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Circadian Rhythm

Cellular and Molecular Mechanisms

Edited by Mohamed Ahmed El-Esawi

CIRCADIAN RHYTHM - CELLULAR AND MOLECULAR MECHANISMS

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Meet the editor

Dr. Mohamed Ahmed El-Esawi is currently a visiting research fellow at the University of Cambridge in the United Kingdom and an associate professor of Molecular Genetics at Botany Department of Tanta University in Egypt. Dr. El-Esawi received his BSc and MSc degrees from Tanta University and his PhD degree in Plant Genetics and Molecular Biology from Dublin Institute of Technology in Ireland. Afterwards, Dr. El-Esawi joined the University of Warwick in the UK, University of Sorbonne (Paris VI) in France and University of Leuven (KU Leuven) in Belgium as a visiting research fellow. His research focuses on plant genetics, genomics, molecular biology, molecular physiology, developmental biology, plant-microbe interaction and bioinformatics. He has authored several international journal articles and book chapters and participated in more than 60 conferences and workshops worldwide. Dr. El-Esawi is currently involved in several research projects in biological sciences.

Contents

Preface

Circadian clocks are endogenous and temperature-compensating timekeepers that provide temporal organization of biological processes in living organisms including cyanobacteria, plants, animals and humans. Circadian rhythms allow living organisms to adapt to the daily light cycles associated with Earth's rotation and to anticipate and prepare for precise and regular environmental changes. The endogenous circadian rhythms are adjusted to the environment by different surrounding cues, such as temperature, light and redox cycles. Biological clocks regulate several biological functions, including hormone levels, body temperature, cell regeneration, metabolism, photoperiodism and other biological activities.

This book discusses the fundamental advances of how the circadian clock regulates critical biological functions as well as the cellular and molecular mechanisms controlling circadian rhythm in living organisms. It also provides new insights into and sheds new light on the current research trends and future research directions related to circadian rhythm. This book provokes interest in many readers, researchers and scientists, who can find this information useful for the advancement of their research works towards a better understanding of circadian rhythm regulatory mechanisms.

The book includes eight chapters. The first introductory chapter "Circadian Rhythms and Their Molecular Mechanisms" presents an introduction to the history, importance and molecular mechanisms of circadian rhythms in living organisms. The second chapter "Circadian Rhythm and Chronobiology" discusses the recent progresses related to circadian biological research as well as understanding how biological clocks govern the human metabolism. The third chapter "Circadian Clock Gene Expression and Drug/Toxicant Interactions as Novel Targets of Chronopharmacology and Chronotoxicology" discusses the interactions of circadian clock genes with drugs and/or toxicants to better understand the importance of circadian clock gene expression as novel targets in pharmacology and toxicology. The fourth chapter "Quantification of Irregular Rhythms in Chronobiology: A Time Series Perspective" compares wavelets and SSA analysis for the quantification of irregular rhythms at different frequency scales and discusses their respective advantages and disadvantages for application in chronobiology. The fifth chapter "Jet Lag" describes the phenomenon of jet lag and its associated symptoms. The sixth chapter "Features of Circadian Rhythms in Patients with Cerebrovascular Diseases" describes in detail the pathogenetic role of desynchronosis in the development of cerebrovascular diseases (CVD). The seventh chapter "The Chronobiology of Acid-Base Balance under General Anaesthesia in Rat Model" reviews the status of acid-base balance and ion concentration in arterial blood under commonly used anaesthesias in experiments in dependence on the light-dark cycle in breathing rats. The eighth chapter "Sudden Death of Circadian Rhythm in Chagasic Patients

Compared to Non-Chagasic Patients" compares the circadian rhythm of sudden death in Ch versus non-Ch patients.

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Chapter 1

Introductory Chapter: Circadian Rhythms and Their Molecular Mechanisms

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1. Introduction

A circadian rhythm is a biological process which shows an endogenous and entrainable oscillation of about 24 h. These 24-h rhythms are regulated by a circadian clock and are widely displayed in different organisms, including plants, fungi, animals, and cyanobacteria [1]. The endogenous circadian rhythms are adjusted to the environment by different surrounding cues, such as temperature, light, and redox cycles. In 2017, Jeffrey C. Hall and his colleagues have awarded the Nobel Prize in Physiology or Medicine for their discoveries of molecular mechanisms controlling the circadian rhythm. Circadian system runs as a result of four main components: (i) photosensitive retinal neurons and retinohypothalamic tract through which light signals come from the environment, (ii) internal circadian oscillator, generating rhythms and synchronizing them with the environment, (iii) signal paths transmitting information from the central regulator to peripheral rhythm generators, and (iv) peripheral rhythm generators (clock genes and proteins in peripheral cells).

In 1729, the French scientist Jean-Jacques d'Ortous de Mairan reported the first observation of an endogenous circadian oscillation and found that 24-h patterns in the movement of the leaves of the plant species *Mimosa pudica* continued even when the plants were kept in constant darkness [2, 3]. In 1896, Patrick and Gilbert reported that during a prolonged sleep deprivation period, sleepiness can increase and decrease within a period of approximately 24 h [4]. Furthermore, in 1918, J.S. Szymanski reported that the animals have shown their capability of maintaining 24-h activity patterns even in the absence or changes of external factors such as light and temperature. Circadian rhythms were also reported in the bees rhythmic feeding times in the early twentieth century. In 1935, circadian rhythms were also noticed in the fruit fly *Drosophila melanogaster* [5, 6]. In 1954, Colin Pittendrigh reported that temperature played a crucial role in eclosion rhythm, and the eclosion period was delayed without stopping when the temperature decreased, indicating that circadian rhythm is controlled by an internal

biological clock [6, 7]. The first clock mutant was identified in *Drosophila* and was called "period" (*per*) gene, which is the first discovered genetic determinant of behavioral rhythmicity [8]. Konopka, Jeffrey Hall, Michael Roshbash, and their team reported that *per* locus represents the center of the circadian rhythm and that loss of *per* locus stops circadian activity [9, 10]. Michael W. Young's team also demonstrated similar roles of *per*, which covers 7.1-kilobase (kb) interval on the X chromosome and encodes a 4.5-kb poly(A) + RNA [11, 12]. The key genes and neurons in *Drosophila* circadian system were also discovered, for which Jeffrey C. Hall and his colleagues received the Nobel Prize in Physiology or Medicine 2017. Moreover, Joseph Takahashi identified the first mammalian circadian clock mutation (*clock*) in mice in 1994 [13]. However, recent reports revealed that the deletion of *clock* does not result in a behavioral phenotype, which questions its potential role in rhythm generation [14, 15].

2. Importance and molecular mechanisms of circadian rhythms

Circadian rhythms enable organisms to better prepare and capitalize on environmental factors (e.g., light and food) as compared to those that are not able to predict such availability. They are also important in regulating and coordinating internal physiological processes [16]. Photoperiodism, the physiological reaction of organisms to the length of day or night, is essential to both plants and animals, and the circadian system plays a crucial role in the measurement of day length. The rhythm is linked to the light-dark cycle. Plant circadian rhythms tell the plant what season it is and when to flower to better attract pollinators. A better understanding of plant circadian rhythms has applications in agriculture, such as helping farmers to extend crop availability and secure it against massive losses due to weather. In addition, *Bmal1* and *clock* proteins are accumulated during daytime forming the *bmal1*/*clock* complex which helps in activating the transcription of the *per* (*per1, per2,* and *per3*) and *cry* genes (*cry1* and *cry2*). The *per* and *cry* proteins also form a *per*/*cry* dimer which moves to the cell nucleus and inhibits the activity of the *bmal1*/*clock* complex, then leads to a reduction in *per* and *cry* protein expression. During nighttime, *per*/*cry* complex is destroyed, and the 24-h cycle begins. Another clock gene involved in the regulation of this cycle is *rev-erb*-alpha. The *bmal1*/*clock* complex activates the transcription of such a gene, which leads to the accumulation of *reverb-alpha* protein which in turn inhibits the transcription of the *bmal1* gene. In conclusion, this work would discuss the circadian rhythm phenomena and their molecular mechanisms in different organisms.

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Chapter 2

Circadian Rhythm and Chronobiology

Hülya Çakmur

Additional information is available at the end of the chapter

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Abstract

All photosensitive organisms have a biological clock to cope with daily and seasonal circle of the earth. Biological clocks create circadian rhythms and regulate their processing according to cycle of the world. Circadian rhythm is an autoregulatory system and commands almost every physiological, biological, and biochemical functions of the mammalians. Therefore, biological clocks operate rhythmically with a period for a day, and this phenomenon is called as circadian rhythm. The process ongoing approximately 24 h rhythm in accordance with the meaning of the word (circa (approx.) dies (1 day). The essential purpose of biological clocks (in other word the organism's innate timing device) is the adaptation of the living organism to environment. Circadian rhythms refer to changes in the organism's approximately 1 day's physiological, biochemical and biological processes. In a molecular level, there are thousands of biological clocks in the human body. The main clocks in the human brain coordinate all these cellular clocks. Thus, the rhythmical phenomenon works in a harmony with the master clock and solar cycle. However, the natural factors within the body produce circadian rhythms the environment cues also affect them. The light is the main cue that affects biological rhythm. These light–dark cycles can control of the molecular structure of biological clocks. Changing the light–dark cycles leads to lengthen, shorten or completely absent of circadian rhythms. Dysfunction of circadian cycle leads to many health problems. The studies in chronobiology provide better understanding of the rhythmic metabolism and disrupted circadian rhythms. In recent years, numerous spectacular researches have been conducted in the field of chronobiology. These researches were intended to understand metabolic process of human body. However, the molecular and genetic mechanisms of circadian clocks still not clearly known. In this study, we aimed to investigate and summarize the recent progress about circadian biological research and understand how biological clocks govern the human metabolism.

Keywords: biological clocks, circadian rhythms, chronobiology

1. Circadian rhythms

Circadian rhythms are well-defined biological rhythmic activities ongoing 24-h time in human metabolism. Biological cyclical variation (circadian-biological-rhythm) is a physiological and kinetic event. It is clearly known that there is a molecular and neuronal connection between circadian clock and human metabolism. Thus circadian rhythms operate almost every functions of the metabolism from sleep/wake to fasting/feeding behavior through control of the cellular and organic system [1–3]. Biological rhythm is not exclusive to human being. Almost every living organism from plant and microorganism, to animal (in other words from single cell to multicellular) has own circadian rhythm [3]. Biological rhythms provide adaptation for living organism to environment both inside and outside. Circadian rhythms are the essential component of homeostasis in human body. Biological clocks are responsible to control physiological, homeostatic, behavioral and endocrine balance in organism [4]. In a nutshell, the repetitive fluctuations in biological, physiological and biochemical functions of the organisms within the period of 24 h are called circadian rhythms. Biological clock is a physiological system, which has the capacity to measure the passage of time in the living organism. Weger et al. have been shown that how the circadian clock contributes to adult stem cell function especially in the brain and neurogenesis [5]. The main issue is to provide the organism adaptation for daily and seasonal changes [6]. Biological rhythms are responsible for secretion of the hormones, the electrical activity of the hearth, body temperature, respiratory motion, and sleep-wakefulness. The main determinative circadian rhythm of the mammalian is sleep and wakefulness cycle [7]. Circadian rhythms, which take those 24 h, are examined in two parts: as nocturnal and diurnal rhythm. Nocturnal rhythm describes changes in the biological rhythm of the night, and diurnal rhythms refer to the biological rhythms that occur during the day. Human being has a diurnal activity pattern [8]. The other classification of the circadian rhythms is the infradian and ultradian rhythms. Infradian rhythms are the long rhythms that last more than from 24 h to weeks, months (examples are lunar −29.5 days- and semi lunar −14 days- rhythm. Ultradian rhythms are the short rhythms than 24 h (examples are tidal rhythms −12 h-). Ultradian rhythms are the important part of the circadian rhythms which regulate physiological functions of the body. When circadian rhythm regulated by natural environmental conditions, it is called entrained rhythm. In the case of the regulation isolated from environment, it is named as free-running rhythm or inner rhythm. There are well-defined patterns of physiological indices such as brain wave activity, cardiovascular, respiratory function, body temperature, and circadian rhythmicity [7]. The relatively new science “chronobiology” examines this biological cyclic phenomenon in living organisms. Chronobiology investigates the underlying mechanisms of chronomes which structures timing in organisms [8, 9]. Medical chronobiology is concerned with the chronopathology and chronopharmacology. Chronophysiology, chronopathology, and chronopharmacology are defined and still developing other fields about circadian rhythm. Chronophysiology examines how fitting in time setup the transaction of organisms and biological systems. Because of circadian rhythm underlie homeostasis of the body in case of dysrhythmia, the function of individual cells and so whole organisms affects negatively [10]. It has been shown that many diseases are related to dysfunction of the circadian rhythm. Consequently, chronopathology is concerned with the effect of biological rhythm in diseases formation. It deals with

the pathology of diseases caused by disrupted rhythm. Chronopharmacology search for the timing of administration of medications and effects of pharmacological agents to biological rhythm [9, 11]. It has been proved that circadian dysrhythmia has significant impact on occurring and prognosis of the many health problems [12]. For this reason, comprehending that how biological clock works could provide amelioration for obesity, metabolic diseases, mental disorders, cardiac disorders, sleep disorders, and other health problems.

1.1. How does it work

Biological clock is a self-regulating clockwork mechanism that synchronizes oxidative and reductive cycles according to the solar cycle [13]. It provides controls of whole organism from the cell to the organ system. The main goal of circadian clock is to maintain the oscillations in a molecular level which enables light-sensitive organisms to coordinate nutrient storage and use it in accordance with the daily period of activity and rest [14]. Scientists' key goal is to understand how such circadian oscillations could be possible. In recent years scientists has been focused on set light to molecular mechanism of circadian clocks in humans [1, 3, 4, 8, 15]. It is well known that biological clocks are composed of specific proteins (molecules) throughout the body. Almost every cell contains an autonomous molecular clock. In mammals, the main circadian clock is located within the suprachiasmatic nuclei of the hypothalamus. The master clocks (suprachiasmatic nuclei (SCN) in the human brain coordinates all these cellular clocks [16]. Approximately 100,000 neurons have been identified that cluster in the suprachiasmatic nuclei of the hypothalamus extending from bilateral nuclei just above the optic chiasma. This location provides the SCN to receive the light for keeping the cycle in time [2, 7, 17–19]. The light directly from the retina adjusts biological clock and synchronizes within a daily cycle. There are special photoreceptive ganglion cells in retina, which contain light-sensitive pigment (it is called melanopsin). The melanopsin cells are stimulated by natural daylight especially short wavelength (blue light) [18]. Exposure to natural daylight stimulates nerve pathway in SCN. Moraes et al. reported that melanopsin cells play a role in synchronizing the central circadian clock with the day. They reported that melanopsin cells convey information about ambient light to the hypothalamic suprachiasmatic nucleus [18]. Blind people also have photoreceptive cells in their eyes. Because of that, they can usually respond to daylight. The light–dark signals are transmitted through the optic nerve to the suprachiasmatic nucleus, which uses them to reset the circadian clock each day [18]. Biological clocks not only need daylight to maintain its process, but also genes that influence the circadian rhythms. Biological clocks need both light and genes to keep it on track [19]. The brain needs lights to reset itself each day and to stay on the 24-h cycle. When humans kept in continuous darkness, the body's daily cycle tends to lengthen to about 25 h. Also people who lack genes which control the clock's cycle have could be lengthened cycles or absent completely. Main clock allows all the tissues in the body to synchronize with each other [17–19]. It has been reported that this mechanism is implemented by feedback loops of transcription and translation of core clock proteins [17]. This is an autoregulatory transcriptional feedback mechanism. It is known that most of genomes have transcriptional regulators, which transcribed in a rhythmic manner [19]. It has been shown that circadian clocks play a key role in mitochondrial oxidative metabolism [13]. It is well known that cellular redox (oxidation and reduction) status influences the activity of clock transcription factors [13]. Peek et al. identified the clock transcription feedback loop that

produces cycles of nicotinamide adenine dinucleotide (NAD+). It is known that NAD+ is an important cofactor in oxidative metabolism. NAD+-dependent deacetylase activity affects protein acetylation in mitochondria. Peek et al. have been reported that circadian control of NAD+ bioavailability modulates mitochondrial oxidative function and organismal metabolism across the 24-h fasting and feeding cycle [13]. Thus, the molecular clock makes up the fluctuations, synchronized with the environment. Essentially circadian rhythms are endogenously generated process. But they could be modulated by external stimuli. Namely it could be affected by external stimuli such as temperature (heat-cold), light (light–dark), sound, food supply, time changing travel (jet lag) and social factors (in other words social jet lag). Circadian system regulates energy metabolism of the human body. Therefore, it governs the glucose, insulin, appetite and lipid metabolism. It has been shown that cellular metabolome—the complete set of small-molecule chemicals found within a biological sample—changes according to time of the day [17]. Dopamine and melatonin are prominent hormones of the circadian biology [20]. Melatonin is the main responsible hormone for human body's daily cycle. Melatonin receptors found a very wide distribution in the body. Suprachiasmatic nuclei control the secretion of melatonin. The primary physiological function of melatonin is to adjust light/darkness cycle in human body. Dark enables the endogenous secretion of melatonin which is constituted by the suprachiasmatic nuclei and entrained to the light/dark cycle. Light suppresses the secretion of melatonin [21]. The daily secretion of melatonin is a biochemical signal of night for preparing the organization to circadian rhythms. Melatonin secretion begins between 9 and 11 pm and peaks between 1 and 3 am. The other physiological functions of the h depend on melatonin signal. Melatonin affects cell surface receptors in central nervous system thus regulates sleep/wake cycle. Suprachiasmatic nuclei also drive the release of cortisol and growth hormone [7]. The biological rhythm of the human body could be summarized as: 00:00 Midnight, 02:00 Deepest Sleep, 04:30 Lowest Body Temperature, 06:45 Sharpest Rise in Blood Pressure, 07:30 Melatonin Secretion Stops, 08:30 Bowel Movement Likely, 09:00 Highest testosterone Secretion, 10:00 High Alertness, 12:00 Noon, 14:00 Best Coordination, 15:30 Fastest reaction time, 17:00 Greatest cardiovascular efficiency and Muscle Strength, 18:30 Highest Blood Pressure, 19:00 Highest Body Temperature, 21:00 Melatonin Secretion Starts, 22:30 Bowel Movements Suppressed [8, 10, 21]. Human physiological processes (including cerebral, renal, cardiac, hormonal and metabolic) are performed according to this cycle. As clearly seen in this cycle the human being could be arranged for feeding, sleeping, and activation time properly to their biological clocks.

1.2. Genes of circadian biology

Several genes have been identified in operation of biological clocks. In recent years, researchers have been focused on describing and analyzing clock gene expression [2, 15, 19]. It has been reported that one-third of all gene activity is regulated by the biological clock [2]. The circadian light receptors are encoded by the essential elements called cryptochromes (cryptochromic genes, CRY1 and CRY2). It has been accepted by the researches that cryptochromes play a fundamental role and they are the most important part of the circadian rhythm. In recent years, scientists defined myraid genes that govern circadian clocks such as BMAL1, CLOCK, CRY, PER, and TIM (specifically identified for the sleep process). It has been shown that these genes were found within the cells of nearly all body tissues but particularly active within the suprachiasmatic nuclei [22, 23]. The clock proteins which encoded by these genes

in the human body could control the activity of these genes. Clock gene is the first identified gene (Vitaterna et al. 1994; Antoch et al. 1997). King et al. have identified BMAL1 gene (1997). BMAL1 gene is accepted as the heterodimeric partner of CLOCK gene. So CLOCK-BMAL1 accepted as transcriptional activator complex (Gekakis et al. 1998). Takahashi defined Period and Cryptochrome genes (Takahashi 2000). In 2002, it was defined "core circadian clock genes" (BMAL1, CLOCK, CRY1, CRY2, PER1, PER2) (Preitner et al. 2002). In the periodicity of the circadian rhythm, it has been found to be important of the regulation of the stability of the PER and CRY proteins by specific E3 ubiquitin ligase complexes [15, 22–25]. All these models describe the circadian clock in mammals. Increased recognition of the responsible genes in the circadian rhythm also provides an understandable processing mechanism of the human body. It has been known that the human body possesses internal time regulators which are genetically determined. Consequently, it is well known that a genetically manifested clock in the human body governs fundamental rhythmicity and enables homeostasis of the organism. It is clearly understood that the circadian rhythmicity is responsible for physiological, biological, and biochemical integrity of the human body. However, it is still unclear that how does circadian rhythmicity integrate with the physiologic systems. Although the numerous genes have been defined in process of the biological rhythmicity, the genetic and molecular mechanisms of circadian clocks also remain unclear. Learning more about the responsible genes for circadian rhythmicity will also help us to comprehend biological patterns of the human body.

1.3. Disrupted biological clocks

In mammals, the biological clock impulse energetic cycles to maintain physiologic stability. Biological rhythms are responsible for secretion of the hormones, the electrical activity of the hearth, immunity, hearth rate, blood pressure, coagulation, body temperature, hemodynamic, respiratory motion, and sleep-wakefulness [4, 12, 17, 19]. Therefore, circadian rhythmicity enables physical and mental goodness of the human body, irregular rhythms lead to various acute (delirium, hallucination) or chronic health problems (obesity, depression) [7, 27, 28]. It has been proved that many physiological variables are related to circadian rhythm. Each cell produces building blocks of amino acids. When desirable concentration is reached, the production stops. Clock genes govern these processes. In recent years, there is growing evidence about microbiome regulates that circadian clock genes [26]. It has been identified that the murine microbiome has circadian behavior and linked it to host feeding time. Liang et al. reported that the host circadian system influences the rhythmicity of the total load and taxonomic abundances in the fecal microbiota. They have reported that disruption of the host circadian clock by deletion of BMAL1 altered the fecal microbial composition [26]. It is well known that chronic sleep restriction alone could disrupt circadian transcription [29]. Circadian dysrhythmia directly causes diseases by cellular and visceral dysfunction in the human body. Cryptochromes play a key role in the diseases caused by disrupted biological clocks. It is well known that the mutation of the cryptochrome gene completely disrupts the circadian clock. Circadian dysrhythmia causes various abnormalities via impaired major metabolic proteins which play a key role in biological clocks [30]. It has been shown that circadian rhythms were severely disrupted in hospitalized patients. Disrupted circadian rhythm affects whole system in the body from sleeping/waking to digestive systems. Studies show that disrupted circadian rhythm contributes to several diseases [27–29]. Mostly circadian dysrhythmia and

metabolic disorders such as obesity and metabolic syndrome are seen together [27, 29]. It has been reported that disrupted biological clocks lead to intolerance of glucose, insulin, and lipid metabolism [27–29]. Because of the glucose homeostasis is dependent on daily light–dark cycle, in case of the desynchrony glucose regulation impaired and this situation leads to metabolic syndrome even diabetes mellitus [12, 23, 29]. In addition, it has been reported in many studies that depressive and affective disorders were accompanied by disrupted sleep–wake patterns [10, 31]. In case of circadian dysrhythmia, the normal morphology of biological rhythms are changes in one or more of the aspects of the normal cycle. This could be a change in degree of fluctuation (mostly decreased amplitude), a phase shift (mostly phase delay), or disintegration of the cycle in a chaotic pattern. It has been shown that the severity of illness was correlated with degree of circadian disruption [7]. Scientist gradually understands that variety of the diseases are rhythmic disorders [10, 28–31]. The sleep disturbance is the first and overt symptoms of circadian dysrhythmia. The core temperature is one of the stable rhythms. It decreases consistently around 5 am. However, in chronic diseases fluctuates. It has been shown a correlation between severity of illness and degree of circadian disruption [7]. Cardiovascular system is a good example of organizing according to the oscillation of biological clocks. Most cardiovascular functions change with the circadian cycle. The longer-term effects of disrupted circadian rhythms are increased risk of obesity, metabolic problems, depression, mental disorders, cardiac and neurological events and even cancer. In recent years, there is a growing awareness about the negative effects of the technology to biological rhythm. Chronic exposure to artificial light causes numerous health problems from simply sleep disorders to many cancers [32]. It is known that sleep–wake disruption common in industrialized society depends on lifestyle. The main issue is how human being could keep their biological clocks in a regular system. As understood clearly in the biological cycle of human body, the daytime is optimum for food intake because of the biological rhythms peaks in the morning and afternoon [12]. Froy et al. showed that well-being could be achieved by resetting the circadian clock. They reported that timed feeding also arranges circadian rhythms [30]. In a similar way, it is obvious clearly the sleep and activation time. It is well known that there is a strong correlation between disrupted circadian rhythms and many health problems person. Based on this knowledge, scientists (consider the contrary) begun to search whether healing be guided by circadian rhythms as well. It has been shown that chronobiological interventions improved the clinical outcomes through amelioration of delirium and sleep disturbance [7, 30]. Understanding what makes biological clocks tick may provide treatments for many health problems from sleep disorders, obesity, mental health disorders, to jet lag. It can also improve ways for individuals to adjust to nighttime shift work. Learning more about the genes, which is responsible for circadian rhythms, will also help us understand biological systems and the human body.

2. Summary points

- Human being has a diurnal activity pattern.
- In a molecular level, there are thousands of biological clocks in the human body.

- The main clocks in the human brain coordinate all these cellular clocks.
- Biological clocks provide adaptation for living organism to environment both inside and outside.
- Circadian rhythms are the essential component of homeostasis in human body.
- Biological clocks are responsible for controlling physiological, homeostatic, behavioral and endocrine balance in organism.
- Circadian rhythm is an autoregulatory system and drives almost every physiological, biological, and biochemical functions of the mammalians.
- The main purpose of circadian rhythm is to provide the organism adaptation for daily and seasonal changes.
- Chronobiology investigates the underlying mechanisms of chronomes.
- Cryptochromes play a key role in the diseases caused by disrupted biological clocks.
- A genetically manifested clock in the human body governs fundamental rhythmicity and enables homeostasis of the organism.
- There is a molecular and neuronal connection between circadian clock and human metabolism.
- Essentially circadian rhythms are endogenously generated process. But they could be modulated by external stimuli.
- Chronic exposure to artificial light causes numerous health problems from simply sleep disorders to many of cancer.
- The daytime is optimum for food intake because of the biological rhythms peaks in the morning and afternoon.
- Disrupted circadian rhythm affects whole system in the body from sleeping/waking to digestive systems.
- Understanding what makes biological clocks tick may provide treatments for many health problems from sleep disorders, obesity, mental health disorders, to jet lag.

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Chapter 3

Circadian Clock Gene Expression and Drug/Toxicant Interactions as Novel Targets of Chronopharmacology and Chronotoxicology

Jie Liu, Huan Li, Shangfu Xu, Yunyan Xu and Chang Liu

Additional information is available at the end of the chapter

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Abstract

Circadian rhythms are driven and maintained by circadian clock gene networks in both brain and peripheral organs. In the liver, circadian rhythms produce oscillation in drug Phase-I, Phase-II, and Phase-III (transporters) metabolism genes, which in turn would affect drug disposition and detoxication, resulting in diurnal variations of efficacy and toxicity when drugs are given at different times of the day. On the other hand, drugs and toxicants could affect circadian clock gene expression to produce biological effects leading to therapeutic or toxic outcomes. This chapter reviewed the relevant literature and a dozen of publications from our work, discussed the interactions of circadian clock genes with drugs and/or toxicants to better understand the importance of circadian clock gene expression as novel targets in Pharmacology and Toxicology.

Keywords: circadian clock gene expression, liver, drug metabolism oscillation, chronopharmacology, chronotoxicology, brain

1. Introduction

Organisms on earth developed the ability to predict and restrict their activity to the night or day by endogenous circadian clock [1, 2]. The mammalian circadian clock system is timed to a 24-h solar time period and maintains rhythmic physiology. In mammals, the circadian clock influences nearly all aspects of physiology and behavior, including sleep-wake cycles, cardiovascular activity, endocrine function, body temperature, kidney function, physiology of the gastrointestinal tract, hepatic metabolism, immune function, detoxification, and the

reproductive system [3, 4]. Disruption of biological rhythms produces negative effects in the short and long terms leading to various diseases [4]. For example, clock dysfunction accelerates the development of liver diseases such as fatty liver diseases, hepatitis, cirrhosis, and liver cancer. Liver disorders also, in turn, disrupt circadian clock function [5].

Circadian oscillations are generated by a set of genes forming a transcriptional autoregulatory feedback loop. In mammals, these include the core clock regulators (Clock, Bmal1, and Npsa2), the clock feedback loop regulator genes (Per1, Per2, Per3, Cry1, and Cry2), and the clock target genes (DBP, Rev-erbα (Nr1d1), RORα, Tef, CK1δ, etc.) [6, 7]. The central clock is located in the suprachiasmatic nucleus in the hypothalamus and peripheral clocks in all tissues. Peripheral clocks in the liver have fundamental roles in maintaining liver homeostasis, including the regulation of energy metabolism and the expression of enzymes controlling the absorption and metabolism of xenobiotics [8]. Over the past three decades, researchers have investigated the molecular mechanisms using global clock-gene knockout mice, or clock gene mutant mice, or other genetic and molecular biology tools to elucidate molecular architecture of circadian clock in mammals [9].

Chronopharmacology and chronotoxicology is a new interdisciplinary science aimed at studying the influence of circadian system on drug disposition, efficacy, and toxicity. Xenobiotics absorption, distribution, metabolism, especially by P450, and excretion [10–14], all under circadian regulation. Circadian variations on these hepatic drug processing genes [15] greatly influence therapeutic effects and toxicity of drugs [10, 16–18]. The chronotherapy of anticancer drugs gives an excellent example [18]. This chapter will focus on the general aspects of circadian rhythms on drug/toxicant disposition and biological effects, and will also discuss the effects of drugs/toxicants on circadian clock gene expression as a novel target of chronopharmacology and chronotoxicology. A dozen of our publications in recent 5 years were also included for discussion.

2. Circadian rhythms affect Phase-I, Phase-II, and transporter gene expressions in the liver

Liver is the major site of xenobiotics metabolism and disposition. Accumulating evidence clearly indicates that circadian rhythms affect the gene/protein expression encoding xenobiotics uptake (Oatps and Ntcp), Phase-I metabolism (P450) and detoxication (Nrf2, MT-1, and GSH systems), Phase-II conjugation (glutathione S-transferases, UDP-glucuronosyltransferases, and sulfotransferases), and efflux transporters (Mrps and MDR) (**Figure 1**).

Diurnal variation of hepatic uptake transporters. In the liver, the major uptake transporters are organic anion transporting polypeptides (Oatp1a1, Oatp1a4, Oatp1b2, and Oatp2b1), organic cation transporter (Oct1), organic anion transporters (Oat2 and Oat6), and others [19]. The expressions of Oatp1a1, Oatp1a4, Oatp1b2, Oct1, and Oat2 display diurnal oscillations, with higher expression in the morning, while Oatp2b1 did not show circadian variation [20]. Na^+-taurocholate cotransporting polypeptide (Ntcp and Slc10a1) is a major bile acid uptake transporter that localizes to the basolateral membrane of hepatocytes, and displays apparent circadian rhythm, with higher expression in the afternoon [20–22].

Diurnal variation of hepatic Phase-I P450 metabolism enzyme genes. Hepatic cytochrome P450 is the major enzyme catalyzing the Phase-I drug metabolism. Most drugs are metabolized by

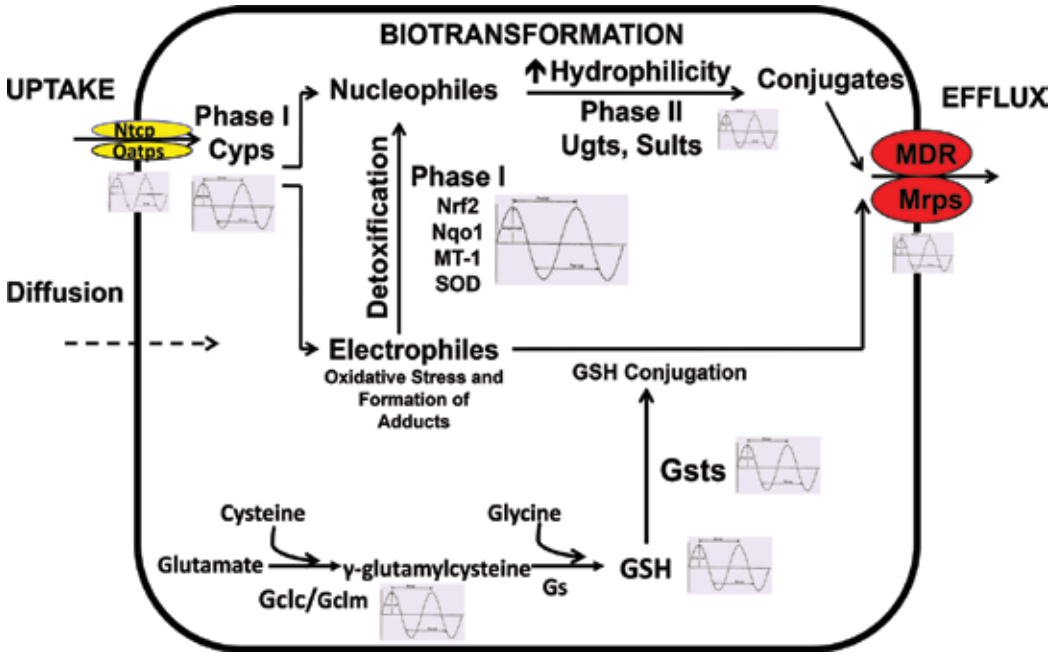

Figure 1. Drug metabolism (Phase-I, Phase-II, transporter) and detoxication (GSH, Nrf2, MT-1) gene expression show circadian oscillations.

P450 1–4 family enzymes. P450 enzyme genes and corresponding nuclear receptors display diurnal oscillations: AhR and Cyp1a1, 1a2 are higher in the morning; CAR and Cyp2b10 are higher in the afternoon and evening; PXR is higher in the afternoon but Cyp3a11 and Cyp3a25 are higher in the morning; PPARα is higher in the morning but Cyp4a10 is higher in the evening [23]. Cyp7a1 is a rate-limit enzyme gene for bile acid synthesis, displays a typical circadian rhyme, with the peak around 18:00 [21–24]. Bile acid synthesis is controlled by the circadian clock and Rev-erbα is a major clock gene controlling bile acid homeostasis [25].

In the liver, circadian rhythm serves to synchronize the metabolism of bile acid, glucose, and lipid, and their disruption could lead to diseases and affect chronotherapy [26]. Indeed, the liver is the key organ to maintain energy metabolism which is greatly influenced by feeding, diets, and diurnal variation [5]. For example, Peroxisome proliferator-activated receptor-gamma coactivator (PGC1α) stimulates the expression of clock genes, notably Bmal1 (also called Arntl) and Rev-erbα (also called Nr1d1), through coactivation of the ROR family of orphan nuclear receptors. Mice lacking PGC-1α show abnormal diurnal rhythms of activity, body temperature and metabolic rate [27]. Circadian clocks regulate metabolic processes not only by simply in response to daily environmental/behavioral influences but also by synchronizing the cell with its environment to modulate a host of metabolic processes [27–29].

Diurnal variation of hepatic detoxification enzyme genes. Many antioxidant enzyme genes display diurnal variations, such as the Nrf2 detoxication pathway genes [30], enzymatic detoxication components such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px1) and non-enzymatic protein such as metallothionein (MT) [31]. GSH is low in the afternoon which is partially responsible for acetaminophen hepatotoxicity when given in the afternoon [23].

Diurnal variation of hepatic Phase-II metabolism gene/proteins. Glucuronide and sulfate conjugations are major Phase-II pathways in the biotransformation and elimination of a wide variety of endogenous compounds, drugs, and other xenobiotics. Diurnal variations of these Phase-II reactions were reported in the 1980s [32]. Consistent to the variation in the conjugation reactions, the expression of Ugt1a5, 2a3, 2b34, 2b36 and UDP-gpb, as well as Sult1a1, 1a5, and Sult5a1, all show diurnal oscillations [20]. Hepatic GSH has the trough at dusk [30], and the activities of GSH S-transferase [33] were lower at the dark phase and the expression of Gst1a1/1, Gst1a4, Gstm2, and Gstt1/2 display diurnal rhythms which are generally lower in the dark phase [20].

Diurnal variation of hepatic Phase-III efflux transporters. P-glycoprotein is the major efflux pump in the liver, and its expression shows circadian variation together with the diurnal expression of Abcb1 [34]. In addition to P-glycoprotein, hepatic multidrug-resistant protein 2 (MRP2), breast cancer resistant protein (BCRP) also show circadian oscillations [35]. Diurnal variations in hepatic mRNA expression of multidrug-resistant gene 1a (Mdr1a), Mrp2, and Bcrp were also evident [20, 35].

Diurnal variation of hepatic Phase-I, Phase-II, Phase-III, and the nuclear transcription factors would affect the xenobiotic metabolism when administered at the different times of the day to impact their efficacy and toxicity, the time really matters [15].

3. Circadian rhythm disruption affects therapeutic effects and toxicity of xenobiotics in the liver

Table 1 gives a few examples of how the disruption of circadian clock could affect drug effect and toxicity. Most of the examples used genetic models with disruption of circadian clock genes or administration of drugs at different times.

Carbon tetrachloride is a commonly used hepatotoxicant. In SD rats, administration of CCl_4 in the afternoon showed more toxicity than administrated in the morning, the increased toxicity was accompanied by the lowest hepatic GSH levels in the afternoon [36]. Acute CCl_4 toxicity was increased in Per2−/− mice. At the 12-h time point after CCl_4 treatment, more vacuolations were observed in the liver tissues of Per2-null mice as compared to wild-type (WT) mice, and at 24 h after CCl_4 treatment, more severe hepatic necrosis was evident than that occurred in WT mice. A deficit of the Per2 gene enhanced Ucp2 gene expression levels in the liver leading to reduced ATP and increased production of toxic CCl_4 derivatives. The absence of Per2 also caused an increased expression of Clock gene [37]. Per2-null mice were not only sensitive to CCl_4-induced acute hepatotoxicity, but also to CCl_4-induced chronic toxicity and fibrosis. CCl_4 caused much more severe liver fibrosis and activated hepatic stellate cell (HSC) in mPer2 null mice as compared to WT mice. Per2-null mice exhibited less efficiency in fibrosis resolution and apoptosis resistance in HSC. Transfection of Per2 cDNA into CCl_4-exposed HSC restored apoptosis sensitivity with up-regulation of the TRAIL-R2/DR5 signaling pathway [38].

Acetaminophen hepatotoxicity also displays diurnal variations. When given acetaminophen in the afternoon, toxicity was greater than that given in the early morning [23, 39]. At 8:00, there

Drug/toxicant	Animal models	Chronotoxicology	References
Carbon tetrachloride	SD rats	18:00 toxicity >6:00, with lowest GSH levels	[36]
Carbon tetrachloride	Per2−/− mice	Acute toxicity increased in Per2−/− mice	[37]
Carbon tetrachloride	Per2−/− mice	Chronic toxicity, fibrosis increased in Per2−/− mice	[38]
Acetaminophen	KM mice	18:00 toxicity >6:00	[23]
Acetaminophen	Per2−/− mice	Toxicity decreased in Per2−/− mice	[40]
Acetaminophen	Clock−/− mice	Toxicity decreased in Per2−/− mice, with prolonged PBST	[41]
Acetaminophen	$Bmal1^{fx/fx}Cre^{Alb}$ mice	Reduced toxicity, reduced protein adducts, altered APAP metabolism	[42]
Dixon (TCDD)	$Per1^{Idc}$,$Per2^{Idc}$ mice, cells	Increased TCDD induction of Cyp1a1, Cyp1b1	[43]
Dixon (TCDD)	$Per1^{Idc}$,$Per2^{Idc}$, Per1/$Per2^{Idc}$ mice	Abolished diurnal variation of TCDD induction of Cyp1a1	[44]
Benzo[a]pyrene	Clock mutant (Clk/Clk) mice	Abolished diurnal variation of B[a]P induction of Cyp1a1	[45]
Bile duct ligation	Per2−/− mice	Increased BDL-induced liver injury and fibrosis	[46]
Cholestyramine diet restricted feeding	$Per1^{-/-}$/$Per2^{-/-}$ mice	Lost diurnal variation in bile acid metabolic enzyme genes	[47]
Isoniazide	Swiss mice	Isoniazid hepatotoxicity at ZT1 > ZT9, ZT17	[48]
Chlorozoxazone	Wistar rats	Diurnal variation in CYP2E1 affect its half-life	[49]
Alcohol	Per1−/−, Per2−/− mice	Less susceptible to alcohol toxicity	[50]
Diethylnitrosamine (DEN)	$Clock^{mut}$ mouse hepatocytes	Decreased DEN metabolism and apoptosis tolerance	[51]
Cadmium	ICR mice	Toxicity at ZT 8 > ZT 20, corresponding to low level of GSH at ZT8	[52]

Table 1. Circadian clock gene expression as novel targets in toxicology.

was no difference of acetaminophen toxicity between Per2-null and WT mice, but at 20:00 when the Per2 expression is highest, Per2-null mice had less liver injury, with less Cyp1a2 expression to bio-activate acetaminophen [40]. In another study, acetaminophen toxicity is greater at Zeitgeber time (ZT)14 than at ZT2, and clock-deficient mice are resistant to the toxicity at ZT14, with prolonged pentobarbital sleep time (PBST), indicating the reduced activation of acetaminophen [41]. Use Bmal1 mutant mice ($Bmal1^{fx/fx}Cre^{Alb}$), the acetaminophen toxicity at ZT12 was decreased, along with decreased APAP protein adducts and altered acetaminophen metabolism kinetics (increased AA-Gluc), possibly due to decreased NADPH-cytochrome P450 oxidoreductase gene expression and activity at ZT12, as compared to WT mice [42].

In Per1, Per2-deficient mice, the ability of AhR ligand dioxin (TCDD) to induce the Cyp1a1 and Cyp1b1 was enhanced, especially with targeted interruption of Per1 [43]. TCDD induction of Cyp1a1 was 23–43 fold greater during the night time (ZT18) than at the day time (ZT6) in WT mice. However, the diurnal variation in the TCDD induction of Cyp1a1 expression was abolished in $Per1^{ldc}$, $Per2^{ldc}$, and $Per1^{ldc}/Per2^{ldc}$ mutant mice, suggesting that Per1, Per2 and their timekeeping function in the circadian clockworks mediate the diurnal variation in TCDD induction of Cyp1a1 [44]. Clock mutant Clk/Clk mice failed to show typical oscillation of AhR expression, and BaP (an AhR ligand) induction of Cyp1a1 was disrupted [45].

In Per2−/− mice, bile duct ligation (BDL)-induced liver injury and fibrosis was increased, along with increases in TNFα, TGFβ1, Col1α, and TIMP1 in livers of Per2-null mice as compared to WT mice [46]. In Per1−/− and Per2−/− mice fed on 2% cholestyramine diet, and/or restricted feeding (phase-shift peripheral clock), liver bile acid levels were increased, and the nuclear receptors CAR and PXR were activated, together with the increased serum enzyme AST levels, indicative of liver damage. In these Per1−/− and Per2−/− mice, the circadian expression of key bile acid synthesis and transport genes, including Cyp7a1 and Ntcp, was lost [47].

The hepatotoxic potential of antituberculosis drug isoniazid varied when it was administered at ZT1, ZT9, and ZT17, and the toxicity was highest when isoniazid was given at ZT1 [48]. Chlorzoxazone is a CYP2E1 metabolized drug, and its kinetics and half-life were altered with the diurnal variation of CYP2E1 activity. The value of chlorzoxazone half-life in plasma of the light phase group was significantly longer than the dark phase group, with an increase of 6-hydroxychlorzoxazone production [49]. Acute alcohol-induced higher toxicity at ZT13 than ZT1 when Per1 and Per2 were highly expressed. Per1−/− and Per2−/− mice were less susceptible to alcohol hepatotoxicity, especially in Per1 null mice. Per1 null mice had decreased expression of peroxisome proliferators-activated receptor-gamma and its target genes related to lipid metabolism such as Srebp1, fatty acid synthase (Fas), CD36, diacylglycerol O-acyltransferase 2 (Dgat2), AP2, and adipsin [50]. In primary hepatocytes isolated from Clock mutant Clk/Clk mice and WT mice, diethylnitrosamine (DEN) induced apoptosis and cell death were reduced in Clock-deficient mice, probably due to decreased DEN metabolism [51]. Cadmium hepatotoxicity is independent of metabolic activation; while its mortality was high at ZT8 than ZT20 when the hepatic GSH level was lowest [52].

Thus, alterations of diurnal oscillations would affect drug metabolism, efficacy, and toxicity. On the other hand, drugs could target circadian clock gene expressions to produce biological effects, which will be discussed below.

4. Drugs/toxicants could affect both central and peripheral circadian clock gene expression

The circadian clock is located in both brain and peripheral tissues [8]. The central clock pacemaker is located in the suprachiasmatic nucleus (SCN) of the hypothalamus, while the peripheral clock is distributed in all peripheral tissues. The liver is the main peripheral tissue under circadian clock regulation [7–9]. Drugs/toxicants could affect both central and peripheral clock gene expression. For example, Mn is a well-known neurotoxicant

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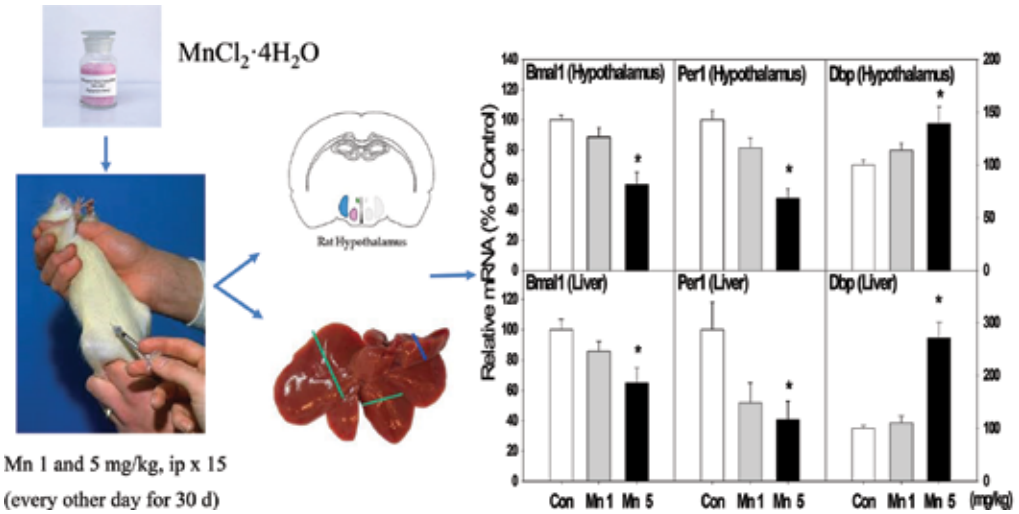

Figure 2. Neurotoxicant manganese intoxication produced aberrant expression of circadian clock genes in both central (hypothalamus) and peripheral (liver). Adapted from Li et al. [53].

producing a Parkinson-like syndrome, but it also produces liver injury. In an attempt to examine the effect of Mn on the central and peripheral clocks, rats were given Mn 1 and 5 mg/kg, ip, every 2 days for 1 month, and the hypothalamus and liver were removed to examine the clock gene expression (**Figure 2**). The results showed that Mn-induced aberrant expression of circadian clock genes in both hypothalamus and liver, and liver was more sensitive to Mn-induced decreases in clock gene Bmal1, Per1, and increase in Dbp, indicating that both central and peripheral clocks could be disrupted by drugs/toxicants [53]. Another example is chronic alcohol administration. Chronic alcohol consumption produced disruption of circadian clock gene expression in both central (hypothalamus) and peripheral tissues (liver and colon) [54], and the liver appeared to be more susceptible than brain in alterations of metabolic genes and core molecular clock disruption. In addition to the fatty liver and affected the diurnal oscillations of metabolic genes (alcohol dehydrogenase 1, carnitine palmitoyltransferase 1a, Cyp2e1, Phosphoenolpyruvate carboxykinase 1, pyruvate dehydrogenase kinase 4, Ppargc1a, Ppargc1b and Srebp1c), the diurnal oscillations of core clock genes (Bmal1, Clock, Cry1, Cry2, Per1, and Per2) and clock-controlled genes (Dbp, Hlf, Nocturnin, Npas2, Rev-erbα, and Tef) were altered in livers from ethanol-fed mice. In contrast, ethanol had only minor effects on the expression of core clock genes in the suprachiasmatic nucleus (SCN) [55].

5. Drugs affect circadian clock gene expression as a novel target of chronopharmacology

Many drugs/toxicants could affect central and peripheral circadian clock gene expression as targets of chronopharmacology and chronotoxicology [10]. **Tables 2** and **3** provide some examples including our work in the field.

Drugs	Animal model (dose, route, time)	Chronopharmacology	References
Atorvastatin	KM mice; 10–100 mg/kg, po × 30 days	Swollen hepatocyte and feather-like degeneration; increased Cyp7a1, FXR, decreased bile acid transporters; increased expression of Bmal1, Npas2, decrease Per2, Dbp.	[56]
Metformin	C57 mice; 164 mg/kg in drinking water for 6 weeks	Increase in serum leptin and decreased glucagon levels. Increase in PGC1α, PPARα, AMPK; decrease in ACC in liver; Phase advance circadian clock and metabolic genes in liver and activation of liver casein kinase Iα (CKIα)	[58]
Oleanolic acid	Apoe−/− mice on HFD, F344 rats; 0.01% OA × 11 weeks	Increased lipid droplets with no change in oxidative stress; increased Bmal1, Clock, and Elovl3, Tubb2a, and Cldn1 decreased Per3, Amy2a5, Usp2, and Thrsp.	[59]
Resveratrol	C57 mice; fed normal or HFD; 0.1% Res × 11 weeks	Ameliorated HFD-increased plasma leptin, lipids, and BW. Restored rhythmicity of Clock, Bmal1, and Per2; and clock-controlled lipid metabolism genes (Sirt1, PPARa, Srebp-1, Acc1, and Fas).	[60]
Sea cucumber saponin (SCS)	ICR mice; 0.03% SCS diet night feeding × 2 weeks	Improve serum lipid profile; restore rhythmicity of PPARα, Srebp1, Cpt, and FAS; restore nighttime feeding-disrupted clock gene expression.	[61]
Zuotai	KM mice; 10 mg/kg, po × 7 days	Decreased the amplitude of Clock, Npas2, Bmal1; increased Dbp, Nfil3 at 10:00, and increased Nr1d1 at 18:00. No effect on Cry and Per genes.	[62]
Polyporus and Bupleuri radix	ICR mice, $Per2^{Luc}$ mice 500 mg/kg, po × 3 days, at different ZT and light/dark	Polyporus and Bupleuri radix were effective in manipulating the peripheral circadian clock phase acutely, with stimulation time-of-day dependency in vitro as well as in vivo.	[63]
Jiao-Tai-Wan	SD normal and model (HFD + PSD × 4 weeks) rats 2.2 g/kg, po × 4 weeks	Increased total sleep time and slow wave sleep time; reversed model rat-induced inflammation markers; increased Cry1, Cry 2, and decreased NF-κB in PBMC.	[64]

Table 2. Circadian clock gene expression as novel targets in pharmacology.

Examples of drugs Atorvastatin is an HMG-CoA reductase inhibitor used for hyperlipidemia. It is generally safe but may induce cholestasis. Repeated administration of Atorvastatin (10–100 mg/kg, po) to mice for 30 days produced hepatocyte swollen and feather-like degeneration, indicative of cholestatic injury, with increases of inflammation markers Egr1 and MT-1, and increased Cyp7a1, FXR, SHP, decreased bile acid transporters Ntco, Bsep, Oastα, and Ostβ. Since Cyp7a1 is a clock-driven gene, its effects on circadian clock gene expression were also examined. Atorvastatin increased the expression of Bmal1, Npas2, decreased the expression of Per2, Per3, Dbp, and Tef, but had no effect on Cry1 and Nr1d1 [56]. The similar effects on the circadian clock gene expression were also observed when atorvastatin was given at the low dose (10 mg/kg) but for a longer period of 90 days, although to a less extent [57].

Metformin is commonly used for type 2 diabetes. In C57 mice, metformin in the drinking water for 6 weeks led to increased serum leptin and decreased glucagon levels. The effect of metformin on liver and muscle metabolism was probably mediated through AMPK activation,

	Animal model (dose, route, time)	Chronotoxicology	References
Carbon tetrachloride	BABL/C mice 0.6 ml/kg, ip, 2/week × 4 weeks	Chronic CCl_4 produced liver fibrosis, altered the amplitudes, meros, acrophases of clock gene expression; circadian rhythms of Cry2, PPARα and POR were lost.	[65]
Diethylnitrosamine	KM mice DEN 100 mg/kg, IP+ CCl_4 + EtOH × 16 weeks	Produced HCC, Markedly increased α-fetoprotein; at 10:00, expression of Bmal1 decreased, expressions of Dbp and Rev-erbα increased.	[66]
Manganese	SD rats 1 and 5 mg/kg, IP, × 4 weeks	Produced neuroinflammation and dopaminergic neuron loss; decreased expression of Bmal1, clock, Per1, Per2, while increased expression of Dbp and Nr1d1	[53]
LPS + Rotenone	SD rats LPS 5 mg/kg, IP ×1, 200 days later, rotenone 0.5 mg/kg, sc × 20	Produced neuroinflammation and dopaminergic neuron loss; at the mRNA and protein levels, reduced expression of Bmal1, clock, Per1, Per2, Dbp, Nr1d1, while no effect on Cy1.	[70]
LPS	ICR mice, LPS 1 mg/kg, IP at ZT4, 10, 16, 22 or at 2, 8, and 26 h after ZT 4 injection	Produced increases in serum TNFa, heart and liver apoptosis; Decrease Per1, Per2 2 h after dose at ZT4 in heart and liver; Increased Per2 8 and 26 h after LPS in heart and liver	[71]
Alcohol	C57 mice, $Per2^{Luc}$ mice Lieber-DeCarli diet for 30–37 days	Produced steatosis, increased serum TG; diurnal oscillations of Bmal1, Clock, Cry1, Cry2, Per1, and Per2 and clock-controlled genes (Dbp, Hlf, Nocturnin, Npas2, Rev-erba, and Tef) were altered in livers of ethanol-fed mice	[55]
Alcohol	WT and $Clock^{\Delta 19}$ mutant mice received Nanji liquid alcohol diet at ZT4 for 10 weeks	Altered the expression of circadian and metabolism genes in hippocampus, liver, and colon from array analysis; $Clock^{\Delta 19}$ affect inflammation and metabolism gene.	[54]

Table 3. Circadian clock gene expression as novel targets in toxicology.

resulting in the inhibition of acetyl CoA carboxylase (ACC), the rate-limiting enzyme in fatty acid synthesis. Metformin-activated liver casein kinase I α (CKIα) and muscle CKIε, known modulators of the positive loop of the circadian clock, thud resulting in phase advances in the liver and phase delays in the muscle for clock and metabolic gene expressions [58].

Examples of active ingredients from herbal medicine. Oleanolic acid is a triterpenoid used to reduce hyperlipidemia. Dietary oleanolic acid supplementation (0.01%) was provided to Apoe- and Apoa1-deficient mice and F344 rats. In Apoe-deficient mice, oleanolic acid supplementation increased hepatic lipid droplets, increased circadian clock genes, together with increases in lipid metabolism genes (fatty acid elongase 3, tubulin beta-2A chain, and claudin 1), while the expression of per3, amylase 2a5, ubiquitin-specific peptidase 2, and thyroid hormone-inducible hepatic protein (Thrsp) were decreased [59].

Resveratrol is an active ingredient in grapes and red wine and shows beneficial effects in metabolic disorders. In HFD-fed mice, resveratrol restored high-fat diet-induced disorders about

the rhythmic expression of clock genes and clock-controlled lipid metabolism, ameliorated the rhythmites of plasma leptin, lipid profiles and whole body metabolic status (respiratory exchange ratio, locomotor activity, and heat production). Meanwhile, resveratrol modified the rhythmic expression of clock genes (Clock, Bmal1, and Per2) and clock-controlled lipid metabolism-related genes (Sirt1, Ppara, Srebp-1c, Acc1, and Fas) [60].

Dietary sea cucumber saponin (SCS) has been shown to have beneficial effects on glucose and lipid metabolism, which is related to the circadian clock. Dietary SCS caused an alteration in rhythms and/or amplitudes of clock genes was more significant in the brain than in liver. In addition, the peroxisome proliferator-activated receptor (PPARα), sterol regulatory element binding protein-1c (SREBP-1c), together with their target genes carnitine palmitoyl transferase, and fatty acid synthase showed marked changes in rhythm and/or amplitude in SCS group mice [61].

Examples of mixtures from traditional medicine. Zuotai is an essential component of many popular Tibetan medicines. Mice were orally given Zuotai (10 mg/kg, 1.5-fold of clinical dose) daily for 7 days, and livers were collected every 4 h during the 24 h period to examine its effects on circadian clock gene expression. Zuotai decreased the oscillation amplitude of Clock, Npas2, Bmal1 at 10:00. For the clock feedback negative control genes, Zuotai had no effect on the oscillation of Cry1, Per1, Per2, and Per3. For the clock-driven target genes, Zuotai increased the oscillation amplitude of Dbp, decreased nuclear factor interleukin 3 (Nfil3) at 10:00, but had no effect on thyrotroph embryonic factor (Tef); Zuotai increased the expression of Nr1d1 at 18:00, but had little influence on Nr1d2 and RORα [62].

Polyporus and Bupleuri radix were popular traditional medicines. Polyporus (Zhulin) is used as a diuresis in the treatment edema, while Bupleuri radix (Chaihu) is used for chronic hepatitis. The $Per2^{Luc}$ mice were used to screen their effects on the circadian clock, and Polyporus was more effective than Bupleuri radix in manipulating the peripheral circadian clock phase-shift, and in promoting time-of-day dependency in vitro as well as in vivo [63].

Jiao-Tai-Wan (JTW), composed of Rhizome Coptidis and Cortex Cinnamomi, is a classical traditional Chinese prescription for insomnia. In obesity-resistant (OR) rats with chronic partial sleep deprivation (PSD) model, 4 weeks of administration of JTW increased total sleep time and total slow wave sleep (SWS) time in OR rats with PSD, and reversed the mode rats elevated serum markers of inflammation and insulin resistance, and these changes were also associated with the up-regulation of Cry1 mRNA and Cry 2 mRNA and the down-regulation of NF-κB mRNA expression in peripheral blood monocyte cells [64].

6. Toxicants affect circadian clock gene expression as a novel target of chronotoxicology

Table 3 lists some examples of known toxicants which disrupted circadian clock gene expression as a mechanism of their acute and chronic toxic effects to both brain and liver.

Examples of hepatotoxicants. Chronic carbon tetrachloride administration in C57 mice (0.6 mL/kg, IP, twice a week for 4 weeks) produced liver injury and fibrosis. The expression of clock genes and metabolic genes in fibrosis livers was altered. The amplitudes of circadian expressions of

Bmal1 and Per1 were attenuated and the mesors in the expressions of Clock and Per1 were increased. Acrophases for the expressions of Clock, Per1 and Cry1 were significantly delayed. Circadian rhythm of Cry2 expression was lost in fibrosis group. The circadian rhythm of PPARα and cytochrome P450 oxidoreductase (POR) was also lost [65].

Chronic diethylenediamine (DEN) administration not only produce hepatocellular carcinoma and markedly enhanced expression of Afp, but also decreased the expression of Bmal1, increased the expression of Dbp and Rev-erbα (Nr1d1) [66]. Circadian disruption is well-known to promote carcinogenesis [67]. In the end-stage of human hepatocellular carcinoma, the expressions of the clock genes, including Bmal1, Per1, Per2, Cry1, and Cry2 were decreased, alone with decreases in clock targeted MT-1, MT-2, and MTF1 (which are considered as biomarkers of HCC). On the other hand, the expression of clock target genes Nr1d1 and Dbp was upregulated as compared with Peri-HCC and normal livers. Peri-HCC also had mild alterations in these gene expressions [68].

Examples of neurotoxicants. As mentioned in **Figure 2**, repeated Mn administration disrupted both central and peripheral liver circadian clock genes, with decreases in Bmal1, Clock, Npas2, Per1, Cry1, but increases in Dbp and Nr1d1. Mn-induced aberrant expression of these clock genes in the brain was consistent with that in the liver, and liver appeared to be more sensitive than hypothalamus to Mn-induced disruption of circadian clock [53].

Chronic neuroinflammation would aggregate neurotoxic effects of toxicants. Rats received a single injection of LPS at the dose of 5 mg/kg, and 200 days later given repeated injection of low dose of rotenone (0.5 mg/kg, sc, 5/week for 4 weeks), and produced neuroinflammation and loss of dopaminergic neurons in Substantia Nigra, replicate the model of Parkinson's disease [69]. In this PD model, aberrant expression of circadian clock genes in brain cortex was evident, as evidenced by decreases of core clock gene Bmal1, clock, and Naps2, decreases in circadian clock feedback gene Per1 and Per2, but had no effect on the expression of Cry1 and Cry2, as well as the decreased expression of clock target gene Dbp and Nr1d1 [70].

LPS not only produces inflammation in the brain but also in the liver. ICR mice received LPS (1 mg/kg, IP) at ZT4, ZT10, ZT16, and ZT22, and liver and heart were harvested 2 h later for gene expression analysis. Hepatic expression of Per1 and Per2 was decreased after LPS injection at ZT6, but Per1 was increased 8 and 26 h after LPS injection. Heart speared to be more sensitive than the liver to these changes as at ZT4, both Per1 and Per 2 in the heart were decreased [71].

Examples of chronic ethanol toxicity. Alcoholic liver diseases are a major concern as it produced metabolic disruption. In C57 mice and Per2 mutant mice, ethanol administration altered the expression of clock genes in the liver, but not in the brain. Diurnal oscillations of core clock genes (Bmal1, Clock, Cry1, Cry2, Per1, and Per2) and clock-controlled genes (Dbp, Hlf, Nocturnin, Npas2, Rev-erba, and Tef) were altered in livers from ethanol-fed mice [55].

In clock mutant mice, altered clock and metabolism genes were evident in hippocampus, liver, and colon. Of particular interest was the finding that a high proportion of genes involved in inflammation and metabolism on the array was significantly affected by alcohol and the Clock gene mutation in the hippocampus [54].

Thus, drugs/toxicants could affect central and peripheral circadian clock gene expression as targets of their therapeutic effects and/or toxicity [10].

7. Summary and perspectives

The importance of chronopharmacology has been reviewed 10 years ago [16]. Circadian rhyme governs many physiological functions, and the RNA-Seq revealed that over 3000 genes in the liver showed circadian oscillation [72]. Over the past two decades, research has investigated the molecular mechanisms linking circadian clock genes with the regulation of hepatic physiological functions, using global clock-gene-knockout mice, or mice with liver-specific knockout of clock genes or clock-controlled genes. Clock dysfunction accelerates the development of liver diseases such as fatty liver diseases, cirrhosis, hepatitis, and liver cancer, and these disorders also disrupt clock function. Similarly, clock dysfunction clearly affects drug efficacy and toxicity.

In the liver, Phase-I is composed mainly of cytochromes P450 involved in detoxification and hormone and lipid metabolism [11], which are regulated by nuclear receptors. Phase-II enzymes modify the phase-I metabolites by conjugation reactions, while phase-III includes membrane transporters responsible for the elimination of modified xenobiotics. Phases I–III of drug metabolism are under strong circadian regulation [15]. The rhythmic control of xenobiotic detoxification provides the molecular basis for the dose- and time-dependence of drug toxicities and efficacy, and makes the circadian clock gene expression as a target for chronopharmacology [10], not only for drugs but also for traditional medicines [73]. Circadian rhythms also greatly affect drug toxicity at the different times of administration [74]. Circadian rhythms are controlled, regulated and maintained by clock gene networks, which are the emerging targets of chronopharmacology and chronotoxicology.

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Conflict of interest

The authors do not have conflict of interest.

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Chapter 4

Quantification of Irregular Rhythms in Chronobiology: A Time-Series Perspective

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Additional information is available at the end of the chapter

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Abstract

In optimal conditions of youth and health, most—if not all—physiological systems obey regular circadian rhythms in response to the periodic day-night cycle and can be well described by standard techniques such as cosinor analysis. Adverse conditions can disturb the regularity and amplitude of circadian cycles, and, recently, there is interest in the field of chronobiology to quantify irregularities in the circadian rhythm as a means to track underlying pathologies. Alterations in physiological rhythms over a wide range of frequency scales may give additional information on health conditions but are often not considered in traditional analyses. Wavelets have been introduced to decompose physiological time series in components of different frequencies and can quantify irregular patterns, but the results may depend on the choice of the mother wavelet basis which is arbitrary. An alternative approach are recent data-adaptive time-series decomposition techniques, such as singular spectrum analysis (SSA), where the basis functions are generated by the data itself and are user-independent. In the present contribution, we compare wavelets and SSA analysis for the quantification of irregular rhythms at different frequency scales and discuss their respective advantages and disadvantages for application in chronobiology.

Keywords: singular spectrum analysis, SSA, wavelets, spectral analysis, Fourier analysis, data-adaptive, model-free

1. Introduction

Circadian rhythms are physical, mental and behavioral variations that follow an approximately 24-hour cycle in response to the periodic alternation between day and night. In the last

few decades, it has become well established that most—if not all—physiological systems exhibit these regular circadian rhythms, and it has been discovered that they are largely controlled by a central clock and several peripheral oscillators [1]. More recently, it has been observed that adverse conditions, such as "healthy" and pathological aging, illness, stress and medication use, can disturb the regularity and amplitude of the circadian rhythm. Consequently, the focus in the field of chronobiology is shifting from a description of periodic cycles to the quantification of irregularities and the study of the mechanisms underpinning their disruption and normalization [2–5].

The traditional method to analyze circadian rhythms is cosinor analysis, which quantifies the circadian 24 h cycle, and/or other specific infra- or ultradian periodic cycles, by means of examining the degree of "fit" between the experimental data and a user-defined model consisting of a superposition of cosine functions [6, 7] and allows to calculate the *circadian parameters* of mesor, amplitude, period and acrophase [8]. However, data where the patterns are irregular, or where the statistical properties vary over time (nonstationary time series), such as having a dominant trend [9–11], or time-varying amplitudes, frequencies or phases [12–15], are much harder or impossible to describe using models based on these periodic functions. Recently, more specialized techniques have been developed to study circadian rhythms; in particular, wavelets have been applied to study the irregular aspects of circadian rhythms [13–15]. Wavelets however are, as with cosinor analysis, model-based in the sense that the results obtained may depend on the particular wavelet basis functions (mother wavelet) selected by the user. Between the most recent developments in the field of time-series analysis are data-adaptive decomposition techniques such as singular spectrum analysis (SSA) [16–20], empirical mode decomposition (EMD) [21, 22] and nonlinear mode decomposition (NMD) [23, 24]. The basic idea of these data-adaptive techniques is to decompose a time series as a sum of modes that describe separately non-oscillating trend, (quasi-)periodic components and high-frequency noise. These techniques are nonparametric because, in contrast to the classical Fourier decomposition, the modes are not model dependent and do not need to be periodic sine or cosine functions. Instead, the modes are derived from the data itself, and they are not limited to a single time scale or a limited range of scales, but describe the data at all scales present. Recently, we applied SSA to quantify irregular rhythms in actigraphy data in the case of persons that suffer from acute insomnia [25], and SSA was applied as well to study irregular patterns in neural and locomotor activity in hamsters [26], but apart from these studies, data-adaptive time-series methods have not been applied in chronobiology. The lack of accessible specialized software to carry out data-adaptive time-series analysis may be one of the reasons that these techniques, to date, have not been applied to circadian rhythm research; fortunately, several open-source implementations have recently become available in multiple platforms such as Mathematica, MATLAB, R, Python, and so on, for SSA [27–30], EMD [31–34] and NMD [35].

Another disadvantage of the cosinor method is that it is unable to measure *rhythm fragmentation* [2, 36, 37]. In actigraphy, rhythm fragmentation was originally defined as the deterioration of the regular circadian rhythm by the occurrence of daytime naps and/or nocturnal activity episodes. On the other hand, spontaneous moment-to-moment fluctuations are a characteristic property of actigraphy time series in particular and of physiological variables in general, and a

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moderate level of rhythm fragmentation may be indicative of a healthy physiological capacity to respond to random and unforeseen events at multiple time scales. Spectral analysis and other time-series decomposition techniques are ideal tools to study such rhythm fragmentation in actigraphy and other physiological data because they quantify the relative contribution of different time-series components at different time scales to the total variance of the experimental data [25]. A $1/f$ fractal power law may be an indication of such an optimal level of rhythm fragmentation, because it has been observed empirically over a wide range of ultradian time

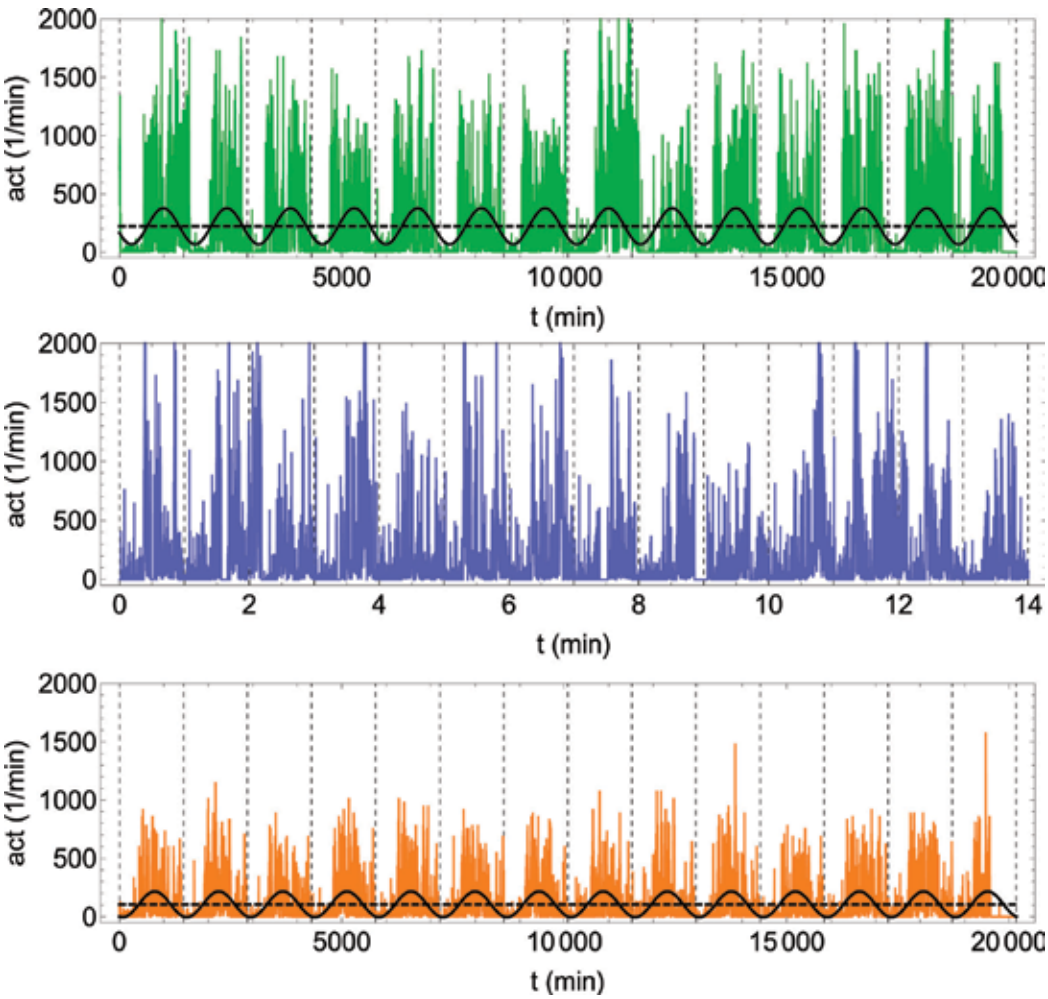

Figure 1. Two-week continuous actigraphy time series of number of movements per minute for (a) young female adult A (23 years old) with regular circadian cycle, (b) young male adult B (22 years old) with irregular circadian cycle (c) older male adult C (82 years old) with regular circadian cycle. All subjects are asymptomatic controls. Vertical gridlines indicate midnight. Also shown are estimations using cosinor analysis of the circadian 24-h cycle (continuous curve) around a constant mesor (broken line) (see Eq. (2)). All time series have a length of $N = 20160$ data points (corresponding to 7×24 h) (data from the public database of Ref. [43]).

scales in heart rate time series and actigraphy, whereas adverse conditions such as aging [38], cardiovascular disease [39], dementia [40–42] and insomnia [25, 43] have been found to correlate with deviations of this power law.

The purpose of the present contribution is to illustrate how Fourier-based spectral analysis, wavelets and data-adaptive methods describe irregular circadian rhythms and rhythm fragmentation. Of the data-adaptive methods mentioned, in the present work, we prefer SSA because of its closeness to standard Fourier analysis and the availability of graphical tools such as the scree diagram that can be interpreted as a generalization of the well-known Fourier power spectrum. We will illustrate the advantages and disadvantages of these methods using selected 2-week continuous actigraphy time series for three nonsymptomatic control subjects of the public database of Ref. [43] (see **Figure 1**). These actigraphy time series show the number of movements per minute for a total duration of 20,160 minutes (7 × 24 h). The time series were chosen upon visual inspection: subject A is a young female adult (23 years old) with a regular circadian rhythm, subject B is a young male adult (22 years old) with an irregular circadian rhythm and subject C is an older male adult (82 years old) with a regular circadian rhythm.

2. Cosinor analysis

The traditional method to study the periodic aspects of circadian rhythms is cosinor analysis [6, 7]. The cosinor approach is based on regression techniques and is applicable to equidistant or non-equidistant time series $x(n)$ of N discrete data points:

$$x(n) = \{x_1, x_2, \ldots x_N\}. \tag{1}$$

The procedure consists of fitting a continuous periodic function $y(t)$ to time series $x(n)$:

$$y(t) = M + A\cos\left(2\pi t/T + \phi\right) \tag{2}$$

where M is the average value or mesor around which the function oscillates, T is the period, A is the amplitude and ϕ is the phase which defines the value at which the function begins at the start of the monitoring at $t = 0$. By taking into account trigonometric rules, the cosinor function of Eq. (2) can be rewritten as $y(t) = M + B\cos(2\pi t/T) + C\sin(2\pi t/T)$, where B and C are amplitudes and where the phase is given implicitly by the superposition of the sine and cosine functions. Function $y(t)$ is fitted to the data by minimizing the summed square residual errors $e_n^2 = \left(x_n - y_n\right)^2$ for all data points $n = 1, 2, \ldots, N$, and the value of period T for which the amplitude A maximizes can be considered to be the circadian period (see **Figure 2**). For organisms exposed to the natural day-and-night cycle, this period can be expected to be $T \approx 24$ h = 1440 min. Once chosen period T, the other circadian parameters M, A and ϕ are determined as well [8]. Phase ϕ does not give any physiological information on the monitored individual, because time series may start at an arbitrary time of the day. Instead, a more interesting variable is the acrophase ϕ_0, which can be defined as the time of the day when the

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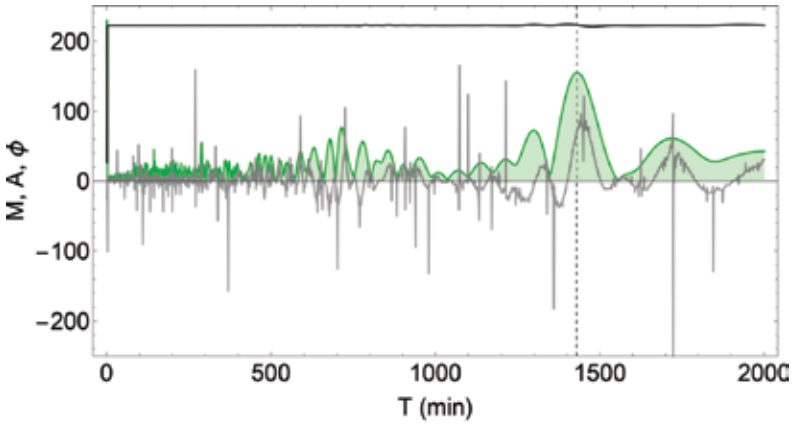
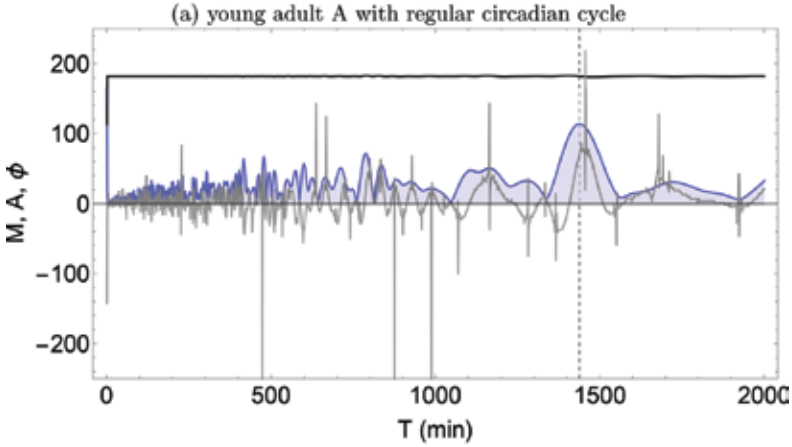
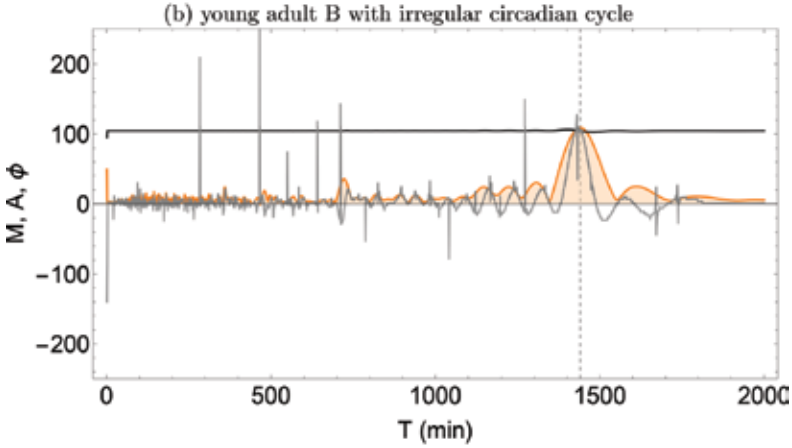


(a) young adult A with regular circadian cycle



(b) young adult B with irregular circadian cycle



(c) older adult C with regular circadian cycle

Figure 2. Regression analysis of Eq. (2) to the time series of subjects A, B and C of Figure 1. Period T is varied from 1 to 2000 min, and amplitude A (shaded curve), mesor M (black line) and phase ϕ (gray curve) are plotted as a function of T. Local maxima near $T = 24$ h = 1440 min are indicated with a vertical line and are located at $T = 1430$ min (subject A), $T = 1438$ min (subject B) and $T = 1440$ min (subject C).

		Cosinor			Fourier filter			DWT			SSA		
		A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
Mean (M)	(1/min)	222.39	182.81	104.77	222.3	181.9	104.7	222.3	181.9	104.7	227.8	181.8	106.2
Mean (T)	(min)	1430	1438	1440	1409.7	1444.3	1428.1	1409.3	1446.7	1433.3	1421.4	1396.1	1431.7
Mean (A)	(1/min)	154.88	113.66	109.14	174.7	145.4	112.9	151.4	142.0	108.3	165.2	137.5	112.1
Mean (ϕ_0)	(°)	230.75	240.75	198.75	229.3	217.0	198.6	218.9	210.9	200.1	226.3	220.6	199.9
Mean (ϕ_0)	(hh:mm)	15:23	16:03	13:15	15:17	14:28	13:14	14:36	14:04	13:20	15:05	14:42	13:20
SD (M)	(1/min)	—	—	—	—	—	—	0.8	1.3	0.4	39.3	49.0	15.4
SD (T)	(min)	—	—	—	116.4	261.9	78.0	127.9	326.5	72.5	105.8	190.0	60.2
SD (A)	(1/min)	—	—	—	108.0	87.7	35.5	35.9	46.9	14.8	53.9	59.8	17.9
SD (ϕ_0)	(°)	10.46	2.09	0	30.9	72.5	15.1	36.6	68.8	13.7	27.8	73.2	11.1
SD (ϕ_0)	(hh:mm)	00:42	00:08	00:00	02:04	04:50	01:00	2:26	04:35	00:55	01:51	04:53	00:44
R^2		0.112	0.058	0.207	0.193	0.141	0.238	0.174	0.129	0.230	0.202	0.161	0.241

Table 1. Circadian parameters mesor M, circadian period T, amplitude A and acrophase ϕ_0 for subjects A, B and C according to cosinor analysis, Fourier filter, discrete wavelet transform (DWT) using the Daubechies [4] mother wavelet and singular spectrum analysis (SSA) with parameter $L = 1440$. Presented are average values (mean), standard deviation (SD) and the coefficient of determination R^2.

circadian cycle obtains its maximum, with respect to a fixed moment in time which is the same for all subjects, e.g. taking midnight as a reference, and which can be expressed as hours and minutes (hh:mm), or alternatively, as an angle (°, taking into account the relation $360^\circ = 24$ h), relative to this reference time. The fitted function may be generalized to include more than one period, $y(t) = M + \sum_k A_k \cos\left(2\pi t/T_k + \phi_k\right)$, where the sum usually runs over a small number k of different periods. In the present case, **Figure 2** shows many ultradian ($T < 1440$ min) and infradian periods ($T > 1440$ min) that have nonzero amplitudes, but in the present case, there are no other periods than the circadian period $T \approx 24$ h that are clearly distinguishable from the neighboring values to warrant their inclusion in the model function $y(t)$. An important result in cosinor analysis is the coefficient of determination R^2, which compares the variance of the residual errors e_n around the fitted model y to the variance of the time series $x(n)$ around its average value:

$$R^2 = 1 - \frac{\mathrm{Var(e)}}{\mathrm{Var(x)}} \tag{3}$$

$$= 1 - \frac{\sum_{n=1}^{N} \left(x_n - y_n\right)^2}{\sum_{n=1}^{N} \left(x_n - \mathrm{mean}(x)\right)^2}, \tag{4}$$

such that R^2 is a measure for the fraction of the variance of the time series that can be explained by model $y(t)$.

Results for the circadian parameters of the cosinor model function *y(t)* of Eq. (2) for subjects A, B and C are presented in **Figure 1**. In the present of one single period T, function $y(t)$ is periodic, and mesor M and amplitude A are constant and capture the average properties of the time series without the possibility to describe day-to-day variability. If $T \equiv 1440$ min, such as for subject C, also acrophase ϕ_0 is constant, and there is no variability for ϕ_0. If, on the other hand, $T \neq 1440$ min, there is a day-to-day phase advance ($T < 1440$ min) or phase delay ($T > 1440$ min), respectively, as is the case for subjects A and B, and one can calculate an average value and variability measures for ϕ_0.

3. Fourier spectral analysis

Fourier spectral analysis makes the supposition that the fluctuations of time series $x(n)$ with $n = 1, 2, \ldots, N$ may be interpreted as the superposition of $\kappa_{\max} = N/2$ independent harmonic oscillators, where $2\pi/T_{\kappa_{\max}}$ is the Nyquist frequency and where each harmonic oscillator corresponds to a periodic function (see Ref. [44]):

$$y(t) = \sum_{\kappa=1}^{\kappa_{\max}} A_\kappa \cos\left(2\pi t/T_\kappa + \phi_\kappa\right) \tag{5}$$

$$= \sum_{\kappa=1}^{\kappa_{\max}} B_\kappa \cos(2\pi t/T_\kappa) + C_\kappa \sin(2\pi t/T_\kappa), \tag{6}$$

which establishes a link between the time domain t and the frequency domain $f_\kappa = 2\pi t/T_\kappa$. Here, the first term $\kappa = 1$ corresponds to $f = 0$ or $T = \infty$ and is the direct current (DC) term around which the other terms $\kappa > 1$ oscillate and may be interpreted as the equivalent of the mesor M of cosinor analysis. One of the most important results of Fourier spectral analysis is the power spectrum, which gives the power $P(f_\kappa)$ as a function of frequency f_κ. The variance of time series x is given by

$$\mathrm{Var}(x) = \frac{1}{N}\sum_{n=1}^{N} (x_n - \mathrm{mean}(x))^2, \tag{7}$$

and Parseval's theorem establishes that the variance in the time domain is identical to the variance of the oscillations of all components around the DC term in the frequency domain, i.e.

$$\mathrm{Var}(x) = \frac{1}{(N-1)}\sum_{\kappa=2}^{\kappa_{\max}} P(f_\kappa), \tag{8}$$

which allows us to interpret the power $P(f_\kappa)$ of the component with frequency f_κ as a *partial variance*, and we can focus our attention to the components that concentrate most of the variance of the time series x.

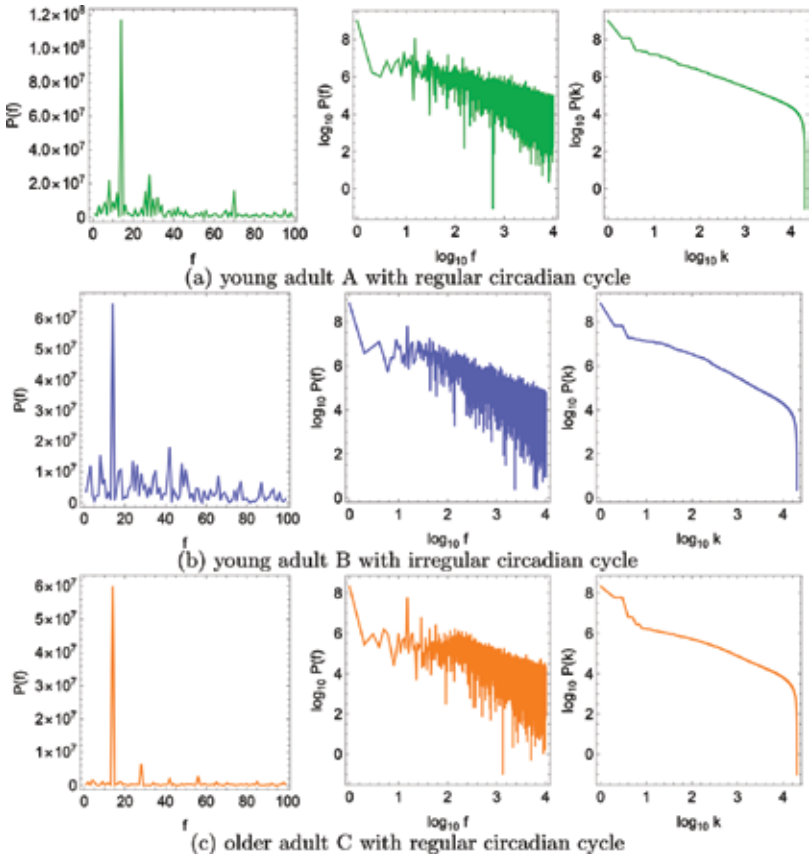


Figure 3. Fourier spectral analysis for subjects A, B and C. Power spectrum $P(f)$ as a function of frequency f in linear scale where the DC term has been omitted for reasons of visibility (left-hand column), logarithmic scale (middle column) and as a scree diagram $P(k)$ ordered according to magnitude in logarithmic scale (right-hand column). Frequency units are the number of oscillations during the whole duration of the time series.

Results from the Fourier spectral analysis of subjects A, B and C are shown in **Figure 3**. The variance of the time series is Var(A) = 107,417, Var(B) = 110,405 and Var(C) = 28,725. Power spectra $P(f)$ as a function of frequency in linear scale show for all subjects a dominant peak at the circadian frequency of $f \approx 14$ oscillations during the 2-week duration of the time series. Apart from the circadian frequency, for subject C, only discrete peaks at higher harmonics are visible, whereas for subject A, many low-frequency components are present, which are even more predominant in the case of subject C. logarithmic scale allows to focus not on the dominant peaks but on the continuum of spectral contributions at a wide range of frequency scales, which for subjects A and B show an approximate $1/f^{\beta}$ scaling with spectral exponent $\beta \approx 1$ and which for subject C appears to be truncated at about $f = 10^2$ oscillations, below which the power spectrum flattens out. A drawback of the power spectrum representation $P(f)$ as a function of f is that it has a lot of dispersion because of which it may be difficult to make an

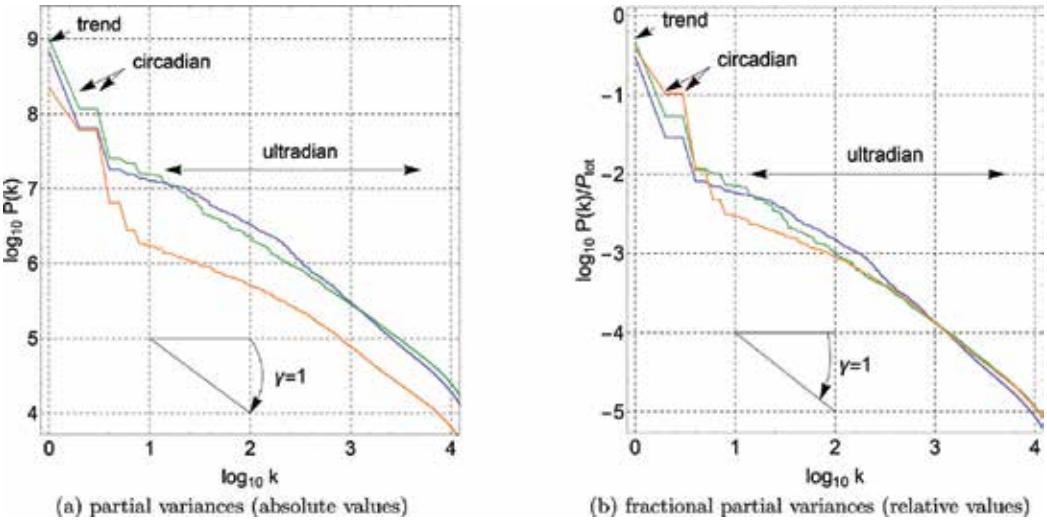


Figure 4. Comparison of the Fourier scree diagrams $P(k)$ for subjects A (green), B (blue) and C (orange), using (a) absolute values and (b) relative values where the total variance of all time series has been normalized Var(x) = 1.

accurate estimate of the value of the spectral exponent β. The power spectrum $P(f)$ can be re-ordered in the shape of a so-called *scree diagram* or *Zipf*-type plot $P(k)$, where the partial variances have been ordered from the most to the least dominant. The advantage of scree diagrams $P(k)$ is that much less dispersion is present, such that power laws $1/k^{\gamma}$ can be more easily determined, and scaling properties are conserved between both representations, i.e. $\beta = \gamma$. The disadvantage is that the exact frequency ordering is lost: low-frequency oscillations tend to cluster to the high-magnitude side of the scree diagram and high-frequency oscillations to the low-magnitude noisy tail, but not all components follow this tendency. Scree diagrams for subjects A, B and C are compared in **Figure 4**. In absolute values (panel a), partial variances of time-series components are comparable for subjects A and B at all scales, whereas those for subject C are smaller. In relative values (panel b), on the one hand, subject C has a larger and subject B a smaller contribution of the circadian cycle in comparison with subject A, but on the other hand, subject C has less and subject B more rhythm fragmentation over a wide range of ultradian frequency scales. Overall, ultradian components appear to scale according to a $1/f$ power law ($\gamma \approx 1$) for subject A, whereas this power law appears to be slightly broken below $k = 10^2$ for subjects B and C. Fourier spectral analysis (Eq. (5)) allows to filter the spectral components of time series. Coefficients of unwanted spectral contributions can be put zero, $A_\kappa = 0,$ or $B_\kappa = C_\kappa = 0$, for selected κ, to construct a low-pass, high-pass, bandpass or band-stop filter. **Figure 5** shows results for a bandpass filter applied to the time series of subjects A, B and C where arbitrary limiting frequencies $f_{\min} = 1$ and $f_{\max} = 20$ were chosen (to include frequencies above a period of 12 h to obtain a circadian cycle with a single maximum per day). The resulting circadian fit describes variability in time and in amplitude, and corresponding circadian parameters are listed in **Table 1**.

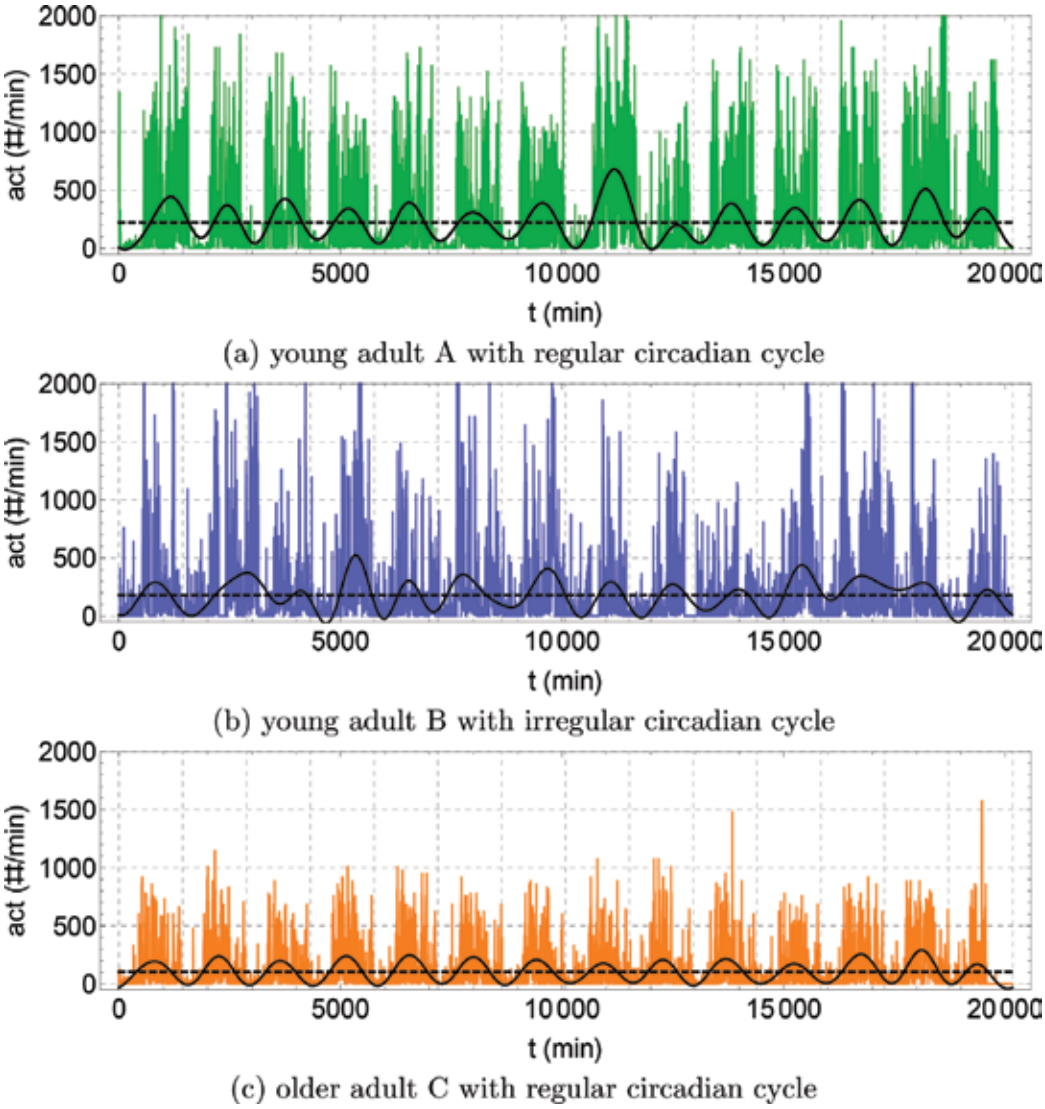


Figure 5. Filtered time series of subjects A, B and C of **Figure 1** using a Fourier filter between frequencies $f_{\min} = 1$ and $f_{\max} = 20$ in units of the number of oscillations for the whole duration of the time series of 2 weeks.

4. Wavelet analysis

4.1. Continuous wavelet transform (CWT)

The basic idea of wavelets is to decompose a time series in terms of a time-frequency set of orthonormal functions [45]. The continuous wavelet transform (CWT), also called analytic

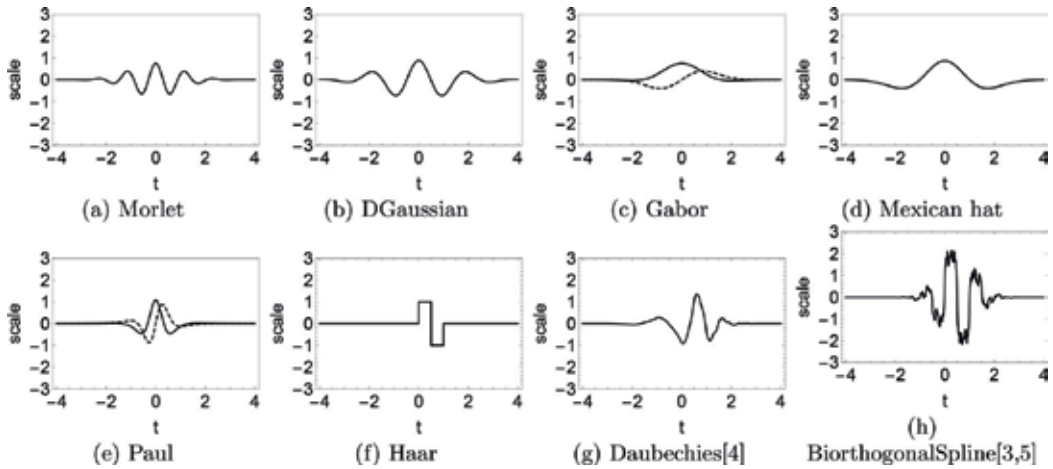


Figure 6. Mother wavelet basis functions, for use with CWT, are (a) Morlet, (b) DGaussian, (c) Gabor, (d) Mexican hat and (e) Paul and with DWT are (f) Haar, (g) Daubechies [4] and (h) BiorthogonalSpline [3, 5]. Basis functions can be real (continuous curve), or complex, in which case the real part (continuous curve) and the imaginary part (dashed curve) are plotted separately.

wavelet transform (AWT), is a measure of similarity (in the sense of similar frequency content) between a basis wavelet function $\Psi(t)$, called "mother wavelet", and the signal $x(n)$ itself. The resulting CWT depends on Ψ (see **Figure 6** for a representation of some popular mother wavelets), but an appropriate choice of basis functions allows to analyze nonstationary time series. The Morlet mother wavelet has been suggested to be one of the most adequate bases for spectral studies [23, 24]. To evaluate CWT, the mother wavelet is translated to be centred at t and scaled by a factor s:

$$W(t,s) = \int_{-\infty}^{\infty} \Psi^* \left(\frac{u-t}{s} \right) x(u) du, \tag{9}$$

where the asterisk denotes the complex conjugate, and the result is the scalogram $W(t,s)$, which represents at each time t the instantaneous period T (represented by scale s) and the instantaneous intensity or power W of the signal [14]. At high frequencies, CWT has a good scale resolution but a poor frequency resolution, while at low frequencies, the frequency resolution is improved but time resolution is lost. One of the most important properties of a scalogram is the so-called ridge, which corresponds with the dominant behavior of the time series and in the present case can be expected to be the circadian rhythm. The extraction of the time-series component corresponding to the ridge can be not obvious, in particular when there are multiple ridges, and is the topic of the data-adaptive method of nonlinear mode decomposition (NMD) [23, 24]. The present discussion of CWT will be limited to the scalograms.

The left-hand panels of **Figure 7** show the scalograms of the time series of subjects A, B and C using the Morlet mother wavelet. In the three cases, there is a high-intensity ridge near

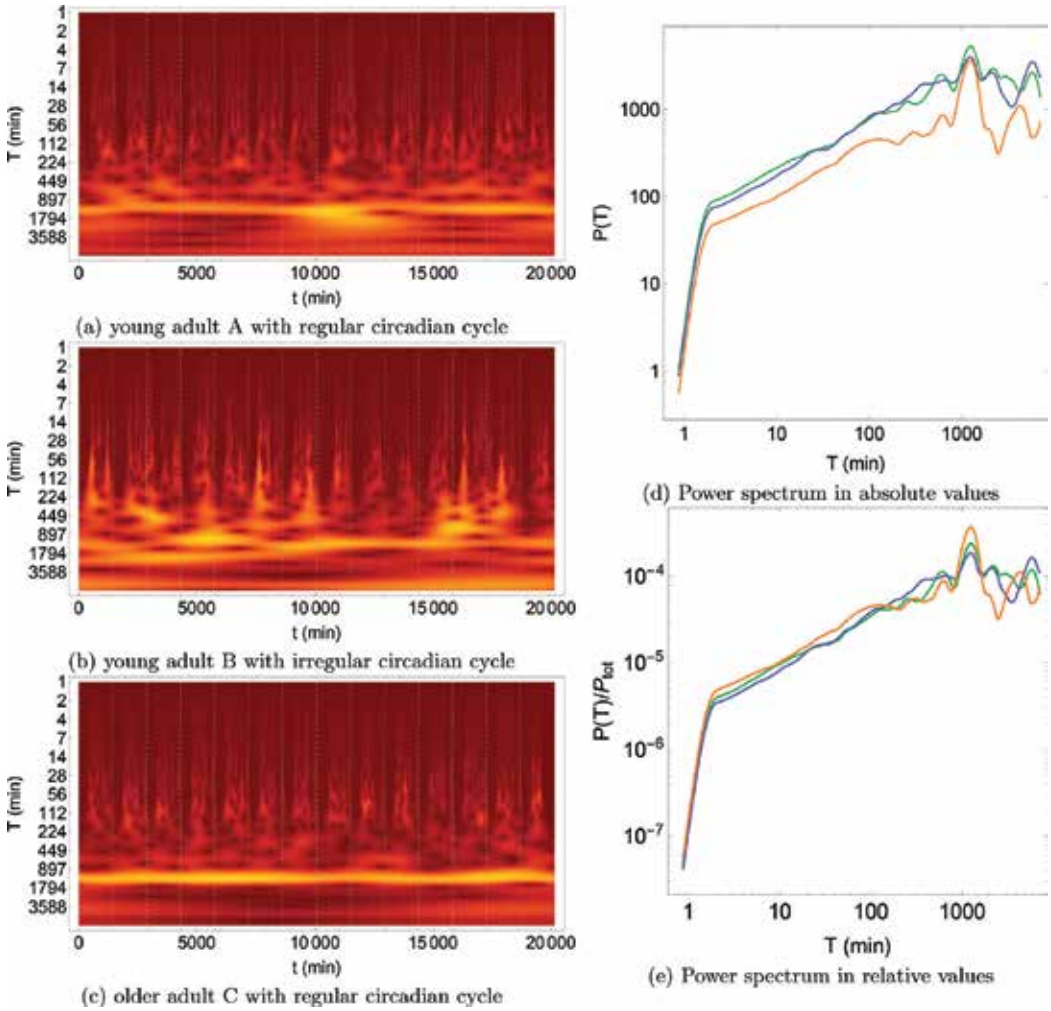


Figure 7. CWT analysis of time series of subjects A, B and C of **Figure 1** using a Morlet mother wavelet. Scalograms are presented in (a)-(c), where vertical gridlines indicate midnight. Power spectra (time-averaged projections on the period axis) are presented in (d) in absolute values and in (e) in relative values (unit variance).

$T \approx 1440$ min, but the intensity and the instantaneous value of T vary in time, reflecting the time and amplitude variability of the circadian rhythm. At ultradian frequencies, $T < 1440$ min, there are fringes of higher and lower intensities that alternate, reflecting day and night with high and low activities, respectively. The scalogram of subject C contains only a circadian ridge and fringes, whereas the scalogram of subject B shows a wide variety of competing and simultaneous features, and the scalogram of subject A is in between both the extremes. The right-hand panels show the average behavior of the scalograms over time,

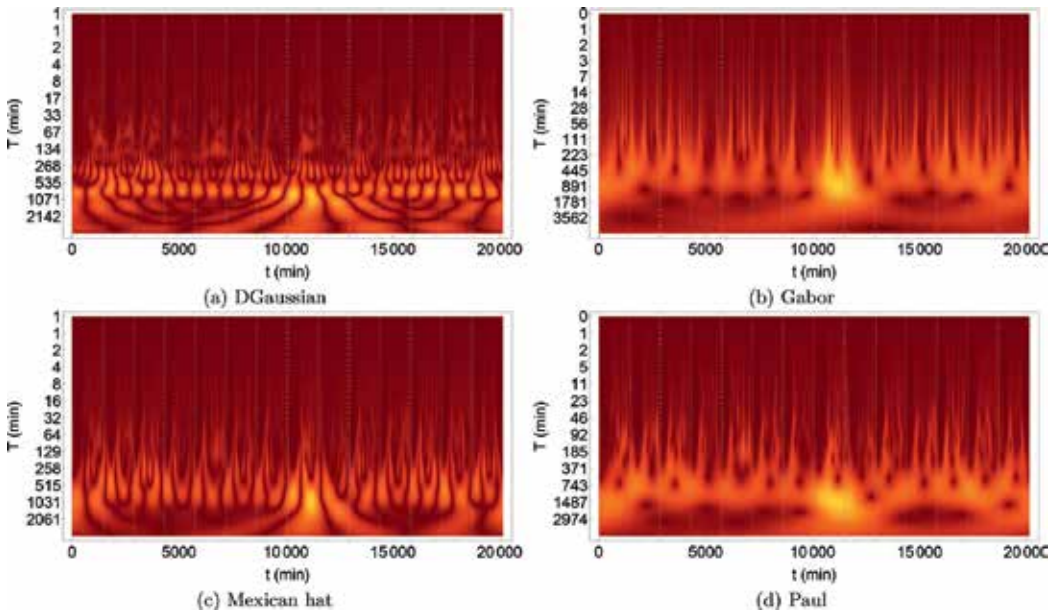


Figure 8. CWT analysis of time series of subject A of **Figure 1** using different mother wavelets: (a) Gaussian, (b) Gabor, (c) Mexican hat and (d) Paul. Vertical gridlines at midnight.

corresponding to a projection of the intensity values $W(t,s)$ on the period axis, which are similar to the Fourier spectral analysis of **Figure 4**. For the three subjects, one can appreciate the dominant power of the circadian period of $T \approx 1440$ min and the $1/f$ scaling behavior at ultradian time scales. In absolute values, all scales have less power for subject C than for subjects A and B, but in relative values, the circadian cycle contributes much more for subject C than for the other subjects. Finally, to illustrate how CWT analysis depends on the wavelet basis, **Figure 8** shows scalograms for the time series of subject A for other choices of the mother wavelet.

4.2. Discrete wavelet transform (DWT)

The discrete wavelet transform (DWT) starts with the partitioning of the signal into an approximation (smooth) and a detailed part, which both together yield the original signal itself. This subdivision is such that the approximation signal contains the low frequencies, whereas the detailed signal collects the remaining high frequencies. By repeatedly applying this subdivision rule to the approximation part, the details of increasingly finer resolution are then progressively separated out, while the approximation itself grows coarser and coarser. This procedure in effect offers a good time resolution at high frequencies and good frequency resolution at low frequencies, since it progressively halves the time resolution of the signal [14, 46]. The result of the DWT decomposition may depend on the wavelet basis chosen (see

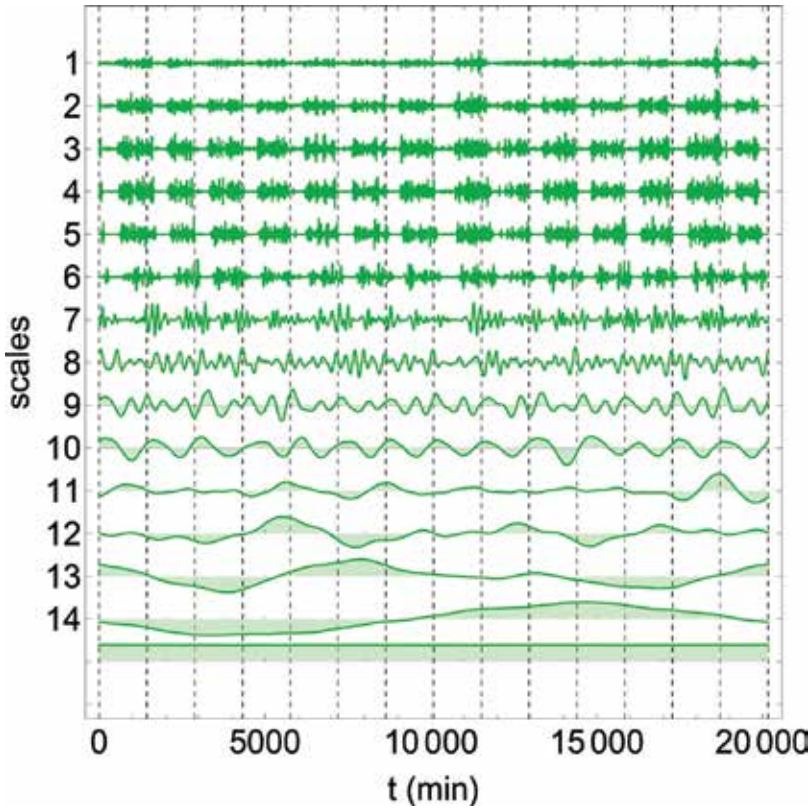


Figure 9. DWT time-series components for subject A using Daubechies [4] mother wavelets. $\mathrm{Log}_2(\mathrm{dim}) \approx 14$ scales have been used in the decomposition, and components are ordered from smallest (scale 1) to largest (scale 14). Components are shown with a common time axis (left-hand column). The 10th scale oscillates approximately once every 24 h and can be identified as the circadian cycle, whereas the smaller scales (1–9) correspond to ultradian rhythms and the larger scales (11–14) to infradian rhythms. The last component, without order number, is the residual after all wavelet components (scales 1–14) have been subtracted and can be interpreted as the mesor.

Figure 6 for an example of some popular mother wavelets), and the results may depend also on the number of scales into which the time series is decomposed. Ref. [15] suggests to use the Daubechies (4) mother wavelet for application of DWT to actigraphy data because of the discontinuous character of these time series.

Figure 9 shows the decomposition of the time series of subject A with DWT using a Daubechies (4) mother wavelet and a maximum number of scales, $\log_2(N) = \log_2(N) \approx 14$. The decomposition of the time series of the other subjects B and C is similar to the example shown for subject A. Visual inspection of the decomposition allows to identify the 10th scale, which oscillations about once every 24 h, as the circadian cycle. **Figure 10** shows the circadian fit for subjects A, B and C. It can be appreciated that the fit reflects the variability in time and in amplitude of the time series. Corresponding circadian parameters are listed in **Table 1**. **Figure 11**

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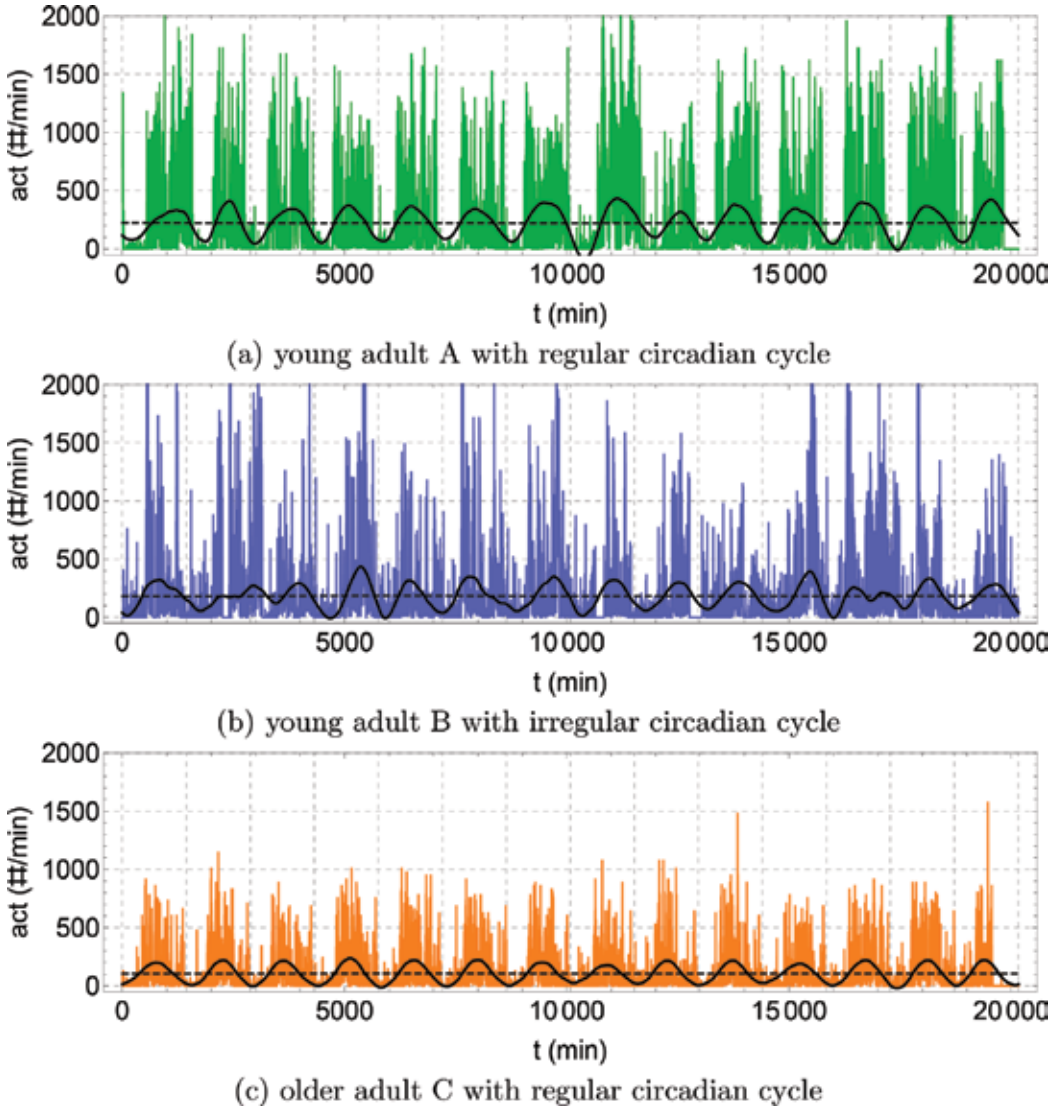

Figure 10. Estimation of the circadian cycle for subjects A, B and C according to the DWT analysis with Daubechies [4] mother wavelets, using the 10th of 14 scales. Vertical gridlines at midnight.

compares the partial variance carried by all 14 scales for the three subjects. It can be seen that both subject A and C present dominant circadian rhythms, whereas subject B is characterized by a large rhythm fragmentation at ultradian scales. Finally, **Table 2** shows coefficients of determination R^2 for circadian fits for different choices of the DWT mother wavelet. Of all choices tried, Daubechies (4) appears to maximize the coefficient of determination R^2.

DWT	A	B	C
Haar	0.154	0.101	0.205
Daubechies (1)	0.154	0.101	0.205
Daubechies (2)	0.179	0.132	0.220
Daubechies (4)	0.174	0.129	0.230
Daubechies (6)	0.143	0.104	0.223
BiorthogonalSpline (1,3)	0.158	0.103	0.212
BiorthogonalSpline (2,2)	0.095	0.046	0.185
BiorthogonalSpline (2,6)	0.106	0.057	0.194
BiorthogonalSpline (3,5)	0.150	0.096	0.201
BattleLemarie (2)	0.119	0.045	0.195
BattleLemarie (3)	0.127	0.075	0.212
BattleLemarie (4)	0.152	0.103	0.222
BattleLemarie (15)	0.128	0.079	0.210

Table 2. Coefficient of determination R^2 according to DWT analysis using different types of mother wavelet. As suggested in Ref. [15], the Daubechies (4) basis is the most adequate to describe circadian cycles in actigraphy data, and the values given here are the same as listed in **Table 1**.

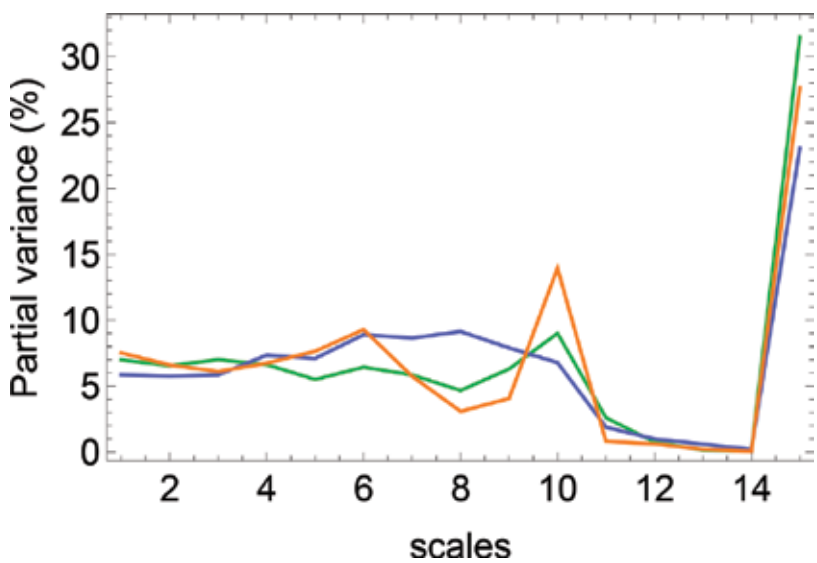

Figure 11. Partial variance (%) carried by the scales 1–14 of the DWT analysis for subjects A, B and C, using Daubechies [4] mother wavelets. The last (largest) partial variance corresponds to the residual after all wavelet components have been subtracted of the time series.

5. Singular spectrum analysis (SSA)

SSA has been discussed in detail in a number of textbooks [16–18]; a short and very accessible introduction can be found in Ref. [19], whereas a larger and very complete review article is in Ref. [20]. We have discussed the SSA method previously in Ref. [25]. In brief, SSA can be explained as a three-step process: (i) the time series is transformed into a matrix which represents the underlying phase space of the time series, (ii) singular value decomposition (SVD) is applied to decompose this matrix as a sum of elementary matrices or—equivalently—to decompose the original phase space in a superposition of "subphase spaces" and (iii) each of the elementary matrices or "subphase spaces" is transformed back into a time-series component. Unlike Fourier analysis which expresses a time series as a sum of predefined sine and cosine functions, SSA can be considered to be *data-adaptive* or *model-independent* because the basis functions are generated from the data itself. It can be shown that the sum of all time-series components is identical to the original time series. Below, a summary is given of the most important outcomes of SSA analysis. When applying SSA to a discrete time series $x(n)$ with length $n = 1, \ldots, N$ (see Eq. (1)), a particular window length L must be chosen as an initial parameter, with $2 \leq L \leq N/2$, which allows to fix the number of components r into which the time series will be decomposed:

$$x(n) = \sum_{k=1}^{r} \sigma_k g_k(n), \tag{10}$$

where $g_k(n)$ is the time-series component, σ_k is the *singular value* that serves as weights for the components and $r \leq \min(K, L)$ with $K = N - L + 1$. Only (quasi-)periodicities with average length $T \lesssim L$ will be resolved into separate time-series components, whereas those with lengths $T > L$ will be absorbed in the trend component. One can chose L as a multiple of the (average) periodicity of the data, i.e. $L = mT$, where m is an integer number. In the case of circadian data, the obvious choice would be $L = T = 24$ h = 1140 min. It can be shown that in the limit for $L \to N/2$, SSA converges to Fourier spectral analysis [20], where a time series is always decomposed as the superposition of $N/2$-independent oscillators (Nyquist theorem). Whereas the Fourier power spectrum of a quasiperiodic time series with average period T would correspond to a broad Gaussian peak around the central frequency $f = 1/T$, intuitively, it can be understood that for an adequate choice of the parameter L the neighboring Fourier components of this broad Gaussian peak can be "compressed" within a single SSA component. One of the main results of SSA analysis is the so-called scree diagram that visually represents the *partial variances* $\lambda_k = \sigma_k^2$, ordered according to magnitude from the most to the least dominant, where λ_k can be interpreted as the variance of the "subphase space" of time-series component $g_k(n)$ and where $\lambda_{tot} = \sum_{k=1}^{r} \lambda_k$ is the total variance of the phase space of the original time series $x(n)$. The dominant partial variance λ_1, associated with component $g_1(n)$, usually corresponds to the trend. Dominant periodicities can be recognized as "steps" in the scree diagram, i.e. two successive partial variances λ_k and λ_{k+1} that are nearly degenerated and clearly distinguishable from the neighboring partial variances, where the corresponding components $g_k(n)$ and $g_{k+1}(n)$ are the Fourier equivalents of a sine and cosine function with the same

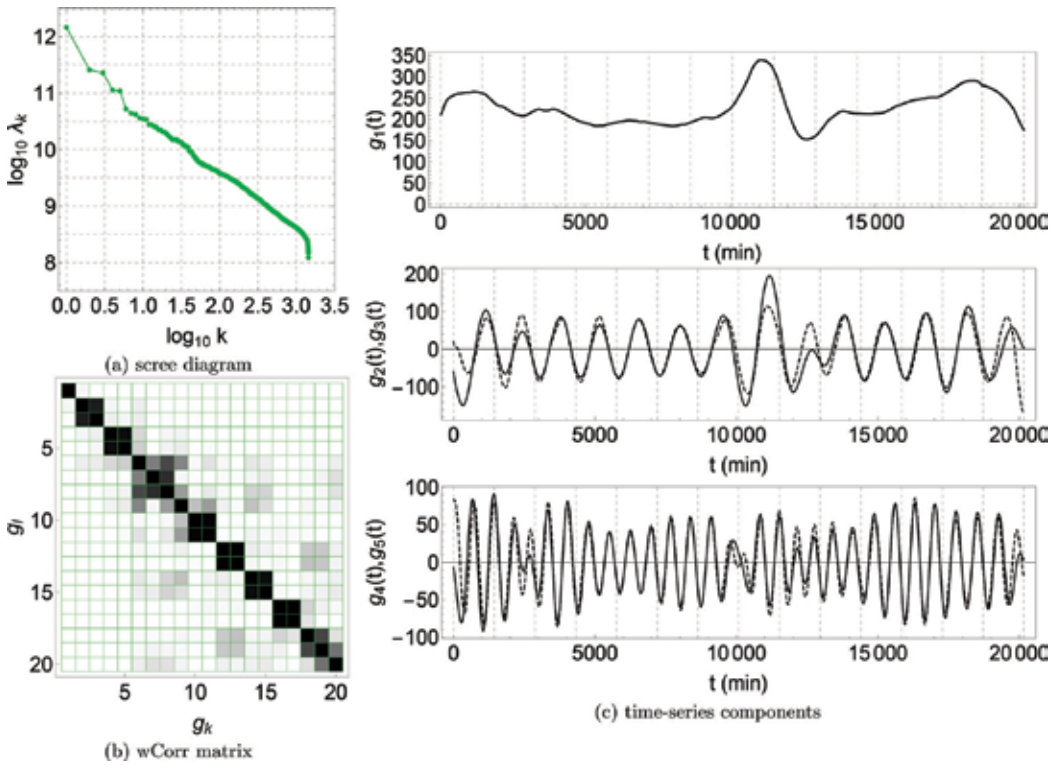

Figure 12. SSA analysis of time series of subject A of **Figure 1**. Shown are (a) the scree diagram of partial variances λ_k, (b) the matrix of weighted correlations between the first 20 time-series components $g_k(t)$ and $g_l(t)$ with $k, l = 1, \ldots, 20$ and (c) the first five time-series components $g_k(t)$ with $k = 1, \ldots, 5$, of which $g_1(t)$ is the trend component, $g_2(t) + g_3(t)$ is the circadian rhythm and $g_4(t) + g_5(t)$ is the 12 h ultradian rhythm.

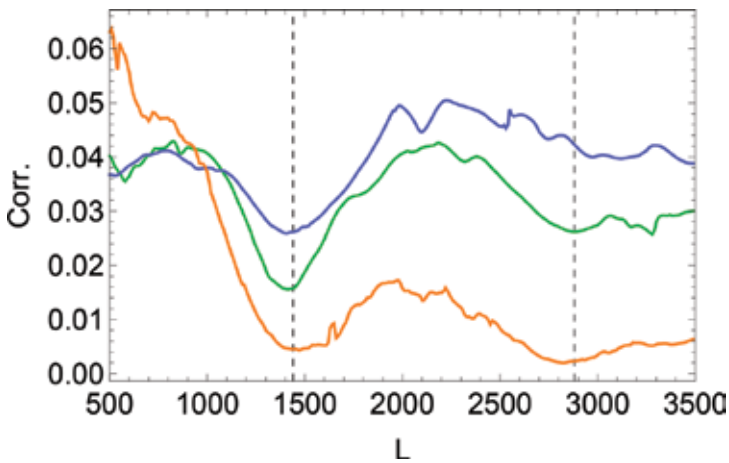

Figure 13. Average correlation the circadian mode $g_2(t) + g_3(t)$ with the neighboring components $g_1(t), g_4(t), \ldots g_{20}(t)$ as a function of parameter L for subjects. Average correlation is lower for subject C (orange curve) than for subject A (green curve), which is lower than for subject B (blue curve). Gridlines indicate multiples of the 24-h period, 1440 and 2880 min.

frequency. Higher-order partial variances λ_k tend to have values that decrease gradually and continuously with k, indicating that at these scales it is impossible to distinguish any individual time-series components $g_k(n)$ that separately can be assigned physical significance.

Figure 12 shows some details of the decomposition of the time series of subject A. In the scree diagram, a trend mode $g_1(t)$, a mode $g_2(t) + g_3(t)$ which we will identify as the circadian (24 h) cycle and a mode $g_4(t) + g_5(t)$ which we will identify as an ultradian (12 h) cycle, can be distinguished with distinctive partial variances, and there is a long tail with components with similar partial variances that appear to obey a power law $1/k^{\gamma}$ with $\gamma \approx 1$. The weighted correlation matrix shows that the trend mode and the circadian mode are uncorrelated from the higher-order modes, but the ≈12-h mode does seem to have non-neglectable correlations with other components. The waveforms of before-mentioned modes confirm that they

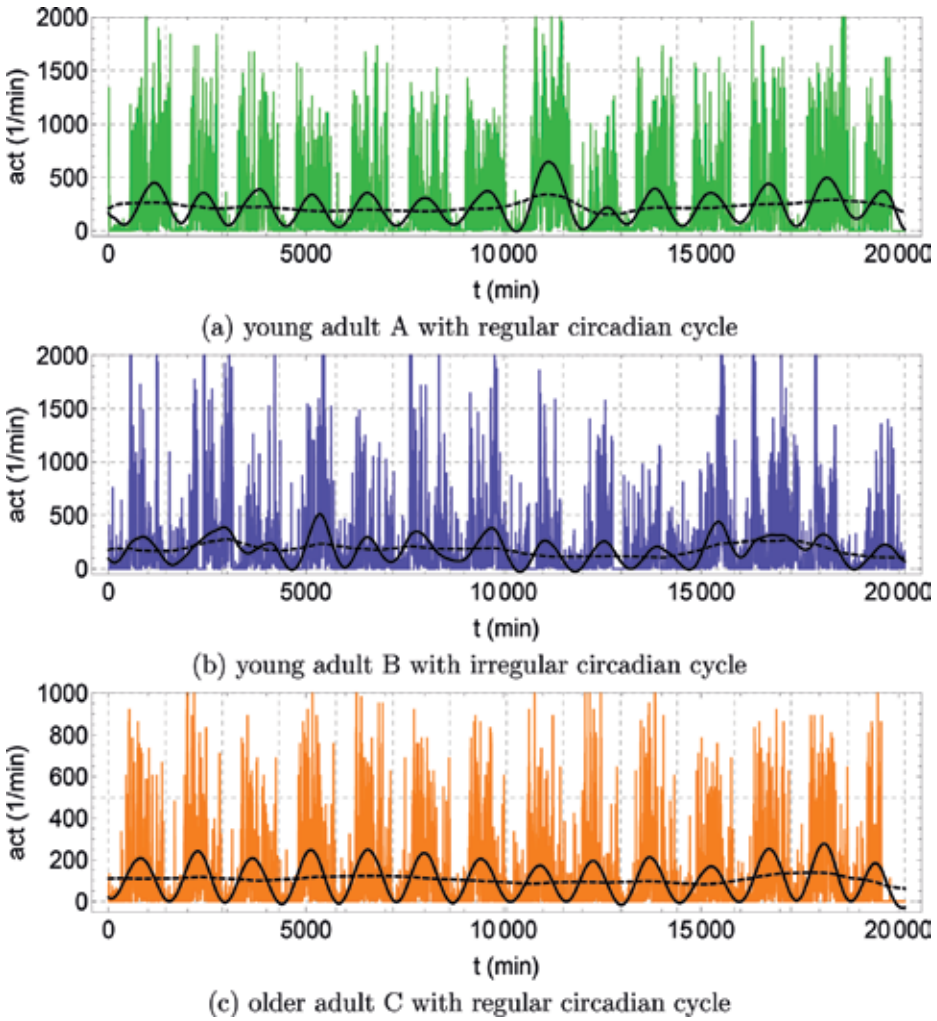

Figure 14. Estimation of the circadian cycle of subjects A-C according to SSA analysis using $L = 1440$.

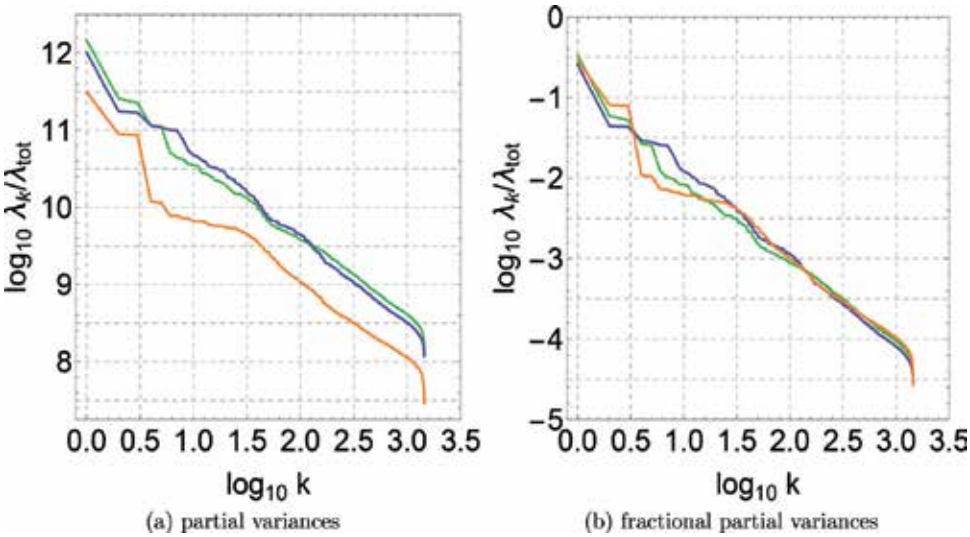

Figure 15. Scree diagram of SSA analysis of subjects A-C, showing (a) partial variances and (b) fractional partial variances.

correspond to the trend, the circadian rhythm and a ≈12-h ultradian mode. **Figure 13** shows the average correlation of the circadian mode with other SSA components, and broad minima can be observed for L being equal or a multiple of the circadian periodicity, $L = m \times 1440$ min. The more regular the circadian rhythm, the less correlated it is with the other time-series components and the better it can be isolated. **Figure 14** shows the circadian fit with SSA for subjects A, B and C using $L = 1140$ min, and it can be appreciated that it describes the variability in time and amplitude of the time series. Corresponding circadian parameters are listed in **Table 1**. **Figure 15** compares the scree diagrams for subjects A, B and C and is similar to **Figures 4** and **7(d-e)**. In absolute values, it shows that time series of subjects A and B have comparable variances, which are much larger than the variance of the time series of subject C. In relative values, it shows that subject C has the strongest circadian rhythm and subject B the weakest. At the highest ultradian frequencies ($10^{1.5} \leq k \leq 10^{3.0}$), all subjects show a very similar behavior with a $1/f$ scaling. At the lowest ultradian frequencies ($10^{0.5} \leq k \leq 10^{1.5}$), $1/f$ scaling continues for subject A, whereas rhythm fragmentation is increased for subject B and decreased for subject C.

6. Discussion

The interest of the field of chronobiology is shifting from a description of the periodicity of the circadian cycle to a quantification of deviations from regularity. The objective of the present contribution is to compare several methods in their description of irregular rhythms: the

cosinor analysis, the Fourier filter, the continuous (CWT) and discrete wavelet transform (DWT) and the singular spectrum analysis (SSA). We are interested in irregular rhythms at the circadian time scale and rhythm fragmentation over a wide range of ultradian scales. Our aim is to illustrate the differences, similarities, advantages and disadvantages of the different methods using selected actigraphy time series.

We will first discuss the circadian time scale. According to the coefficient of determination R^2 of **Table 1**, the Fourier filter, DWT and SSA describe the circadian cycle better than the cosinor analysis with one single period, and SSA gives the best description of all methods discussed here. One of the reasons may be that cosinor cannot take into account the variability in time and amplitude of the experimental time series, whereas the other methods can. It is less clear why SSA analysis results in the best fit, the average amplitude and the variability of the circadian parameters tend to be larger for the Fourier filter than for SSA, but they tend to be smaller for DWT. The goodness of fit to the data for DWT depends on the specific mother wavelet used, but we chose the Daubechies (4) mother wavelet because of the maximized R^2 for all the different mother wavelets that we experimented with. On the other hand, the number of DWT scales might be increased or decreased, in order to adjust the variability of the circadian mode for a better fit to the experimental data, but there is no rule of thumb that says how many scales to choose. It is possible that the Fourier filter description may be improved by carefully adjusting $f_{\min}$ and $f_{\max}$, but it is not a priori clear which limiting frequencies will result in the best R^2. **Figure 13** suggests that it might be possible to slightly improve the SSA description by fine tuning the parameter value L to a value for which a global minimum is obtained in the correlation of the circadian mode $g_2(t) + g_3(t)$, but on the other hand, the broad minima basins suggest that the calculations are rather stable. Thus, as long as $L \approx 1440$, the precise value of L and the number of components into which the time series is decomposed have little influence on the description of the circadian cycle by SSA. **Table 3** compares the similarity of the description of the circadian cycle between the different methodologies. Results for the Pearson product-moment correlation r and Spearman's rank correlation ρ are very similar. When the circadian cycle is very regular, as for subject C, descriptions by different methods resemble very much. On the other hand, when the circadian cycle is very irregular, as for subject B, the different methods of cosinor, Fourier filter, DWT and SSA give different results.

A		r				B		r			C		r		
		cosinor	**filter**	**DWT**	**SSA**		**cosinor**	**filter**	**DWT**	**SSA**		**cosinor**	**filter**	**DWT**	**SSA**
ρ	**cosinor**		0.758	0.845	0.840	ρ		0.646	0.757	0.791	ρ		0.933	0.968	0.976
	filter	0.793		0.881	0.949		0.638		0.830	0.906		0.930		0.964	0.977
	DWT	0.843	0.912		0.950		0.758	0.820		0.949		0.960	0.963		0.992
	SSA	0.842	0.953	0.950			0.790	0.894	0.947			0.968	0.976	0.991	

Table 3. Correlation between the calculation of the circadian cycle according to the cosinor method, the Fourier filter, DWT and SSA, for subjects A, B and C. Results are given for the Pearson product-moment correlation r (upper-right triangle) and Spearman's rank correlation ρ (bottom-left triangle).

We will now discuss the ultradian rhythm fragmentation. The Fourier scree diagram, the CWT power spectrum and the SSA scree diagram suggest a trade-off effect. If subject A is taken as a reference, then subject C would seem to exhibit a very strong circadian rhythm associated with an important reduction of rhythm fragmentation at a wide range of ultradian scales, and, as a consequence, there is a flattening of the $1/f$ power law over the same frequency range, resulting in an overall rigid rhythm; subject B, on the other hand, is characterized by a reduced circadian contribution and an increased rhythm fragmentation over a wide range of ultradian scales, which leads to an increased power law slope over the same frequency range, resulting in an overall more random rhythm.

Of course, the three time series studied in the present contribution are not sufficient to draw any definite conclusions on the average values of the circadian parameters and their variability or on rhythm fragmentation at ultradian scales; therefore, a much larger statistical study is needed, but we have shown that circadian and ultradian scales can be studied within the same approach, and we hypothesize that partial variances are related over wide circadian and ultradian scales.

7. Conclusions

In recent years, there is a shift in interests in chronobiology where a larger emphasis is now put on an accurate quantification of irregularities of circadian rhythms and ultradian rhythm fragmentation to follow underlying pathologies. Wavelet analysis has probably been the method of choice to describe irregular rhythms at different time scales, but wavelet analysis has the drawback to depend on the choice of a mother wavelet which is arbitrary and user dependent. Data-adaptive time-series decomposition, where the basis functions are generated by the data itself, such as singular spectrum analysis (SSA) may offer an alternative. In the present contribution, we have shown that SSA is at least as versatile and accurate as wavelet analysis in the description and quantification of irregular rhythms at the circadian and ultradian time scales and may be a useful method to be adopted in the field of chronobiology.

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Chapter 5

Jet Lag

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Additional information is available at the end of the chapter

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Abstract

This chapter describes the phenomenon of Jet Lag and the symptoms associated with it, which vary not only from person to person, but also according to how many times zones are crossed, and in which direction. Homeostatic and circadian influences on sleep and vigilance are explained on the basis of Borbély's two-process model. Jet Lag is reasonably well explained scientifically today: rapid changes of time zones disturb the functioning of the body clock, which remains stubbornly set on departure times for a while. This can make sleep shallow or non-existent for substantial parts of the night while vigilance is less than optimal during parts of the day. Two main lines of research are described: one endeavors to accelerate the adaptation process; the other helps to fight insomnia and sleepiness between arrival and adjustment to the new time zone. Besides practical things that can be done to reduce the burden of Jet Lag, the adjustment process can be speeded up using bright light and melatonin. Sleeping pills and neurostimulants may be added to compensate for insomnia and sleepiness.

Keywords: chronobiology, circadian, circadian clock, homeostatic, Jet Lag, sleep debt, sleep inertia, sleepiness, time zone, vigilance, light, melatonin

1. Introduction

Jet Lag is a phenomenon that annoys to varying degrees the millions of people who travel across time zones each year. Its causes are relatively easy to understand.

Jet Lag results from a mismatch between the actual time in the arrival zone, and the internal time, which is set by the internal body clock (see below), itself reflecting the preceding time zone.

Its main symptoms include sleepiness at odd times during the day, and insomnia at night. They vary according to several variables, from individual sensitivity to age, to flight direction (East or West), to number of meridians crossed, to length of stay and to seasons.

Jet Lag treatment remains difficult, as no simple remedy is available, yet. As long as the earth keeps spinning round and our genome is not modified, the bad news is that Jet Lag is here to stay. Probably, some day it will be possible to turn the body clock forward or back and reset it immediately to the new time zone, but such a procedure has yet to be discovered. The natural process of adjustment to new time zones is efficient but slow.

In this chapter, we review the scientific literature on Jet Lag origin, describe its symptoms in more detail, and see what can be done about it.

2. What is Jet Lag?

2.1. Definition

The American Academy for Sleep Medicine (AASM), in the second edition of its International Classification of Sleep Disorders [1] defined Jet Lag (circadian disorder, Jet Lag type), as a disorder "related to a temporal mismatch between the timing of the sleep-wake cycle generated by the endogenous circadian clock produced by a rapid change in time zones." In other words, it results from crossing time zones too rapidly for the circadian clock to keep pace.

2.2. Sleep propensity in humans: circadian and homeostatic

2.2.1. Circadian influences

In mammals, periods where sleep is favored alternate with periods where it is not. The timing, the duration and the frequency of these periods are regulated in part by the circadian body clock (see below). Timing, duration and frequency also vary across species.

Mice for instance sleep in between 40 and 120 bouts of a few minutes per day-night periods (polyphasic sleep). Human infants also show polyphasic sleep, as infants frequently wake up at night and sleep partly during the day. Most of the time, sleep episodes progressively converge to monophasic sleep with growing age, until one large sleep episode occurs at night. This monophasic organization of sleep is somewhat challenged in older life and there may be a return to forms of polyphasic sleep.

Sleep propensity in humans is maximal at the beginning of the night, moderate in the afternoon, and minimal during the morning and the evening. In parts of the world where working is difficult in the afternoon for climatic reasons, sleep has been observed to come in two phases (biphasic sleep). As our distant ancestors came from tropical areas where work is harder when the sun is high, this biphasic organization of sleep may well have been the first one to have appeared, which might explain a biphasic design.

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2.2.2. Homeostatic influences

The propensity to sleep is also a function of the time lapse since the last sleeping episode. The longer the time since the last sleeping episode, the stronger the pressure to enter sleep.

2.2.3. Result of two processes

Propensity to sleep is thus the result of two processes, one circadian and one homeostatic. The combination of these two processes has been first modelized by Borbély [2]. The relative weight of the two processes against each other has not been determined to the day, but I assume that they are roughly equivalent.

The intensity of both processes (circadian and homeostatic) is minimal in the morning after a normal episode of sleep, which favors awakening. As the homeostatic pressure has already decreased and the circadian influence allows for some sleep, a tendency to nap may be observed in the afternoon. The processes are at odds in the evening—the homeostasis favoring sleep but the circadian factor opposing it—which explains why it is difficult to enter sleep at that time of the day. The processes converge at the beginning of the night, hence favorizing sleep.

2.3. Body clock (inner clock, internal clock, circadian clock)

Like other mammals, human beings have a circadian clock, a paired group of nuclei in the base of the hypothalamus—the suprachiasmatic nuclei. They receive information from the retina about external light. These data allow for a daily-based correction and synchronization with the daily cycle of light and dark. The suprachiasmatic nuclei are considered together to represent the "master clock" of the brain, synchronizing a series of hormones (such as melatonin, cortisol and growth hormone), behaviors and other more specialized body clocks (core body temperature, rapid eye movement sleep, for instance).

The actions of the suprachiasmatic nuclei monitor the change from day to night. They have a useful stabilizing function on the body's biological rhythms, but they are slow to react to changes in lighting schedules, so that after time zones have been crossed rapidly, the endogenous signals for sleep and wakefulness do not match the local light-dark and social schedules.

How is the body clock indexed by external time? The major factor is the alternation of dark (night) and light (day) periods. The light signals are transmitted to the hypothalamus and support alignment of the inner body clock to the external time. There are also minor time clues (often called by their original German name, "Zeitgebers"). Minor Zeitgebers include social schedules, eating and drinking patterns, external temperature and physical activity.

Core body temperature is one of the best markers of the body clock. It is important as it has been linked with sleep and its rapid eye movement (REM) and non-rapid eye movement (NREM) components. "The ease of getting to sleep and staying asleep depends not only on previous wake time, but also on associations with the circadian rhythm of core temperature. Sleep is easiest to initiate when core temperature is falling rapidly or is at its lowest and most

difficult when body temperature is rising rapidly or is high. Waking is the opposite of sleep initiation, because it occurs when core temperature is rising or is high. Sleep is favored when body temperature is low or rapidly descending, as at the end of a normal evening" [3].

Some of the biological cycles adjust in a few days, while others, such as the core body temperature, take more time [4].

2.4. When the body clock is not in phase with external time

2.4.1. Body clock resilience: shift work

One fundamental aspect of the circadian influence is its resilience over time. A good example is shift work. If someone with a regular work schedule abruptly shifts to a night work, that person will usually find it quite difficult to enter sleep in the morning.

This would not make sense if there was only a homeostatic force at play. Indeed, the homeostatic pressure will be maximal after a night with no sleep, since the last sleep episode ended 24 h earlier. The homeostasis hence pushes to enter sleep as soon as circumstances will allow for it.

Sleep will however be difficult to find because the body clock insists that it is time to wake up. As a consequence, sleep, if it happens, will be shallowed and fragmented. It is only after several days that the new schedule will be integrated.

And then it could be time to change the work timetable again. Continuous shift work is an unending, impossible to stabilize, and most likely unhealthy process that modern day society is unfortunately not ready to reduce.

2.4.2. Body clock resilience: Jet Lag

Jet Lag is roughly the equivalent of shift work when people are transported in a fast way (jet airplanes) across one or several meridians, that is, east- or westwards. No Jet Lag should be expected in purely north- or southwards trips. When meridians are traversed, the internal clock remains indexed, for a few days at least, to the old schedule. It is thus not adjusted yet to the new timetable.

2.4.3. Shift work, Jet Lag and Zeitgebers

There is one substantial advantage of Jet Lag over shift work. In shift work, everything around the worker reminds her that it is presently night and that other people sleep. Most shops are closed, the sky is dark, there are less sounds from the city. The time clues are working against her and do not help synchronize the biological clock. In Jet Lag, what seems strange is the daytime sleepiness and the insomnia at night, although it is basically the same phenomenon. That is because the Zeitgebers here continuously act in favor of adjustment: external light, eating and other social schedules help adjust as fast as possible.

2.4.4. Jet Lag and travel fatigue

Jet Lag must be distinguished from travel fatigue, that happens every time transport has been long and uncomfortable, be it in car, train, boat or jet. Travel fatigue vanishes after a good night sleep. Jet Lag does not.

2.4.5. Sleep debt

An important factor in the understanding of Jet Lag is the issue of sleep debt (cumulative sleep loss). Insomnia of an hour or two during the first night will add to 1 or 2 h on the second night, and so on. The result after 2–3 days is increased sleepiness, which will favor sleep at times where a fast adaptation would indicate to stay awake (long naps at inappropriate times for instance, staying asleep too long in the morning or going to bed too early). This will delay the adaptation process.

One hour of sleep debt has been compared to the absorption of at least 33cl of beer [5]. A cumulative debt of 4 h should thus be compared to the absorption of four-five times 33cl. Sleep debt has also been found to parallel alcohol intoxication [6]. It is easy to understand that driving a car, for instance, should be avoided. Similarly, other important activities will be negatively impacted.

2.4.6. Sleep inertia

And then there is sleep inertia. Coming out of sleep is not comparable to an electrical switch, it is a biological process that has its complex rules. If you have slept enough and have no circadian problem, then you should feel fully awake after about 15–30 min. But if you have a sleep debt, or are suffering from the effects of shift work or Jet Lag, or have just had too long a nap, your brain may be half asleep, sometimes for hours, unable to sleep but unable to work properly either.

3. Jet Lag symptoms

3.1. Descriptive data

Jet Lag symptoms are frequent. Rogers and Reilly [7] indicated that 74% of surveyed travelers reported some form of Jet Lag. Of these respondents, 50% reported above-average tiredness and fatigue and 28% indicated some disruption in normal sleeping patterns. And 5% reported difficulties in concentration, while 5% reported eating problems. Gisquet [8] reported 80% of sleep disorders among travelers: difficulty in falling asleep, insomnia, premature awakening and daytime sleepiness. Among very frequent travelers such as flight personnel and international business executives, the disorder may be recurrent or even chronic [9].

The most obvious Jet Lag symptoms for the majority of travelers are the inability to sleep during destination night and to remain alert during destination day. It also includes difficulty in concentrating, clumsiness, difficulty with memory, general weakness, dizziness and lethargy [10]. General malaise, dysphoric mood, headaches and gastrointestinal disorders (indigestion, loss of appetite, bowel irregularities) have also been described but cause less harm.

Symptoms logically vary according to the time of day where assessments are made. They will be lighter when day-time overlaps with a day-time at home (or when night-time overlaps with night-time at home). They will be worse when day-time is superimposed on night-time at home (sleepiness) or when night-time is superimposed on day-time at home (insomnia).

Morning-type people may find it more difficult to adjust westwards and evening-type people have more trouble traveling eastwards [11]. People with more rigid sleeping habits suffer more [12]. Short sleepers understandably adjust easier. Age over 60 results in greater difficulty in adjusting to Jet Lag [13]. Gender does not make a significant difference [14].

3.2. Number of time zones crossed

As could be expected, symptoms of Jet Lag are generally worse the more time zones are crossed. Although traveling over one to four time zones is not universally considered Jet Lag, I propose here to do so for the sake of consistency. If the normal sleep schedule is partly included in night-time at destination (more or less from ±1 to 7–8 h), there is at least some overlap with the usual sleeping hours: the problem will either be to have difficulties in keeping on sleeping (having flown westwards) or in falling asleep (eastwards) but some sleep will be found at least. When more than seven or eight time zones are crossed, then sleepiness will be experienced during the whole day and sleep will always be difficult to find at night. This spectrum of symptoms also explains why Jet Lag treatment standardization is difficult.

Flying west is usually considered easier in terms of Jet Lag. This is intuitive, as it is easier to stand some sleepiness late at night than trying to force oneself to sleep when there is no urge to do so. To almost fully adjust, and as a rule of thumb, it is generally considered that 1 day is needed for an hour eastwards, and 2 or 3 days for an hour westwards. Six hours eastwards (e.g., New York to Brussels) thus mean six days to adjust. Six hours westwards (Brussels to New York) mean 4 days. Above 8 h, time zone difference, it is not clear whether you will adjust by advancing or retarding the body clock and there may be individuals who do either one or the other [15].

3.3. Sometimes severe adverse effects

Jet Lag is usually medically benign and self-limited. It may though occasionally cause serious misjudgments in business and professional dealings. As mentioned above, it may ruin holidays, especially if they are of short duration. Athletes will not perform at their highest level if the timing of contests does not coincide with their circadian peak and training occurs at inappropriate times [16].

But Jet Lag can sometimes be a more serious health issue. Loss of sleep is usually translated into excessive daytime sleepiness, and sleepiness is clearly associated with reduced performance and cognitive reaction time [17, 18]. Pilots on long-haul east-west flights have, for instance, been shown to microsleep during the “cruise” phase of a flight and even during the critical transition from the cruise phase to the descent phase, the point at which the planned descent to final approach altitude is initiated [19].

Countermeasures, such as controlled short cockpit naps, crew alternation or alertness monitoring have been proposed [20]. It has been estimated that 21.9% of all road accidents are related to sleepiness in general [21]. Business people have been observed to function at only 80% of their perception after traveling [22]. Studies in shift workers, who also suffer repeated episodes of dyssynchrony, suggest even more severe consequences, including rates of cancer,

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cardiovascular disease and female reproductive problems [23, 24]. The part played by Jet Lag in performance degradation and accidents has not been estimated precisely so far but might be substantial.

4. Remedies

4.1. General considerations

4.1.1. The chronobiologist and the military

There are two main pathways followed by scientists to counter Jet Lag. Most studies have been performed by chronobiologists and have as their main goal to speed up the adjustment of the body clock to the new schedule, in particular using timed exposure and avoidance of bright light, as well as the timed use of the hormone melatonin. At the present time, chronobiologists have often succeeded in accelerating and thereby reducing the adjustment process. Their recommendations still make for a slow and not fully satisfactory result, especially when many time zones are crossed.

The second school of research is more pragmatic. Here we start from the point of view that we want to be as fully operational as we can and as soon as possible. So the body clock is more or less left to itself and allowed to take its time without much interference. The aim is to counter insomnia and fatigue or sleepiness with substances that help sleeping and waking until the moment the adjustment is complete and the body clock has taken over. Obtaining enough sleep at more or less the right time, albeit somewhat artificial through the use of sleep inducers, is crucial to be in shape the next day, helping to adjust to Jet Lag, and is also far more comfortable than insomnia. On the other hand, fighting excessive daytime sleepiness has been studied in diseases that make people abnormally sleepy during the day, such as narcolepsy. This is not Jet Lag, although many symptoms are shared, but this information may be useful. However, the pragmatic approach has only been fully developed in the military, as they understandably need realistic answers to the issues of sleep, fatigue and vigilance, so that soldiers and pilots are at their best on the battlefield 365/24/7 if needed and do not risk their lives unduly because of lack of vigilance. The services need to control Jet Lag as fast as possible and have a long and valuable experience with drugs, from which civilians can probably benefit.

4.1.2. What do people usually do?

Taking sleeping pills during flights and for a few days when at destination is in fact what many travelers do today, as an off-label (i.e., unapproved) treatment. This helps at least to obtain a subjectively better sleep, hopefully to be more awake the next day. Most people also use caffeine, some use alcohol and some even use stimulants. But most travelers do not know which medications to use, at which dosage and when to take them, in large part because one Jet Lag is not another Jet Lag.

Several general remedies have been proposed: low or high carbohydrate "Argonne" diets, relaxation, fresh nutrients, baths, aromatherapy, Bach flowers, essential oils, Ayurveda, homeotherapy, avoiding the stress of packing, adjusting to seasonal differences, physical exercise, foot massage, and optimal flight schedules, to name a few). They may prove to be effective at some point and hence be considered part of the proposals against Jet Lag, but I consider today that the evidence is not there yet.

4.1.3. Breaking the issue down

In fact, situations vary considerably depending on whether three or ten time zones are crossed. They also depend on whether the travel is done eastwards or westwards. I like to divide Jet Lag into three clusters. Unless one moves across more time zones than the amount of hours usually taken to sleep, part of the destination night overlaps with the normal sleep schedule.

Going westwards means resisting sleepiness in the evening, falling asleep rather easily and waking up too early (Group A). Going eastwards means finding it hard to fall asleep and making for difficult waking (Group C). Travel between West+8 h and East-8 h zones make for a clearly worse problem since there is no overlap at all (Group B).

In more detail, there are 23 different cases (24 time zones minus one - the one where the travel begins). Each of these situations actually deserves a specific « treatment », from the very easy, corresponding to changing the clock for the sake of daylight saving in the spring or autumn, to the real challenges of flying to the antipodes.

4.2. No-nonsense measures

4.2.1. Fast psychological adjustment

It may be interesting to set the wristwatch at the future new schedule as soon as the plane takes off and follows the destination schedules (meals, etc.) right after landing. These new time clues may help the body clock to adjust faster (to what extent has not been measured scientifically). But it cannot harm.

4.2.2. Exercise

It is usually recommended to do some physical exercise to adjust to the new schedules. It could be especially recommended at those times of the day when sleepiness is present. However, it is not recommended to undertake physical exercise less than 2 h before bedtime especially in eastwards flights, where sleep should be difficult to find.

4.2.3. Naps

Sleeping partly during daytime is the rule for babies, infants and many children. As mentioned earlier, this is also the case for adults in many parts of the world: by shifting some of the main sleep load from nighttime to naptime (siestas), working hours are adjusted to more favorable weather conditions. The circadian clock seems less rigid in the afternoon than in

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the morning or the evening and may allow for individual variations that become the usual circadian pattern in the long run.

Naps may produce surprisingly powerful waking effects. Sleeping just a few minutes during the daytime may considerably reduce the urge for sleep that was present immediately before and made concentration difficult. Napping more than 20–25 min, however, can paradoxically be less favorable and induce a slight degree of drowsiness (because of sleep inertia, as we have seen) sometimes for hours. Longer naps also pull some sleep pressure off from the coming night, and thus may delay the adaptation to local time.

If there is a good indication for a nap, its optimal duration should thus be somewhere between 15 and 25 min. This is probably linked to the sleep stages that occur during the nap. Very light sleep (stage 1 sleep) is probably not very useful for recovery. Stage 2 sleep includes loss of awareness of the external world and seems to be quite beneficial. Stage 3 (deeper sleep) is even more effective but may include the negative impact of sleep inertia. REM sleep should be an exception in naps, but can happen when the body clock is still set to the home time, as REM sleep also depends on the circadian clock. We should thus focus on finding some stage 2 sleep: it will happen spontaneously if one manages to lose awareness during the nap. To make what is commonly called a powernap, a timer or an alarm clock can be launched for 15–25 min.

When there is a strong sleep debt, or if the nap timing corresponds to sleep time at home, it may be difficult to wake up after the programmed 15–25 min. Naps are thus a double-edged sword and make for a difficult issue.

4.2.4. Launch window

In spaceflight, a launch window is a time period during which a particular vehicle (rocket, space shuttle, etc.) must be launched in order to reach its intended target. If the rocket is not launched within the "window," it has to wait for the next opportunity.

Using this as a metaphor, and as seen above, there are times where day-time at destination overlaps with day-time at home. Jet Lag symptoms should be minimal at these times. The launch window is large when few time zones are crossed. It will be shorter—and split into two parts—when the globe is crossed. Knowledge of the launch window(s) may be crucial to plan and do the important things that should be done at destination, be it museum visits, family or business meetings, or political negotiations. It will of course be easier to choose meeting times as a powerful CEO than if one is looking for a job.

4.2.5. Stay duration

I could not find comprehensive statistics on worldwide average stay duration abroad. It would be important because if a lot of time is available ahead (weeks, months), Jet Lag is very likely to be a minor issue. But since most people do not have extensible holidays or inexhaustible extra time, I suspect that the majority of travel across time zones will last a week or two, or even less (weekend shopping trips to New York or Milan for instance). Here, and especially if a considerable distance is traveled for a round trip, Jet Lag may bother seriously. The good news is that readjustment is faster after short stays once back home.

For very short stays (2–3 days), and if the launch window is comfortable enough (see above), one may just as well decide to keep home schedules. For a westwards trip, this means going to bed early, at the usual home times. That will make for an early wake-up that can be used for doing some homework or early visits. For an eastwards trip, this means going to bed late at night and stay asleep late in the morning.

4.2.6. Discipline and wake-up time

As human circadian clocks take several days to adjust, exercising discipline for about 1 week will help saving much time, as the body clock will adjust faster. This means going to bed when there is little need to and waking up even if it is uncomfortable.

If not, chances are that Jet Lag will last longer and the body clock will swing between home and destination times.

4.2.7. Preflight scheduling (anticipating phase shift)

Although it is neither practical nor easy, it is theoretically possible to adopt destination timetables prior to departure. This can be useful when crucial meetings are scheduled and Jet Lag should absolutely be avoided. It means going to bed earlier or later—and waking up accordingly of course. Perhaps, it is only valuable with very little time difference (1–2 h). More than that would be heroical most of the time.

4.2.8. Sleep in two parts

Sleeping in two half nights can be useful when the destination is at the antipodes and the stay duration is short [25]. It would be comparable with a long nap (corresponding partly to sleep at home times) and a short sleep at night. This is still largely theoretical at this date but would be just as physiological and healthy as long as the total number of hours is slept.

It is possible that people have slept in two chunks for ages, for instance when they got to sleep early in the winter, then woke up and did several activities, to go back to sleep again until morning [26].

4.3. Accelerating the phase adjustment

4.3.1. The natural way is fine but slow

The body clock adjusts spontaneously and seamlessly to destination time. The only problem is that the change is slow and holidays or business stays abroad may be too short for its full and timely implementation.

As has been mentioned earlier here, it takes about one day by time zone when flying eastwards and two-thirds of a day when flying westwards to adjust for sleep and vigilance. Flying from London to Shanghai (eight time zones difference) may hence demand 8 days until Jet Lag symptoms become almost undetectable—the reverse course from Shanghai to London would take between 5 and 6 days.

Hormones such as cortisol may take up to 3 weeks to fully adjust—but this is fortunately not translated into obvious clinical symptoms [27].

Adjusting the body clock is a natural and complex process, based on the reception of time-givers. The most important of them is exposure to light above a certain threshold of physical intensity (bright light being apparently far more effective than dim light, although the issue is not closed). As mentioned before, other time-givers also play a less important part: social activity, meals, psychological adjustment, physical activity, and so on.

4.3.2. How to interact with the body clock?

Bright light has a direct time-setting action on the body clock supra-chiasmatic nuclei. It also inhibits the production of melatonin [28]. Melatonin in turn helps to adjust the body clock to the external day/night 24-h cycle. There are receptors for melatonin in the body clock, which allows exogenous artificial melatonin to act on it.

Exposure to bright light on the one hand and melatonin on the other hand are two complementary ways that can be used to push the body clock and adjust faster to the destination time. Bright light should also be avoided in certain conditions to prevent the unwanted opposite effect. Bright light especially needs to be administered at precise timings for every different kind of Jet Lag difference.

4.3.3. Bright light

Bright light is the main time-giver to the body clock. Its effects (phase-response curve) on the body clock were modeled 30 years ago [29]. Exposure to bright light in the morning should have the effect of advancing circadian rhythms: it helps to sleep earlier (useful when flying eastwards). Exposure to bright light in the evening should do the opposite, retarding the circadian rhythms and helping to sleep later (useful when flying westwards).

The switch-point is between retarding and advancing actions in the midst of the night (of the body clock). This has to be seriously taken into consideration. If bright light is taken at the wrong time when at destination, it may have the opposite effect contrary to what is wanted.

It is the retina (eyes) that matters, not body exposure, which is irrelevant here. Importantly, and as a result of retina automatic adaptation, artificial (dim) light may give a subjective impression of being almost as powerful as external light. In fact, when measured with physical instruments, common artificial lighting is usually more than 10 times weaker than daylight. To the brain and for that matter, living under artificial light is almost like living in the dark. So, to benefit from really activating light (bright light), the choice is between exposure to external solar light (it works even when the weather is cloudy most of the time), and the use of artificial lighting, such as powerful halogens (500 W), specially designed light boxes or a light visor— appliances that are easily portable and are worn on the head like a baseball cap. To avoid light when it is counterproductive, sunglasses or even an eye mask could be used if the circumstances allow.

4.3.4. Melatonin

Melatonin is a hormone produced by the pineal gland, a small brain structure in the epithalamus of almost all vertebrates (slightly behind the hypothalamus). Its secretion in humans is normally high at night and close to zero during the day, as it is inhibited by light. Its secretion thus begins in the evening and lasts until dawn. It follows a circadian cycle (very close to the external 24-h light-dark cycle). Its production is synchronized by the body clock with the light-dark cycle. Its effects are contrary to those of exposure to light [30].

The primary function of melatonin seems to be regulation of the day-night cycles. It also has a sleep-inducing action, especially at larger doses. It also works as an antioxidant and shows moderate anti-inflammatory properties.

The melatonin produced by the body is called endogenous. The one prescribed as a medical compound (artificial) is exogenous. Although melatonin is a natural hormone, using it exogenously at the dosages suggested here is not natural, since it will amount to about 5–25 times the 100–150 micrograms normally produced.

When exogenous melatonin is taken in the evening (before the onset of its natural endogenous secretion), it advances the body clock to an earlier time, which is useful when traveling eastwards. To benefit from both the phase-resetting and the sleep-inducing effect, long-acting larger doses (2.5 mg) can be used about 2 h before going to bed. But taking melatonin at nighttime when traveling westwards does not make much sense, since it will overlap with the endogenous secretion and probably won't do any good. When it is taken in the morning (of the home schedule), after endogenous levels have fallen, it will reset the clock to a later time. It may thus be preferable to take a short-acting small dose (0.5 mg) later at night [9].

Prescription of melatonin a few days before departure, at a time that coincides with bedtime at destination, has been suggested on theoretical bases but it remains unclear whether anticipatory treatment provides a substantial advantage over treatment that is administered after arrival at destination.

Melatonin is the most extensively studied treatment for Jet Lag so far. It has shown effects superior to placebos. In a meta-analysis, the subjective benefit has been estimated at around 40% (eastwards) and 50% (westwards) [31].

No major or consistent adverse events have been reported in the clinical trials that have been performed. But larger studies are still needed to confirm the drug's effectiveness and safety. Driving is not recommended after absorption of long-acting (extended release) dosages for about 5 h.

Melatonin is also the precursor of a new class of drugs—chronobiotics. Agonists such as Ramelteon, seem to possess sleep-inducing as well as phase-adjusting properties and is marketed in the US for insomnia with difficulties initiating sleep. Tasimelteon, another melatonin agonist, improves sleep latency, sleep quality, sleep maintenance and provides a shift in circadian rhythms after an abrupt advance in sleep time [32]. Agomelatin is another melatonin agonist, which also has antidepressant properties.

4.3.5. Seasons

Although a bit neglected in the scientific literature, the issues of seasons and latitude (north-south) inescapably increase the level of complexity. Although daylight duration is very similar in the summer and the winter close to the equator, it varies considerably closer to the north or south poles. There, as seasons change without interfering much with sleep duration, and as most people do not sleep behind opaque curtains or blinds, we must postulate some kind of mechanism which adjusts the body clock progressively to longer or shorter durations of external bright light and varying durations of melatonin secretion. It is likely that the other time-givers, such as meals and social life, help to achieve this adaptation.

What I want to insist on here is how complex it may be to determine the best timing for the administration of melatonin and bright light. The greater the north-south difference, the less unpredictable the reaction will be, as this area needs more research.

4.4. Sleep inducers

4.4.1. Drugs of all kinds

There is nothing more restorative than plain, normal, drug-free sleep. But there is also little doubt that sleep induced by sleeping-pills is more restorative than insomnia.

Over the centuries, humans seem to have experimented with all kinds of compounds including opioids, alcohol, cannabis and many others to obtain sedation (reducing excitation and anxiety), alter consciousness or induce sleep (among other effects). In addition to these, many such compounds are now produced artificially from raw chemicals.

4.4.2. Alcohol

Alcohol-containing beverages (from beer to whisky) work on several parts of the brain and not necessarily in the same order in all of us. To some, alcohol is mostly felt as a sedative; but to others, it may be felt as a stimulant, or as a social disinhibitor, a trigger to violence, and so on. But whatever the particular effect it has during wake time, it is metabolized rather rapidly by the liver and, as such, may elicit a rebound (awakening) effect during the night, so that sleep may become lighter and more irregular after a few hours [33, 34]. In the case of Jet Lag, and especially if flying westwards and risk waking up at night, absorbing alcohol is not a very good idea. Going east across a few time zones should be less of a problem, since the body clock increases the propensity to sleep in the later parts of nights.

4.4.3. Benzodiazepines

Among all the pharmacological classes that may induce sleep, benzodiazepines and z-drugs are the most studied for their sleep-inducing properties. Z-drugs are compounds that lack the chemical benzodiazepine structure but act, albeit probably more specifically, at the same brain location. They are effective and their side effects are well known and benign when used carefully on a short-term basis.

Side effects for short-time use may include sleepiness, lack of concentration, reduced memory, attention and muscle weakness. These should be a minor nuisance to most but may be substantial to others, as they vary from person to person and depend on the dosage, as well as the patient's health, age, weight, gender, and so on.

Their action duration, best described by their half-life, varies considerably, from about an hour to more than 48 h. This is determined by pharmacokinetic properties, such as the rate of absorption, extent of the distribution and elimination time. Sometimes, metabolites prolong the action of the original substance.

Short-acting drugs are most useful when it will be difficult to induce sleep, as in flying westwards. Mid-range duration drugs will be more useful when the problem lies in waking up too early.

4.4.4. Melatonin and its derivates as sleep-inducing substances

Beside their usefulness as body clock resetters, melatonin and its agonists (e.g., Ramelteon) have demonstrated action as sleeping pills. They may prefer to benzodiazepines because of their more "natural" origin (though the proposed dosages are not natural, as seen above). Hey should be taken 2 h before bedtime for their sleep-inducing effect. However, patient appraisal shows large divergences about their effectiveness as sleeping-pills proper (see [35] for instance).

4.5. Neuro-stimulants

4.5.1. Sleepiness and stimulants

When the body clock presses for sleep when it is plain morning, middle of the day or early evening at your destination, it may be difficult and sometimes impossible to enjoy the stay or do what was planned. Sleepiness may be very difficult, often painful to resist and hamper the normal functioning.

The burden on human activities and the heavy load of casualties caused by fatigue, whether military, civil or personal, have been very convincingly described in Caldwell and Caldwell's Fatigue in Aviation [36]. This great book also discusses in detail the various ways used by the military to counteract fatigue, among them being the use of stimulants and sleeping-pills (see also [37]).

As mentioned in the previous chapter, the first thing is to make sure an adequate number of hours of sleep is obtained. If not, homeostatic pressure and sleep debt will add to the circadian issue, as we have seen above. So the first thing to do is to sleep enough (see preceding chapter).

The use of stimulants must be seen as a last resort, after everything else has been tried.

Stimulants (caffeine, modafinil) have been recommended in Jet Lag situations for short (2 or 3 day) stays, since melatonin and bright light do not have time to suppress adaptation time [25].

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Unfortunately, both caffeine and modafinil have a rather long duration of action (5–8 h for caffeine, 5–10 h for modafinil). This does no harm when sleepiness due to Jet Lag manifests itself in the morning or the afternoon (Groups B and C). But this long action makes them trickier to use when sleepiness is felt in the evening (Group A) and sleep is needed a few hours later.

4.5.2. Caffeine

A mild stimulant from the xanthine family, it is arguably the most widely used psychoactive drug in the world. It is found in coffee of course, but also in tea, cocoa, (chocolate), soft drinks, energy drinks, in some medications and over-the-counter preparations.

Its absorption is irregular, varies from person to person, and is influenced by food intake. Peak effects are observed after 1 h, while residual effects can be perceived from 5 to 8 h afterwards [38, 39], and even more in case of pregnancy or when taking hormonal contraceptives.

Three hundred milligram of slow-release caffeine has been shown to increase alertness and reduce other Jet Lag symptoms after eastwards flights across seven time zones [40]. But long-acting tablets of caffeine are not easy to find nowadays.

A cup of filter or instant coffee contains 100–150 mg caffeine. An expresso contains 50–75 mg. Black tea (or iced tea) contains 15–70 mg. Green tea contains 25–50 mg. Decaffeinated coffee or tea contain less than 15 mg.

Caffeine mildly increases neural activity in several parts of the brain. It is also diuretic, stimulates striated muscles and acts on the cardiovascular system. It is used to reduce physical fatigue and to prevent or treat drowsiness. It produces increased wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination. The amount of caffeine needed to produce these effects varies from person to person, depending on body size and degree of tolerance. Caffeine has the desired effect of delaying or preventing sleep, but does not affect all people in the same way. It also improves performance during sleep deprivation [41]. In shift workers, it leads to fewer mistakes caused by drowsiness [42].

Side effects include restlessness, irritability, nervousness, shakiness, headache, lightheadedness, sleeplessness, nausea, vomiting.

The problem with caffeine is that most of us already take caffeine on a daily basis (e.g., 90% of the adult population in the US). The average consumption of adults is 300 mg/day (some of us 500 mg/day), that is, pretty much the dosage suggested to combat sleepiness in Jet Lag, as we have seen above. Fighting Jet Lag using caffeine basically only works for people who do not use it regularly. One option would be to wean oneself off caffeine about a week before the travel.

4.5.3. Energy drinks

So-called « Energy drinks » may contain between 80 and 200 mg caffeine and about 2–3 g taurine. It is unclear whether taurine adds efficacy to caffeine and the opposite may even be

true [43]. All in all, one can have an energy drink that has about the same effect as 1–3 cups of coffee.

4.5.4. Modafinil

Modafinil was originally developed as a treatment against narcolepsy, a disease that has sleep attacks and severe sleepiness as one of its core symptoms. Modafinil has been shown to have a moderate but significant effect on reducing sleepiness and the number of car accidents in shift work [44]. Recently, it has been shown to improve driving in real conditions in patients with excessive daytime sleepiness [45].

Modafinil thus increases wakefulness, alertness, concentration and decreases fatigue and sleepiness. It could slightly reduce appetite. It is assumed not to prevent subsequent sleep.

Compared with caffeine, modafinil has demonstrated less cardiovascular stimulation, and less interference with scheduled sleep. But it was also shown to be less effective than caffeine on the Stanford vigilance test [46].

4.6. Tailoring remedies

As seen above, Jet Lag means very different things as a function of the time zone differences, the direction of the flight, the duration of the stay, the purpose of the stay, the seasons, the differences in longitude. One must also add the still largely unexplored individual differences in sensitivity to it.

Thus, there is no way presently to universally suppress Jet Lag and its consequences. The best way to adjust nowadays is to break the issue down and to look for tailored solutions, especially taking into account the difference in time zones and flight directions. For each one, specific adjustment strategies can be proposed, and they will be more efficient and less harmful than universal ones [47].

5. Conclusions

Jet Lag is a phenomenon that is best explained by a mismatch between the circadian body clock and actual destination time. After fast (airplane) transportation to destination, the body clock remains set to departure schedules for a number of days that depend mostly on the amount of time zones crossed.

The circadian clock slowly adjusts to new schedules, thanks to external time-givers, especially the alternation of outside night and dark periods but also social meetings and meals.

Several remedies have been tried to speed up or bypass this adaptation, from no-nonsense tricks to bright light and melatonin to sleeping pills and stimulants. But, all in all, these remedies still tackle the issue in an unsatisfactory way. The best manner to cope presently seems to tailor the solutions to individual travels and demands.

Conflict of interest

There is no conflict of interest.

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Chapter 6

Features of Circadian Rhythms in Patients with Cerebrovascular Diseases

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Additional information is available at the end of the chapter

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Abstract

The chapter describes in detail the pathogenetic role of desynchronosis in the development of cerebrovascular diseases (CVD). The data of domestic and foreign literature on the study of desynchronosis are presented. The role of melatonin in the regulation of circadian rhythms (CR) is shown. Pathological changes in CR affect sleep disturbance, emotional and cognitive disorders. It is demonstrated the need of the further study of the prevalence and structure of desynchronosis in patients with CVD. The search of the most significant factors of desynchronosis development in patients with vascular diseases is of great scientific and practical significance. The importance of creating and introducing diagnostic and therapeutic algorithms for chronodiagnostics and chronotherapy of CVD into everyday practical activities. The effectiveness of melatonin for the normalization of sleep and CR in patients with insomnia, acute stroke, depressive disorders is shown. Complex therapy of the patients with CVD taking into account chronobiological disorders allows to eliminate the adverse effect of sleep disorders and CR on the regulation of the cardiovascular system and improve the efficiency of rehabilitation.

Keywords: circadian rhythms, desynchronosis, chronobiology, SCN, melatonin, cerebrovascular disease, stroke, sleep disorders, cognitive disorders, phototherapy, chronodiagnostic, chronotherapy

1. Introduction

Physiological processes in living systems undergo rhythmic fluctuations, called biological rhythms. Among the great variety of biological rhythms in maintaining the health and functioning of the organism, circadian rhythms (CR) with a period of oscillations of about 24 hours are particular important. Evolutionally formed synchronization of the CR as an indicator of

internal and external synergism, indicates a health status [1]. In the case of discrepancy of the CR, there is desynchronosis – a form of circadian pathology, a nonspecific manifestation of pathological conditions characterized by changes in the structure of the rhythm: an increase (decrease) in amplitude; inversion of acrophases; change the duration of the period [2]. The manifestation of many diseases, such as myocardial infarction, stroke, sudden death, etc., is closely associated with certain periods of the day [3–5]. The diurnal rhythms of biochemical processes and physiological functions are synchronized in time, or synchronous. Thus, the number of heart rate (HR) and respiration rate are correlated as 4:1 (72:18, 80:20), that ensures optimal oxygen supply to tissues and is consistent with the rhythms of metabolism. There are several theories about the nature of endogenous factors. In 1976, the chronohypothesis was developed. According to it there is a site in the DNA structure – "chronon," controlling biorhythms. According to the multi-oscillator model of biorhythms, there are many drivers of rhythm-pacemakers in the body [6].

External factors of general synchronization include geophysical factors: photoperiods (day-night), fluctuations in the geomagnetic field of the Earth, changes in the temperature of the environment, etc. For a modern person, the change in the phylogenetically formed stereotype under the influence of social factors is very important [7].

In the process of evolution, complex mechanisms of nervous and humoral regulation of biorhythms, their optimal synchronization were developed. The launching of circadian oscillations and their interconnection is carried out by the activity of the central nervous mechanism performing the pacemaker function, which is realized through the humoral regulating link [2]. Light is the main factor that determines the activity of suprachiasmatic nuclei (SCN) as a biological clock. The information on the light mode is fed into the SCN from the retina of the eye. They also receive signals from other parts of the brain (afferent inputs) and send impulses to various brain structures (efferent inputs) [8].

Through the efferent pathways, the SCN are involved in the regulation of the rhythmic activity of the endocrine system, blood circulation, eating behavior and other functions. Another structure important for the rhythmic organization of functions is the epiphysis – neuroendocrine transducer, an organ that transmits information about the illumination of the environment from the nervous system to the endocrine. Biologically active substance-melatonin is synthesized in epiphysis cells [9].

There are different methods for detecting biorhythmological personality: measuring body temperature, blood pressure, heart rate, breathing, sleep-wake cycle, metabolic rate during the day, determining the level of melatonin in the blood or its metabolites in saliva or urine [10]. The prevalence of sleep and wakefulness disturbances in patients with cardiovascular diseases (CVD) is very high. After a stroke, patients often experience sleep disorders such as insomnia, daytime sleepiness, fatigue, behavioral disturbances in the sleep phase with rapid eye movements and the restless legs syndrome, obstructive sleep apnea syndrome [11–16].

To detect sleep disorders, semi-quantitative scales and questionnaires, polysomnography are used [10]. Examination of Daily blood pressure and Holter monitoring of ECG allow to establish violations of daily dynamics of blood pressure and heart rate. Holter monitoring of ECG

enables to evaluate the circadian index (CI), which is an informative method for assessing circadian diurnal fluctuations in the heart rhythm [17].

The study of CR in patients with CVD has a great practical interest, since CRs are highly sensitive to various types of external influences and their disturbances can be the first symptoms of beginning abnormalities in the vital activity of the organism. There is a lot of data on the existence of chronobiological patterns in the development of stroke and myocardial infarction [11–16, 18–26].

The great scientific and practical interest is the study of the chronotropic activity of melatonin, the leading biochemical marker of CR. There is clear CR of melatonin production in the epiphysis and suppression of its secretion in the light [8].

The role of melatonin in the regulation of diurnal fluctuations of blood pressure is proved [27]. It has anti-inflammatory and antioxidant, as well as possible epigenetic activity [28, 29]. A number of studies have shown the effectiveness of melatonin for normalizing sleep and circadian rhythms in patients with insomnia, acute stroke, depressive disorders, arterial hypertension, etc. [29–35]. Chronotropic or rhythm-organizing activity of melatonin determines the origin of two leading, official indications for its use: treatment of sleep disorders and desynchronosis [29, 31, 36].

The analysis of published data shows the high scientific and practical relevance of further study of chronobiological disorders in patients with diseases of the cardiovascular system. It allows to reveal the pathogenetic relationship of comorbidity of disorders and to substantiate complex approaches to therapy [37–39].

2. Morphofunctional, molecular, genetic and biochemical basis of circadian rhythm regulation

2.1. Morphofunctional features of suprachiasmatic nuclei and their connections with epiphysis

Circadian rhythms (CR) are physiological and behavioral cycles, which are provided by the internal oscillator and remain in the absence of an external "regulator". The ability to maintain a 24-hour rhythm is a fundamental characteristic of a circadian system that allows the body to adapt to environmental conditions [1].

Circadian system operates due to four key components:

1. Photosensitive retinal neurons and retinohypothalamic tract through which light signals come from the environment;
2. Internal circadian oscillator, generating rhythms and synchronizing them with the environment;
3. Signal paths transmitting information from the central regulator to peripheral rhythm generators;
4. Peripheral rhythm generators – clock-genes and proteins in peripheral cells [10].

The central circadian oscillator is the suprachiasmatic nuclei of the hypothalamus (SCN), which are heterogeneous in structure and neurochemical organization and are subdivided into the rostral and caudal divisions [8, 9].

Most of the SCN neurons are GABA and secrete different peptide neurotransmitters. GABA provides a link between the neuronal populations of the ventral and dorsal sections of the SCN. It participates in stabilizing the activity of the SCN and maintaining high-frequency oscillations of neurons in the CR. Many of the individual SCN neurons exhibit electrical and molecular rhythms in isolation, but the rhythms are weaker and less stable [8–10].

It is found, that light stimuli trigger the intra- and intercellular cascade of gene expression first in the center of the SCN, whence elements of peripheral parts are involved in the process through the GABA-ergic signaling pathways. Specific neuropeptides, gap junctions, astrocytes and GABA-ergic signaling realize interrelation between the SCN neurons. Vasoactive intestinal peptide (VIP) and arginine-vasopressin (AV), involved in the regulation of rhythms, are most studied. Studies show that VIP maintains and synchronizes rhythms of the SCN, while AV participates in maintaining high amplitude of the output signal from the SCN and in re-input pulse modulation [8–10].

Rhythmicity and synchronism of the nuclei operation in the diurnal regime is maintained in this way. The physiological role of the SCN, which reduces to the generation of circadian signals and the subordination of the activity of neighboring brain structures and peripheral organs, is entirely determined by the nature of their afferent and efferent connections [8].

Among the afferent projections of the SCN, the retinohypothalamic tract, which provides the nucleus with information about the state of photoperiodic processes, is of particular importance. It transmits to the SCN the main stream of optical impulses and is represented by collaterals of retinal ganglion cells. Its damage affects the dynamics of the CR in the form of a phase shift [8].

Another significant afferent input for SCN is the ascending axons of the neurons of the seam nuclei projecting here. The existence of direct raphohypothalamic tracts explains the high content of serotonin in the SCN. The electrical stimulation of the seam nuclei clearly inhibits the rhythmic of the hypothalamic neurons. In experiments on isolated SCN neurons, agonists and antagonists of serotonin receptors when applied locally, simulating the effect of light, were shown to be able to shift the phase of CR cells [8, 9].

The SCN forms neural connections with the nuclei of the stem, responsible for the regulation of sleep and wakefulness processes [10]. The SCN have direct connections with supraventricular and preoptic regions, dorsomedial divisions of the hypothalamus, arcuate and paraventricular nuclei. The direct and inverse relations of the SCN with the various elements of the limbic system and the motor centers have great functional significance. In particular, some nuclei of the amygdala and septa are projected onto the SCN [40].

A special place in the temporal organization of adequate adaptive behavior and the genesis of affective disorders is attributed to the interaction of the SCN with the **epiphysis and emotiogenic limbic structures.** Epiphysis is an important relay station and the leading link in the realization of circadian signals in relation to different functional indicators [8, 9].

The SCN almost entirely determines dependence of brain activity on the state of external illumination. During the day, light entering the retina activates its photosensitive ganglion cells, the information from which is transmitted through the retinohypothalamic tract and further into the SCN. Signals from the SCN are transmitted to the paraventricular nucleus of the hypothalamus, and further, trough the intermediolateral column of the spinal cord, reach the upper cervical ganglion. Sympathetic postganglionic noradrenergic fibers innervate melatonin-secreting cells in the epiphysis. Norepinephrine acts on postsynaptic beta-1 and alpha-1-adrenergic receptors in the cells of the epiphysis, which trigger the synthesis of **melatonin**. There is a clear daily periodicity: the production of melatonin begins with the onset of darkness, reaches a maximum at midnight and stops in the light. In the light phase of the day, this process is replaced by an increased synthesis of **serotonin** [7, 8, 41].

There are reciprocal relationships between the SCN and the epiphysis, and melatonin is able to make certain corrections to circadian dysrhythmia, including inhibiting the discharges of SCN neurons. Under the influence of melatonin, the CR phase shift is also described in humans, which allowed recommending it for the correction of latitudinal desynchronosis [29, 41].

By obeying the signals of the SCN, the epiphysis through melatonin can directly interfere with the functional activity of the limbic structures of the brain. Hyperactivity of the latter causes the development of dysrhythmia, accompanied by an increased level of anxiety. With steady stressing, the anxiety transforms into a depressive state. The SCN due to its direct efferent projections into the subcortical limbic nuclei, and indirectly (via melatonin) limits hippocampal excitability. Probably, this is one of the ways to realize anti-anxiety properties of the epiphyseal hormone. Thus, the disturbance of the interaction of SCN with the epiphysis is one of the pathogenetic factors of anxiety and depression [42, 43].

In addition to managing the CR of the psychoemotional state, along with other circadian fluctuations, the SCN provide regulation of the basal cycle of calm-activity. It is known, the patients with depression are characterized by night sleep disturbance and phase structure of sleep disorder. One of the probable causes is legitimately sought in violation of the normal activity of the central pacemaker. It has been established that the SCN lesion in animals along with other CR disturbance significantly disturbs the sleep [42, 43]. Insomnia in humans is often combined with neurodegenerative pathology (Alzheimer's disease (AD), etc.), which is usually accompanied by the SCN lesion. On the other hand, a rhythmic change in the states of sleep and wakefulness is quite an autonomous process and persists in people deprived of external time sensors, which emphasizes the dependence of sleep on the activity of the leading pacemaker [44–48].

According to modern concepts, the periodic nature of the sleep-wake cycle is determined by the co-operation of the brainstem formations in the ascending awakening system of the brain and the hypnogenic pathways, the impulse from which, following to the forebrain, along with other structures, involves ventrolateral preoptic nuclei. The latter provide alternating excitation of activating and inactivating (hypnogenic) mechanisms with rhythmic change of sleep and wakefulness states during 24 hours, demonstrating a switching function. The weakness of the rhythm-organizing properties of the SCN can be determined by the pathological reorganization of intranuclear processes at the molecular level. An important reason for this is often the changes in the circadian oscillations of the clock genes [49, 50].

2.2. Molecular mechanisms of circadian oscillations

The molecular basis for the CR regulation is provided by the hour genes, whose work is carried out on the principle of loops of positive and negative feedback. The BMAL1 and CLOCK proteins accumulated during the day form the BMAL1/CLOCK complex. The BMAL1/CLOCK dimer activates the transcription of the PER genes (PER1, PER2, PER3) and CRY (CRY1, CRY2). Synthesized PER and CRY proteins also form a PER/CRY dimer acting on the principle of negative feedback. PER/CRY moves to the cell nucleus and inhibits the activity of the BMAL1/CLOCK complex, which leads to a decrease in the expression of PER and CRY proteins. During the night, the PER/CRY complex is destroyed, and the 24-hour cycle begins anew [49, 50].

Another clock gene involved in the regulation of this cycle is REV-ERB-alpha. The BMAL1/CLOCK complex activates the transcription of the gene, which leads to the accumulation in the cell of the protein REVERB-alpha. The REVERB-alpha protein in turn inhibits the transcription of the BMAL1 gene and presumably the CLOCK and CRY1 genes [51].

2.3. Melatonin involvement in the circadian rhythms regulation

The leading regulator of biological rhythms is the epiphyseal hormone melatonin (N-acetyl-5-methoxytryptamine) acting on circadian systems via MT1- and MT2-melatonin receptors in the hypothalamus SCN [1, 28, 29].

The melatonin donor is the amino acid tryptophan, which participates in the synthesis of the neurotransmitter serotonin, which under the influence of the enzyme N-acetyltransferase turns into melatonin (**Figure 1**) [29].

Melatonin is an indole derivative of serotonin and is produced at night with the participation of N-acetyltransferase and hydroxyindole-O-methyltransferase enzymes [29].

Extrapineal sources of melatonin synthesis are enterochromaffin cells of the gastrointestinal tract (EC-cells), the main depot cells of serotonin (contain up to 95% of all endogenous serotonin). The

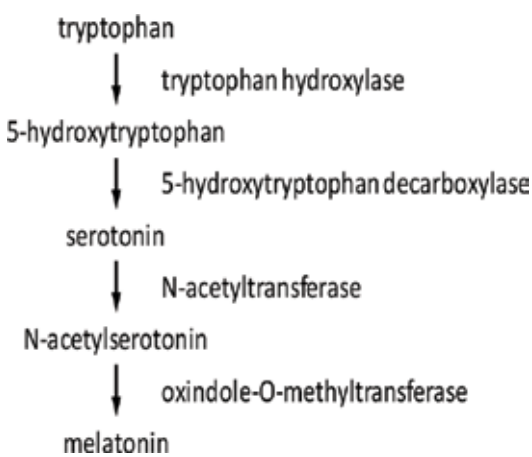

Figure 1. Melatonin synthesis scheme.

synthesis of this hormone has been found in many neuroendocrine cells of the airways, lungs, in the cortical layer of the kidneys and along the boundary between the cortical and medullary layer of the adrenal glands, under the hepatic capsule, in the paraganglia, ovaries, endometrium, prostate gland, placenta, gallbladder and inner ear. In recent year's studies, melatonin synthesis is found: in blood cells – mast cells, lymphocytes – natural killers, thrombocytes, eosinophilic leukocytes, in the thymus, pancreas, cerebellum, retina. Functionally, many melatonin-producing cells belong to the so-called diffuse neuroendocrine system – a universal system for adapting and maintaining the body's homeostasis. Thus, two links of melatonin-producing cells are distinguished: central (includes the pineal gland and cells of the visual system), in which the rhythm of melatonin secretion coincides with the rhythm of light-darkness, and peripheral – all other cells where the secretion of the hormone does not depend on illumination [1, 2, 29].

Melatonin is transported by serum albumin, after liberation from albumin it binds to specific receptors on the membrane of target cells, penetrates into the nucleus and performs its action there. The biological half-life of melatonin is 45 minutes. This makes it difficult to collect material for research purposes. Melatonin is rapidly hydrolyzed in the liver and excreted in the urine (80–90%), the main metabolites are 6-hydroxymelatonin-sulfate (6-SOMT) and 6-hydroxyglycuronide. The concentration of melatonin metabolites in saliva and/or urine correlates well with the total level of melatonin in the blood during the sampling period [1, 10, 30].

It has been found that the effect of melatonin is realized through MTNR1A (MT_1) receptors, which are expressed mainly on the cells of the anterior lobe of the pituitary gland, the hypothalamus SCN and in many peripheral organs; as well as MTNR1B (MT_2) receptors, expressed in some parts of the brain, in the retina and in the lungs. The nuclear receptors of melatonin of the subfamily RZR/ROR of retinoid receptors have recently been discovered. Many immunostimulatory and antitumor effects of melatonin are mediated through them [52].

During the first years of life, peak concentrations of melatonin increase and reach a maximum by 2–4 years, after which they begin to decrease and reach the plateau by the time of puberty. The secretion of melatonin continues to decrease yearly after the end of puberty [10]. Both basal and peak concentrations of melatonin decrease with age, the daily curve of melatonin secretion is smoothed and the peak of night secretion decreases [10, 52–54].

The daily fluctuations in the melatonin level in the blood (melatonin curve) looks like the following. Its concentration is minimal by day (1–3 pg./ml), it starts to increase 2 h before the usual time for going to sleep (if there is no bright light). After turning the light off in the bedroom, the concentration of melatonin increases rapidly (up to 100–300 pg./ml). In the prehour hours, a recession usually begins, which ends after awakening. For each person, the melatonin curve is stable from night to night, while in different people of the same gender and age the curves differ significantly, so one can speak of an individual curve [10, 52].

In a number of experiments on animals, the *antioxidant properties* of melatonin have been demonstrated. The mechanism of antioxidant action is manifested in the fact that melatonin has a pronounced ability to bind free radicals, including those formed during peroxidation of hydroxyl radical lipids, and exogenous carcinogens, and it also activates glutathione peroxidase, a factor protecting the body from free radical damage. The main functions of the melatonin antioxidant action are aimed at protecting DNA [10, 29, 52, 55]. To a lesser extent on the protection of

proteins and lipids. Its addition to the ration of rats resulted in an increase in life expectancy and testosterone levels in males [52, 56]. In the study of V.A. Lesnikov and W. Pierpaoli transplantation of the pineal gland from young to older individuals increased their lifespan by 42% and, conversely, transplantation of the epiphysis of older individuals reduced it by 29% [57]. Against the background of the use of melatonin in aging mice, not only the duration of life but also the volume of thymus, adrenals and testes increased, which was accompanied by an increase in the level of testosterone and thyroid hormones in the blood. Thus, a decrease in melatonin synthesis probably plays an important role in aging processes [53].

Reducing melatonin concentrations in the elderly is probably one of the main factors in the development of age-related neurodegenerative diseases. A retrospective analysis of 6-year-old data in patients with depression revealed a disruption in the regulation of the synthesis and metabolism of catecholamines, neurotransmitters, melatonin and immunological proteins [42]. It has also been shown that melatonin supports the optimal mitochondrial membrane potential and preserves mitochondrial functions. In addition, mitochondrial biogenesis and its dynamics are also regulated by melatonin. Mitochondrial dynamics demonstrates an oscillatory pattern that corresponds to the CR of the secretion of melatonin in the pinealocytes and, possibly, in other cells [28, 52, 55]. A number of recent scientific studies have identified the neuroprotective effect of melatonin, which is manifested by affecting the proliferation and differentiation of neural stem cells, increasing the content of myelin and oligodendrocytes [58, 59].

In other studies, melatonin demonstrated a *neuroprotective effect* in neurodegenerative diseases. Melatonin reduces the toxicity of beta-amyloid and prevents the death of cells in experimental AD models, and also reduces oxidative stress in PD models [44–46, 48].

In addition, the experiment demonstrated the effect of melatonin *on the proliferation and differentiation of stem nerve cells.* Depending on the dose of melatonin introduced into the mice cortex, the proliferation rate of oligodendrocytes, the percentage of the main myelin protein, as compared with the control group, increased. Thus, melatonin may have a potential therapeutic effect for some neurological diseases associated with oligodendrocyte pathology and myelinopathy [59].

In recently published papers it is reported that melatonin synchronizes not only central but also peripheral biorhythms, which allows to synchronize biological functions by means of CR with respect to periodic changes in the environment and, therefore, facilitates adaptation of the individual to the external environment [28, 52].

The large number and diversity of the main effects of melatonin opens up important prospects for measuring the level of melatonin as a biomarker for the purpose of clinical, preventive and therapeutic use [10, 32].

3. Violation of CR and cerebrovascular diseases (CVD)

3.1. Desynchronosis as a risk factor for stroke

The presence of chronobiological disorders in patients with CVD is noted by many researchers [11–26, 60–86].

In a comparative analysis of autonomic control of the rhythms of the cardiovascular system (CVS) in young and elderly healthy people in Ukraine, it was shown that circadian regulation of blood pressure and heart rate is impaired in elderly people. [25].

There are reports that chronobiological disorders are detected in patients with arterial hypertension [70–72], diabetes mellitus [73–75], cardiac ischemia [11, 12, 14, 61, 66], dementia [67–70], etc. Nowadays there is a lot of data on the existence of chronobiological patterns in the development of stroke and myocardial infarction (MI) [11, 12, 14].

It is known that Ischemic stroke (IS) develops more often in the early morning hours [10]. This may be due to an increase in the activity of the coagulating system of blood at this time [71], as well as with a violation of the daily regulation of blood pressure and heart rhythm in these patients [72, 73]. In epidemiological studies, increased frequency of sudden cardiac death, MI and transient myocardial ischemia, pulmonary embolism and critical ischemia of the lower extremities, as well as rupture of the aortic aneurysm at dawn.

The second small peak of incidence is noted in the early evening [74]. European researchers point to an increased incidence of stroke and MI in winter [75].

In the epidemiological study conducted in Hawaii, it was found that the MI in local population of the Caucasoid race occurs most often between 04:00 and 12:00, and in Japanese visitors – from 12:00 to 16:00, which corresponds to the morning hours in Japan [76]. Similar daily dynamics of MI and stroke development early in the morning and between 12:00 and 18:00 was noted in a prospective study conducted in India involving 158 elderly patients [77]. Such a pattern of development of MI and stroke in the morning can be associated with an increase in platelet aggregation capacity in the morning hours with a peak at 09:00 [71–73]. Also in the early morning, endothelial cells reduce the synthesis of tissue activator plasminogen, nitric oxide and prostacyclin, the tone of the myocytes of the vascular wall is reduced, which promotes thrombosis [71].

In addition, there is a seasonal and cyclical decompensation of the CNS. As a rule, exacerbations occur in the spring and autumn. There is evidence that hemorrhagic stroke (HS) often manifests in winter and spring, and IS in summer and autumn [78]. Daylight saving time transgresses the CR and shifts the picture of the diurnal variation at the beginning of the stroke, but the effect on the IS frequency is unknown.

Effects of 2004–2013 daylight saving time (DST) transitions on IS hospitalizations and in-hospital mortality were studied nationwide in Finland. Hospitalizations during the week following DST transition (study group, n = 3033) were compared to expected hospitalizations (control group, n = 11,801), calculated as the mean occurrence during 2 weeks prior to and 2 weeks after the index week. DST transitions appear to be associated with an increase in IS hospitalizations during the first 2 days after transitions. Susceptibility to effects of DST transitions on occurrence of ischemic stroke may be modulated by gender, age and malignant comorbidities [79].

Disorders of CR are associated with an increased risk of IS. A monitoring of blood pressure for 5 days after a previous IS or HS, conducted in 50 patients (India), indicates a decrease in natural circadian fluctuations with an increase in blood pressure during the night [77].

According to the Stockholm population cohort study, 48-hour heart rate monitoring in 678 practically healthy people aged 55–75 years allowed to reveal a statistically significant risk of MI development in patients with reduced nighttime heart rate variability. Some authors point to a direct relationship between the frequency and severity of MI and the severity of violations of daily BP regulation. They divide patients into groups of "dippers" and "non-dippers". It was established that the activity of the central link of the sympathetic nervous system was increased in patients with "non-dipper", i.e., those who do not have a decrease in blood pressure during night sleep or less than 10% of the daytime sleep. These people are less active in endothelium-dependent vasodilation, and a possible cause of high pressure is the damage to the baroreceptor reaction. As a result, "non-dippers" are characterized by increased sympathetic activity during sleep and, as a consequence, have a high risk of general and cardiovascular mortality [80].

The dependence between the amount of brain damage and the degree of decrease in night-time blood pressure (BP) is established: the greater the amount of brain damage, the less it decreases at night. Thus, in patients with lacunar stroke, in contrast to patients with non-lacunar stroke, a greater BP reduction is detected during monitoring at night. This may indicate the safety of the mechanisms of regulation of circadian rhythms of pressure in the case of lacunar stroke [81].

The results of the large clinical trial in Japan with a 24-hour outpatient BP measurement in 515 patients and parallel magnetic resonance imaging (MRI) of the brain showed that increasing the pulse pressure during sleep and mean BP on waking, especially in the elders, are independent predictors of MI in elderly hypertensives. In this case, the effect of pulse pressure and the mean value of BP on stroke risk differs in separate phases of the sleep-wake cycle. Thus, an increase in the pulse pressure for every 10 mmHg. in a sleep independently increases the risk of stroke by 43% ($p = 0.001$), while the average BP index during sleep is not so significant. At the same time, the mean BP increase for every 10 mmHg. on waking, independently increases the risk of stroke by 48% ($p < 0.001$), and the level of pulse pressure upon awakening is not a significant factor [82]. The study of night-time heart rate variability may have prognostic value for the stroke prevention.

Our study with the inclusion of 226 patients with cardiovascular diseases (CVD) has shown a high incidence of sleep disorders and desynchronosis in these patients. A comparative analysis of the nature of sleep disorders in patients with cardiological disease (myocardial ischemia, essential hypertension) and CVD showed that sleep disorders due to anxiety-depressive disorders prevail in patients with CVD in the structure of detected sleep disorders, and after a stroke – sleep disturbances due to desynchronosis. This may indicate deeper violations of the adaptive mechanisms regulated by nonspecific brain systems, which leads to disturbances in the sympathetic and parasympathetic links in the vegetative status.

Patients with MI also showed sleep disorders, which may indicate the role of cardio-cerebral interactions in the regulation of sleep mechanisms.

Patients with CVD showed a decrease in the level of 6-SOMT, among which predominate the patients with MI [83,84]. The presence of cognitive disorders, sleep disorders and chronobiological

disorders (daily regulation of heart rhythm and BP) was related with a low level of 6-SOMT in daily urine examined [85, 86].

The study of sleep characteristics in patients with CVD revealed high frequency of occurrence of sleep disorders and desynchronosis in these patients and their positive correlation with the development of behavioral and affective disorders ($r = 0.57$, $p = 0.002$), as well as their effect on the daily profile of the cardiac rhythm and BP ($r = 0.46$, $p = 0.008$). Therefore, timely diagnostic and complex psycho-pharmacological correction of sleep disorders and desynchronosis in patients with CVD will improve the psychological and emotional status of patients, normalize daily profile of heart rhythm and BP. A positive correlation between desynchronosis and stroke was proved ($r = 0.39$, $p = 0.013$). This suggests that desynchronosis is a risk factor for stroke in patients with CVD [25].

3.2. Stroke and sleep disorders

According to the polysomnographic study sleep disorders in stroke reach 100% of the cases and are manifested as insomnia, disturbance of the "sleep-wake" cycle and respiratory distress in sleep as the type of "sleep apnea" syndrome [87]. It was found that sleep disorder is one of the etiological factors of stroke, also increases the risk of recurrent stroke and prevents recovery after it [18–25, 62, 87–95].

Large population studies (more than 3000 patients) indicate that a reduction in sleep duration (less than 6 hours) is associated with an increased risk of hypertension, especially among women compared to men, and is stronger in premenopausal women than in postmenopausal women. The revealed relationship does not depend on the socioeconomic status, traditional cardiovascular risk factors and psychiatric comorbidity, and is stronger in premenopausal women. Consequently, a decrease in the duration of sleep increases the risk of developing hypertension, which can lead to the cardiovascular pathology (CVP) development in women [62].

In the Danish cohort population study over 12 years, which included 20,432 men and women aged 20–65 years, a high incidence of CVP was found in people with insufficient duration and quality of sleep [15]. In the Australian study, among 218,155 people 45 years of age or older, it was found that sleep duration of less than 6 hours and more than 9 hours is associated with a high risk of diabetes, stroke, hypertension and coronary heart disease (CHD). A prospective study of 1986 patients aged 55–69 years (Great Britain) showed that IS is more likely to develop in patients with disturbed sleep at night, and MI is associated with increased daytime sleepiness [25].

In 2016, scientists from the University Clinic of Essen published a meta-analysis of 29 scientific papers evaluating sleep disorders that may be associated with stroke. A total of 2.343 patients with IS, HS or transient ischemic attack (TIA) participated in the studies. Sleep disorders in these patients were divided into 2 groups: (1) disturbance of breathing during sleep (obstructive sleep apnea); (2) sleep and wakefulness disorders, which reduces the duration of sleep. It was revealed that sleep disturbance was observed in 72% of patients with IS, in 63% of patients with HS and in 38% of patients with TIA. A lot of patients had a sleep disorder before stroke. This allows to believe that sleep disorders increase the risk of stroke [22].

The authors also proved that not only insomnia, but hypersomnia and restless leg syndrome increase the risk of stroke. To date, specific mechanisms for increasing cardiovascular risk in restless legs syndrome have been described: (1) periodic movements of limbs in a dream, accompanied by a significant increase in the heart rate and BP; (2) fragmentation of sleep and lack of sleep, invoking changes in the regulation of nervous and vascular systems, metabolism, oxidative, inflammatory processes; (3) iron deficiency, which creates new risks for CVP [90–92].

In general, the exact mechanisms by which sleep disorders can lead to stroke are not disclosed. Nevertheless, it is shown that sleep has an important restorative function of the brain and affects the processes of neuroplasticity. Sleep disorders can persist after a stroke, and without appropriate correction may obstruct the after stroke rehabilitation.

3.2.1. Characteristics of sleep disorders in patients with stroke

Insomnia in stroke patients is characterized by a change in the duration of sleep, frequent nocturnal awakenings, lack of satisfaction at night sleep, and the appearance of "heaviness" in the head [22, 87]. According to the polysomnographic study, there is an increase in stages 1 and 2, a decrease in phases 3 and 4 of the slow-sleep phase (SSP), and often a reduction in the phase of fast sleep (FSP) [10, 13].

Sleep-wakefulness disorders in patients with stroke are caused by damage of the hypothalamic structures associated with the "internal clock", or their connections. Clinically manifested by disturbance of night sleep, pathological daytime drowsiness or a combination of both. This is more common in multiple lacunar stroke. In patients with severe cognitive impairment after stroke, the inversion of the sleep-wake cycle with sleeplessness at night and daytime drowsiness is often observed. As a rule, these conditions are accompanied by behavioral disorders. At the same time the patient is nervous, cannot understand where he is, tries to get out of bed, go, resists the actions of medical staff. These conditions cause the difficulties in rehabilitation of these patients in the hospital [22, 87].

Studies of recent years have shown that obstructive sleep apnea (OSAS) is an independent modifiable risk factor for stroke. To date, it has been established that respiratory events associated with OSAS are involved in cyclical episodes of hypoxemia and hypertension, increased platelet aggregation, reduced fibrinolysis, endothelial dysfunction, increased intracranial pressure, decreased cerebral blood flow and local cerebral ischemia. In the acute period of IS the incidence of OSAS is 36% [93]. Respiratory disturbances in sleep in patients with stroke, cause the worst efficiency of the rehabilitation process. It is shown that the presence of OSAS is accompanied by greater functional insufficiency and a longer period of hospitalization of patients [94–100].

In general, for all stages and forms of stroke, changes in both the mechanisms of sleep generation and the mechanisms of its maintenance are typical. The cause of these violations are not only the damage and death of brain tissue of a local nature, but also disorders of general and local hemodynamics, the appearance of edema and displacement of the brain substance, the ingress of blood into the cerebrospinal fluidways, and as a result – the irritation of various structures located in the brainstem [18].

It is believed that the greatest impact on sleep is the nature, size, localization of the process and the stage of the disease.

3.2.2. Features of sleep disorders depending on the type, localization of the focus, stage of stroke and development time of the stroke

HS in comparison with IS leads to the most severe disorders of night sleep. Characterized by a deep reduction in the duration of sleep, frequent and prolonged awakening, an increase in the representation of the first stage. However, with a favorable outcome of the disease, the degree of recovery of the structure of sleep is faster than in IS. In IS there is a focus of necrotic decay of brain tissue, while with hemorrhage, damage occurs as a result of the stratification of brain structures with blood. Therefore, the restoration of both the clinical picture and night sleep is better and for a shorter period in HS [22, 101].

The size of the focus of stroke plays a significant role in the formation of sleep disorders. A large focus leads to a common swelling of the hemisphere, sometimes even the opposite, the emergence of processes of compression of the brain stem. Hence the most severe disorders of sleep are observed in large foci of stroke. In the available studies it was shown that the maximum proximity of the focus to the median structures and the liquor-bearing pathways (medial arrangement) leads to more severe sleep disorders. Not only quantitative but also qualitative changes in the structure of sleep are noted. Thus, the medial focus with the capture of thalamic structures is characterized by the disappearance on the side of the lesion of "sleepy spindles" (electroencephalographic signs of stage II of sleep). The lateral processes are accompanied by less severe sleep disorders [19].

The localization of the lesion in the hemispheres or in the brain stem causes specific changes in the structure of sleep. Greater disorders observed in the *right hemispheric processe*s: decreases the duration of d-sleep and FSP, lengthen the waking period and stage I, the duration of falling asleep; the number of awakenings increases. The reason for such sleep disorders in right hemisphere patients is the damage of the deep mechanisms of the relationship between the right hemisphere and the hypnogenic structures of the brain. In addition to sleep disorders, these patients notice marked changes in vegetative regulation, which is manifested by tachycardia, various types of cardiac arrhythmia, high BP numbers. The *left hemisphere* is most closely associated with the activating systems of the brain. There is an opinion that this is the cause of frequent impairment of consciousness in left hemispheric strokes [19].

When a process occurs in the area of the pons, the duration of the FSP dramatically decreases, and its latent period increases. Bulbar symptoms are accompanied by a decrease in the duration of d-sleep.

The acute stage of stroke (week 1) is characterized by a number of clinical and polysomnographic features. In this period, there are difficult to control hemodynamic, general cerebral and local neurological processes. Depending on the direction of the disease, a different picture is observed in polysomnography. Severe disturbances of consciousness (sopor, coma), as a rule, are accompanied by a diffuse slow wave activity, which excludes the possibility of isolating individual stages of sleep. The emergence of separate stages and sleep phenomena against

a background of diffuse cerebral electrical activity is a prognostically favorable sign. With conserved consciousness in the most acute period, polyphase and inversion of the "sleep-wakefulness" cycle due to circadian disorders are often enough. In the first case, patients fall asleep several times during the day; in the second – the cycle "sleep-wakefulness" shifts, there are daytime sleep and night wakefulness. In the presence of general cerebral symptoms, frequent awakenings, a decrease in d-sleep and the absence of FSP are observed [19].

The structure of sleep in patients with stroke also differs depending **on the time of its onset**. A characteristic feature for a stroke that occurred during sleep is a high FSP presence, which, along with the "vegetative storm" in this phase, can be one of the causes of stroke at night. According to statistics, in patients with a "morning stroke" in comparison with "daytime" and "night", the shortest FSP time is noted [19].

Investigation of the night sleep structure in patients with stroke showed the premorbid sleep problems (frequent awakening, long sleep, dissatisfaction with sleep, early awakening), which are associated with worse parameters of sleep quality after stroke [18, 101]. Thus, the initial feature of the regulation of the "sleep-wake" cycle influences the formation of structural changes in sleep after stroke.

3.2.3. Sleep disorders and recovery from stroke

The quality of sleep can serve as a prognostic criterion for the possibility of recovering patients with stroke. So, changes in the structure of sleep in the acute period of stroke have an important prognostic value. If a normal pattern of sleep does not return within 7–10 days after a stroke, the prognosis is considered unfavorable [10]. A multicenter-observational and correlation study involving 280 patients with mild and moderate severity of stroke showed initially high rates of affective and cognitive impairment (26.9%) and sleep disorders (567%) in patients in the early recovery period of stroke. In patients with sleep disorders, regardless of the severity of the stroke, recovery of the neurological deficit, cognitive functions proceeded more slowly compared to patients without sleep disorders, primarily with regard to improving the Berg balance scale. It was found that sleep disturbance after stroke has a negative effect on functional recovery, especially on improving the balance in the group of moderate stroke [102].

Restoring and maintain a natural biorhythm sleep-wakefulness with the use of physical and medicinal methods for the stroke prevention and treatment are recommended.

Currently, in the correction of sleep in patients with stroke, the leading place is the drug therapy. The strategy of pharmacotherapy of sleep disorders in patients with stroke is reduced not to achieving a one-time hypnogenic effect, but to normalizing the adaptive-compensatory potential of the central nervous system. Based on such positions the advantages of melatonin as a hypnotic are estimated. As a natural chronobiotic, melatonin synchronizes CR, provides normalization of desynchronized CNS activity. Therefore, the correction of sleep disturbances with melatonin (as opposed to "classical" hypnotic drugs) becomes not only as the result of a hypnogenic effect, but due to normalizing the activity of various brain structures that support the processes of complex central regulation. Exogenous melatonin, taken in the evening,

stabilizes the work of the SCN and marks the starting point for determining the subjective dark time of the day [29, 31, 32].

A number of clinical studies have shown the positive effect of melatonin on daytime sleepiness, a reduction in the period of falling asleep and the number of nocturnal awakenings, the restoration of disturbed sleep initiation in patients with stroke [33–37].

In one of the major studies to correct the disturbances of the sleep-wake cycle, patients with stroke were prescribed a melatonin (Melaxen, Unipharm, Inc., USA) in a dose of 3 mg at bedtime for 10 nights. Against the background of taking Melaxen, there was an increase in the time of night sleep and a decrease in the number of sleep episodes during the day. That is, there was a normalization of the distribution of the sleep time in the 24-hour sleep-wake cycle [35].

When studying the condition of the CNS in 60 patients in the acute period of stroke, it was found that the inclusion in the scheme of complex therapy of Melaxen at a dose of 6 mg per day contributed to a faster and more complete recovery of motor disorders, improvement of the cranial nerves function. In addition, while Melaxen's administration, a rapid normalization of a number of electrophysiological parameters during the recording of an electroencephalogram was observed: changes in activity in the EEG delta and theta bands, which were accompanied by a change in the BIS and ITA indices [104].

We evaluated the effectiveness of chronotherapy (Melaxen) on the dynamics of sleep disorders, cognitive and emotional disorders, neurotrophic brain factor (BDNF), the level of melatonin secretion (6-SOMT) in patients in the early recovery period of cerebral stroke. 112 patients were examined in the early recovery period of the stroke (mean age 58.0 ± 9.74 years). The main groups of patients, along with the standard treatment regimen, received phototherapy and Melaxen 3 mg per day for 3 months. The effectiveness of the therapy was assessed by the dynamics of sleep disorders, psychoemotional status, the concentration of the neurotrophic brain factor BDNF, the level of 6-SOMT in the urine. The study demonstrated a high effectiveness of chronotherapy (Melaxen, phototherapy) in the rehabilitation of patients in the early recovery period of stroke. The presence of cognitive disorders, sleep disorders and emotional disorders correlated with a low level of 6-SOMT in urine in the patients.

Complex therapy with Melaxen, revealed a significant increase in the level of excretion of 6-SOMT in patients by the end of the 3-month follow-up period. An increase in the concentration of BDNF after 3 months of therapy and throughout the observation period may indicate activation of the synthesis of growth regulators and differentiation of the nervous tissue (**neurotrophic effect**). Increased concentrations of BDNF, 6-SOMT in the urine correlated with improved sleep, cognitive and emotional status, motor disorders and quality of life of patients.

In another our study, with 132 outpatients (59 men and 73 women) aged 61.4 ± 4.7 years in the early recovery period of ischemic stroke (IS), OSAS was detected in 52 (39.4%) cases. Light and medium OSAS was diagnosed in 49 cases, in 3 patients – severe OSAS, which required selection of CPAP therapy. Patients with OSAS of mild and moderate severity (49 persons) were divided into 2 groups, comparable by sex, age and neurological manifestations.

All patients received drug therapy according to the standards of specialized medical care; positional therapy, exercise therapy, mechanotherapy, psychotherapy. Patients of the main group (25 people, mean age 59.5 ± 4.8 years), along with the treatment described above, received melatonin 3 mg per day for 30–40 minutes before sleep for 3 months and used intraoral repositioning applicators. Patients in the control group (24 patients, mean age 62.3 ± 4.2 years) were prescribed only standard therapy. Already a month after the start of therapy, the patients of the main group had a positive dynamic: decrease in daytime sleepiness, snoring, and an expression of morning fatigue. After 3 months, the sleep characteristics of the patients in the main group were statistically significant ($p < 0.05$), differed from the control group by a shorter sleep time (8.8 ± 3.2 vs. 20.9 ± 16.7 minutes), an extended total sleep duration (431, 0 ± 34.7 vs. 386.9 ± 90.4 minutes), greater representation of the 4th stage of slow sleep (12.6 ± 3.5% vs. 8.1 ± 6.7%) and a lower total waking time (8, 0 ± 4.2 vs. 37.5 ± 12.8 minutes). After 6 months of therapy, positive changes in the polysomnography index remained, a reduction in the frequency of obstructive events in the patients of the main group as compared with the control.

Simultaneously with the normalization of sleep, the positive dynamic of clinical and neurological indicators was demonstrated. By the 3rd month. Therapy, the cumulative cognitive parameters of the MoCA test, the psychoemotional functions and the quality of life of the patients in the main group were statistically significantly improved, in contrast to the controls.

Detailed study of night sleep in patients with stroke is not only of scientific interest, but also has serious practical significance in matters of prognosis, secondary prevention, as well as medical and rehabilitation measures.

The use of a standardized criterion for assessing the dynamics of the CR of the heart rate extends the diagnostic capabilities, reveals new pathogenetic links of the CVD, optimizes the treatment regimen for patients with CVP. Identification of CR abnormalities in the management of BP and heart rhythm in combination with sleep disturbances allows to include melatonin drugs in therapy.

Thus, the detection of sleep disorders and desynchronosis in patients with CVD requires that medical, psychological and social aspects is included in complex therapy.

It is advisable to use the following chronotherapeutic approaches:

1. Change of daily regime according to a chronotype of the patient and reduction of a mode of work and rest in conformity with natural photoperiods;
2. Use of physiologically appropriate diet;
3. Optimization of the motor activity regime with the recommendation of walking outdoors and moderate insolation;
4. Inclusion of photo- and color therapy in the complex of rehabilitation;
5. The Chronopharmacological approach in Drug Administration.
6. Melatonin administration at a dose of 3 mg per day. For 30–40 minutes before bedtime.

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4. CR disorders in patients with DVB and development of cognitive disorders

At present, there is enough evidence on the association of early and progressive CR disorders, changes in quality and sleep architecture with an increased risk of developing cognitive impairment (CI) [67–70, 103–112].

When analyzing 12,926 documents from the PubMed, EMBase, ISI WebofScience and PsycINFO databases published before October 28, 2016, among 246,786 patients in 25,847, an average of 9.49 years was observed in dementia. The prognostic role of sleep disturbances, their subtypes (insomnia, OSAS, excessive sleepiness during the day, sleep disorders, and nonspecific sleeping problems) in the development of dementia were evaluated. Compared to those without sleep disorders, patients with sleep disorders had a higher risk of developing dementia. Subgroup analysis showed that insomnia increases the risk of developing AD, but not vascular dementia (VD). In contrast, OSAS was associated with a higher risk of early onset of SI, incl. AD and VD [105].

The relationship between the sleep architecture and the potential risk of developing CI in the community is considered on the basis of Framingham Heart Study (FHS). For 19 years' study (the average follow-up period was 12 ± 5 years), there were 321 patients participating in Sleep Heart Health Study between 1995 and 1998, over the age of 60 at the time of sleep assessment. 32 cases of dementia were traced; 24 cases were due to AD. After adjusting for age and sex, a low percentage of FSP and greater latency of REM sleep were associated with a higher risk of dementia. Each percentage reduction in FSP was associated with an increasing the risk of dementia by approximately 9% ($p < 0.05$). The relationship between the percentage of FSP and dementia was similar for the following adjustments for multiple covariates, including vascular risk factors, depressive symptoms, and drug use. The stages of slow wave sleep were not associated with the risk of dementia [106].

Among 96 patients in the acute period of IS, 79% of patients had heterogeneous post-stroke CI. In 21% of patients they had a dysmnestic character. The concentration of 6-SOMT was lower in patients with IS compared with the control. In the study of chronotypes, it was found that the majority of patients had an early variant, while the social jetlag value was 40 minutes. This indicator decreased with increasing age of patients. In most patients, IS developed in the morning, these patients had the lowest content of 6-SOMT in daily urine and the lowest values for MMSE. Potentially this is associated with a decrease in the protective activity of the melatonin. There was a correlation between chronobiological parameters and cognitive status. Thus, the expression of the "social jetlag" was associated with the semantic coding of memory, reflecting the function of the hippocampus. Patients with a late version of the chronotype were characterized by higher rates of delayed reproduction and semantic verbal fluency. With an increase in the social jostlag, the concentration of 6-SOMT in urine, probably compensatory, increased. The use of melaxen accelerated the recovery of CR, which had a positive effect on the rehabilitation of patients. It has been suggested that in elderly and senile patients, a high concentration of 6-SOMT in the acute period of IS was a marker of dysregulatory cognitive impairment, whereas its low content in the presence of a cognitive deficit may indicate a mixed, hippocampal type of CI [86].

Possible prospects for the use of melatonin in elderly patients with CI are due to its antioxidant, neuroprotective and nootropic effects. The positive effect of exogenous forms of melatonin on sleep in elderly patients is confirmed by the results of two placebo-controlled studies in which more than 500 patients over the age of 55, with primary insomnia [1107].

Using 6 mg of melatonin once a day before bedtime for 10 days in patients with moderate CI (MCI) led to significant improvement in memory and regression of depressive symptoms simultaneously with normalization of the sleep-wake cycle [103]. H. Jean-Louis et al. [108], Peck et al. [109], observed 26 patients with MCI syndrome received similar results. While 1 mg melatonin administration just before sleep for 4 weeks, there was a significant decrease in forgetfulness in the auditory memory modality and improvement in night sleep compared with placebo. By Cardinali et al. there was made the retrospective analysis of the effect of melatonin therapy on cognitive functions, night sleep and wakefulness in 96 patients with MCI (61 patients received melatonin in doses of 3–9 mg once daily for 9–18 months). It has been proven that melatonin therapy contributes to significant cognitive improvement and regression of depressive symptoms [110].

The high efficiency of the combination of memantine and melatonin in the correction of MCI was shown in the experiment [111].

There is a discussion about the importance of light therapy in correcting of CI. Most of the studies demonstrate the stabilization of CR sleep-wakefulness and a reduction in the time of sleep in dementia with melatonin and light therapy [112].

The prevalence and correction of sleep disorders in patients with CVD need further study in randomized clinical trials in large groups of patients for understanding their impact and establishing cause-effect relationships in the development of CI. These investigations results will help to develop a treatment strategy.

5. Conclusion

Thus, literature data show that stroke has a peculiar organization in time. The vegetative dysrhythmia and failure in the work of the Central control of biorhythms regulation play a key role in these processes.

The night sleep structure investigation is essential part in patients with stroke and patients with cardiovascular disease risk factors. The CR violation leads to the syndrome of desynchronosis—mismatched dynamics of different indicators of the internal environment. This is a potential basis for the cerebrovascular and cardiovascular pathology.

Therefore, including the restoration of the CR with the chronotherapeutics methods is need for the vascular diseases prevention and treatment. Distribution of daily cases of stroke depends on individual properties of CR hemostasis and cerebral hemodynamics, and the specific of the night sleep structure of the of patients. In this regard, there must be a differentiated approach in the treatment of “day” and “night” strokes. The main aspect of reducing the probability of primary and secondary cardiovascular “accidents” development should be the timely detection of sleep disorders and desynchronosis, as one of the leading risk factors.

The use of the personalized chronotherapeutics approaches allows to neutralize the negative impact of desynchronosis.

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Conflicts of interest

The authors declare no conflicts of interest regarding the content of the manuscript.

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Chapter 7

Chronobiology of Acid-Base Balance under General Anesthesia in Rat Model

Pavol Svorc

Additional information is available at the end of the chapter

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Abstract

The design and development of experimental, in vivo, chronobiological animal models may help reveal some of the relationships between circadian rhythms and biological functions. In vivo experiments require the use of appropriate anesthesia, which should be selected according to their particular effect on the organism. The aim of study was to review the status of acid-base balance and ion concentration in arterial blood under common used general anesthesias in experiments in dependence on the light-dark (LD) cycle in spontaneously breathing rats. The experiments were performed using 3- to 4-month-old pentobarbital(P)-, ketamine/xylazine(K/X)-, and zoletil(Z)-anesthetized female Wistar rats after a 4-week adaptation to an LD cycle (12 h light and 12 h dark). We concluded that P anesthesia disturbs LD dependence of acid-base balance compared to K/X and Z anesthesia, but LD differences in plasma ion concentrations are disturbed under all type of general anesthesia. P anesthesia is not the most appropriate type of anesthesia in rat chronobiological experiments. It eliminated LD differences and also produces a more acidic environment, more pronounced hypercapnia and hypoxia than K/X and Z anesthesias. This should be taken into account because the altered internal environment may affect the activity of systems whose functions are primarily dependent on acid-base balance.

Keywords: Chronobiology, electrophysiology of the heart, general anesthesia, internal environment, rat

1. Introduction

At the end of the eighteenth and early nineteenth century, the white rat became the most commonly used experimental animal in biomedical research because it was recognized as the preeminent model of the mammalian system. Currently, rat models are widely used not

only because of their low costs but also for their ability to mimic several human pathologies. These models are used to analyze basic physiological mechanisms, for preclinical and toxicological studies and/or the evaluation of therapeutic approaches [1, 2]. Rats are also useful model animals for studying acid-base balance, especially in relation to the cardiovascular and respiratory systems [1].

The design and development of experimental, in vivo, chronobiological animal models may help reveal some of the relationships between circadian rhythms and biological function, which is sometimes exceedingly difficult to study in humus. Popilskis et al. [3] referred to the fact that "nonhuman primates are important models for a wide variety of biomedical and behavioral research because of their close phylogenetic relationship to humans and they are useful models for experimental surgical studies." However, in the design and development of such chronobiological in vivo rat models, several problems may be encountered. First is the fact that homeostatic regulatory mechanisms are not eliminated; therefore, the responses of the animal as a whole are only a reflection of these mechanisms at a particular time of day. Second is that the circadian rhythms of the observed function itself are not accounted for. Finally, the initial state of the internal environment and the parameters of the function being observed—after the induction of general anesthesia—are often not considered.

In vivo experiments require the use of appropriate anesthesia, which should be selected according to their particular effect on the organism. Moreover, an increasing number of rat and mice studies have acknowledged that the toxicity and efficacy of some anesthetic agents fluctuate in circadian dependence. For example, the toxicity of barbiturates is higher in the early morning [4], and mortality after halothane anesthesia moves from 5% during the day to 76% at night [5]. The toxicity of althesin is highest around 10:00 h [6], and the effective time of althesin anesthesia is 20% longer at 12:00 h than at 06:00 h [5]. Nevertheless, anesthesia has played an important role in ensuring humane surgical/interventions in experimental animals, particularly in long-term in vivo protocols requiring animal survival. Presently, anesthetic practice is primarily based on physiology. The importance of the application of physiological principles in anesthesia has been reaffirmed and emphasizes the need for progress in systemic physiology [7].

2. Acid-base balance, anesthesia, and circadian rhythms

To survive, all living organisms need to maintain acid-base balance and oxygenation. The key role of homeostatic maintenance in all living organisms is not at odds with the observation that various biological parameters are dynamic. Rhythmic changes observed in humans that occur regularly play an important role in adaptation to dynamic environments. Chronobiology affects the activities and functions of the organs and tissues and is also a driver of anatomical, physiological, and molecular changes [8]. Control of acid-base balance depends on the concentration of H^+ and HCO_3^- ions in bodily fluids. In healthy wakeful mammals, including humans, compensatory mechanisms exist for the maintenance of the acid-base balance necessary for normal enzymatic activity, electrolyte diffusion, hemoglobin saturation, and heart contraction, all of which leads to normal functioning of vital organs [9].

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The problem of acid-base balance in anesthesia was addressed by several authors in the early decades of the twentieth century. It was then pointed out that patients under general anesthesia experienced metabolic acidosis due to the ineffective metabolism of carbohydrates in states of unconsciousness [10]. However, later works began to report that this acidosis has a respiratory origin due to disordered respiration [11, 12]. In 1955, Lucas and Milne [13] highlighted the respiratory origin of acidosis in 166 patients who underwent surgery. Respiratory acidosis has been shown to be detrimental during surgery, because it predisposes to shock and the occurrence of problem reflexes. It has been shown that in deep general anesthesia with spontaneous breathing, respiratory acidosis invariably occurs regardless of the anesthetic used. If controlled breathing is used, significant respiratory alkalosis is common with a normal arterial CO_2 pressure of approximately 20 mmHg. For anesthesiologists, metabolic acidosis associated with hypothermia and circulatory arrest is particularly important in cardiac and peripheral vascular surgery [14]. Monitoring of acid-base balance is recommended, especially for prolonged surgical procedures. There are studies indicating that patients undergoing inhaled anesthesia are affected by metabolic acidosis, which depends not only on the duration of the operation but also on the duration of anesthesia. As the duration of general anesthesia is prolonged, pH decreases significantly [15, 16]. This most likely also applies to animal models involving general anesthesia. Therefore, the choice of anesthetic and its effect on the respiratory and cardiovascular system is critical [17, 18].

Changes in the functional efficiency of these systems lead to changes in acid-base balance, and vice versa, changes in acid-base parameters affect the functional state of these systems. Similarly, changes in acid-base balance also reflect 24 h fluctuations in respiratory and cardiovascular functions. Therefore, reference values for acid-base balance can cause problems because the parameters of acid-base balance and ion concentration reflect the current state of the organism at a given time. Results are often compared with average reference values and often regardless of their dependence on the circadian rhythm.

However, rats are typical night animals, which adapt to a natural or controlled artificial light-dark (LD) cycles, which are the strongest synchronizers of endogenous rhythms. This means that their physiological functions exhibit circadian rhythmicity (i.e., fluctuate over a 24 h period).

If we focus on the respiratory system, data confirm that ventilation and metabolism in rats exhibit circadian rhythms and rebut the hypothesis that breathing is affected only by the current state of wakefulness or sleeping. The effects of circadian rhythms on breathing in sleep and wakefulness, as well as the rate of metabolism, are additive in the rat [19]. Some measures that reflect the mechanical properties of the lungs, such as functional residual capacity, forced expiratory volume, and respiratory airways resistance, vary periodically with the time of day. Additionally, resting pulmonary ventilation, tidal volume, and respiratory rate are governed by circadian patterns. Circadian oscillations of the respiratory pattern occur independently of the daily rhythms of other activities or states of wakefulness or sleep. Recent measurements of breath patterns over an extended time period in intact animals have shown that circadian changes occur in a close time phase with changes in oxygen consumption, carbon dioxide production, and body temperature. However, none of these variables can fully explain the circadian pattern of breathing, the origin of which remains unclear [20]. Selected parameters

of the cardiovascular system (e.g., heart rate, blood pressure) in rats also demonstrate circadian rhythmicity [21, 22], which are regulated by various mechanisms, including those part of the autonomic nervous system [23, 24]. Vulnerability of the rat myocardium to ventricular arrhythmias during normal pulmonary ventilation demonstrates a defined 24-h course, with higher vulnerability during the light period of the day. The acrophase, calculated using the population cosinor test, was 22:53, with a confidence interval from 19:20 to 00:28 [25].

The problem of circadian variation of acid-base balance parameters, therefore, remains. Circadian rhythms of acid-base balance and blood gases have been studied in humans, and the following acrophases were found: pH at 16:05; stHCO_3^- at 18:45 h; HCO_3^- at 22:55 h; buffer bases (BB) at 19:03; pCO_2 at 2:47 pm; pO_2 at 04:39 h; HbO_2 08: 07 h; and Hb at 2:16 pm [26]. In rats placed in constant darkness, diurnal rhythms were found in glycemia, pH, and pCO_2. Light pulses of 30 min duration increased blood glucose levels but did not affect plasma pH and pCO_2. These circadian rhythms are most likely under the control of the suprachiasmatic nuclei in the hypothalamus, while the hyperglycemic reaction to light is not controlled by circadian clocks and, thus, may involve retinal inputs to areas of the suprachiasmatic nuclei that are not sensitive to visual inputs [27].

3. Ion concentrations, anesthesia, and circadian rhythms

Ion concentrations neither can be neglected nor is there question whether they are affected by anesthesia or whether their circadian rhythm is maintained under anesthesia. These states can change significantly, for example, in myocardial excitability, which also changes over a 24 h period and is dependent on ion distribution. Based on ion status in the body and their particular role, especially in electrophysiological processes occurring in vital tissues, determination is essential. Potassium, for example, is an essential mineral micronutrient and is the primary intracellular ion for all types of cells, providing vital maintenance of fluid and electrolyte balance in humans and animals [28, 29].

There is clear evidence of the presence of circadian rhythm in potassium and sodium concentrations [30–35]. In all the examined species in which these rhythms occur, overlap of the peak excretion of potassium and sodium occurs essentially at the same time during a 24 h period. It is assumed that the peak of sodium excretion corresponds to reduced sodium reabsorption, and the peak in potassium concentration corresponds to an increase in potassium secretion. Studies involving squirrels, monkeys [36], and rats [37, 38] indicate that cyclic changes in potassium excretion are independent of changes in plasma potassium concentration. However, the correlation between plasma potassium and cyclic potassium excretion has been observed in humans [39]. Maintenance of stable plasma potassium ion (K^+) concentration is extremely important because K^+ controls muscle and nervous activity. In humans, urinary excretion of K^+ peaks in the early morning (05:30–07:30 h), with a minimum at night (21:00–05:30 h) [40]. Circadian rhythmicity has also been demonstrated in thoroughbred racehorses, in which plasma K^+ exhibited a significant rhythm, with acrophase during dark periods [41]. Similar results were found in plasma K^+ concentration in mice, in which based on measurement of urinary excretion, investigators found that peak excretion occurred in the resting

period [42]. Circadian variation of plasma sodium ion (Na^+) in the rat was also demonstrated in a study by Sotak et al. [43]. Electrogenic Na^+ transport in the rat colon was significantly higher during the subjective night than during the subjective day. Transporters and channels operating under the control of NaCl absorption exhibit diurnal regulation, and the role of the intestinal clock in coordinating intestinal NaCl absorption is presumed.

Because the above described events occur primarily in the kidneys, renal function is influenced by circadian clocks through two types of circadian inputs. The first is onset of renal rhythms through external circadian signals such as rhythms of hormones, food intake, activity, and body temperature. The second is the activity of the internal renal circadian clock. For example, Doi et al. [44] reported that the circadian time system controls the reabsorption of sodium in the distal nephron and in the collecting channel via the effect of aldosterone production in the adrenal glands. On the other hand, Rohman et al. [45] reported that internal renal clocks directly regulate Na^+/H^+ activity in the proximal tubule. Gumz et al. [46] reported that the circadian repressor period 1 is able to regulate expression of epithelial sodium channels in the cells of the collecting channel. A study by Roelfsema et al. [47] reported that the maximum excretion of potassium, phosphate, and magnesium is only slightly affected by the dietary regimen, indicating that it depends mainly on endogenous rhythm. In contrast, the minimum excretion of these ions is determined by food intake. Maximum calcium levels, as well as minimal excretion, correlate with dietary regimen. The sodium excretion pattern differs from the calcium, potassium, phosphate, and magnesium patterns, indicating that it is controlled by another mechanism. Unless this fact is taken into account, we can encounter distortions in which the final results are interpreted from a state that does not correspond with the physiological state before administration of the anesthetic.

Sodium ions are necessary for the generation of nerve impulses and for the maintenance of electrolyte and fluid balance. In animals, sodium ions are necessary for these functions and for heart activity and certain metabolic functions [28]. Symptoms of hyponatremia can vary from none to severe [48, 49]. Mild symptoms include a decreased ability to process information, headaches, nausea, and poor balance [50]. Severe symptoms include confusion, seizures, and coma [48, 49]. Hypernatremia can evoke a strong feeling of thirst, weakness, nausea, and loss of appetite [51]. Severe symptoms include confusion, muscle twitch, and bleeding in or around the brain [51, 52].

Calcium ions also play a vital role in the physiology and biochemistry of organisms and the cell. They play an important role in signal transduction pathways [53, 54], where they act as a second messenger in neurotransmitter release from neurons, in the contraction of all muscle cell types and in fertilization. Many enzymes require calcium ions as a cofactor, those of the blood clotting cascade being notable examples. Extracellular calcium is also important for maintaining the potential difference across excitable cell membranes, as well as proper bone formation. Symptoms of hypercalcemia may include abdominal pain, bone pain, confusion, depression, weakness, kidney stones, or abnormal heart rhythm and cardiac arrest [55]. Hypocalcemia can be associated with disorders of hemocoagulation, numbness, muscle spasms, seizures, confusion, or cardiac arrest [56]. Chloride is an essential electrolyte located in all bodily fluids and is responsible for maintaining acid-base balance, transmitting nerve impulses, and regulating fluid in and out of cells.

What is the effect of anesthetics on ongoing ion-dependent processes? Evidence from voltage-clamp studies of individual nerve fibers suggests that, for example, molecules of local anesthetic interact with sodium channels directly from the inside of the nerve membrane. Anesthetics bind to sodium channels, which open during membrane depolarization and prevent normal sodium flow. Anesthetic molecules can separate from open channels, but not from channels that remain closed when the nerve is kept in the resting state. The "gate" properties, which regulate the opening and closing of sodium channels, are reversibly adjusted during anesthesia [57]. Despite the significant advances in chronobiological studies, the mechanisms of circadian regulation of ion channels remain largely unknown. By exploring and understanding the circadian regulation of the ion channel in detail, progress in the development of therapeutic effective strategies for the treatment of sleep disorders, cardiovascular diseases, and other diseases associated with circadian desynchronization [58] will be developed.

4. Chronobiology of anesthesia

Anesthesia is often required in in vivo experiments to ensure comfort and to eliminate pain in animals. However, in small animals, the use of anesthesia can cause certain problems, and therefore, it is necessary to recognize the effect of anesthesia on the internal environment and to account for LD changes in the individual parameters of homeostasis. However, from experimental practice, we know that experiments are performed mostly during working hours (i.e., during light). Thus, if rats are synchronized to the light and dark modes corresponding to the annual season, experiments are performed in the light period of their regimen day (i.e., during their inactive period, when many physiological functions are inhibited). Experiments are, therefore, essentially performed on "sleeping" animals, and questions regarding function during the active part of their regimen day will remain. However, most methodologies do not specify the time of day at which the experiments are performed or the factors responsible for changes in the particular monitored parameters over time. Instead, they focus primarily on current mechanical and metabolic changes, often regardless of the functional status of the body systems over a 24 h period, which may be a problem from a chronobiological point of view [59, 60]. Animal adaptation should, therefore, be taken into account, particularly in in vivo experiments.

Normative data regarding arterial acid-base balance and plasma ion concentrations would help to identify healthy animals suitable for experiments [1], and there are studies that have examined the reliability of these data [61]. **Tables 1** and **2** summarize the ranges of some acid-base balance parameters and ion concentrations in arterial rat blood, which have been described in several published studies. However, the time at which the experiments were performed or the time of blood sampling for evaluation of blood gases, pH, bicarbonates, and some ions, or the synchronization of animals to the LD cycle, was not considered in the methodologies of these studies.

Although chronobiological studies investigating the interactions between general anesthesia and circadian rhythms are scarce, they all suggest that general anesthesia has a significant

Author(s) (year of publication)	pH	pCO_2 (kPa)	pO_2 (kPa)	HCO_3^- (mmol/l)
Lewis et al. [62]	7.43	5.47	12.13	
Pepelko and Dixon [63]	7.446–7.486	5.24–5.74	11.77–12.71	
Brun-Pascaud et al. [64]	7.45–7.49	4.2–4.99	11.26–12.72	24–27
Girard et al. [65]	7.46–7.47	4.57–4.71	12.72–13.02	25–25.8
Hess et al. [66]	7.43–7.51	3.33–4.67	12.2–15.4	
Dettmers et al. [67]	7.38–7.46	5.19–5.99	9.4–11	
Chi et al. [68]	7.27–7.37	4.78–5.77	13.8–17	
Ohoi and Takeo [69]	—	4.66–5.32	13.3–17.3	
Schultz et al. [70]	7.35–7.45	3.33–5.32	10.6–14.6	
Sun and Wainwright [71]	7.40–7.45	4.64–5.32	11.3	
Forkel et al. [72]		5.16–6.39	12.85–15.48	
Valenza et al. [73]	7.41–7.43	5.18–5.48	—	25.3–27.1
Subramanian et al. [1]	7.26–7.4	5.05–7.51	10.76–14.60	21.5–28.1
Peralta-Ramírez et al. [74]	7.2–7.46	5.62–6.20	—	23.2–25.8
Luo et al. [75]	—	5.58–6.08	10.37–12.19	
Range*	**7.369–7.452**	**4.75–5.298**	**10.75–14.184**	**23.8–26.78**

*Ranges were calculated as the mean value from the lower and upper limits of the ranges reported in these studies.

Table 1. Values of pH, blood gases, and bicarbonate in the arterial blood of rats published in previous studies.

Authors (year of publication)	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+} (mmol/l)	Cl^- (mmol/l)
Menegon et al. [76]	142.1–143.9	3.6–3.8		
Costa et al. [77]	138.9–141.1	4.74–4.86	6.72–7.38	
Valenza et al. [73]	132.4–140	4.1–4.42		102.9–107.7
Subramanian et al. [1]	140.7–145.6	3.08–4.02		
Peralta-Ramírez et al. [74]	134.6–137.3	3.93–4.25	1.23–1.29	104.4–108.1
Range*	**137.4–140.7**	**3.86–4.21**	**?**	**103.7–107.9**

*Ranges were calculated as the mean value from the lower and upper limits of the ranges reported in these studies.

Table 2. Arterial plasma ion concentrations in the arterial blood of rats according to previously published studies.

effect on biological functions [78]. Some have pointed to the temporal dependence of some anesthetic effects on the [78] circadian rhythm. For example, in locomotor activity, a phase shift of circadian rhythm occurred after administration of selected anesthetics, indicating its dependence on time. Pentobarbital injections induced both advanced and delayed phase shifts in the circadian rhythm of movement activity in SK mice; however, no phase shifts were observed in any circadian time with pentobarbital injections in C57BL mice. This suggests

that differences in phase shifts after the use of pentobarbital are not quantitative but qualitative [79], and that pentobarbital-induced phase shifts are not the result of increasing levels of activity [80].

In a study by Pang et al. [81], pentobarbital had no apparent effect on melatonin release and did not affect plasma levels of cerebral natriuretic peptide in rats, in which both hormones are at a relatively low level at 02:30 h [82]. Naguib et al. [83] described the effects of anesthesia on melatonin production. Anesthesia disrupts the circadian rhythm of melatonin, the major humoral transmitter of suprachiasmatic nuclei activities in the hypotalamus [84–86]. It appears that intravenous anesthetics with different behavioral profiles act on different and specific ligand-bound ion channels to create specific anesthetic behavior. Whether the anesthetic effect of melatonin is due to a direct effect on melatonin receptors remains largely unknown. Melatonin receptors, as such, are not commonly considered to be molecular targets for general anesthetic effects. However, there is evidence to suggest that the central effects of melatonin include at least partial facilitation of GABAergic transmission by modulation of GABA receptors [87–89]. In a study by Mihara et al. [90], pentobarbital demonstrated no effect on melatonin secretion or on movement activity, regardless of the time of dosing. On the other hand, in rats under general propofol anesthesia, the plasma concentration of melatonin decreased over the first 4 h after anesthesia induction and increased after 20 h. Thus, general propofol anesthesia abolishes the circadian rhythm of melatonin in rats adapted to an LD cycle [91].

Results of a study by Kana et al. [92], involving the inhalation anesthetic sevoflurane, reported that sevoflurane had the greatest efficacy in suppressing mPer2 expression (mPER2 acts as a positive rhythm transcription regulator in hypothalamic suprachiasmatic nuclei) in the morning. The investigators proposed that, in the morning, this biochemical reaction is inhibited by anesthesia, which can lead to suppression of mPer2 expression and effectively reflect circadian clocks. However, at the phase delay of movement cycle activation, sevoflurane acted independently of time.

Prudian et al. [93] and Pelissier et al. [94] reported a disrupting effect of ketamine on circadian rhythms; however, this effect was associated only with a modification of acrophase, amplitude or mesor, without loss of daily rhythmicity. To date, however, there is no literature evidence supporting the effect of general anesthesia on acid-base balance and ion concentration in arterial blood, depending on circadian rhythmicity or LD cycles. This highlights the fact that different anesthetics may have different effects on the circadian rhythms of many parameters.

5. Aims

The specific objective of the present in vivo study is to investigate chronobiological aspects of the status of acid-base balance and plasma ion concentrations in arterial blood (i.e., existence

of possible circadian variations) and to determine whether there are differences between types of anesthesia after the immediate application of the most common anesthetics in in vivo rat experiments, pentobarbital (P), ketamine/xylazine (K/X), and zoletil (Z) in spontaneously breathing rats.

6. Materials and methods

The present study conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication number 85–23, revised 1996). The study protocol was approved by the Ethics Committee of the Medical Faculty of Safarik University (Kosice, Slovak Republic) (permission numbers 2/05 and ŠVPS SR: Ro-4234/15–221).

The present study was performed using female Wistar rats (mean [±SD] weight 310 ± 20 g), 3–4 months of age after a 4-week adaptation to an LD cycle (12 h light:12 h dark [intensity of artificial illumination 80 Lux]; 40–60% humidity; cage temperature 24°C; two animals/cage; *ad libitum* access to food and water). The effect of the light period on the monitored parameters was examined after adaptation to an LD cycle, with the light period from 06:00 to 18:00 h. The effect of the dark period was monitored after adaptation to the inverse setting of the LD cycle (i.e., with the light period from 18:00 to 06:00 h) (**Figure 1**).

The animals were divided into one of three experimental groups according to anesthetic agent used (**Table 3**). Approximately 20 min after administration of anesthetic agent, the spontaneously breathing animals were fixed supine to an experimental table. pH and blood gases from blood samples obtained from the femoral artery were examined using a blood-gas analyzer

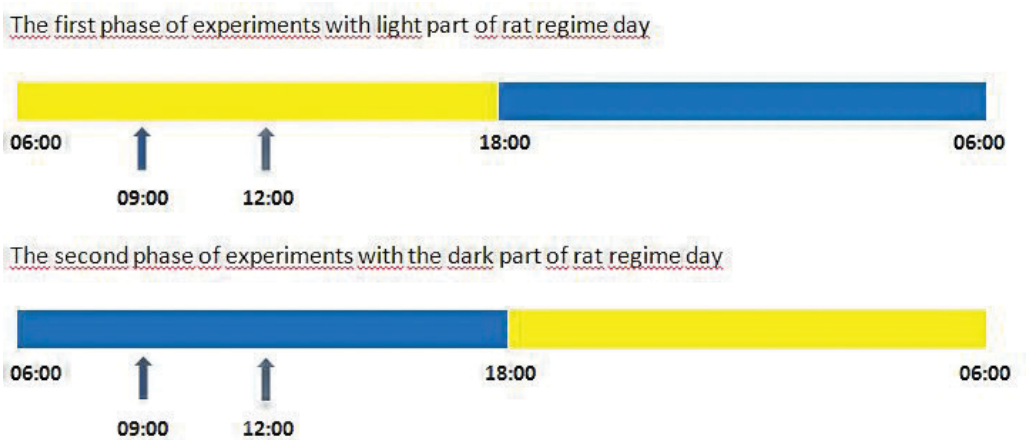

Figure 1. Scheme of adaptation to the light-dark (LD) cycle. Arrows indicate the time of the experiment. The experiments were performed once in each animal in the course of a single LD period (the first animal at 09:00 h and the second at 12:00 h).

	Experimental period	Number of animals	Anesthesia	Route of administration
Group 1	Light	16	*Pentobarbital* (40 mg/kg, SPOFA, Prague, Czech Republic)	Intraperitoneal
	Dark	27		
Group 2	Light	11	*Ketamine* (100 mg/kg, Narkamon) + *xylazine* (15 mg/kg, Rometar, SPOFA, Prague, Czech Republic)	Intramuscular
	Dark	13		
Group 3	Light	10	*Zoletil* (30 mg/kg, VIRBAC, France)	Intraperitoneal
	Dark	12		

Table 3. Experimental groups.

(ABL 800 Flex, Radiometer Medical, Copenhagen, Denmark) in the Department of Laboratory Medicine, Faculty Hospital Louis Pasteur in Kosice. The depth of anesthesia was estimated according to whether painful stimuli evoked noticeable motor or cardiovascular responses.

6.1. Statistical analysis

The data were analyzed using GraphPad InStat (GraphPad Software, USA) and presented as mean ± SD. ANOVA was used to detect significant differences within a single end point. The Tukey-Kramer test was used to identify significant differences between groups; $p < 0.05$ was considered to be statistically significant. The experiments were performed over the course of an entire year, and the results were averaged independent of season and estrous cycle.

7. Results

7.1. pH

Under P anesthesia, significant LD differences in arterial pH were not found, and values remained at the same levels. Under K/X ($p < 0.001$) and Z ($p < 0.001$) anesthesias, the pH was significantly higher in the dark (active) versus the light part of the rat regimen day (**Table 4**, **Figure 2**). In the light part of the day, the pH values reflect acidosis, compared with the range calculated from other authors (**Table 1**) in all types of anesthesia, and there was no significant difference between individual types of anesthesia. In the dark part of the day, mean pH values were significantly higher in K/X ($p < 0.05$) and Z ($p < 0.05$) anesthesias compared with P anesthesia. The pH was acidic under P anesthesia, from normal to alkaline under K/X anesthesia and from acidic to normal under Z anesthesia.

7.2. pCO_2

Significant LD differences in pCO_2 were found under K/X anesthesia but not under P and Z anesthesias (**Table 4**). In both light parts of the rat regimen day, significant hypercapnia

	Pentobarbital		Ketamine/xylazine		Zoletil	
	Light	**Dark**	**Light**	**Dark**	**Light**	**Dark**
Acid-base parameter						
pH	7.31 ± 0.04	7.31 ± 0.07	7.33 ± 0.04	7.43 ± 0.14**	7.32 ± 0.05	7.38 ± 0.06***
pCO_2	8.58 ± 1.22	8.64 ± 1.49	6.76 ± 1.84	2.88 ± 0.72**	6.75 ± 0.93	6.65 ± 1.11
pO_2	8.36 ± 1.64	8.89 ± 2.86	7.17 ± 0.37	10.75 ± 1.84***	10.06 ± 2.31	8.46 ± 2.08*
HCO_3^-	31.56 ± 2.73	31.31 ± 2.09	28.02 ± 4.57	15.55 ± 5.62***	25.28 ± 1.3	28.8 ± 2.11***
$stHCO_3^-$	26.67 ± 1.72	26.55 ± 2.01	24.16 ± 1.24	19.22 ± 5.14*	22.95 ± 1.37	27.0 ± 1.91***
$ctCO_2$	32.61 ± 4.15	31.66 ± 3.48	28.4 ± 2.69	15.91 ± 5.93***	22.58 ± 1.05	25.6 ± 2.23***
BE	3.66 ± 2.12	3.43 ± 2.38	0.06 ± 1.71	−5.23 ± 6.34*	−1.41 ± 1.58	2.21 ± 1.34***
BB	51.56 ± 2.23	51.13 ± 2.82	48.34 ± 1.71	42.44 ± 6.65*	46.39 ± 1.56	50.21 ± 1.34***
ctO_2	9.22 ± 2.29	10.28 ± 2.72	11.76 ± 5.11	20.05 ± 0.56*	18.58 ± 1.34	19.17 ± 1.05
$SatO_2$	87.25 ± 8.86	87.8 ± 10.34	84.66 ± 2.96	93.29 ± 7.42**	89.96 ± 5.46	89.25 ± 4.64

Data presented as mean ± SD.*$p < 0.05$;

**$p < 0.01$; and

***$p < 0.001$ statistically significant differences between the light and dark parts of the rat regimen day. pCO_2 (kPa) – partial pressure of carbon dioxide, pO_2 (kPa) – partial pressure of oxygen; HCO_3^- (mmol/l) – bicarbonate; $stHCO_3^-$ (mmol/l) – standard bicarbonate; $ctCO_2$ – the sum of carbon dioxide bound to hemoglobin and carbon dioxide dissolved in plasma; BE (mmol/l) – base excess; BB (mmol/l) – total buffer bases; ctO_2 – the sum of oxygen bound to hemoglobin and oxygen dissolved in plasma, $satO_2$ (%) – saturation of hemoglobin by oxygen.

Table 4. Values of acid-base balance parameters for selected type of anesthesia in the light and dark parts of the rat regimen day.

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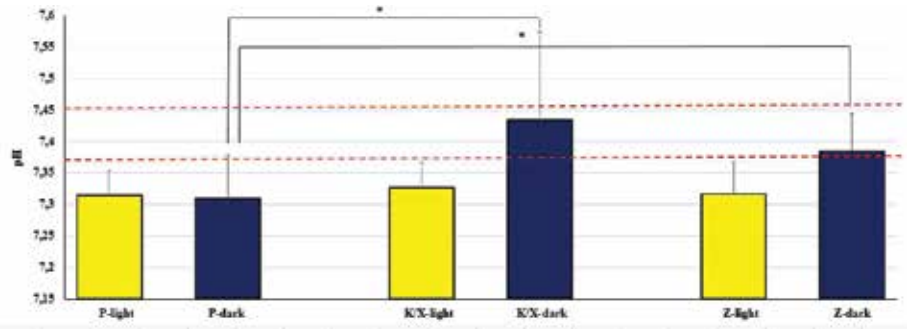

Figure 2. pH in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-, ketamine/xylazine (K/X)- and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. * $p < 0.05$ was considered to be a statistically significant difference between individual types of anesthesia. Red dotted lines represent the ranges reported in **Table 1**.

occurred under P and Z anesthesias. More pronounced hypokapnia was found under K/X anesthesia in the dark part. In the light part, there was a significant difference between P and Z anesthesia ($p < 0.001$), with higher values in P anesthesia. In the dark part of the rat regime day, significant differences between all selected types of anesthesia (P vs. K/X [$p < 0.001$]; P vs. Z [$p < 0.01$]; and K/X vs. Z [$p < 0.001$]) were observed (**Figure 3**). Because the pCO_2 ranges listed in **Table 1** are considered to be physiological compared with these ranges, the mean pCO_2 reported in this study is in the range of hypercapnia for each type of anesthesia in both light parts, except K/X anesthesia in the dark part of the rat day.

7.3. pO_2

Similar to pH, LD differences in pO_2 were only significant in K/X ($p < 0.001$) and Z ($p < 0.05$) anesthesias (**Table 4**). However, it is interesting to note that for all types of general anesthesia used in this study, hypoxia was detected in spontaneously breathing rats in both light parts of

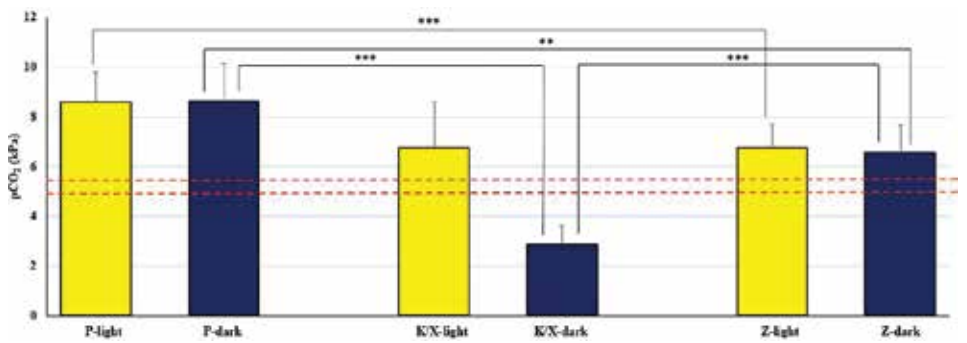

Figure 3. pCO_2 in the light (yellow columns) and dark (blue columns) parts of rat regimen day in pentobarbital (P)-, ketamine/xylazine (K/X)-, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. **$p < 0.01$, ***$p < 0.001$ were considered to be a statistically significant difference between individual types of anesthesia. Red dotted lines represent the ranges reported in **Table 1**.

the rat regimen day. Statistically significant differences were found in a light part between P and K/X ($p < 0.001$), P and Z ($p < 0.05$), and between K/X and Z anesthesia ($p < 0.001$), with the lowest values under K/X anesthesia. In the dark part, more pronounced hypoxia was under Z anesthesia ($p < 0.05$) compared with K/X anesthesia. Differences between P and Z anesthesias were not found (**Figure 4**).

7.4. HCO_3^-

Significant LD differences in HCO_3^- were detected under K/X and Z anesthesias (**Table 4**). Taking into account that the normal range of bicarbonate (from **Table 1**) is from 23.8 to 26.78 mmol/l, increased levels were measured in P anesthesia, which would correspond to metabolic alkalosis in both light parts of the regimen. Normal levels were detected in Z anesthesia in both light parts. In K/X anesthesia, the levels of HCO_3^- were dependent on the cycle of alternating light and darkness. Under this type of anesthesia, in the light part, values moved around the normal range; however, in the dark part of the day, levels were reduced to what corresponds to metabolic acidosis. Between individual anesthetics, significant differences were found, especially in the dark part of the rat regimen day (**Figure 5**).

7.5. BE, BB, and saturation of hemoglobin by O_2

Significant LD differences in total buffer bases (BB) and base excess (BE) were found in K/X and Z anesthesias (**Table 4**). BB moves from 40 to 60 mmol/l in all types of anesthesia and the BE from −8 to +12 mmol/l in both light parts of the rat regimen day under all types of anesthesia. Saturation of hemoglobin by oxygen was practically the same in all types of general anesthesia, and significant LD differences were not found except for K/X anesthesia, with higher saturation in the dark part of the rat regimen day. Significant differences of acid-base parameters between the single type of anesthesias are summarized in (**Table 5**).

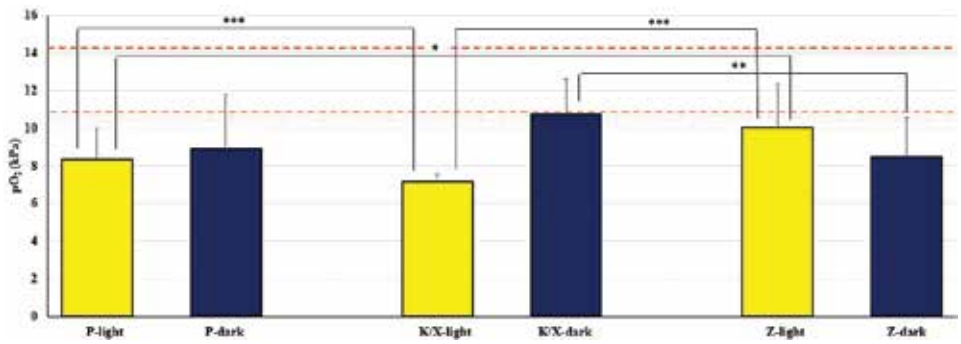

Figure 4. pO_2 in the light (yellow columns) and dark (blue columns) parts of rat regimen day in pentobarbital (P)-, ketamine/xylazine (K/X)- and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent ranges reported in **Table 1**.

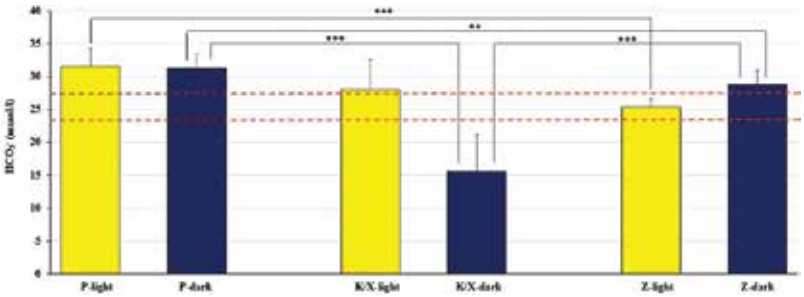

Figure 5. HCO_3^- in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-, ketamine/xylazine (K/X)- and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. **p < 0.01 and ***p < 0.001 were considered to be a statistically significant difference between individual types of anesthesia. Red dotted lines represent the ranges reported **Table 1**.

7.6. Ions

LD differences for plasma Na^+ concentration were not detected under any of the selected general anesthesias (**Table 6**). The highest Na^+ concentrations were under P anesthesia in the both light parts of the rat regimen day (light P vs. K/X, $p < 0.01$; P vs. Z, $p < 0.01$; dark P vs. K/X, $p < 0.01$; and nonsignificantly higher compared with Z anesthesia). In the light part of the day, the highest plasma concentration of Na^+ was recorded under P anesthesia and the lowest concentration in Z anesthesia but with increasing dispersion of values. Based on our findings, it appears probable that the distribution of Na^+ ions is significantly influenced by Z anesthesia (**Figure 6**). Under P anesthesia, regardless of the light or dark part of the day, hypernatremia was detected. In K/X and Z anesthesia, mean plasma Na^+ concentrations moved from hyponatremic to hypernatremic.

Significant (i.e., $p < 0.01$) LD differences in plasma K^+ concentration were found only under K/X anesthesia, with higher values during the dark part of the rat regimen day (**Table 6**).

	pH	pO_2	pCO_2	HCO_3^-	$stHCO_3^-$	BE	BB	$ctCO_2$	ctO_2	$satO_2$
Light										
P-K/X	0.617	**0.001**	0.094	0.166	**0.01**	**0.01**	**0.01**	**0.05**	0.339	0.419
P-Z	0.869	**0.05**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.401
K/X-Z	0.708	**0.001**	0.985	0.252	0.104	0.136	0.064	**0.01**	**0.05**	**0.01**
Dark										
P-K/X	**0.05**	0.137	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.251
P-Z	**0.05**	0.707	**0.01**	**0.01**	0.559	**0.001**	**0.001**	**0.001**	**0.001**	0.730
K/X-Z	0.268	**0.01**	**0.001**	**0.001**	**0.001**	**0.001**	**0.01**	**0.001**	**0.01**	0.119

Bold values indicate statistically significant differences. P – pentobarbital P; K/X – ketamine/xylazine Z – zoletil; pCO_2 (kPa) – partial pressure of carbon dioxide; pO2 (kPa) – partial pressure of oxygen; HCO_3-(mmol/l) – bicarbonate; $stHCO_3$ (mmol/l)-standard bicarbonate; $ctCO_2$ – the sum of carbon dioxide bound to hemoglobin and carbon dioxide dissolved in plasma; BE (mmol/l) – base excess; BB (mmol/l) – total buffer bases; ctO_2 – the sum of oxygen bound to hemoglobin and oxygen dissolved in plasma, $satO_2$ (%) – saturation of hemoglobin by oxygen.

Table 5. P values reflecting the statistical significance of differences in acid-base parameters among individual types of anesthesia in the light and dark parts of the rat regimen day.

Ion	P-light	P-dark	K/X-light	K/X-dark	Z-light	Z-dark
Na^+	145.08 ± 2.13	143.24 ± 1.7	140 ± 6.74	134.17 ± 5.56	133.97 ± 16.06	140.8 ± 7.67
K^+	4.69 ± 0.31	4.91 ± 0.30	6.81 ± 1.42	8.85 ± 1.31**	5.00 ± 0.71	4.68 ± 0.50
Ca^{2+}	1.31 ± 0.05	1.33 ± 0.05	2.14 ± 0.07	—	0.99 ± 0.44	1.00 ± 0.38
Cl^-	100.1 ± 1.21	100.51 ± 2.43	110.2 ± 2.39	—	104.8 ± 5.19	101.1 ± 5.1*

Data presented as mean ± SD.*p < 0.05,

**p < 0.01 statistically significant differences between the light and dark periods. P – pentobarbital P; K/X – ketamine/xylazine Z – zoletil; Na^+ – sodium, K^+ – potassium, Ca^{2+} – calcium and Cl^- chloride anions.

Table 6. Ion concentrations in arterial blood under individual types of anesthesia.

Under this type of anesthesia, the mean value was significantly higher ($p < 0.001$) compared with both P and Z anesthesias in both light parts of the day (**Figure 7**). Moderate hyperkalemia was detected under P and Z anesthesias in both light parts of rat regimen day.

Similar to Na^+, no significant LD differences in plasma Ca^{2+} concentrations were found (**Table 6**). Under P and Z anesthesias, plasma concentrations of Ca^{2+} were practically the same. In the light part of the day under K/X anesthesia, there was a significantly ($p < 0.001$) higher Ca^{2+} concentration versus P and Z anesthesias. In the dark part of the day under K/X anesthesia, the values were out of range of the ABL 800 Flex ion analyzer (**Figure 8**). Although significant differences were found between the different types of anesthesia in both light parts of the day, the animals were in relatively severe state of hypocalcemia, especially when under P and Z anesthesias.

A significant (i.e., $p < 0.05$) LD difference in plasma concentrations of Cl^- was found only under Z anesthesia (**Table 6**). Hypochloremia occurred under P anesthesia in both light parts of the rat regimen day. Normochloremia to hyperchloremia occurred under both K/X and Z anesthesias in both light parts of the rat regimen day (**Figure 9**). In the dark part of the day under K/X anesthesia, the values were out of the detection range of the ABL 800 Flex ion analyzer. Significant differences in ion concentrations between the single type of anesthesias are summarized in (**Table 7**).

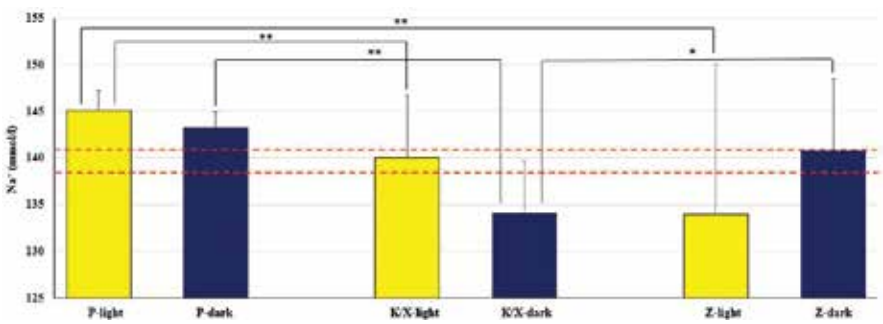

Figure 6. Plasma concentration of Na^+ in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. *p < 0.05 and **p < 0.01 were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent ranges reported in **Table 2**.

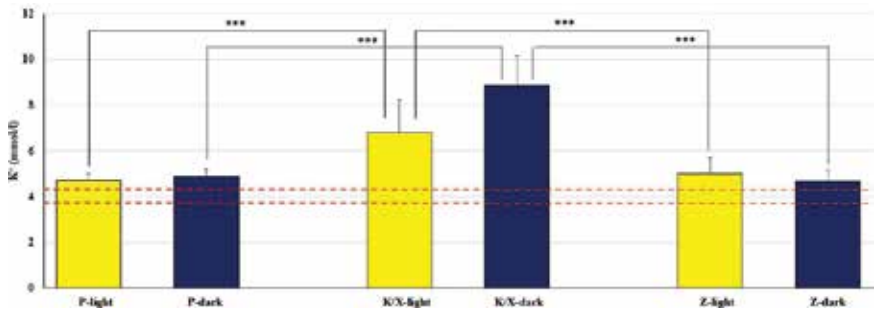

Figure 7. Plasma concentration of K^+ ions in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. ***$p < 0.001$ was considered to be a statistically significant difference between single types of anesthesia. Red dotted lines represent the ranges reported in **Table 2**.

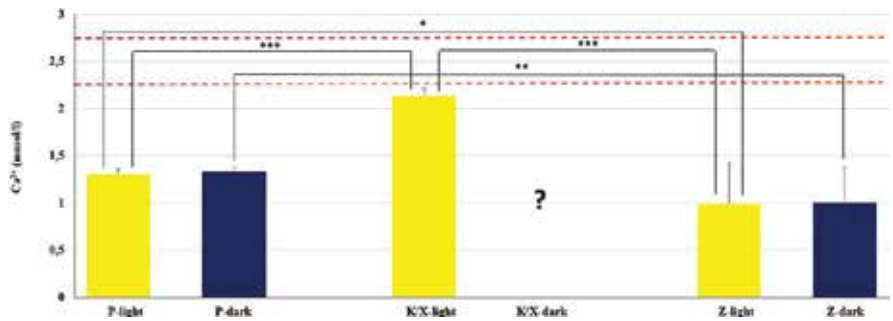

Figure 8. Plasma concentration of Ca^{2+} ions in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent ranges reported in **Table 2**.

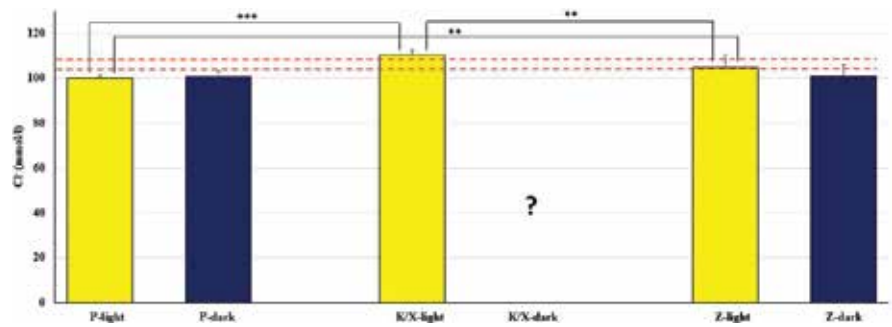

Figure 9. Plasma concentration of cl^- ions in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. ***$p < 0.001$, **$p < 0.01$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent the ranges reported in **Table 2**.

	Na^+	K^+	Ca^{2+}	Cl^-
Light				
P-K/X	**0.01**	**0.001**	**0.001**	**0.001**
P-Z	**0.01**	0.203	**0.05**	**0.01**
K/X-Z	0.202	**0.001**	**0.001**	**0.01**
Dark				
P-K/X	**0.01**	**0.001**	—	—
P-Z	0.246	0.770	**0.01**	0.687
K/X-Z	**0.05**	**0.001**	—	—

Bolded values indicate statistically significant differences.

Table 7. Differences in plasma ion concentrations of individual types of anesthesia in the light and dark parts of the rat regimen day.

8. Discussion

The methodological character of this study was based on the chronobiological perspective of the initial state in acid-base balance and plasma ion concentration in arterial blood after application of commonly used anesthetics in experiments, as well as to differences in parameters of the internal environment between used the selected types of general anesthesia. The methodical characteristics of this study highlight the potential risks of experimental design. Each of the acid-base balance parameters reflects the current state of the internal environment, which can significantly affect the functionality of the monitored system.

If we only hypothetically assume that experiments are performed during working hours (i.e., in the light [inactive], part of the rat regimen day), the values presented in **Tables 1** and **2** are comparable with our results only from the light (inactive) part of the day. In the dark (i.e., active) part of the rat regimen day, the values—although significantly different among the individual types of general anesthesia—may be within the normal range but can also move out of range; this also applies to ion concentrations. In this case, therefore, comparisons are irrelevant.

8.1. pH and blood gases

The cardiovascular system is particularly sensitive to changes in the internal environment. For example, earlier work by Gerst et al. [95] did not detect an impact of respiratory acidosis and alkalosis on the threshold of heart vulnerability to ventricular fibrillation in dogs; however, together with hypoxia, they increased its threshold [96]. Conversely, metabolic acidosis reduces the ventricular fibrillation threshold, reduces the maximum diastolic potential, shortens the duration of action potentials, inhibits excitability, stimulates impulse conduction between Purkinje fibers and muscle tissue [97], worsens atrioventricular (AV) conduction, and inhibits AV node automation [98]. Acidosis affects the mechanical and electrical activity of the

mammalian heart. In this way, acidosis can dramatically prolong the delay of AV conduction. In combination with short cycle times, this may cause partial or complete AV block of conduction and, consequently, contribute to the development of bradyarrhythmias under conditions of local or systemic acidosis [99]. Hypoventilation in rats is associated with systemic acidosis, hypoxia and hypercapnia, decreased mesor, amplitude, as well as altered circadian rhythm of ventricular arrhythmia threshold from one peak to two peaks, with a smaller peak between 15:00 and 18:00 h and higher between 24:00 and 03:00 h [25].

Our results indicate that P, K/X, and Z anesthesias cause acidosis, hypoxia, and hypercapnia, especially in the light period of the rat regimen day. In the dark part of the day, values are closer to physiological ranges, except for P anesthesia [100]. It also appears that differences in pH, pO_2, and pCO_2 differ among each type of general anesthesia, depending on the light period. The decrease in pH, observed in all types of anesthesia, is probably the result of a contemporaneous depression of pulmonary ventilation and decrease in body temperature in the light as well as in the dark part of the rat regime day.

We have confirmed the conclusions of other work investigating the effects of anesthesia on pulmonary ventilation. Induction of anesthesia in rats using P significantly increases pCO_2 and TCO_2, while pH is decreased [64, 101, 102]. P-induced anesthesia caused mild respiratory acidosis accompanied by an increase in arterial lactate levels. Urethane anesthesia leads to partially compensated metabolic acidosis. Hypothermia reduces metabolic acidosis and hypercapnia induced by P anesthesia. In urethane anesthesia, no difference was observed between hypothermic and normal values [103]. Alfaro and Palacios [104] compared acid-base balance in mildly hypothermic (30°C) and seriously hypothermic rats (20°C). The authors found that in the first group of hypothermic animals, respiratory alkalosis occurred with an increase in pH from 7.476 to 7.546 and a decrease in arterial bicarbonate from 22.9 to 16.8 mmol/L; in the second group, from 7.484 to 7.563 with a bicarbonate drop from 20.7 to 14.6 mmol/l. This pattern was clearly different in rats under P anesthesia (mild respiratory acidosis) and under urethane anesthesia (metabolic acidosis). Similar results were reported by Gaudy et al. [105]. Anesthesia may interfere with the development of processes that lead to the acid-base balance pattern observed in conscious animals. In 1997, Alfaro and Palacios [106] supplemented that their observations regarding the blood pH of normothermic anesthetized rats (body temperature Tb = 37°C) was also associated with an increase in plasma anions (lactate and Cl^-). More severe metabolic acidosis in rat blood were detected in urethane-induced hypothermia (Tb = 32°C).

Changes observed in rats anesthetized with the thiobarbiturate inactin were similar to urethane anesthesia, although they were generally less severe. Most subjects treated with barbiturates were significantly hypercapnic. Urethane anesthesia was characterized by a higher and more stable heart rate and greater pulse pressure. Arterial carbon dioxide and bicarbonate values in the urethane group were significantly lower at all sampling times than those obtained in the barbiturate groups [107]. In connection with hypercapnia, it is also interesting to note that mild hypercapnia increases peripheral tissue oxygenation in healthy individuals, which can improve resistance to infections after surgical intervention. Partial pressure of tissue oxygen, blood flow rate through the skin, cardiac index, and saturation of muscle oxygen increases linearly with partial CO_2 pressure. The observed difference in peripheral oxygenation is clinically important because previous work has suggested that a comparable increase in tissue oxygenation reduces the risk of infection from 7–8%, to 2–3% [108].

Considering changes in blood gases from a chronobiological perspective, Ohshima et al. [109] and Iwase et al. [110] reported interesting results regarding the effects of histamine on ventilation and the balance of energy metabolism via H1 receptors in the brain. The hypothesis was tested on mice as to whether the ventilatory response to hypoxia fluctuated between the light and the dark period and whether histamine H1 receptors are necessary for circadian variation. The results demonstrated that during hypoxic conditions, minute ventilation in wild type mice increased during the dark period. Hypoxia reduced metabolism, but O_2 consumption and CO_2 elimination were higher in the dark period. In H1 receptor knockout mice, changes in minute ventilation were minimal because minute ventilation was relatively increased with respect to O_2 consumption in the light period. In this group, HCO_3^- and BE were elevated in arterial blood, and serum levels of ketolate were increased, indicating metabolic acidosis. The results of that study assume that minute ventilation varies between the light and dark periods, and that H1 receptors play a role in the circadian variation of minute ventilation through acid-base balance control and metabolism in mice [109, 110].

Rectal temperature in rats measured before administration of anesthetic agent varies depending on the LD cycle, with significantly higher values in the dark (active) part of the day, indicating the preservation of the circadian rhythm of body temperature. After anesthetic administration, a significant drop in rectal temperature (rectal temperature before anesthetic administration versus rectal temperature 15 min after induction of anesthesia [$p < 0.001$]) has been observed under all types of anesthesia in both light parts of the rat regimen day [100]. Interestingly, LD differences in K/X and Z anesthesias were maintained, except for P anesthesia. These results confirm the well-known fact that thermoregulation is impaired under general anesthesia [111]. This basic process occurs when the body core temperature is redistributed to the surface of the skin by anesthetic-induced vasodilation and depression of hypothalamic thermoregulatory centers [112]. Thus, the loss of LD differences under P anesthesia confirms this fact, and that P likely also acts on the suprachiasmatic nuclei of the hypothalamus.

Sustained anesthesia and hypothermia may be required under certain conditions of critical care. Data suggest that mild hypothermia (35–33°C), in combination with sustained anesthesia, may reduce the need for high levels of breathing volume and respiratory rate without significant changes in arterial oxygenation and acid-base balance. The risk for barotrauma in ventilated rats exposed to conditions similar to critical care could, therefore, be reduced by using lower volume/pressure ventilation in the presence of mild hypothermia and P anesthesia [113]. Moderate hypothermia in rats induced by sustained P anesthesia reduces ventilation but without a change in arterial oxygenation or acid-base balance, measured at normal body temperature. In theory, observations in spontaneously breathing rats indicate that a combination of moderate hypothermia and anesthesia can be safely used to maintain adequate ventilation with relatively low ventilation. It is assumed that such a maneuver, when used during mechanical ventilation, can prevent secondary pulmonary damage by allowing a lower adjustment of the volume and pressure of the ventilator [114].

Metabolism and pulmonary ventilation change over a 24 h period and exhibit circadian fluctuations. Because their changes are always synchronic, blood gases can remain stable in a narrow range. Piccione et al. [115] monitored arterial blood gases, pH, body temperature and respiratory rate in 5 cows and detected a circadian rhythm only for pCO2. In cows, blood

gases remain highly stable for 24 h. Daily body temperature oscillations, respiratory rate, and probably many other factors affecting metabolism and pulmonary ventilation do not exclude excellent blood gas homeostasis.

If respiratory acidosis is induced after anesthesia, it is logical to adjust pulmonary ventilation so that the acid-base balance is adjusted to a physiological range. However, there is a problem with how to set up artificial ventilation to adjust acid-base balance parameters. The method of artificial ventilation for rats under general anesthesia has been in use since 1940 [116–119]. This can be a suitable procedure for creating experimental models observing the effect of pulmonary ventilation disorders on various functional systems. However, artificially controlled ventilation parameters using room air should be adequate to maintain acid-base balance. There are several types of normal artificial ventilation in rats that can be applied to maintain acid-base balance (**Table 8**).

The selection of anesthetic agent may be problematic with respect to the respiratory and cardiovascular systems [17, 18]. Changes in the functional performance of these systems lead to changes in acid-base balance. Conversely, changes in acid-base balance also reflect 24 h fluctuations in respiratory and cardiovascular function. Therefore, acid-base balance reference values may be problematic because acid-base balance only reflects the current state of the organism at a particular time of day. The results are then often compared with the average reference values, often regardless of dependence on the circadian rhythm of changes in acid-base balance. If both pH and partial pressures of the respiratory gases depend on respiratory and cardiovascular

Author (year)	Respiratory rate, breaths/min	Tidal volume, ml/100 g
Fagbeni et al. [120]	54	2
Richard et al. [121]	60	1
Guarini et al. [122]	55	2
Lott et al. [123]	70	1.5–2
Ohoi and Takeo [69]	40–60	1
Godin-Ribuot [124]	54	1.5
Oosting et al. [125]	60	3
Schultz et al. [70]	65–70	Not determined
Sun and Wainwright [71]	54	2
Häfner et al. [126]	30	Not determined
Tanno et al. [127]	44–55	1.5–2.5
Ravingerova et al. [128]	65–70	1.2
Wang et al. [89]	60–70	1.2
Neckař et al. [129]	65–70	1.2
Neckař et al. [130]	69	1.2

Table 8. Previously published artificial lung ventilation parameters to maintain normal acid-base balance ranges in vivo in rats.

activities and demonstrate circadian rhythmicity in these systems, acid/base balance parameters will also exhibit a parallel circadian rhythmicity. The functional efficiency of the respiratory and cardiovascular systems is greater during periods of activity; therefore, pO_2 will also be higher at these times, and CO_2 output will be increased. pH depends on changes of pCO_2. The question, therefore, remains: to what extent are changes in acid-base balance parameters still acceptable in in vivo rat models? Additionally, to what extent should the dependence on circadian rhythms be accounted for in the design of in vivo experiments involving general anesthesia?

8.2. Acid-base balance and ion concentration

When considering parameters of acid-base balance, the most important is bicarbonate concentration. In general, given the impact of some processes on acid-base balance, it is advisable to especially consider changes in the concentrations of the major ions and their equilibrium to evaluate changes in the concentration of bicarbonate. The change in pH is secondary due to the change in the Henderson-Hasselbach equation. Eventual loss or addition of protons is immediately equalized by buffering mechanisms, and the capacity of which are significant with regard to regulating proton concentration.

Bicarbonate content in serum or plasma is a significant indicator of electrolyte dispersion and anion deficiency. Together with pH determination, bicarbonate measurements are used to diagnose and treat many potentially serious disorders associated with acid-base imbalance(s) in respiratory and metabolic systems. Concentration of bicarbonate reflects the acidity or alkalinity of the blood. In metabolic acidosis, the bicarbonate concentration is low, and in metabolic alkalosis, bicarbonate concentration is high. The actual concentration of bicarbonate reflects not only the metabolic component but also the respiratory component. For control of the respiratory component, standard bicarbonate is a better measure of the metabolic component than actual bicarbonate. Standard bicarbonate is inverse to the standard pH, which is pH under standard conditions (pCO_2 = 40 mmHg, temperature 37°C, and 100% oxygen saturation).

8.3. Bicarbonate and acid-base balance

The relationships between acid-base balance and ion management are closely connected. The main reason is that one part of the bicarbonate buffer has no charge (H_2CO_3 [i.e., CO_2]), while the second component is charged (HCO_3^-). Therefore, the bicarbonate anion must be in equilibrium with other ions to preserve electroneutrality in the internal environment. For partial pressure of CO_2, this does not apply, and therefore, its regulation can be largely independent. According to the Henderson-Hasselbach equation, the pH of the internal environment depends on the ratio of bicarbonate concentration to pCO_2. Regarding the regulation of most major ions (Na^+, K^+, Cl^-), these regulations are very sensitive but have only limited possibilities for rapid influence, resulting in serious functional consequences for the organism. In this case, if the concentration of a particular ion alters some pathological process, this change must be compensated by a change in the concentration of another ion to maintain electrical neutrality. Often, this compensation is afforded by changes in bicarbonate concentration. Biocarbonates, regardless of blood pH, alter the transcellular distribution of K^+, reflecting the utility of hydrogen carbonate therapy in hyperkalemia, even in conditions of compensated blood pH [131].

Our measurements indicated elevated levels of bicarbonate under P anesthesia, which, compared with the normal range (23.8–26.78 mmol/l in rats), would correspond to metabolic alkalosis, unless there were changes in other parameters of acid-base balance in both light parts of the day. However, under P anesthesia, we also found relatively severe acidosis, hypercapnia, hyperkalemia, and hypochloremia, which could signal the compensation of this state or the replacement of chlorides in the blood by bicarbonates. In this regard, P anesthesia induces more serious disruption of acid-base balance, independent of the cycle of alternating light and darkness. In K/X and Z anesthesias, these changes were more subtle, and when LD differences appear to be preserved, we assume that circadian rhythms are also preserved, and therefore, from a chronobiological point of view, these are appropriate types of general anesthesia.

8.4. BE and BB

BE relates to a true excess of base in the range (above or below) of the total BB. Normally, BB is 48–49 mmol/l. If BB is 40 mmol/l, it means that the buffer base was decreased by almost 8 mmol/l or BE is −8 mmol/l (also known as base deficiency). If BB is 60 mmol/l, it indicates that the base of the buffer is increased by approximately 12 mmol/l, or BE is +12 mmol/l. Fifty percent of BB is produced by bicarbonate and 25% by other buffers (proteins, phosphates, sulfates). In our experiments, BE and the total BB moved within the normal ranges, which would mean that buffering capacity was sufficient not only in the dark but also in the light period of the rat regimen day and under all types of anesthesia.

8.5. Ions

8.5.1. Potassium and acid-base balance

As early as the 1950s and 1960s, the relationship between extracellular potassium, bicarbonates, and blood pH was recognized. Relatively small changes in potassium concentration in the cell compartment can result in large changes in plasma potassium concentration. As a result, plasma potassium concentration may be reduced, normal, or elevated, despite normal stores of potassium in the body. The main regulator of transcellular potassium distribution is the pH of the extracellular fluid, which is reflected in blood pH. It was demonstrated that lowering the pH of blood increases serum potassium levels and vice versa [132–135]. It has recently been found that the concentration of extracellular bicarbonate—apart from its effect on extracellular pH—affects a wide range of metabolic reactions [136–139]. During this time, there was contradictory evidence that changes in blood hydrogen carbonate concentration in isohydric conditions alter plasma potassium concentration [140–143] in normokalemia, and no information regarding the role of bicarbonates in hypokalemia or hyperkalemia was available. At the increase of pH about 0.1, kalemia is increased about 0.5–0.6 mmol/l.

In acidemia, a number of "redundant" protons will enter the cells in which they will buffer. Consequently, a cation is transferred through the plasma membrane, which would in itself lead to a change in membrane potential. Instead of the proton, another cation is transferred from the intracellular to the extracellular space. Because the conductivity of the plasma membrane is highest for K^+ ions, primarily potassium ions will be transferred. Acidemia in this scenario leads to hyperkalemia. The total amount of potassium in the body does not increase,

and it only changes its distribution between compartments. From a whole-body perspective, potassium depletion will be a consequence of acidity, because its renal loss increases (so that heavier and longer-lasting acidosis will be accompanied by depletion of potassium at the current hyperkalemia). Similarly, alkalemia is accompanied by hypokalemia. However, the entire mechanism also works inversely: hyperkalemia causes acidosis and hypokalemia, on the other hand, leads to alkalosis. Simplified, we can imagine that potassium cations that move through the plasma membrane are exchanged for protons.

From the chronobiological point of view, however, this was not confirmed by our results. In each type of anesthesia, hyperkalemia was recorded, irrespective of whether the measurements were made in the light or dark part of the rat regimen day. Acidosis occurred only in the light part of the day under each type of anesthesia, while in the dark part of the day, the pH values also moved within normal ranges, but only under K/X and Z anesthesias These findings should, therefore, be taken into account to avoid application of particular anesthesias in the light part of the rat regimen day because positive correlations between pH and plasma K^+ concentration have been calculated for all types of anesthesia (P light r = 0.41, P dark = 0.16; K/X light r = 0.57, K/X dark r = 0.01, Z light r = 0.79, dark r = −0.22). What this means that the increase in plasma concentration K^+ shifts the pH to the alkalinity, respectively. Alkalosis increases K^+ leakage if the rat is in general anesthesia. In the dark part of the rat regimen day, no pH dependence on K^+ was found under all types of anesthesia.

If we generally consider the consequences of changes in plasma K^+ concentration affecting membrane processes, they touch primarily exciting tissues. In case of hyperkalemia, the concentration gradient decreases so that potassium escapes from the cell more slowly. However, the resting membrane potential becomes less negative and, therefore, in the initial phase of hyperkalemia, excitability increases (the resting potential is closer to the threshold). Increasing the potassium concentration in the extracellular environment by increasing the potential leads to blockage of voltage-gated Na^+ channels, and consequently, excitability decreases.

Considering the electrophysiology of the heart, hyperkalemia affects the production and conduction of impulses, which can lead to ventricular fibrillation through several mechanisms:

- the concentration gradient of K^+ in the direction from the intracellular into extracellular space is the key factor determining the value of the resting membrane potential. Increases in the extracellular K^+ concentration leads to a decrease in the gradient to a decreased outward K^+ current and thus to a decrease in the negativity of the membrane potential. In the myocardium, the resting membrane potential is reduced from −90 to −80 mV.
- at decreased negativity of the resting membrane potential, the difference between resting and threshold potential is lower and depolarization is more easily induced. If the negativity of the resting membrane potential continues to fall, the negativity of the threshold potential also begins to decrease.
- the value of the resting membrane potential also determines the number of sodium channels that open during depolarization to allow Na^+ input into the cell. The lower the negative resting membrane potential, the less the Na^+ channels are activated and depolarization occurs slower.

- repolarization is the result of opening K^+ channels and the subsequent outward K^+ current. For unclear reasons, the amount of K^+ from the cell paradoxically increases with increasing extracellular K^+ concentration. In hyperkalemia, therefore, acceleration of repolarization occurs.

Electrolyte abnormalities are becoming an increasingly important cause of arrhythmias. In humans monitored using electrocardiography, spiky and narrow T-waves (acceleration of repolarization) are the most common manifestations, QRS complex enlargement and prolongation of the PQ interval (slow depolarization). If hyperkalemia deepens, atrial activity may disappear, and ventricles are stimulated from AV node with resulting bradycardia. In severe hyperkalemia, the QRS complex expands, with consequent risk for ventricular fibrillation and cardiac arrest.

Although electrocardiographic (ECG) changes in hyperkalemic rats are poorly understood, it is clear that excess plasma potassium may also alter cardiac excitation. In addition, the effects of hyperkalemia on ECG in rats may differ from other species that do not have ST segments and longer QT intervals. At testing, the effects of two local anesthetics (bupivacaine and lidocaine) at normocalcemia and hyperkalemia were found that hyperkalemia with concentration 9.0 mmol/l had little effect on heart rate or AV conduction in the absence of bupivacaine or lidocaine. Nevertheless, the effect of local anesthetics on slowing the ventricular rate was significantly enhanced. For bupivacaine, ventricular deceleration to 50% vs. control, during hyperkalemia, was performed almost completely through inhibition of AV conduction whereas for lidocaine through not only inhibition of AV conduction but also atrial rate. Regardless of the mechanism, hyperkalemia of this grade increased the ventricular slowing effect of bupivacaine and lidocaine [144]. Kuwahara et al. [145] described changes in rat ECG in dependence on K+ levels. In moderate hyperkalemia, an increased amplitude of T wave occurred. The duration of the PR interval and the QRS complex was slightly reduced, and the P wave disappeared in most rats at potassium levels above 8.0 mmol/l. In advanced hyperkalemia (plasma potassium concentration higher than 7.5 mmol/l), conduction was suppressed in all parts of the heart.

As for hypokalemia, except impacts on other functions and systems, heart failure and cardiac rhythm are typical of cardiac symptoms. On the ECG, low, flat, or inverted T-waves and prolongation of the QT interval can be seen. Supraventricular and ventricular extrasystoles occur episodically.

8.5.2. Calcium and acid-base balance

Similarly as the proton is exchanged for the potassium cation, a calcium cation is also exchanged for protons. Plasma proteins play a key role in this mechanism. Blood plasma proteins behave as buffers, primarily due to carboxyl groups and amino groups. As regard the carboxyl groups, these groups are in protonic, nondissociated state (-COOH) in the acidic environment. In the alkaline environment, they begin to buffer and their dissociation into the carboxylate $-COO^-$ occurs, which is able to bind very effective especially Ca^{2+}. It means that in the case of acidosis, the -COOH does not change to $-COO^-$, and in the case of alkalosis, it dissociates to $-COO^-$ and H^+ and the calcium binds to $-COO^-$.

It can also be said that the pH depends on what part of the calcium will be ionized and what part will be nonionized. The practical consequence is that alkalosis leads to ionized hypocalcemia, acidosis, on the contrary, to ionized hypercalcemia. Although total calcium does not change, we have to realize that ionized calcium is metabolically active, especially when it comes to membrane processes.

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Hypercalcemia is a state when the serum Ca^{2+} level is greater than 2.8 mmol/l and ionized Ca^{2+} is greater than 1.4 mmol/l. At values above 4 mmol/l, "chemical death" may occur when cardiac arrest may occur. Hypertension and arrhythmias occur at the hypercalcemia. On the ECG, QT interval is shortened. Hypocalcemia is accompanied by an increase in neuromuscular excitability, but myocardial contractility decreases.

8.5.3. Chlorides and acid-base balance

During Cl^- loss (e.g., vomiting), the concentration of the other major ions is not altered, and for maintenance of the electrical neutrality, the anion deficiency is supplemented by an increase in the bicarbonate concentration. pCO_2 does not change; therefore, ventilation is maintained and hypochloremic alkalosis develops. In summary, substitution of chlorides in the blood occurs at the expense of hydrogen carbonates.

9. Conclusions

After summarizing the results from the analysis of acid-base balance parameters (**Table 6**), we concluded that there are differences in the final status of the rat internal environment that depend on the LD cycle and on the type of anesthesia.

In the light part of the day, under P anesthesia, the rats are in a state of acidosis, hypercapnia, and hypoxia, and elevated levels of bicarbonate have been reported. Similarly, it is also in the dark, but with mild acidosis, hypercapnia and hypoxia with a moderate decrease to normal pO_2 values but with elevated levels of bicarbonate. Saturation of hemoglobin by oxygen was at the same level in both light parts of the rat regimen day, and at approximately 87%, the efficiency of the buffer system was not impaired because the values were within the normal range.

Under K/X anesthesia, we found a dependence on LD cycle in all monitored parameters. In the light part of the day, unambiguous acidosis, pCO_2 ranging from normocapnia to hypercapnia, pO in the hypoxic range, relatively large range of bicarbonate (from reduced to increased levels) levels and lower saturation (around 85%) were observed. In the dark part of the day, from normal to alkaline pH, hypocapnia, moderate decreased to normal pO_2 but with a reduced level of bicarbonate. Different values were in saturation of hemoglobin by oxygen, where higher saturation was during the dark (active) part (around 90%). The efficiency of the buffer system moved within the normal range in both light parts of the day.

Under Z anesthesia, the status was as follows: acidosis, hypercapnia, hypoxia to normoxia, and normal levels of bicarbonate in the light part of the day. In the dark part of the day, the state of the internal environment was from acidic to normal, hypercapnia, and pO_2 moved from mild hypoxia to normoxia at a normal to moderately elevated level of bicarbonate. The saturation of hemoglobin by oxygen fluctuated around 89% in both light parts of the rat regimen day, and BB and BE were also in the normal range; thus, buffering capacity remained intact.

It was concluded that P anesthesia is not the most appropriate type of general anesthesia to use in chronobiological rat models. It is likely to produce a more acidic environment than K/X and Z anesthesias, and although an LD difference in P anesthesia was not recorded, the pH values

were the lowest in both light parts of the rat regimen day compared with K/X and Z anesthesias. Initially, acidosis is induced, irrespective of the synchronization of animals with the LD cycle, and therefore, it is not possible to monitor periodic changes in the functions of individual systems that are primarily dependent on changes in extracellular pH. As a result, P probably and immediately reduces either the activity of the buffer systems or inhibits the regulatory mechanisms associated with the maintenance of isohydria, independently of the LD cycle. In this regard, K/X and Z anesthesias may be more appropriate for general anesthesia because the arterial pH varies within the range of isohydria. This assumption is only valid if the rat experiments are performed under K/X and Z anesthesia in the dark (i.e., active) parts of the day.

Hypoxia modifies circadian oscillations of important variables, such as body temperature and metabolism, and may lead to the expectation that the rhythms of many functions are disrupted by hypoxia according to their relationships and association with the primary variables [146]. This effect appears to be apparent in rats under P anesthesia. From a chronobiological point of view, P anesthesia, therefore, is not a suitable form of general anesthesia. Using this type of anesthesia, with the exception of the initial hypoxia and hypercapnia, the LD differences in pO_2 and pCO_2 are eliminated. As a result, the effect of initial hypoxia and hypercapnia on the circadian rhythms of oxygen-dependent systems, immediately after administration of anesthetics, can significantly affect the end result.

Based on the results of this study, we concluded that general anesthesia affects the circadian fluctuation of arterial acid-base balance and plasma concentrations of some ions (**Table 9**). This should be taken into account, and experiments should start with a normal range of acid-base balance. Even at the beginning of the experiment, the altered internal environment may affect the activity of systems whose functions are primarily dependent on acid-base balance.

Anesthetic	Status
Pentobarbital	
Light	Acidosis, from hypoxia to hypercapnia, increased HCO_3, hypernatremia, hyperkalemia, hypocalcemia, hypochloremia
Dark	Acidosis, from normoxia to hypoxia, hypercapnia, increased HCO_3^-, hypernatremia, from normokalemia to hyperkalemia, hypocalcemia, from hypochloremia to normochloremia
Ketamine/xylazine	
Light	Acidosis, hypoxia, from normocapnia to hypercapnia, from decreased to increased level of HCO_3^-, from hyponatremia to hypernatremia, hyperkalemia, hypocalcemia, from normochloremia to hyperchloremia
Dark	From normal pH to alkalosis, from hypoxia to normoxia, hypocapnia, decreased HCO_3^-, from hyponatremia to normonatremia, hyperkalemia, hypocalcemia,
Zoletil	
Light	Acidosis, from hypoxia to normoxia, hypercapnia, normal HCO_3^-, from hyponatremia to hypernatremia, from hypokalemia to hyperkalemia, hypocalcemia, from hypochloremia to hyperchloremia
Dark	From acidosis to normal pH, from hypoxia to normoxia, hypercapnia, from normal to increased HCO_3, from normonatremia to hypernatremia, hyperkalemia, hypocalcaemia, hyperchloremia

Table 9. Internal environment under general anesthesia dependent on the light-dark cycle in the rat.

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Chapter 8

Sudden Death Circadian Rhythm in Chagasic Patients Compared to Non-Chagasic Patients

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Additional information is available at the end of the chapter

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Abstract

Chagas disease (Ch) affects 8–10 million people in Latin America. Sudden death is the major cause of death in patients with Ch. Objective: To compare the circadian rhythm of sudden death in Ch vs. non-Ch patients. Methods: Retrospective analysis of all the cases of sudden death (SD) is recorded in our department, including autopsied patients from 1963 until 2011. Pattern of death of 266 patients (116 Ch and 146 non-Ch), 56.7% men, average age 54, 6 years old, divided into four groups: Group A: Ch with SD (n = 38), Group B: non-Ch with SD (n = 58), Group C: Ch with non-SD (n = 81), and Group D: non-Ch with non-SD (n = 89). Results: 44.7% (17/38) of sudden deaths in Group A (Ch) occurred between 6 am and 5:59 pm, while for Group B (not Ch) 70.7% (41/58) died in that time ($p < 0.005$). Between 6 pm and 5:59 am occurred 55.3% (21/38) of the SD in Group A (Ch) compared with 29.3% (17/58) in Group B ($p < 0.005$). Conclusions: Circadian rhythm of SD in patient with Ch differs from those patients with non-CH. In CH patients, SD occurs predominantly during the night compared with non-Ch SD that occurs predominantly during the morning.

Keywords: sudden death, cardiomyopathy, circadian rhythm, Chagas disease

1. Introduction

The Chagas disease is a malady caused by the *Trypanosoma cruzi* protozoan, and it represents an endemic disease in Latin America, affecting 8–10 million of patients, most of them being poor [1–2]. It is estimated that 400,000 infected persons live in nonendemic countries, mainly in the United States and Europe [3, 4]. A recent meta-analysis of European studies that, in aggregate, screened 10,000 Latin American immigrants found a positive serological test prevalence of 4.2% [5]. Based on published seroprevalence in Latin American immigrant populations (1.31%), it was

estimated that approximately 300,000 individuals with *T. cruzi* infection live in the United States, with 30,000–45,000 cardiomyopathy cases and 63–315 congenital infections annually [6].

The sudden death circadian variation has been demonstrated in two large-scale studies, the Framingham Heart Study [7] and the Massachusetts Death Certificate Study [8]. Both studies show a peak of sudden deaths between 7 and 11 am with a lower incidence during sleep, which is similar to the rate of ischemic and arrhythmic events [9, 10]. The sudden death is the main cause of death in those patients with the Chagas disease, being responsible of the 55–65% of their deaths [11]. Lopes et al. [12] demonstrated that there is a sudden death circadian rhythm in Chagasic patients. In this study, 50 cases of Chagasic sudden death, along with 473 cases of nonsudden natural death, were compared in several centers. To the best of our knowledge, this is the first report that compares the rhythm of the sudden and nonsudden death of Chagasic patients vs. non-Chagasic patients with cases within a same center.

2. Material and methods

A retrospective study of a consecutive series of sudden death cases, registered within our department between 1963 and 2011, including the ECG records, Holter records from sudden death victims, autopsies, and the Death report by the relatives. The Chagas disease diagnosis was performed through serological studies, or a necroscopic study was performed in the cases of autopsies.

The date and time of death were collected from necropsy protocols and/or emergency clinical histories, as well as data obtained from relatives and witnesses.

Sudden cardiac death (SCD) is generally defined as a sudden and unexpected pulseless event, but noncardiac conditions need to be excluded before the occurrence of a primary cardiac event can be confirmed [13]. A case of established SCD is an unexpected death without obvious extracardiac cause, occurring with a rapid witnessed collapse, or if unwitnessed, occurring within 1 h after the onset of symptoms [13]. A probable SCD is an unexpected death without obvious extracardiac cause that occurred within the previous 24 h [13]. In any situation, the death should not occur in the setting of a prior terminal condition, such as a malignancy that is not in remission or end-stage chronic obstructive lung disease [13]. In our study, we included both established and probable SCD.

A total of 266 cases were analyzed; 56.7% of the subjects were male with an average age of 54.6 years, which were divided into four groups: Group A: Chagasic patients with sudden death, n = 38; Group B: non-Chagasic patients with sudden death, n = 58; Group C: Chagasic patients with non-sudden death, n = 89; and Group D: non-Chagasic patients with non-sudden death, n = 81.

The results were assessed using exploratory data analysis (EDA) and comparison of ratio differential. As the statistic validation rule, a p-value <0.05 was considered as statistically significant.

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3. Results

A total number of cases per hour of sudden death in patients with and without Ch are shown in **Figure 1**. **Figure 2** shows the number of cases per hour of nonsudden death in patients with and without Ch. After analyzing the data divided into 12-h periods (day and night), significant differences were observed. **Figure 3** shows the percentages of cases from the SD groups occurring during night and day. Forty four point seven per cent (44.7%) (17/38) of the sudden deaths in Group A (Ch) occurred between 6 am and 5:59 pm, while for Group B (non-Ch), 70.7% (41/58) of the patients died within that time ($p < 0.005$). Between 6 pm and 5:59 am, 55.3% (21/38) of the deaths of Group A (Ch) occurred in that time compared to 29.3% (17/58) from Group B ($p < 0.005$). **Figure 4** shows the data of nonsudden death cases. 49.4% (40/81) of Group C (Ch non-SD) died between 6 am and 5:59 pm compared to 59.6% (53/89) of Group D (non-Ch, non-SD), (p not significant), while between 6 pm and 5:59 am, 50.6% of Group C (Ch, non-SD) cases died compared to 40.4% (36/53) of Group D (non-Ch, non-SD) (p was not significant).

In order to perform a more detailed analysis, the percentages of cases were grouped within 3-h periods: (6–8, 9–11, 12–14, 15–17, 18–20, 21–23, 24–2, 3–5). **Figure 5** shows the circadian rhythm of sudden death in Chagasic patients (Group A) compared to the non-Chagasic patients (Group B). Within these periods, a higher death percentage in the Chagasic group is observed within the 21–23 h interval (34 vs. 3%, $p = 0.0001$), while the non-Chagasic arm presented a higher percentage of cases within the 9–11 h range (43 vs. 3%, $p < 0.0001$), of 24 to 21 2 h (10 vs. 0% $p < 0.005$), and from 3 to 5 h (7 vs. 0%, $p < 0.005$). The difference of the other analyzed periods was not significant. When comparing the number of cases of non-sudden

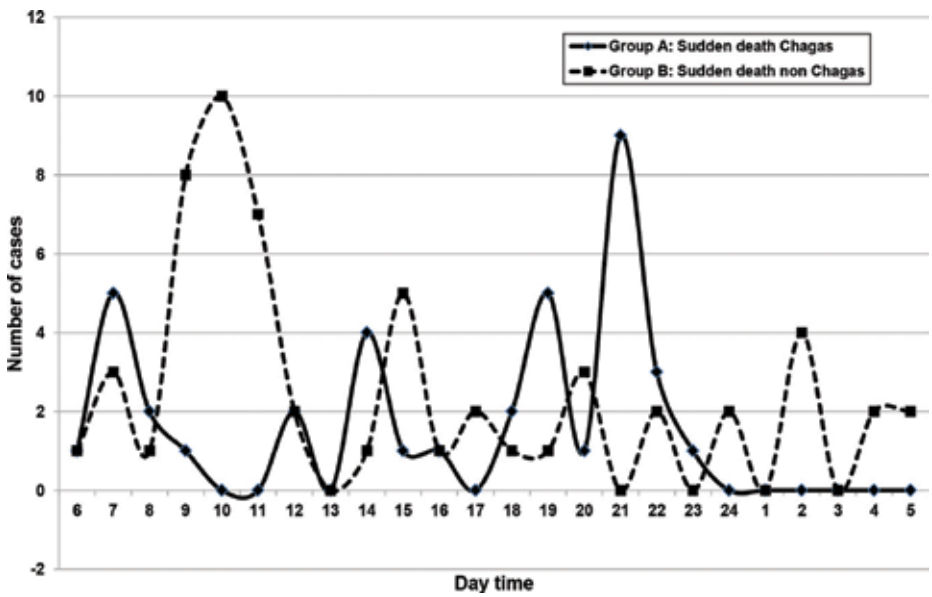

Figure 1. Sudden death of circadian rhythm.

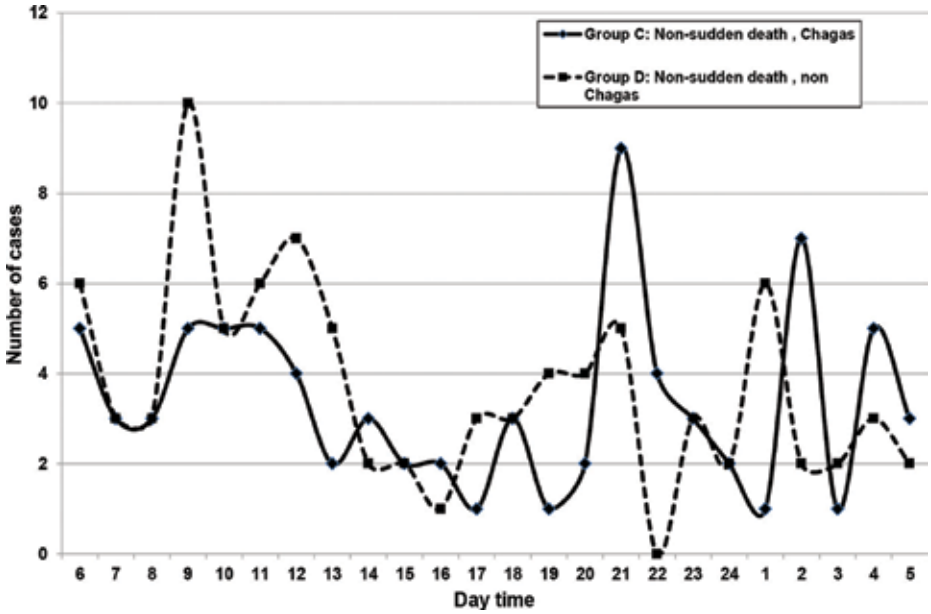

Figure 2. Circadian rhythm of nonsudden death.

	Group A Chagas SD n=38		Group B Non-Chagas SD n=58		Dif %	P-value
	Cases	%	Cases	%		
6:00 am - - 5:59 pm	17	44.7	41	70.7	26%	0.0048
6:00 pm - - 5:59 am	21	55.3	17	29.3		

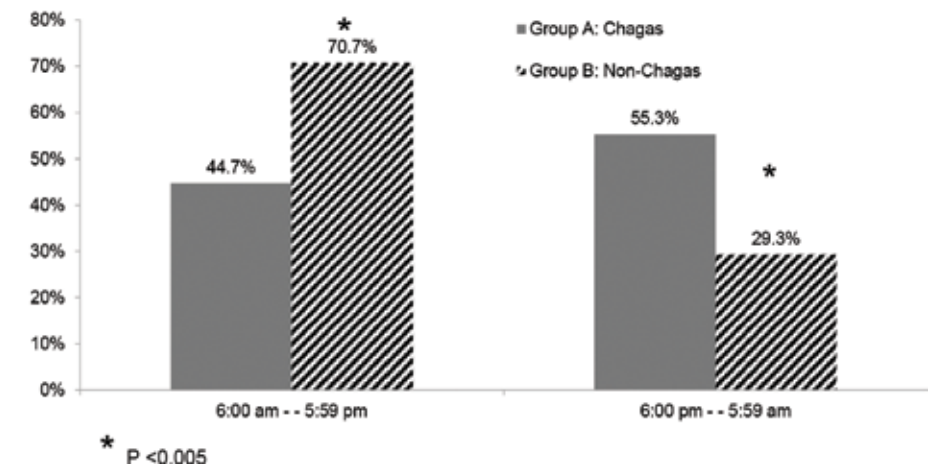

Figure 3. Comparison of 12-h periods of sudden death of Chagasic and non-Chagasic patients.

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	Group C Chagas, non-sudden death, n=81		Group D Non-Chagas, non-sudden death, n=86		Dif %	P-value
	Cases	%	Cases	%		
6:00 am - - 5:59 pm	40	49.4	53	59.6	12%	0.054
6:00 pm - - 5:59 am	41	50.6	36	40.4		

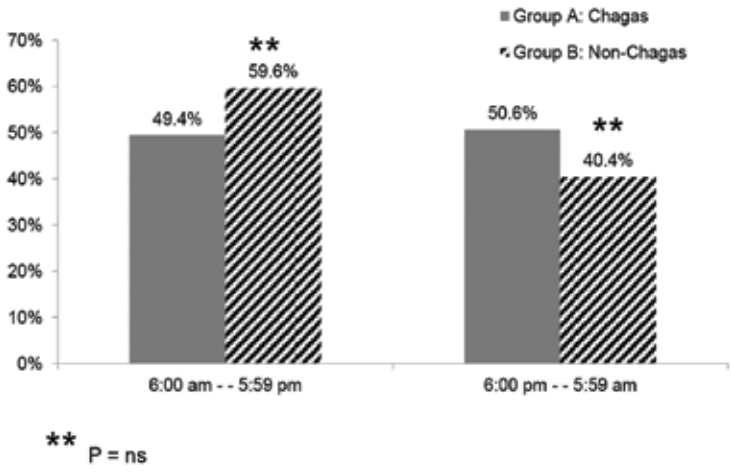
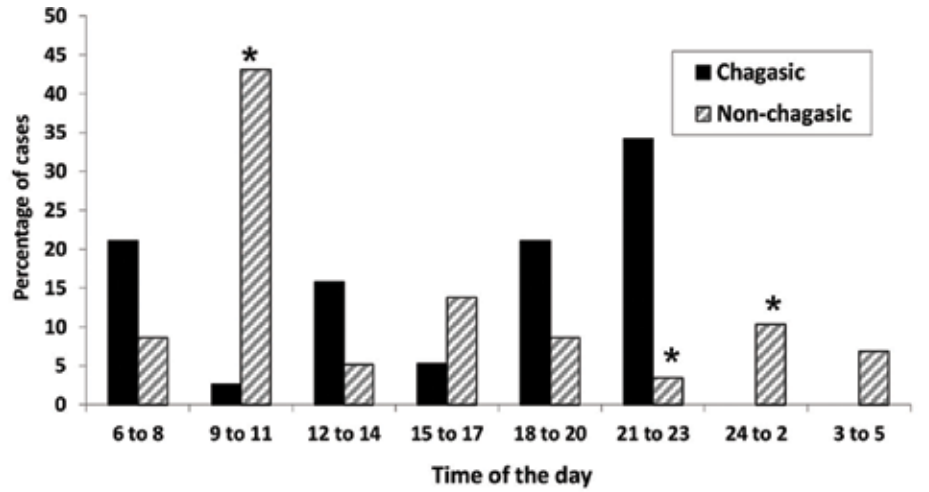


Figure 4. Comparison of 12-h periods of nonsudden death in Chagasic vs. non-Chagasic patients.



Pvalue	0.1006	0.0000	0.1072	0.1413	0.1006	0.0001	0.0097	0.0382

Figure 5. Sudden death circadian rhythm in Chagasic patients compared to non-Chagasic patients.

deaths in Chagasic patients vs. non-Chagasic patients, a significant difference in any of the analyzed ranges was not observed.

4. Discussion

The sudden death is the primary cause of death in patients suffering from Chagas disease, representing around 60% of the total cases [11], hence the importance of its study. Our study clearly evidenced that in Chagasic patients, the higher percentage of cases of sudden death occurred during the nighttime (**Figures 1**, **3**, and **5**). When we analyzed the non-sudden death results, no difference between the Chagasic and non-Chagasic arms was observed (**Figures 2 and 4**). Our results agree with those of Lopes et al. [8], who demonstrated a sudden death predominance in Chagasic patients during the nighttime. On the other hand, our study also agrees with previous studies in the non-Chagasic population in the United States, which were a predominance of sudden death cases occurred in the morning [7, 8]. The importance of our study lies in two milestones:

(1) This study represents the largest series reported to date comparing the sudden death circadian rhythm in Chagasic and non-Chagasic patients. (2) The fact that this study represents a series where all included patients belonged to the same center within a Latin American country, allowing a better group comparison.

The potential mechanisms for the sudden death circadian variation in the general population are not entirely clear, especially since due to the interaction among them, it is difficult to independently determine the importance of each factor. The proposed mechanisms include:

a. Autonomic nervous system alterations.

It is being proposed that both the sympathetic nervous system and the parasympathetic system may stimulate the circadian variation. Using the frequency domain, it has been demonstrated an unfavorable variability profile of the heart rate in the morning time. [14–17] This may be caused by both the sympathetic tone endogen variations and the increasing level of physical activity [15–17]. The use of beta-blockers reduces or removes the morning peak of ischemic and arrhythmic events [18–22], which supports the hypothesis of increasing adrenergic tone, since this same effect is not achieved via antiarrhythmic non-beta blockers medication [23]. Generally, the HRV indexes significantly decrease during the daytime and increase during the night [24–30].On the other hand, the variations of autonomic tone and parasympathetic-sympathetic balance have been proposed as the cause, which have been analyzed through heart rate variations (HRV) [31–35].

b. Morning variations of the electrophysical properties.

In both invasive electrophysiological studies [28] and non-invasive studies using permanent pacemaker telemetry [15, 31], circadian variation of the ventricular refractory has been demonstrated, being the last lower during the morning time and higher during sleep.

This variation does not seem to be related to the potassium or circulating catecholamine levels [31]; on the other hand, it would be aligned with the variations of the maximum QT interval [33].

c. Circadian variation of ischemic episodes.

A peak in the morning and in the afternoon of ischemia-related conditions, such as the myocardial infraction [22, 34, 35], anginal crisis [36–38], and strokes, [39, 40] has been reported. These episodes have been related to morning variations of the endothelial function [41] and of thrombogenesis biochemical markers [42–46]. Durgan et al. [47] demonstrated that there is a relation between the date time and the tolerance to reperfusion-ischemia in cardiomyocytes of isolated mice, being the lowest tolerance during the morning time.

The factors that may bias for the circadian rhythm to be different in patients with Chagas disease are not clear; however, several hypotheses have been posed:

1. The autonomic balance of Chagasic patients, which has been evidenced by several authors [48–50]. Cardiac autonomic dysfunction, characterized mainly by parasympathetic depression, is present in human and experimental Chagas disease, even in patients with minor ECG alterations [51].
2. The endothelial dysfunction [52].
3. The presence of antibodies against the adrenergic receivers may reduce the morning adrenergic activity, hence, suppressing the morning peak [53].

Abello et al. [54], when analyzing 22 Chagasic patients with third-generation implantable defibrillators, demonstrated a ventricular tachycardia circadian rhythm pattern, characterized by a frequency peak between noon and 18:00 h with a nadir between 24:00 and 6:00 h, which would be in line with our results.

5. Conclusion

The sudden death circadian rhythm in Chagasic patients significantly differs from that of the non-Chagasic patients, showing a greater prevalence during the nighttime. Further studies are needed in order to analyze both the prognostic implications and the therapeutic ones.

6. Limitations

Regarding the certainty of the time of death, the study limitation is common to that of all sudden death studies, since the time of death, which is mostly reported by a witness, decreasing the accuracy of the data. In most of the times, we ignored the personal history of the patients (previous pathology, concomitant treatment, etc.) because the death occurred suddenly. Also,

we do not have previous data from other complementary explorations such as echocardiogram, stress test, and so on in much of the cases.

Conflict of interest

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Circadian clocks are endogenous and temperature-compensating timekeepers that provide temporal organization of biological processes in living organisms. Circadian rhythms allow living organisms to adapt to the daily light cycles associated with Earth's rotation and to anticipate and prepare for precise and regular environmental changes. This book discusses the fundamental advances of how the circadian clock regulates critical biological functions as well as the cellular and molecular mechanisms controlling circadian rhythm in living organisms. It also provides new insights into and sheds new light on the current research trends and future research directions related to circadian rhythm. This book provokes interest in many readers, researchers and scientists, who can find this information useful for the advancement of their research works towards a better understanding of circadian rhythm regulatory mechanisms.

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