecutivos de 3 ó 4 días cada uno: antes, durante, inmediatamente después y un poco después de la administración de un ciclo de quimioterapia cronomodulada estándar.

La cantidad relativa de actividad dentro y fuera de cama (I<O), el coeficiente de autocorrelación r24, la amplitud relativa (AR), la estabilidad interdiaria (IS) y la variabilidad intradiaria (IV) se compararon en función del periodo de estudio.

La administración de cronoterapia generó disrupción circadiana (considerada por debajo del umbral de 97,5% para I<O), empeorando significativamente ($p<0,05$). los cinco parámetros rítmicos mencionados. Los valores medios de estos parámetros se recuperaron tras el tratamiento hasta alcanzar valores cercanos a los iniciales. La existencia de disrupción circadiana durante la quimioterapia se asociaba con un mayor cansancio clínicamente relevante ($p=0,028$) y pérdida de peso corporal ($p=0,05$). Además, encontramos cuatro patrones de evolución del sistema circadiano en respuesta al tratamiento: sin cambio en un 9,5% de los pacientes, mejora (un 14,3%), alteración y recuperación completa (31%) o deterioro sostenido (45%), posiblemente debido una dosis de quimioterápico inadecuada y/o un horario incorrecto. Por ello, minimizar la disrupción circadiana personalizando la administración de la cronoterapia podría suponer una mejora en la tolerancia clínica.

CAPÍTULO 5: Evaluación de la fase circadiana mediante la Monitorización Ambulatoria Circadiana en pacientes de cáncer.

La disrupción circadiana basada en la monitorización del ritmo de actividad-reposo predice una menor supervivencia en pacientes de cáncer. Sin embargo, incluir otras variables rítmicas puede ayudar a evaluar de forma integral el estatus del sistema circadiano a lo largo de un ciclo de quimioterapia. Uno de estos procedimientos está basado en el cálculo de la variable integrada TAP que pretende permitir la personalización de la quimioterapia del cáncer según las características circadianas de cada paciente.

De este modo, se registró la variable TAP en intervalos de 10 minutos en periodos de 4 días antes, durante y después de un ciclo de cronoterapia estándar en pacientes con cáncer colorrectal (n=21), pancreático (n=2) o esofágico (n=1) (11 hombres y 13 mujeres, 60,6 ± 2,4 años). Para cada paciente y periodo, se calculó I<O, se efectuaron análisis no paramétricos y se ideó un nuevo biomarcador del grado de mantenimiento del orden temporal interno o de desincronización interna (DI).

Tanto los 3 ritmos individuales circadianos estudiados como TAP presentaron bajos valores de estabilidad y altos de fragmentación durante la administración del tratamiento. Además, para T, A, P y TAP se encontró una gran variabilidad inter- e intra-individual, con diferencias de fase de hasta 12 horas entre pacientes. Igualmente, se encontró una perturbación moderada del orden temporal interno, medida mediante el DI, pero en este caso, la administración de un protocolo de quimioterapia cronomodulada estándar fue capaz de resincronizar parcialmente los ritmos de temperatura y actividad al final del estudio en la mayoría de los pacientes. Se observaron diferencias de género antes del inicio de la quimioterapia y si bien, las mujeres presentaron ritmos más robustos que los hombres, fueron también las más afectadas por la quimioterapia.

La utilización de la variable integrada TAP, al igual que el grado de asincronía entre los ritmos de temperatura y actividad (DI) en pacientes de cáncer permitirá la personalización de la cronoterapia del cáncer considerando los marcadores de fase individuales.

CAPÍTULO 6: Caracterización de los ritmos biológicos en pacientes con deterioro cognitivo leve

Un hecho bien conocido en pacientes de demencia, especialmente en aquellos que padecen la enfermedad de Alzheimer (AD), es la presencia de una arquitectura anormal del ritmo de sueño-vigilia, al igual que ocurre con los patrones circadianos de otras variables rítmicas. Estos hechos se han relacionado con una perturbación de su reloj biológico causada por la enfermedad en sí misma, sin que la edad sea su única causa. Sin embargo, apenas existe información de la organización circadiana en una etapa previa a la aparición de la demencia. Así, el objetivo de este capítulo fue el de dilucidar si ya existen alteraciones en el sistema circadiano de pacientes con deterioro cognitivo leve (MCI) comparado con personas mayores sanas de la misma edad.

Cuarenta sujetos (21 pacientes diagnosticados con MCI, de edad de 74,1 ± 1,5 años y 19 sujetos sanos de edad 71,7 ± 1,4 años) se monitorizaron ambulatoriamente mediante el estudio del ritmo de temperatura de la piel de la muñeca (iButton, ThermoChron®, Data loggers I-button, IDC S.A., Spain) y de los ritmos de actividad motora y posición corporal (Hobo® Pendant G Acceleration Data Logger) durante una semana. Para la caracterización rítmica de cada variable y del TAP se realizaron análisis no paramétricos.

Los pacientes con MCI exhibieron un adelanto de fase significativo en dos marcadores de fase de temperatura: M5 (media ± SEM: 04:20 ± 00:21 vs 02:52± 00:21, sanos frente a MCI, respectivamente, $p<0,05$) y L10 (media ± SEM: 14:35 ± 00:27 vs 13:24 ± 00:16, sanos frente a MCI, respectivamente, $p<0,05$); y dos marcadores de TAP: L5 (media ± SEM: 04:18 ± 00:14 vs 02:55 ± 00:30, sanos frente a MCI, respectivamente, $p<0,05$) y M10 (media ± SEM: 14:30 ± 00:18 vs 13:28 ± 00:23, $p<0,05$, sanos frente a MCI, respectivamente).

Además, la zona de mantenimiento de la vigilia para el ritmo de la temperatura (caracterizado por valores mínimos de temperatura entre las 19:00 y las 21:00h) tendió a desaparecer en sujetos sanos mayores, un fenómeno que fue aún más pronunciado en sujetos con MCI.

Estos resultados prueban que en una etapa temprana de demencia ocurren avances significativos en la fase del reloj biológico, es decir, estos pacientes muestran un declive funcional acelerado comparado con una población sana de la misma edad.

CAPÍTULO 7: Implementación de TAP en una patente internacional (dispositivo consistente en un sensor de posición corporal y actividad, un sensor de temperatura periférica y un sensor de luz para proveer información sobre el estatus del sistema circadiano)

La invención se trata de un dispositivo consistente en al menos un sensor de posición corporal y actividad motora, al menos un sensor de temperatura periférica y al menos, un sensor de luz, configurado para proveer información sobre el estatus del sistema circadiano y para la detección del patrón de sueño-vigilia de un individuo basado en los datos obtenidos de estos sensores.

El dispositivo inventado almacena y procesa la información sobre los parámetros de la temperatura periférica de la muñeca, posición corporal, actividad y luz ambiental. Todos los sensores pueden programarse para registrar información en un amplio rango de intervalos de tiempo.

La implementación preferida es un dispositivo basado en la invención y que se localiza en la muñeca del sujeto. Otra implementación es otro dispositivo consistente en, al menos, un sensor de presión sanguínea que se programa para proveer información sobre el estatus de la presión sanguínea del sujeto. Este dispositivo mejora la precisión diagnóstica de los cambios del ritmo de presión sanguínea circadiana, permitiendo la evaluación objetiva del periodo de reposo del sujeto determinada por los cambios de posición. Otra implementación preferida es que los sensores de actividad motora, posición corporal y presión sanguínea estén localizados en el brazo del sujeto.

Finalmente, otra implementación de la invención es el proceso para determinar el estatus del sistema circadiano y el estatus de sueño o vigilia del sujeto consistente en:

1. La obtención de la temperatura periférica, actividad motora por minuto y valores de posición corporal para el sujeto.
2. Extraer conclusiones sobre el estatus del sistema circadiano y de los estados de sueño y vigilia basados en los cambios de los valores del paso 1.

Del mismo modo, una implementación preferida implica un procedimiento para usar la invención de modo de obtener información sobre el patrón de presión arterial e hipertensión, caracterizado por el hecho de que en el paso 1 las medidas de presión arterial también se recogen para el sujeto y en el paso 2 los valores de presión arterial

se comparan, con el resto de los valores para determinar los cambios en presión arterial en función de estar tumbado o de pie.

CONCLUSIONES

1. La integración de los ritmos de temperatura de la piel de la muñeca, actividad motora y posición corporal en la variable TAP permite la evaluación objetiva, ambulatoria y no invasiva del estado funcional del sistema circadiano humano.
2. La variable integrada TAP es lo suficientemente potente para diferenciar el sueño de la vigilia, comparado con el *gold standard* para los estudios sobre sueño, la polisomnografía. Además, TAP mejora consistentemente las estimaciones para detección de sueño de la actigrafía.
3. La utilidad de la variable integrada TAP para la evaluación del estatus circadiano, la sincronización circadiana y la detección de sueño-vigilia ha demostrado ser tan relevante que se ha registrado una patente internacional que implementa el algoritmo de TAP en un dispositivo de monitorización ambulatoria que ya ha sido comercializado. El dispositivo ya ha mostrado su utilidad a la hora de detectar alteraciones circadianas y de sueño en situaciones tan diferentes como apnea obstructiva de sueño, autismo, trastornos de sueño, ensayos clínicos sobre fármacos...
4. Los tratamientos contra el cáncer basados en principios cronobiológicos, en los que los medicamentos se administran de forma cronomodulada, se relacionan con una alta eficacia y unos efectos secundarios menores y representan una opción interesante a las terapias tradicionales.
5. La administración de quimioterapia contra el cáncer colorectal produce una perturbación general del ritmo de actividad-reposo. Sin embargo, entre los pacientes aparecen patrones de evolución distintos, sugiriendo la necesidad de tratamientos personalizados en función de sus características cronobiológicas.
6. Existen diferencias de hasta 12 horas en los marcadores de fase estudiados en pacientes de cáncer. Además, se ha puesto de manifiesto un alto grado de desincronización interna en estos pacientes. Ambos aspectos tienen efecto sobre la eficacia de los tratamientos y la supervivencia. De este modo, la personalización de los tratamientos sugerida en la conclusión anterior podría

beneficiarse de la utilización de la herramienta TAP para ajustar individualmente este tipo de tratamientos.

7. Los pacientes de deterioro cognitivo leve presentan un avance de fase en sus ritmos circadianos evaluados mediante TAP, demostrando que aunque pequeños, los cambios en el sistema circadiano ya empiezan a aparecer antes del inicio de la enfermedad de Alzheimer. Estos cambios podrían potencialmente ser útiles para estudios poblacionales de detección precoz.

CONCLUSIÓN GENERAL

La monitorización circadiana ambulatoria, no invasiva y fiable del estado funcional del sistema circadiano humano es un procedimiento muy útil para la prognosis de ciertas enfermedades como cáncer, deterioro cognitivo leve y perturbaciones del sueño. La monitorización conjunta y la integración de varias salidas del reloj biológico mejora la consistencia de los resultados y facilita la evaluación de la desincronización interna entre diferentes variables rítmicas.

La variable TAP, basada en la integración de los ritmos de Temperatura periférica, Actividad motora y Posición corporal, ha superado las premisas de alta fiabilidad, aceptación y versatilidad para el estudio ambulatorio y prolongado del estado del sistema circadiano humano en muy distintas situaciones de salud y enfermedad. Así, estos resultados positivos han permitido implementar el registro combinado de diferentes variables circadianas y el empleo de TAP en un dispositivo para la monitorización circadiana ambulatoria que se ha sido objeto de una patente internacional que se encuentra en explotación comercial.