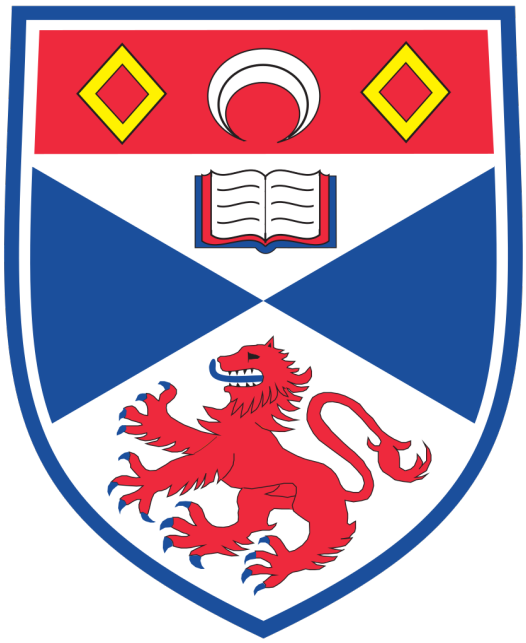
Exploration of the Drosophila circadian rhythm mathematical model



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Declaration

I certify that this project report has been written by me, is a record of work carried out by me, and is essentially different from work undertaken for any other purpose or assessment. The material submitted for assessment is my own work except where credit is explicitly given to others by citation or acknowledgement. This work was performed during the current academic year.

Abstract

The biological rhythm with a period of 24 hours, called *circadian*, plays a crucial role in the process of adaptation to the external environment by regulating the timing of certain physiological processes, such as hormone release and body temperature regulation. The functioning of the rhythm has been extensively studied biologically, however, biological experiments can have limitations in their execution. To overcome such limitations, researchers can use mathematical models to simulate and study the circadian rhythm. The present paper examines the methods employed in research for building mathematical models of circadian oscillations of the fruit fly Drosophila. The first part of the paper explains the simplified structure of the circadian system and the main biological processes involved in its functioning. Then differences between the deterministic and stochastic models of the negative feedback loop of the Drosophila circadian oscillations are presented in order to build an understanding of the circumstances where each of these models can be most useful. Graphical results from implementation of these models in MATLAB simulations are analysed and compared to biological findings. The effect of light on the Drosophila circadian system is then incorporated into the models and explored by variation of the light parameter. Specifically, case studies of jet lag and night shift work are simulated with a clear statement of corresponding assumptions and limitations, confirming the negative effect of such disruptions mathematically. Stochastic simulations of oscillations perturbed by light are also presented along with a description of more advanced modelling methods, such as Extrande, minimising errors in such simulations. The methods used in the present study can lay a foundation for future research on the mammalian circadian rhythm. Results from the paper can be used for a better understanding of the internal clock's functioning, as well as an insight into the employment of different mathematical models for research on the topic.

Contents

1 Introduction

1.1 General Biological and Circadian Rhythms

Adapting to environmental changes has always been crucial for the survival of living organisms. The most drastic variations in environmental conditions happen daily, according to the 24-hour light/dark (LD) cycle, and seasonally. In order to prepare for and adapt to such changes, biological organisms developed cyclical time-keeping rhythms that regulate physiological processes [1]. Biological rhythms can be divided into exogenous, exhibiting a direct link to an external stimulus, and endogenous, driven internally [2]. Both types significantly affect the activity of living organisms. However, rhythms of special significance and interest for researchers are the ones created endogenously with a period corresponding to roughly a day. Such rhythms were named circadian by Franz Halberg in the 1950s from the words circa (about) and dian (day). The word itself implies that this biological rhythm can be close to 24 hours in certain species but does not always exactly correspond to that value. For example, for humans, an average of 24.2 hours was established [3]. It is worth noting at this stage that despite the circadian rhythm being endogenous, light cues still play a role in synchronising the internal rhythm to the external environment.

The first scientific study of the circadian rhythm, which eventually inspired intense research on this topic evolving into the modern field of chronobiology, dates back to 1729, when the French scientist Jean Jacques d'Ortous de Mairan published his observations of leaf movements of the *Mimosa pudica* plant. After conducting an experiment involving observations of daily leaf movements in the presence of a light source and in complete darkness, de Mairan was able to state that the plant's movements continued even when it was not exposed to the cues from the LD cycle, following a circadian rhythm (figure 1). This confirmed the existence of a complex endogenous regulator of the plant's behaviour in contrast to a simpler mechanism of response to the LD cycle [4, 5]. Consequently, research on circadian rhythms was extended to bacteria, animals, and humans.

Research focused on bacteria, in particular on the cyanobacterium *Synechococcus*, established that the presence of a well-functioning circadian rhythm in an organism provides it with a selective advantage [6]. Therefore, the development of an internal clock with a similar rhythm to that of the external environmental cycle of Earth was established to be an outcome of evolution. Subsequently, it was found that the circadian rhythm regulates many aspects of living organisms' physiological functions, including the sleep-wake cycle, hormone secretion, metabolism, body temperature, and reproduction, in order to align internal processes with external environmental conditions [7]. In simpler terms, the circadian rhythm gives diurnal mammals the energy needed for daily activities in the morning and makes them want to rest in the evening to restore their energy.

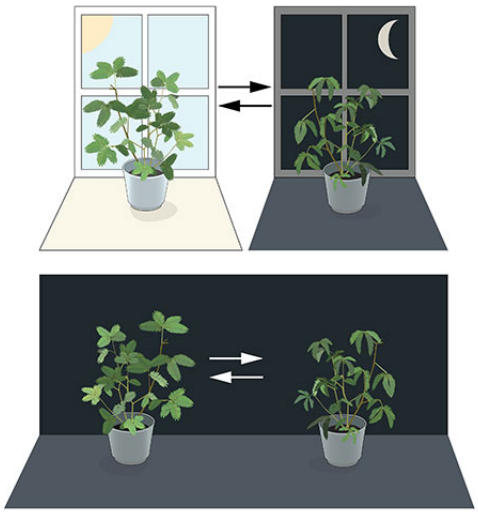

Figure 1: The internal biological clock of the *Mimosa pudica* plant as observed in de Mairan's experiment. The top row shows the observation that leaves open in the presence of light and close in the dark. The second row shows the main idea of the experiment, which is that the explicit foliage movements continue even when the external environment stays constant, confirming the existence of an endogenous regulating mechanism. Picture credit [8].

1.2 Introduction to the Circadian Rhythm in Humans and Importance of Research on This Topic

Human life evolved greatly throughout the centuries, getting further away from the initial, more natural way of living. Modern people generally lead productivity-focused lives filled with technological devices. For example, Thomas Edison's invention of the light bulb in 1879 made it possible for humans to be active at any time of the day. This in a way pushed humans away from all other living organisms. However, even though it may seem that with the invention of electricity and the light bulb humans became free from the influence of biological rhythms, in reality, this is not the case. As demonstrated above with the example of de Mairan's experiment, the circadian rhythm is endogenous. Therefore, new inventions do not have a decisive effect on it, and people's physiology and behaviour are still heavily dependent on the circadian rhythm. It was found that the circadian rhythm regulates humans' blood pressure [9], body temperature [10], metabolism [11], alertness [12], cell regeneration [13] etc. (figure 2). Therefore, alignment with the circadian rhythm is essential for both mental and physical health [14].

Sadly, the intense pace of modern life and lack of awareness of the importance of preserving the circadian rhythm quite often lead to rhythm disturbances or even disruptions. These happen as a result of desynchronisation between the internal clock of the organism and the external environmental cycle. This desynchronisation can lead to the various oscillatory components of the circadian rhythm system going out of phase with each other creating "confusion" in the organism, where the timings of processes do not follow the stable organised pattern presented in figure 2.

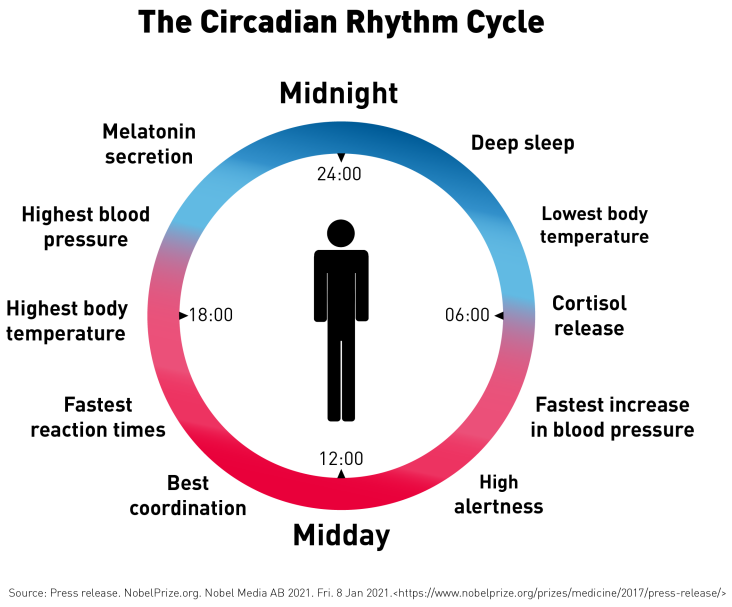

Figure 2: The human circadian cycle. Picture credit [15].

Such misalignments of the natural body rhythm can have a negative effect on the person's general mood and productivity. More drastic and prolonged disruptions, moreover, can lead to additional serious consequences, such as mental illness, metabolic syndrome, cardiovascular problems, and even cancer [16].

There exist many possible sources of circadian rhythm disruption. One of the most common causes of such misalignment in humans is shift work, which can include shifts at any time of the day or night, as well as inconsistent schedules. Unusual shift times disrupt the sleep-wake cycle, and unpredictable schedules make it impossible to establish a routine. Such work circumstances increase the chances of negative effects on the gastrointestinal and cardiovascular systems, including the increased risk of peptic ulcers, coronary heart disease, diabetes, and obesity [17]. Shift work and related health problems are a major source of concern in modern society, as such workers constitute a large part of the workforce, with approximately 20.8% of the European Union workforce currently employed under this type of contract [18].

Another widespread source of circadian rhythm misalignment is jet lag, which happens due to drastic shifts in the external LD cycle when travelling through many time zones at once. In that case, the internal clock becomes desynchronised with the external signals and needs time to adjust to such rapid changes by sensing the new cues and slowly adjusting the internal rhythm to them. Common indicators of jet lag include decreased productivity during the day and sleepiness at unusual times. As a transient disruption of the circadian system, jet lag can have serious negative effects on the body, such as the diseases mentioned in relation to shift work. The extent to which shifting LD cycles affects the physiological functions of a specific person, and the time needed to adjust to the new rhythm depend on the number of time zones crossed, age, and health conditions of the person. Nevertheless, there are ways to facilitate the adjustment, minimising the effect of

the circadian rhythm disruption, such as timed meal consumption, physical activity, melatonin tablets, and timed light exposure [19]. Other common causes of circadian rhythm misalignment include living in regions with unusual external light cycles, and various medical conditions that affect the sleep-wake cycle, such as Alzheimer's disease [20].

The problem of circadian rhythm disruption and associated adverse health effects is quite severe in present times [21]. Potential reasons for this might be increased stress levels due to the competitiveness of the job market, as well as general neglect of the importance of maintaining a healthy circadian rhythm. Scientific research on the circadian rhythm has the potential to contribute to a deeper understanding of the clock's functioning and its relation to other bodily systems. This in turn would add to the knowledge base needed for biological research examining the primal biological principles encoded in humans by nature, as well as studying ways of dealing with diseases arising from misalignment of the clock. Results from research on this topic can help to solve societal problems related to circadian rhythm disruption by finding natural or pharmaceutical means of minimising negative health effects.

In this part, an introduction to the social and medical implications of the circadian rhythm was presented for the reader to understand the importance of research on this subject. To investigate and model the circadian system using mathematical methods, an appropriate level of understanding of the biology behind the oscillations should be achieved by first explaining the general processes involved and gradually building complexity. Providing a biological knowledge base at a level suitable for constructing a mathematical model is one of the objectives of the present paper that will be addressed in section 2.

1.3 Aims of the Study

The present study aims to examine the methods employed in research for building mathematical models that describe oscillatory patterns of the Drosophila circadian rhythm functioning. This is done by an initial description and explanation of the methods and their subsequent application to MATLAB simulations. The approach used for analysis in the paper is graphical. The present study is focused on the Drosophila circadian rhythm, which provides a simpler framework for simulating and analysing circadian oscillations. However, the methods and results of the study can be relevant for research concerning the mammalian circadian system by modifying the code in accordance with the specific features of this system as outlined in section 2.4. Moreover, the section concerning light simulations aims to build a solid framework for simulating disruptions of the rhythm using the Drosophila system that can serve as a foundation for future research on minimising the negative effect of disruptions.

2 Circadian System Functioning

2.1 Simplified Structure of the Circadian System

In order to construct a mathematical model of the circadian system, its functioning needs to be examined in detail and fully understood by first exploring a simplified version providing a framework to build difficulty on.

The synchronisation of internal rhythms of living organisms to external conditions described in the introduction happens not only at the physiological level for the organism as a whole, but also at the molecular and cellular level. A simplified system explaining the functioning of circadian clocks was suggested by Arnold Eskin in 1972. It includes three main components [22]:

- **Input pathways** activated by receptors sensing cyclical signals from the environment called *zeitgebers*. Cues related to light, known as *photic*, are detected by special cells in organisms, such as retinal cells in animals. Photic cues play a major role in synchronisation to the environment. There are other zeitgebers, not related to the LD cycle or non-photic, that can also activate the input pathways, such as time of food consumption, the temperature of the environment, and physical activity. Information concerning the external environment, processed by the input pathways, is then transferred to the central oscillator.
- **The central oscillator** synchronised (entrained) by the incoming processed environmental signals generates self-sustained rhythms of approximately 24 hours. The central oscillator consists of several smaller oscillating systems that respond to one of the types of cues, producing different outputs.
- **Output pathways** consisting of entrained and free-running (non-entrained) rhythms. These rhythms regulate clock-controlled genes, affecting physiological activities of the organism. The outputs from the different smaller oscillators can have different phases, as shown in figure 3.

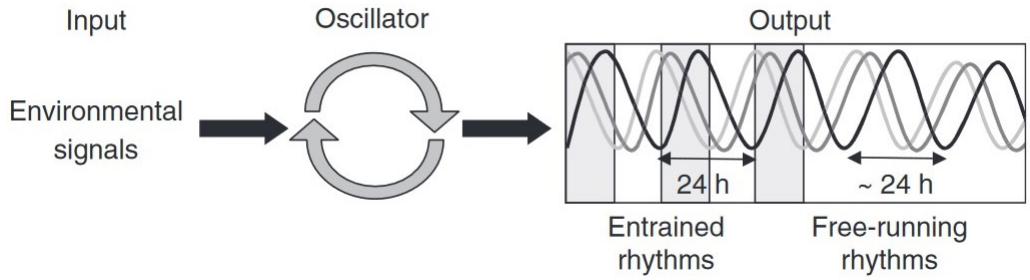

Figure 3: Schematic representation of the three main components of the circadian system in cells. Picture credit [23].

Circadian rhythms following this simplified general structure have been found in a wide range of organisms, from single-celled cyanobacteria to much more complicated mammals. Additional examples include fungi, plants, fruit flies, and mice [23]. The surprising observation of circadian rhythms in unicellular organisms indicates that the circadian system evolved in prehistoric times when life on Earth was just beginning to develop.

As the main purpose of the circadian system consists of generating an internal rhythm in accordance with the external cycle of cues, these two oscillating systems play a major role in describing the functioning of the circadian system mathematically. In particular, the parameters required are the internal circadian cycle period τ and the external zeitgeber cycle period T, along with their relation through the phase ϕ. As shown above, the central circadian clock needs to be entrained by external conditions for correct functioning, which may take several cycles. The response of the central clock to such entrainment mainly depends on the initial phases and the strength of the stimulus, among other factors. Entrainment results in the convergence of the internal circadian rhythm period towards the external zeitgebers period, giving rise to a stable phase relationship $\Delta\phi = \tau - T$. It is worth noting that not all zeitgebers lead to entrainment. Some external stimuli do not receive a systematic response but rather a single intense response called masking, which still helps the organism adapt to external circumstances but will not be explored in this paper [24].

2.2 Molecular Processes Involved in the Circadian Rhythm Functioning

The simplified conceptual model outlined above provides a basic understanding of the general functioning of the circadian clock, however, it leaves many questions unanswered and many details unaddressed. Therefore, after examining the general simplified functioning mechanism of the circadian system, it is sensible to move on to the next level of depth, which is to explore the molecular processes involved in circadian clocks. Due to the wide range of organisms with physiological processes regulated by the circadian rhythm, it should not come as a surprise that the specifics of the system vary drastically between organism classes. In fact, bacterial and plant clocks were found to be significantly different from each other and from clocks of other living organisms. In this part, the similar regulatory system found in fungal and animal cells is explored, and then the focus is shifted to the Drosophila specifically.

At the molecular level, the regulatory framework involves a network of transcription-translation feedback loops (TTFLs) consisting of positive and negative components. The positive arm activates the system in response to external cues, encouraging the transcription of negative arm components. The concentration of negative arm components grows after undergoing transcription, in turn repressing the growth in the concentration of positive arm components [25]. The periodicity of the oscillator is determined by the interrelation of the different elements of the system. These interactions regulate the functioning of the system using the properties of the negative arm feedback loop and the post-transcriptional control of the clock output by the positive arm. It was established that post-transcriptional control mechanisms play a significant role in maintaining

the correct oscillation characteristics of proteins involved in the circadian rhythm functioning [26]. The above-described molecular mechanism regulates the rhythmicity of expression of the so-called circadian-controlled genes, in particular the crucial clock genes. However, it must be stated that even though there is enough evidence in support of this system, certain aspects have been challenged by studies of cyanobacteria and human red blood cells. Therefore, further studies of the regulatory mechanisms in the TTFLs are required.

2.3 Clock Gene in Drosophila Melanogaster

Because of the complicated structure and functioning of the circadian system, an initial prototype offering a clear picture of the mechanism at the molecular level was required. Eventually, in the 1970s, research on the fruit fly Drosophila melanogaster provided a basis for a comprehensive description of the rhythm. The reason for choosing this insect for research lies in the clear restriction of its certain physiological and behavioural activities, such as flight and eclosion (the creation of adult flies from the pupa), to precise times of the day, pointing at the existence of a definite circadian rhythm. Moreover, the fly was found to be responsive to changes in the time of dawn and dusk [27]. The aim of the research on the circadian rhythm of the fruit fly was to identify genes regulating it and then extend the findings to other living organisms. In 1971, Konopka under the supervision of Benzer discovered that mutations in a gene that he eventually called *period* (abbreviated as *per*) led to changes in the usual circadian rhythm of flies [28]. The three *per* mutations identified were the arrhythmic per^0, long per^l, and short per^s, leading to corresponding changes in the daily rhythm of activities. Konopka's discovery was very significant as it created the foundation for understanding the genetic reasons for animal behaviour. The *per* gene was then isolated for in-depth studies that led to the discovery of the PER protein encoded by the corresponding gene. Both gene and protein levels were found to vary periodically following the circadian rhythm for flies with no *per* mutations, no rhythm in arrhythmic per^0 flies, and corresponding long and short periods in per^l and per^s mutated flies [29]. A more detailed picture of the circadian clock's self-regulation mechanism was provided by work in Michael Young's laboratory. He discovered a gene called *timeless* (abbreviated as *tim*) encoding the TIM protein that by forming a complex with PER was able to enter the cell nucleus to inhibit the *per* and *tim* genes activity, thus regulating the cellular protein level [30]. The concentration of these proteins in the body oscillates according to the circadian rhythm by accumulating in the cell nucleus during the night until reaching peak concentration before sunrise and then degrading to restart the process. These genes along with the corresponding proteins lead to the creation of the PER-TIM negative feedback loop, operating as described in part 2.2 for TTFLs, regulating the oscillations of elements involved in the circadian rhythm functioning [29].

2.4 Mammalian Circadian System

The present paper is focused on the circadian rhythm of the Drosophila, however, the similarity between the circadian rhythm functioning in flies and rodents leads to the possibility of extending results from Drosophila studies to mammals. This part explains the principles of mammalian circadian system functioning, which can be useful when extending methods and findings from the present research to the mammalian case.

Research by Moore and Eichler in 1972 showed that damage to a certain part of the rat's hypothalamus led to the absence of its daily sleep-wake cycle [31]. Along with additional research, this eventually confirmed that the circadian clock mechanism in mammals is regulated by a small paired structure consisting of grouped cells located in the hypothalamus, above the optic chiasm, called the *suprachiasmatic nucleus* (SCN). Similar to the Drosophila findings, a clock mutant *tau* contained in the SCN was discovered to be responsible for a shortening of the period of locomotor activities in mammals. The dynamics of the Drosophila and the mammalian circadian rhythms are quite similar, as they both form negative feedback loops. However, the elements of the loops differ. In particular, instead of a PER-TIM loop as observed in the Drosophila, the mammalian system forms a PER-CRY loop where the PER part consists of three mammalian homologs of the Drosophila PER proteins (PER1, PER2, PER3), and the inhibiting complex is formed by their dimerisation with the cryptochrome proteins CRY1 and CRY2 [32].

The main input pathway of the SCN, as defined by the structure in 2.1, is through the photoreceptors contained in the retina of the eye, converting information about external light into electrical signals going to the optic chiasm. From there, the information goes to the back of the brain and the SCN. The other type of special cells contained in the retina is ganglion cells that project information about external light conditions directly to the SCN, inducing entrainment of the central oscillator. After receiving and processing the input information about the length of the LD cycle from the ganglion cells containing the photopigment melanopsin sensitive to blue light, the role of the SCN is to transfer it to the endocrine pineal gland, which in response to the rhythmic input oscillations produces the hormone melatonin [33].

Melatonin plays a significant role in the endogenous regulation of the sleep-wake cycle. The production of melatonin in mammals starts in the evening with the decrease in the intensity of external light leading to a gradual reduction of the SCN activity. The peak of melatonin production happens at night with a gradual decrease as the light day starts. Melatonin forms a feedback loop with the SCN, modulating physiological and behavioural patterns [34]. In simple words, when the retina of the eye senses darkness (absence of light) and the SCN activity is reduced, the pineal gland starts actively producing melatonin, leading to increased levels of sleepiness in the organism that encourage it to rest. On the contrary, when retinal cells sense light, especially wavelengths corresponding to blue light, the pineal gland stops producing melatonin and the organism feels awake and energetic, ready to start daily activities. A schematic representation of this process is presented in figure 4. After entrainment with light cues as described above, the main body clock in the SCN functions endogenously, keeping the pace of circadian oscillations. Despite the scientifically confirmed synchronising effect of melatonin on the endogenous circadian rhythm, its

effect is very subtle. Therefore, it was concluded that melatonin does not play the main role in the central clock entrainment but rather is a part of a more complicated system [35].

Humans, as well as animals, have individual differences in the time when they feel more active and alert. These are connected to differences in entrainment characteristics, in particular in the preferred phase of entrainment. People with clear preferences are commonly referred to as "early birds" and "night owls", however, the more scientific way to refer to such differences is "early" and "late" chronotypes, even though phase differences can lead to a continuum of different chronotypes. People and animals with early chronotypes typically wake up at an early hour and generally feel more alert in the morning, experiencing difficulties with activities in the evening when they feel the need to rest at quite an early evening hour. Late chronotypes, on the contrary, wake up quite late into the day and stay awake until late night hours, leading an almost nocturnal lifestyle. Such differences in alertness hours are considered normal in the natural world but can lead to difficulties for people at work, as shift hours are established without taking into consideration the chronotype of the person. Moreover, it is worth noting that the individual chronotype, despite being determined by genetic factors, is also affected by external factors such as age and sleep schedule [36]. The establishment of the chronotype and specific sleep-wake patterns of mammals are also related to the time of melatonin secretion, so disruption of the circadian rhythm by factors described in part 1.2, such as shift work and jet lag, are in some cases treated pharmacologically by prescription of melatonin tablets. They were shown to decrease the time required to fall asleep, increase the total sleeping time, and improve sleep quality [37]. However, further research is needed for a better understanding of the effect of melatonin on the circadian system.

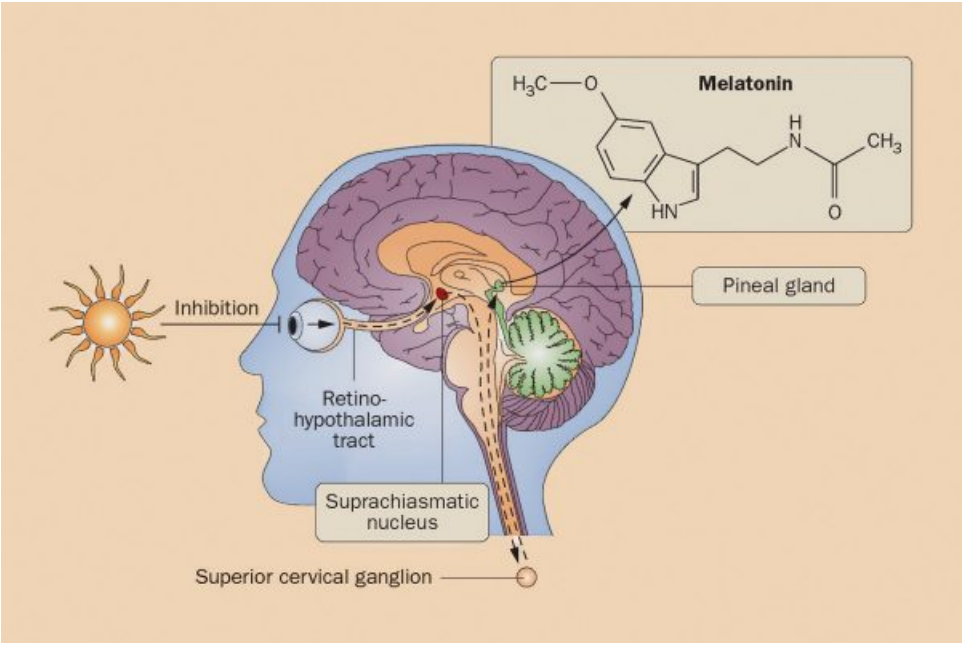

Figure 4: Scheme of the SCN regulation of the circadian rhythm in humans by means of melatonin production. Picture credit [38].

3 Building a Mathematical Description

3.1 Deterministic and Stochastic Models

The circadian system contains many interacting parts that can be difficult to analyse using biological methods. Moreover, certain experiments needed for research are impossible to conduct in laboratory conditions with adequate accuracy. To overcome these limitations, a mathematical model simulating the behaviour of the system can be used. In order to apply methods of mathematical modelling to analyse a system, the real-world problem is first broken down into smaller parts that are expressed by mathematical terms and simplified through assumptions. Then the problem is solved or the needed experiment on the system is conducted using analytical methods or special mathematical software, such as MATLAB in the case of the present study. Finally, after obtaining the results, they are converted back into terms related to the initial real-world problem [39].

Two main types of models can be used in the present study: deterministic and stochastic. The deterministic model considers the time evolution to be continuous and the process to be dependent on the input variables in a systematic, predictable way. This type of model is often governed by ordinary differential equations (ODEs) called *reaction-rate equations*, usually in a system, fully describing the behaviour of the system [40]. In terms of modelling the circadian rhythm, a deterministic model can be used to simulate the functioning of the clock in constant external conditions, to describe the entrainment of the system by photic cues, and to simulate the phase shift induced when subjected to light pulses.

In contrast, the stochastic model regards the system as evolving in an unpredictable way over time. This is useful when the number of genes and proteins involved in the processes is small, which leads to a significant effect of molecular noise that does not allow for fully predictable systematic oscillations [41]. This is due to the fact that systems with a small number of particles experience a larger effect of molecular noise, sometimes called intrinsic noise, which happens due to the randomness of collisions between the molecules. The stochastic model is usually governed by a single differential equation called *master equation*. This type of model usually provides more realistic simulations, however, it usually involves a more advanced level of calculations as well.

The code used in the present paper for both the deterministic and the stochastic model was provided by the dissertation supervisor Giorgos Minas. It is based on a software tool for the analysis of complex biological models called PeTTSy [42]. The initial code received was modified according to the goals of experiments by changing appropriate parameters and incorporating the framework for entrainment by light. The goal of the present study is not to construct a perfectly accurate model from scratch but to understand the methods used for building biologically accurate working models that are not overly complicated to execute. For this reason, the first step for such exploration is to present and analyse the deterministic model of the Drosophila circadian rhythm, after which a comparison to the more complicated stochastic model will be provided.

3.2 Feedback Loop of the Drosophila Circadian Rhythm

In order to construct a model of the circadian rhythm of the Drosophila, the process described from the biological perspective in part 2.3 needs to be broken down into steps, put into mathematical terms, and simplified by means of appropriate assumptions. The deterministic model of Drosophila circadian oscillations used in the present paper involves ten ODEs for the change in concentration of each of the elements from the feedback loop. In particular, the equations are related to the concentrations of the *per* and *tim* mRNAs, three forms each of the PER and TIM proteins, and the nuclear and cytosolic forms of the PER-TIM complex. The corresponding ten kinetic equations are presented in Appendix 1 and their general form is analysed in section 3.3.

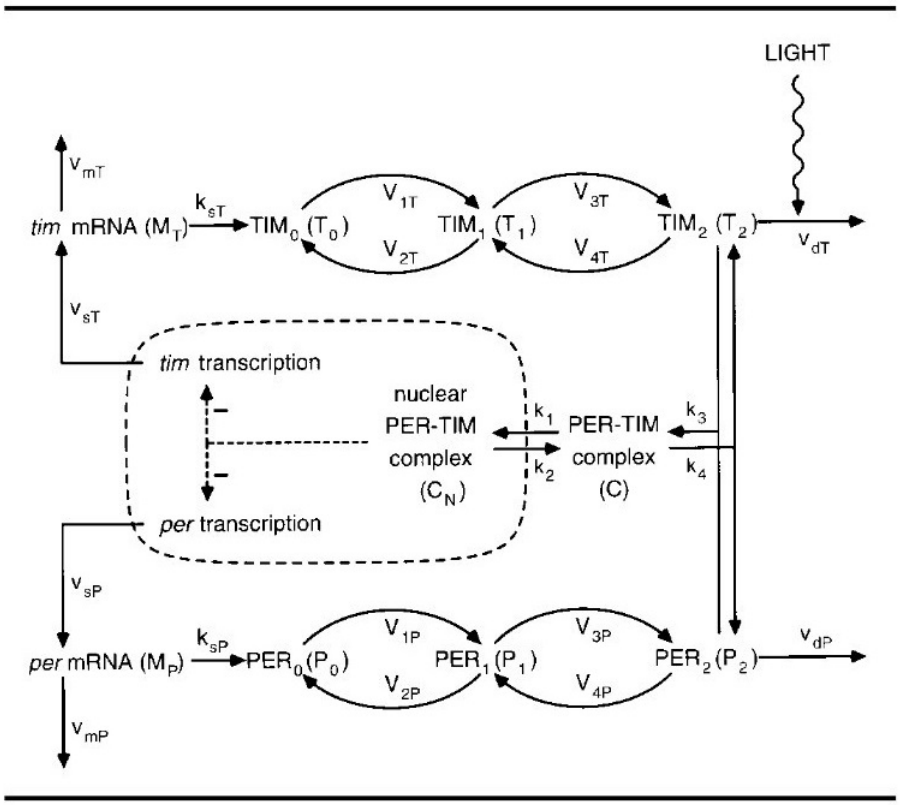

Figure 5: Diagram of the processes involved in the feedback loop of Drosophila. *Per* and *tim* genes are transcribed into mRNAs (M_P and M_T) in the nucleus and moved to the cytosol at maximum rates v_{sP} and v_{sT}. Some of the mRNAs are then lost to degradation at rates v_{mP} and v_{mT}, the rest are translated into proteins P_0 and T_0 with respective rate constants k_{sP} and k_{sT}. The two reversible phosphorylations happen with maximum rates V_{iP} and V_{iT} $(i = 1, 2, 3, 4)$ respectively. The final phosphorylated forms undergo degradation at maximum rates v_{dP} and v_{dT} and form the PER-TIM complex (C) that when transported into the nucleus becomes the nuclear PER-TIM complex (C_N) that inhibits the *per* and *tim* genes transcription. Picture credit [43].

The model of the Drosophila circadian rhythm examined in this paper is based on the negative feedback loop between the concentration of the nuclear PER-TIM complex and the rate of *per* and *tim* gene transcription. A diagram of the analysed model with specified rate parameters of processes is presented above in figure 5.

The process starts with the *per* and *tim* genes undergoing the process of transcription in the cell nucleus to create the corresponding mRNAs. The mRNAs are then transported out of the nucleus into the cytosol where from instructions contained in them, the proteins TIM_0 and PER_0 are created by the process of translation. Modifications of the proteins can still happen at this stage by the addition of a phosphoryl group to them by a process called phosphorylation [41]. In the present model, the proteins PER_0 and TIM_0 are assumed to undergo two phosphorylations each ($PER_0 \rightarrow PER_1 \rightarrow PER_2$ and $TIM_0 \rightarrow TIM_1 \rightarrow TIM_2$). The biphosphorylated forms PER_2 and TIM_2 can decrease in concentration at this point due to degradation, and the remaining proteins can interact with each other to form the PER-TIM complex that enters the cell nucleus to repress the transcription of the *per* and *tim* genes.

3.3 The Goodwin Model

A complete understanding of the equations involved in the mathematical model of the Drosophila used in this paper needs to be gradually built in complexity from simpler models, such as the Goodwin model. The Goodwin model is a mathematical model for simulating oscillations happening within cells, especially useful for simulating negative feedback loops. Since the main regulating process of the Drosophila circadian rhythm is the PER-TIM feedback loop, this model can be quite appropriate for constructing the deterministic model of the Drosophila circadian oscillations. Using the Goodwin model, the feedback loop can be described by means of simulating the time changes of two variables: the mRNA concentration denoted by X and the concentration of the protein it translates into, denoted by Y. The latter inhibits the former in this model. The transcription rate can be described by the function f :

$$f = \frac{K}{(K+Y)},$$

where K is a parameter [44]. From the above equation, it can be seen that the gene transcription rate f decreases when the inhibitor concentration Y increases, which is consistent with the principle of the negative feedback loop. The remaining two equations of the Goodwin model are responsible for the rates of change in X and Y with time:

$$\frac{dX}{dt} = \alpha_1 \frac{K}{K+Y} - \delta_1$$

$$\frac{dY}{dt} = \alpha_2 X - \delta_2,$$

where K, α_1, α_2, δ_1, and δ_2 are parameters that need to be defined. Upon integration, the last two equations give self-sustained oscillations of the concentrations of X and Y in time. It is important to note that the reaction rates in the above two equations do not depend on their own concentrations.

Such type of reactions is said to have zero-order kinetics. This simplifies calculations, however, in this case, element concentrations can become negative, thus biologically invalid. To overcome this problem, Michaelis-Menten terms should be added to the expressions to relate the rate of change in the concentration of the element to the concentration of the element itself. By doing so, the equations become:

$$\frac{dX}{dt} = \alpha_1 \frac{K}{K+Y} - \delta_1 \frac{X}{K_1+X}$$

$$\frac{dY}{dt} = \alpha_2 X - \delta \frac{Y}{K_2+Y}.$$

The Michaelis constants K_1 and K_2 are connected to the damping of the oscillations. In terms of relating the model to the Drosophila circadian rhythm, the X in this case would correspond to the concentration of the *per* and *tim* mRNAs and Y to the nuclear PER-TIM complex. However, since the present paper is exploring a much more complicated system than a two-parameter feedback loop, the reaction-rate equations are expected to have more terms, as intermediate steps need to be included between the transcription of mRNAs and the formation of the PER-TIM complex. The ten kinetic equations used in the paper presented in Appendix 1 have the general form given by the Goodwin model with appropriate Michaelis-Menten terms, degradation constants, and rate constants. As mentioned above, the equations describe the change in concentration of the *per* and *tim* mRNAs, the three corresponding forms of proteins each, and the cytosolic and nuclear complexes. Because the elements involved in the feedback loop are interconnected, the concentration of each element is influenced by other elements of the loop.

3.4 Stochastic Chemical Kinetics Model

The Goodwin method described above provides a good framework for an initial understanding of methods used to describe the evolution of a reacting system deterministically with ODEs. However, to make the simulation more realistic, the model needs to account for the discreteness and randomness of the reacting molecules' behaviour. As mentioned previously, stochastic simulations are particularly useful for modeling reactions in cells, as the small number of interacting particles leads to a significant influence of molecular noise on the system, deviating the time evolution of the system from the prediction of the deterministic model. A fundamental parameter for the stochastic model is the system size Ω. For large values of Ω, the large system size leads to a smaller effect of molecular noise on the system, so the time evolution is expected to be similar to the deterministic case. In contrast, for smaller Ω, the smaller system size leads to a larger effect of molecular noise on the system, so the time evolution is expected to differ from the deterministic case quite significantly [45]. In the stochastic model, positions and velocities of individual molecules are not taken into account, being replaced by considerations of the changes in molecular populations instead. This is due to the assumption that the system is *well-stirred*, meaning that the elevated number of elastic collisions between particles not leading to chemical reactions leads to their random positioning, and velocities follow the Maxwell-Boltzmann distribution [46]. Nonreactive collisions are not considered in the model to reduce the processing time. Only reactions that change the molecular population

size are taken into account. Therefore, the stochastic model is mainly governed by the laws of statistics and probability rather than by explicit tracking of particles.

Information about a specific reaction channel R_j is given by two main parameters: the state-change vector v_j and the propensity function a_j. The ith component of the state-change vector, denoted by v_{ji}, gives the change in molecular population caused by one reaction in the specified reaction channel. The propensity function multiplied by an infinitesimal time interval $a_j(x)dt$ gives the probability of a reaction occurring in this reaction channel in the time interval between t and t+dt. The propensity function itself consists of the product of other parameters:

$$a_j(x) = c_j h_j(x),$$

where c_j is the specific probability rate constant for the channel and $h_j(x)$ is the number of molecular combinations in state x involved in reactions [47]. The propensity function holds special importance in stochastic kinetics as missing information about the system can be determined from it using the laws of probability. From these two functions, the chemical master equation (CME) specifying the change in probability of reaction occurrence with time can be derived [48]:

$$\frac{\partial P(x,t|x_0,t_0)}{\partial t} = \sum_{j=1}^{M}(a_j(x-v_j)P(x-v_j,t|x_0,t_0) - a_j(x)P(x,t|x_0,t_0).$$

The form of the CME shows that every index of reacting molecules leads to a separate term in the equation, making the equation long and complicated. Moreover, if at least one of the propensity functions is non-linear, the CME cannot be solved. Therefore, an alternative strategy presented by Gillespie [46] is to simulate trajectories of a specific state of the system X(t) with time. For this purpose, a new probability function $p(\tau, j|x,t)$ should be defined. Then, $p(\tau, j|x,t)d\tau$ will denote the probability of the next reaction occurrence in a specific reaction channel in the time interval between $t+\tau$ and $t+\tau+d\tau$. Here j is the index of the next reaction and τ is the time to the next reaction. By using the laws of probability and the propensity function definition, the new probability function can be defined as:

$$p(\tau, j|x,t) = a_j \exp^{-a_0(x)\tau},$$

where a_0 is the sum of all the values of the system propensity function [49]:

$$a_0(x) = \sum_{j'=1}^{M} a'_j(x).$$

Therefore, it can be seen that the probability of the next reaction is decreasing exponentially as the time between reactions increases.

The CME used in the present paper for the stochastic model of the Drosophila circadian rhythm was constructed in a way to correspond to the reaction rate equations mentioned previously, in the limit $\Omega \to \infty$, where the effect of molecular noise is negligible. In this way, results from simulations provide a clear demonstration of how molecular noise perturbs the system further from the deterministic prediction.

4 Results from Simulations

4.1 Results from the Deterministic Model

By plotting the dependence of the concentration of the nuclear PER-TIM complex on the concentration of *per* mRNA (figure 6) deterministically, oscillations are seen to evolve towards a closed trajectory. This closed trajectory forms a limit cycle of the system, meaning that the concentrations of the values plotted eventually evolve to that cycle from initial conditions in its neighborhood. The oval shape of the graph is explained by the repressing behaviour of the complex (y-axis) on the mRNA (x-axis). When following the cycle counterclockwise, it can be seen that as the concentration of the complex grows strengthening the repression, the concentration of mRNAs diminishes. And vice versa: as the concentration of the complex diminishes and the repressing behaviour weakens, the concentration of mRNAs grows. Since the periodic oscillations were obtained using a deterministic model, thus not accounting for molecular noise, the limit cycle consists of a single line. The graph confirms the accuracy of the deterministic mathematical model in simulating the behavior of the biologically described feedback loop.

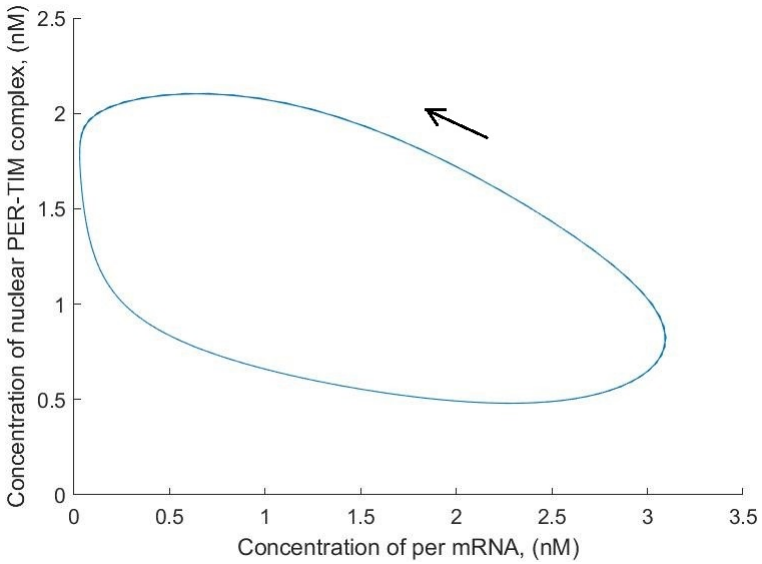

Figure 6: The limit cycle of the concentration of PER-TIM complex against concentration of *per* mRNA of the ten variable deterministic model of the Drosophila circadian rhythm.

The motivation for interpreting the limit cycle in the counterclockwise direction can be deduced from the graph of the two concentrations plotted against time in figure 7 by looking at the respective phases of maxima and minima. Since the peak of nuclear complex concentration closely follows the peak of mRNA concentration, this should correspond to the shorter path on the limit cycle between those points, which is the top part taken counterclockwise. The respective evolution of the concentrations with time provides a clear way to ascertain once again the repressing behaviour of

the complex on the transcription of *per* mRNA by following the red line and seeing the respective changes in the blue line.

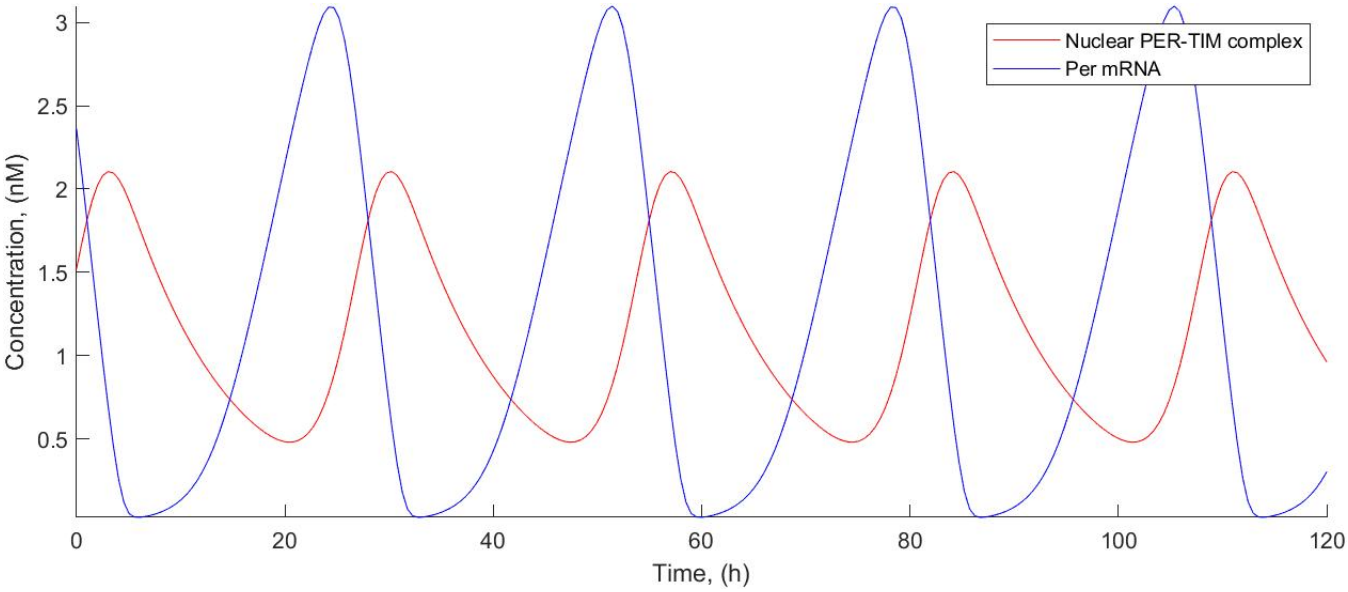

Figure 7: Oscillations in concentration of the nuclear PER-TIM complex and of the *per* mRNA with time corresponding to the deterministic limit cycle.

4.2 Results from the Stochastic Model

Stochastic simulations of the Drosophila circadian rhythm were executed using the Gillespie method described in part 3.4. The effect of molecular noise on the system was investigated by taking three different equidistant values of the system size parameter, $\Omega = 200, 600, 1000$. It is worth remembering that smaller values of system size correspond to a more significant impact of molecular noise. The simulations were performed for a time of 260 hours corresponding to roughly 100 cycles of the system, providing a balance between producing a detailed graph of the behaviour of the system and not taking excessive time to compute. Results obtained from stochastic simulations should then be compared to the deterministic ones.

From figures 8-10 it can be seen that stochastic simulations yield the expected oval shape of the limit cycle. However, due to stochasticity and molecular noise, many lines are present in the graphs, and each cycle is perturbed. The increased number of lines increases the thickness of the limit cycle shape as the value of the system size decreases. The case of Ω=1000 in figure 10 yields the closest to the deterministic case result as expected because the system size is large and the effect of molecular noise is small. This is confirmed by the quite precise, thin line of the limit cycle produced in this case. As the system size decreases, molecular noise gradually starts having a larger impact on the system by thickening the curve, as can be seen in figure 9 for $\Omega = 600$. At

the smallest considered system size $\Omega = 200$ in figure 8, noise has a very large effect on the system, so the graph looks very perturbed, however, it still follows the expected shape. As the system size decreases further, on the order of $\Omega = 10$, we expect noise to ultimately take over rhythmicity and disrupt the definite oval shape of the limit cycle.

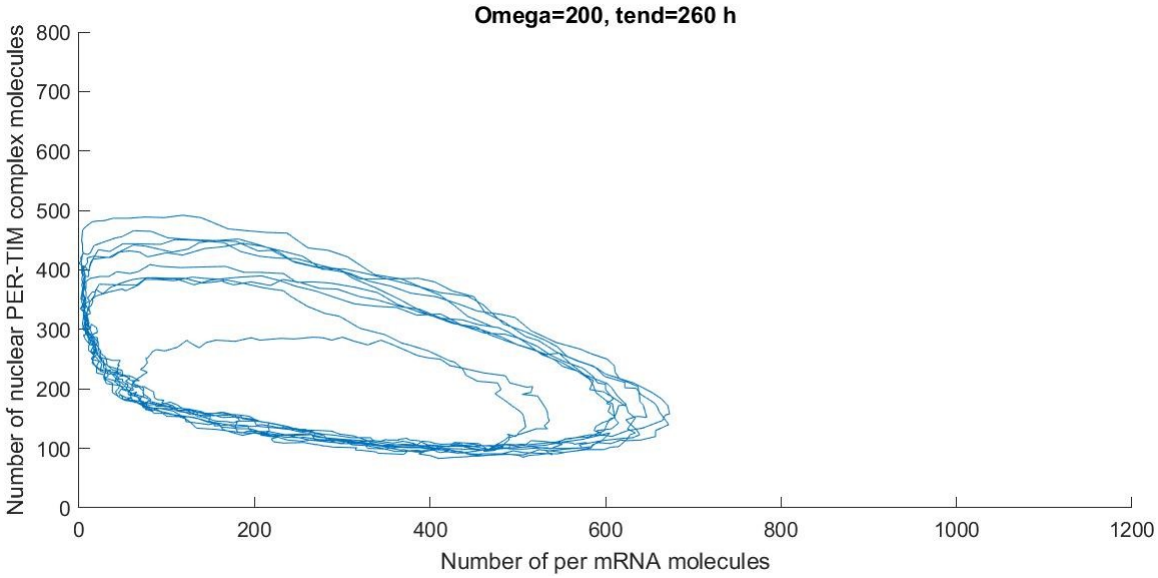

Figure 8: Limit cycle of the number of PER-TIM complex molecules as a function of the number of *per* mRNAs of the stochastic model for small system size $\Omega = 200$.

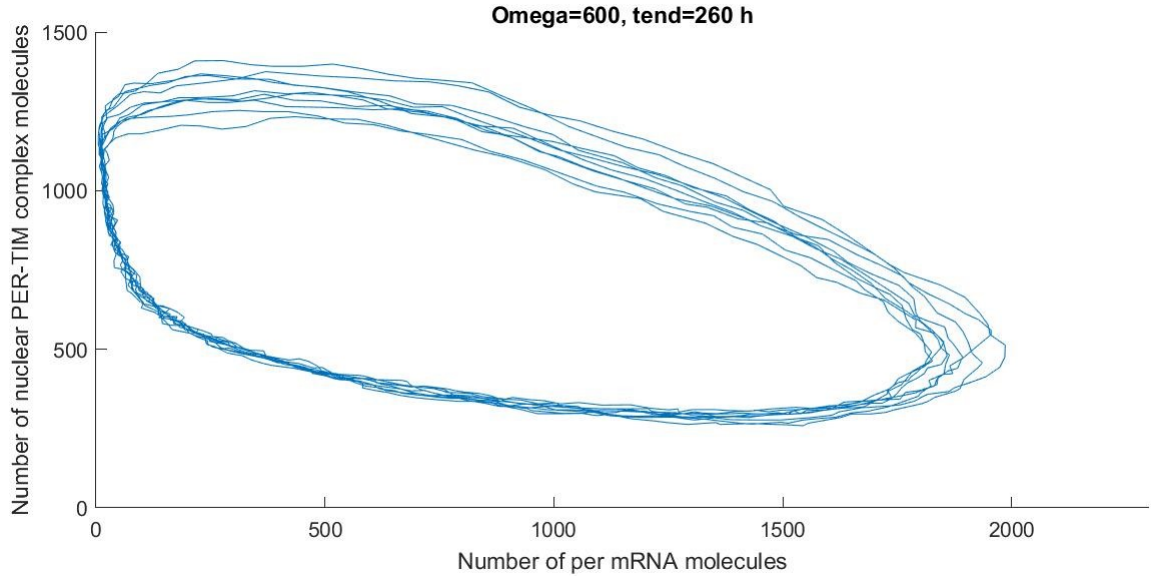

Figure 9: Limit cycle of the number of PER-TIM complex molecules as a function of the number of *per* mRNA of the stochastic model for system size $\Omega = 600$.

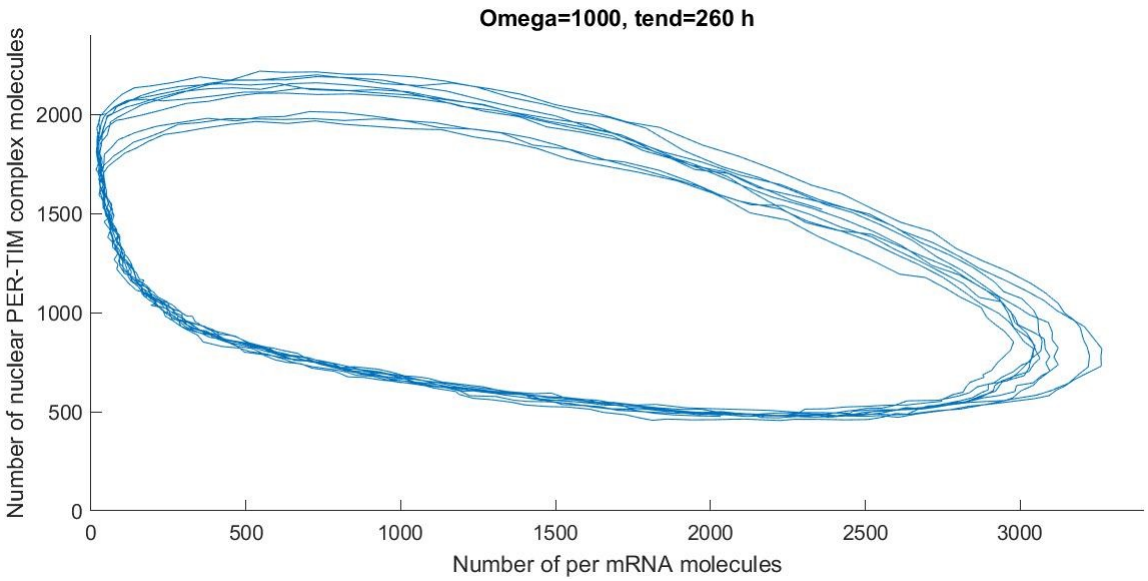

Figure 10: Limit cycle of the number of PER-TIM complex molecules as a function of the number of *per* mRNAs of the stochastic model for system size $\Omega = 1000$.

Oscillations of the concentration of *per* mRNA molecules with time for the deterministic and stochastic cases are compared for different values of the system size in figures 11-13. It can be seen that for large system sizes $\Omega = 1000$ with a small impact of molecular noise the two graphs behave very similarly and coincide for the first two peaks. As the system size decreases to $\Omega = 600$ and the effect of molecular noise becomes more relevant, stochastic oscillations start to present visible deviations from the deterministic prediction. In the graph for the smallest system size examined in this paper, $\Omega = 200$, the two graphs are clearly seen to not coincide. Due to the strong effect of molecular noise, the stochastic graph presents a significant phase drift in relation to the deterministic prediction, which can potentially cause large errors in the long-time limit. The stochastic simulation can be improved in accuracy by the implementation of more advanced methods, such as the *phase-corrected Linear Noise Approximation* method [50].

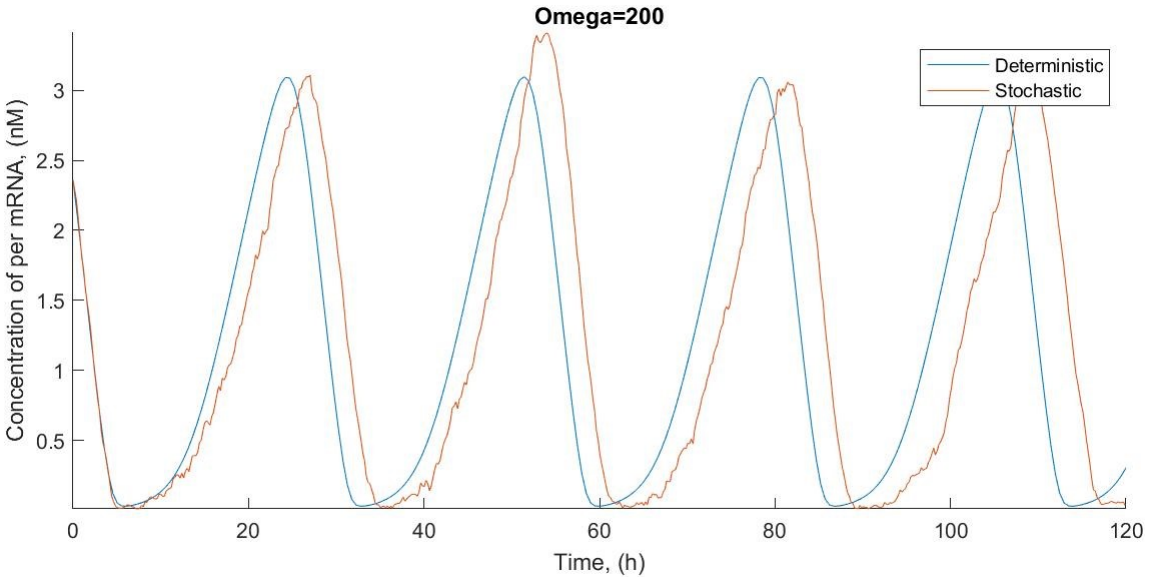

Figure 11: Oscillations of *per* mRNA concentration with time corresponding to the deterministic and stochastic models for $\Omega = 200$.

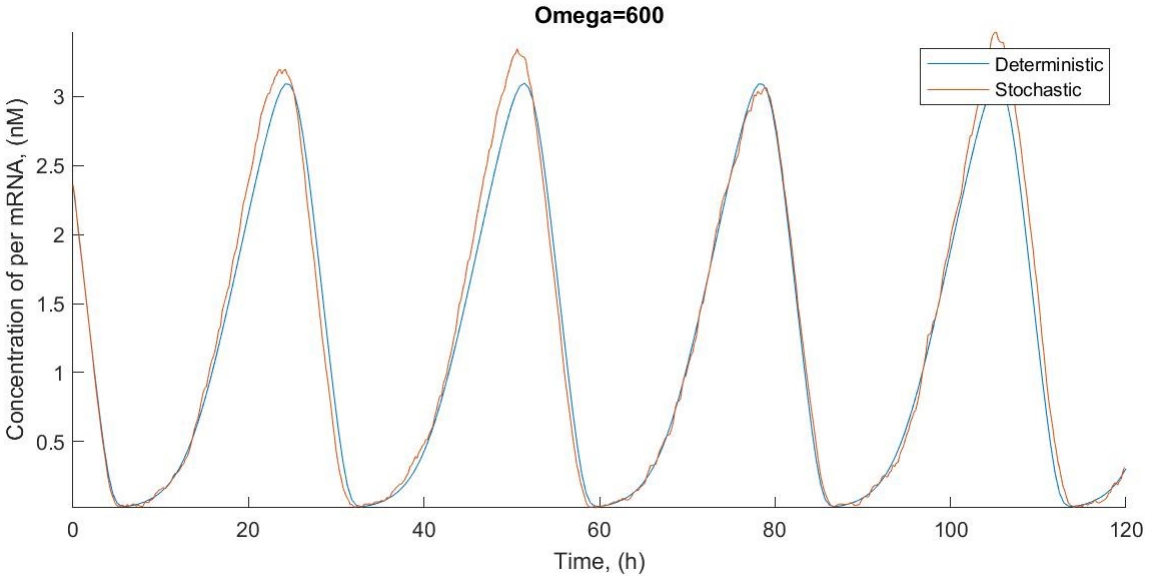

Figure 12: Oscillations of *per* mRNA concentration with time corresponding to the deterministic and stochastic models for $\Omega = 600$.

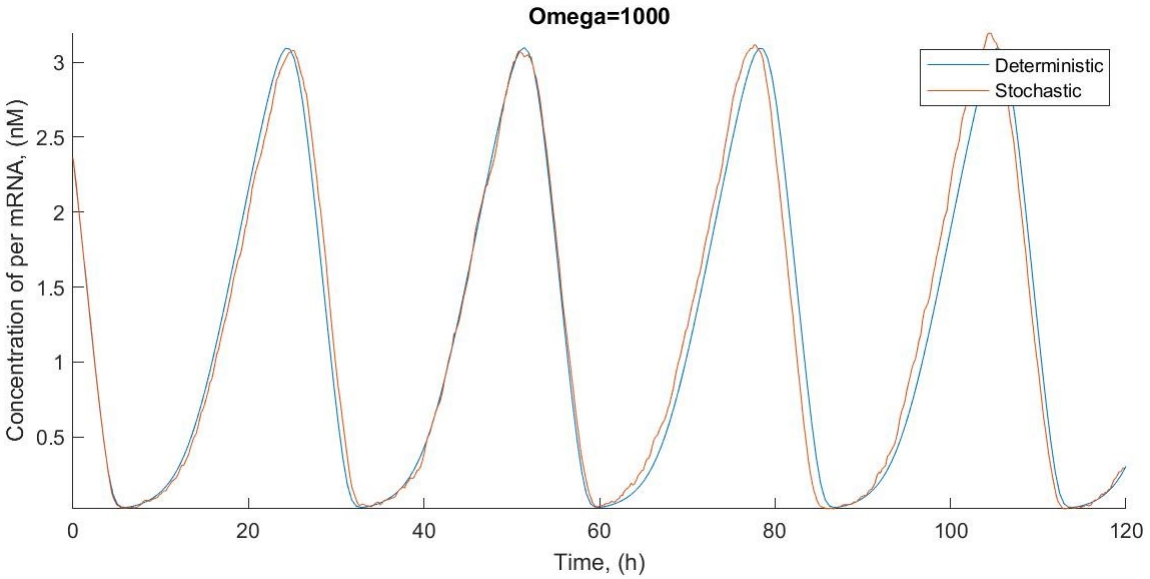

Figure 13: Oscillations of *per* mRNA concentration with time corresponding to the deterministic and stochastic models for $\Omega = 1000$.

5 Simulation of the Effect of Light on the Drosophila Circadian Rhythm

5.1 Deterministic Light Effect Simulations

Light can have a significant effect on circadian rhythm oscillations as it is connected to the rate of decay of the biphosphorylated form of the TIM protein T_2 [51] via the parameter v_{dT}, reflecting the maximum rate of degradation induced by light, as shown in figure 5. Therefore, the parameter v_{dT} can be varied according to specific goals of the analysis to reflect the conditions of the LD cycle. Including a framework for the light-controlled parameter variation in the model of the Drosophila circadian rhythm can be useful for simulating the entrainment of circadian oscillations by photic cues from external light conditions.

The first case examined is the case of constant darkness (DD), which can be modelled by setting v_{dT} to a sufficiently small constant value. In this paper, v_{dT} is set to 2 for darkness.

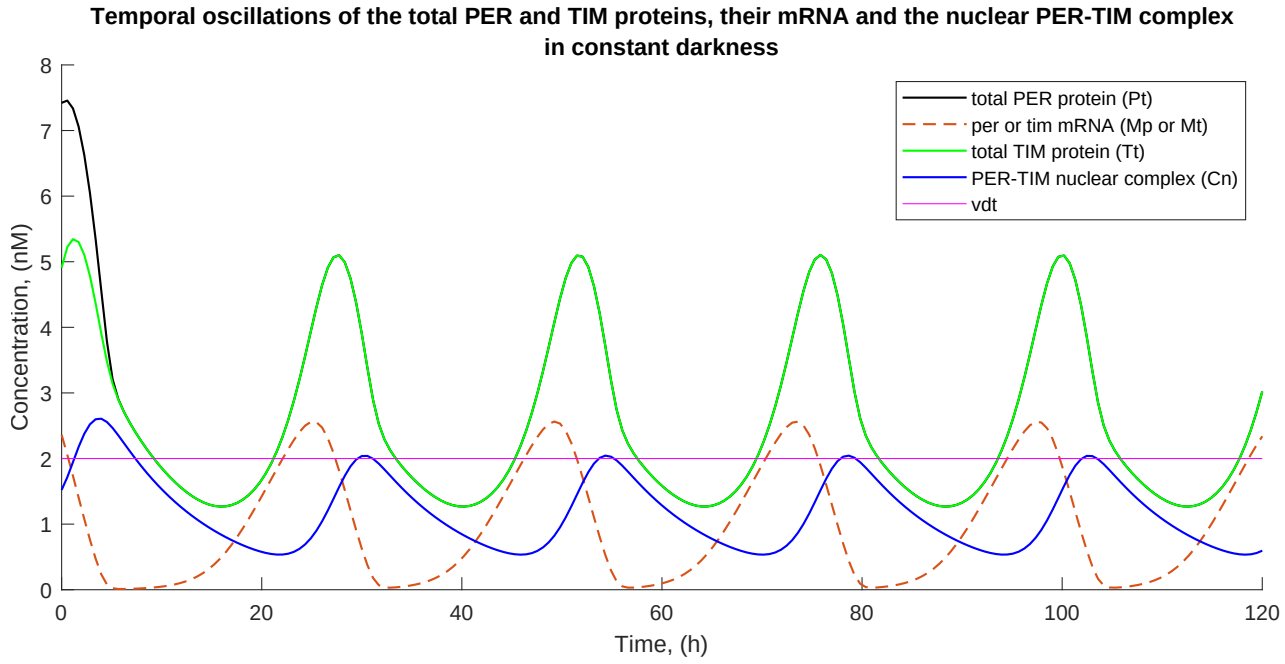

Figure 14: Temporal oscillations of the total PER and TIM proteins (P_t and T_t), their mRNAs (M_p and M_t) and the nuclear PER-TIM complex (C_N) in the condition of constant darkness (DD).

The total quantities of PER and TIM proteins (P_t and T_t) are defined as the quantities of the proteins in all the phosphorylated forms summed with their quantity present in the complexes:

$$P_t = P_0 + P_1 + P_2 + C + C_N,$$

$$T_t = T_0 + T_1 + T_2 + C + C_N.$$

Figure 14 demonstrates oscillations of the total proteins along with their mRNAs and the nuclear complex in constant darkness (DD). This figure, similarly to other figures in the analysis, is plotted for the symmetrical case, meaning that the rate constants of processes are assumed to be identical for the corresponding steps in the PER and TIM cycles. Since the rate constants are the same and there is no additional degradation of T_2 due to light in the constant darkness case, we see that the oscillations of the mRNAs are identical for *per* and *tim*. The graph also shows that the initial conditions for the PER and TIM proteins differ, which is related to the algorithm used by the model to retrieve initial conditions from their evolution on the limit cycle. Oscillations of the system after a short period of adjustment settle to a rhythm that continues in time undisturbed. This corresponds to the settlement of the system to a limit cycle. In the following parts, such settled oscillations will be the focus of the analysis.

The parameters used for the analysis of the symmetrical case are presented in Appendix 1. These parameters were found to be biologically appropriate for defining a period close to 24 hours in constant darkness for the symmetrical case. In the present model, the periods of C and C_N were found to be approximately 23.5 hours. The parameters can be modified to explore the asymmetrical case, in which the corresponding processes of the PER and TIM cycles are taken to have different rate constants. The extension to the asymmetrical case would bring the model closer to its real-world functioning, as it was shown that there are roughly 5 times more *tim* mRNAs than *per* mRNAs in the circadian cycle [52]. The present paper, however, is focused on the symmetrical case analysis.

In the following part, the focus will be shifted to the TIM protein, as it is directly affected by light. Figure 15 shows oscillations of the different forms of TIM involved in the cycle (T_0, T_1, T_2), along with the cytosolic and nuclear complexes for the DD case. The three forms of TIM can be seen to be very similar in their concentration and behaviour. As the parameter v_{dT} is increased to 4, which is the value taken in this paper for the condition of light, we can see from figure 16 that T_2 decreases in its concentration quite significantly (from 0.965 to 0.290 nM), which corresponds to the expected effect of light starting the degradation of T_2. This confirms the finding that light affects the concentration of T_2 and checks that the mathematical model used indeed behaves in accordance with this biological observation.

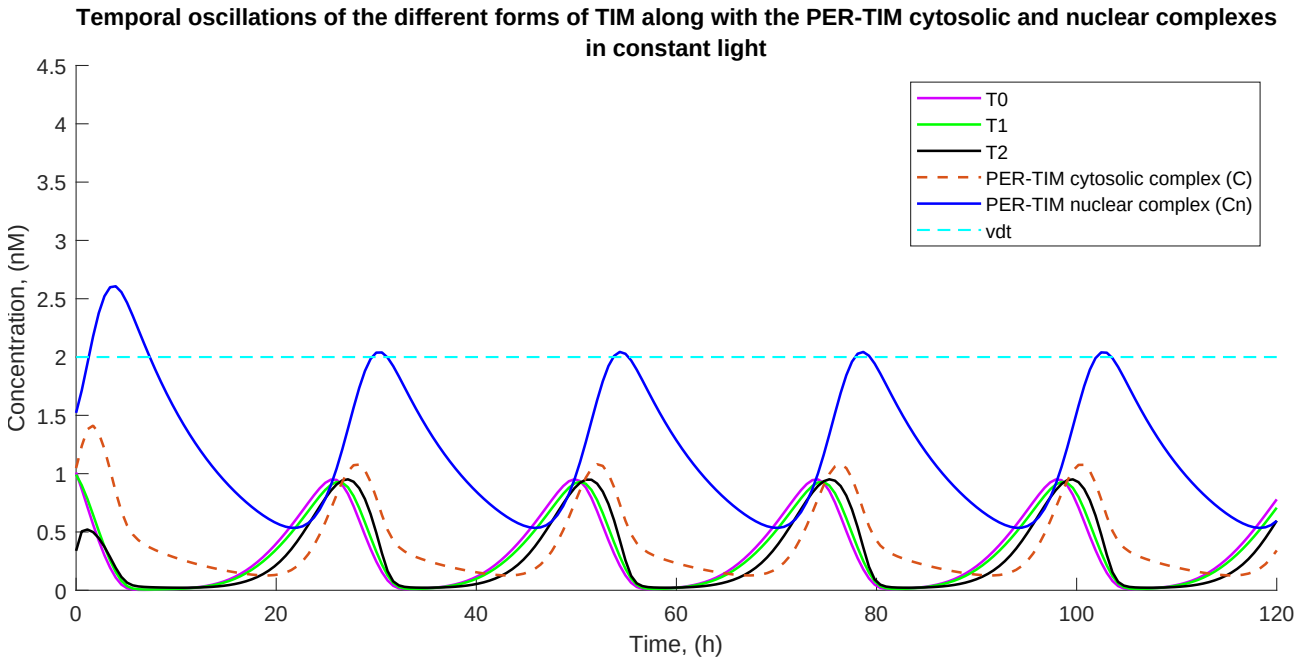

Figure 15: Temporal oscillations of the three different forms of TIM (T_0, T_1, T_2), and the cytosolic (C) and nuclear PER-TIM complex (C_N) in the condition of constant darkness (DD).

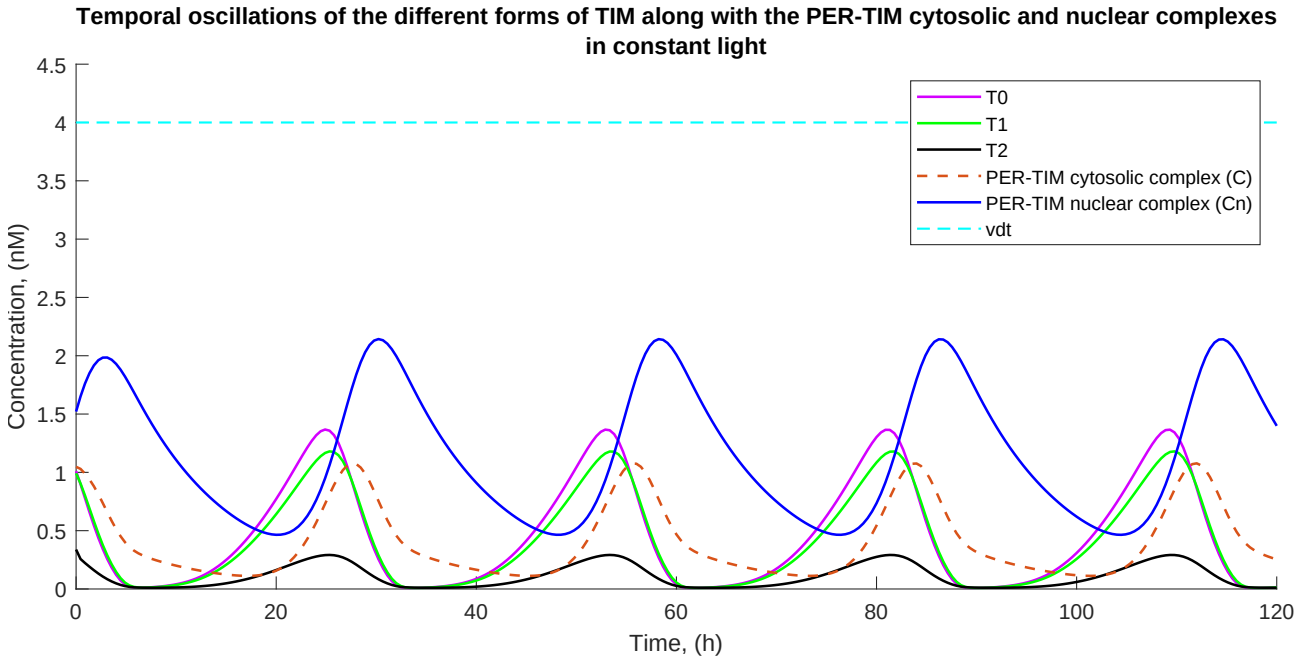

Figure 16: Temporal oscillations of the three different forms of TIM (T_0, T_1, T_2), and the cytosolic (C) and nuclear PER-TIM complex (C_N) in the condition of constant light (LL).

For research purposes regarding the entrainment of circadian oscillations by the LD cycle, external conditions need to change cyclically to simulate daily patterns of day and night. The first such pattern examined in the present paper is the cycle of 12 hours of light and 12 hours of darkness (12:12 LD) with the parameter v_{dT} being equal to 2 for darkness and 4 for light as before. For this case, a square-wave variation of the parameter v_{dT} would be the most appropriate starting point. However, it is worth noting that such a shape of the light profile does not account for the gradual changes in light during the periods of sunrise and sunset. The results of applying the 12:12 LD cycle to the circadian oscillations of the Drosophila can be found in figure 17.

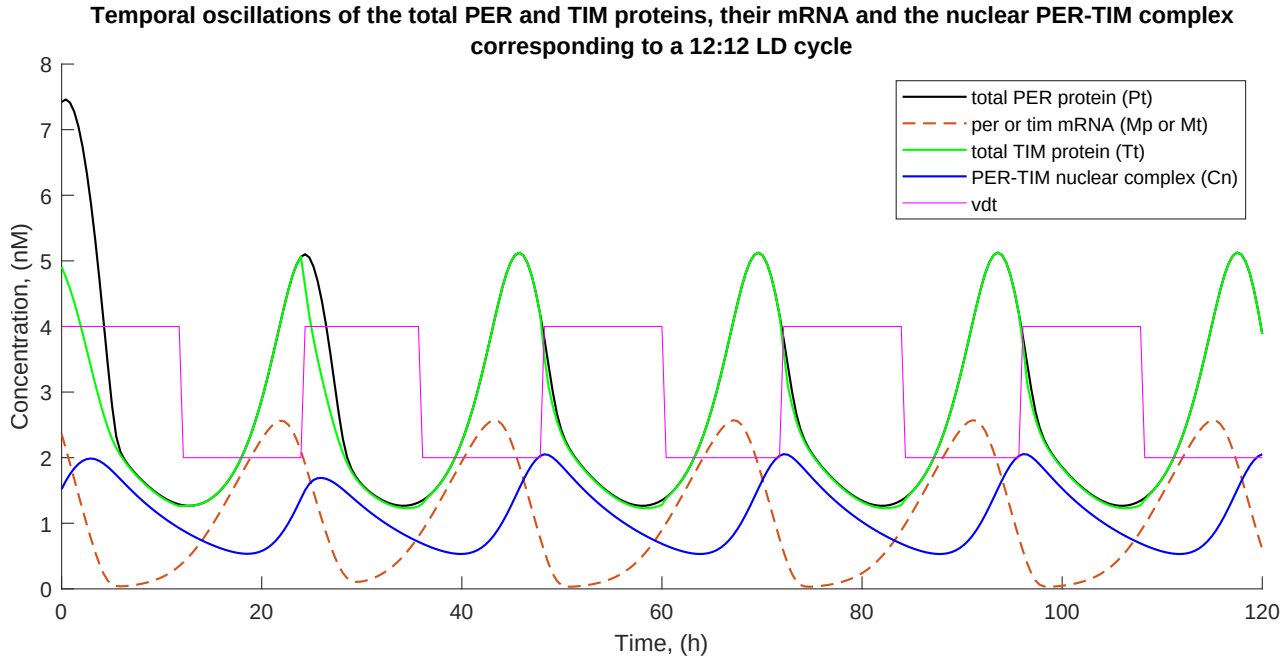

Figure 17: Temporal oscillations of the total PER and TIM proteins (P_t and T_t), their mRNA (M_p and M_t) and the nuclear PER-TIM complex (C_N) in the condition of the 12:12 square-wave LD cycle.

As can be seen by comparing figure 17 to figure 14, applying a 12:12 LD cycle to the oscillations does not affect the settled oscillations of the system in a significant way. The largest effect seen from the graph is the difference in the widths of the oscillations of total proteins: the downward part of T_t is steeper than that for P_t, which diminishes in a slightly more gradual way. This makes sense since the TIM protein is the one directly affected by light changes. However, it is worth noting that the difference in their widths is very subtle after the second cycle, which is when the system starts having settled oscillations. Despite the underwhelming effect of the 12:12 LD cycle application to the oscillations, the graph obtained contains useful information about the daily variations of the cycle components as well as the phase relationships between them. As seen in figure 17, the two proteins accumulate in the organism during the dark phase (night), reaching their maximal value in the late second part of the dark period, and start decreasing in concentration just before the light phase (day) starts. The timing of the rise and fall of the concentrations of

PER and TIM found using the mathematical model agrees with biological experimental results [53].

For the case of the 12:12 LD entrainment cycle, an increase in the amplitudes of P_t and T_t and their mRNA oscillations was expected due to the more drastic T_2 degradation under the influence of light. Since T_2 decays more strongly due to v_{dT}, and the concentration of TIM reduces, a consequent reduction in the formation of the PER-TIM complex is expected, and hence less inhibition of gene transcription, leading to a larger quantity of *per* and *tim* mRNAs, and of the total concentrations P_t and T_t of the two proteins. However, these changes are so subtle that they cannot be clearly seen graphically.

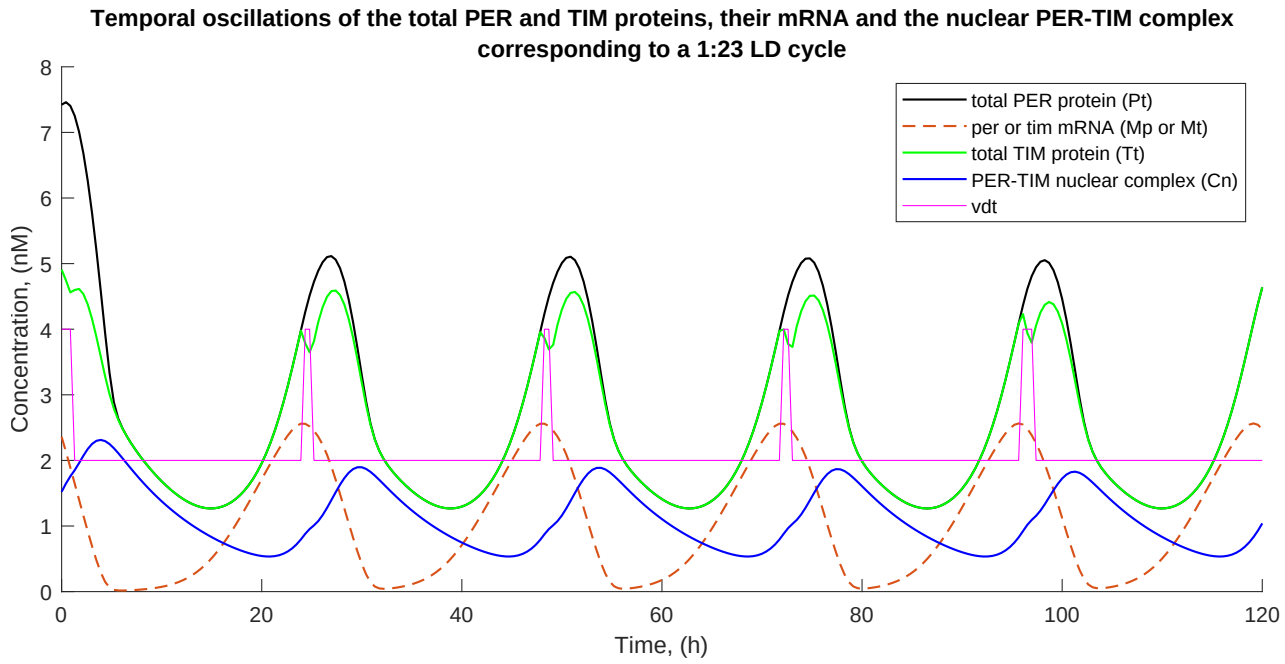

Figure 18: Temporal oscillations of the total PER and TIM proteins (P_t and T_t), their mRNA (M_p and M_t) and the nuclear PER-TIM complex (C_N) in the condition of the 1:23 square-wave LD cycle.

The framework of the square-wave light perturbation can be modified to simulate any shape of the LD cycle. In figure 18, the effect on the system of one-hour light pulses followed by 23 hours of darkness is represented. It can be seen that in this case the pulses have quite a significant visible effect on T_t by drastically bringing its concentration down in the hour of light, thus not letting the oscillations reach the previously obtained maximal value.

The significance of the introduction of the framework for simulating the effect of light on the circadian rhythm of the Drosophila lies in the possibility to model and research real-life situations. Disruptions introduced in part 1.2 (shift work and jet lag), can be modelled using appropriate light wave shapes.

5.1.1 Jet lag simulations

As mentioned above, jet lag occurs when the organism crosses multiple time zones in a short time. As a result, the travelling subject experiences a change in duration of the light part of the LD cycle if travelling by day or of the dark part if travelling by night. The change in the LD cycle depends on the direction of travel. If travelling from later time zones (..., UTC-10, UTC-9,...) to earlier ones (..., UTC+9, UTC+10,...), the difference in time is subtracted from the duration of the day. On the contrary, if travelling from earlier time zones to later ones, the difference in hours is added to the duration of the day.

In this paper, the effect on the circadian rhythm of day and night flights to two different popular destinations is modelled. The flights analysed are London (UTC) - New York (UTC-4) and London (UTC) - San Francisco (UTC-7). The reason for taking flights from an earlier time zone to later ones lies in the fact that in this case the plane speed and the time of dawn in different time zones do not play a crucial role as in the opposite flying direction case, so the effect of the flights can be sensibly modelled by a prolongation of the light period (or dark period for night flights) of 16 and 19 hours respectively. For simplification of the model certain assumptions have been made: days are taken to be 12:12 LD cycles in both the departure city and the destination, the flight departs as soon as the sun rises, the speed at which the crossed regions get exposed to sunlight is larger than the speed of the plane, which provides light exposure from the plane window for the duration of the flight. Sunrise is taken to happen at 0, sunset at 12.

Figure 19 shows the profile of oscillations for the effect of the London (UTC) - New York (UTC-4) day flight and subsequent days for adaptation. The flight duration is 8 hours and upon arrival the time in New York is 8-4=4, leaving 12-4=8 more hours of light. The sum of the hours passed in the plane and the remaining hours of light upon arrival in New York gives the number of hours in the prolonged day (8+8=16). The prolongation of the day affects the phase relationships between the oscillating components, creating disruption. From figure 20, containing the magnified version of the effect of the 16 hours light perturbation, the prolonged day (48-64 h in the graph) can be seen to affect the phases of the oscillating components with respect to the light profile. This of course does not happen because of an actual shift in the oscillating components but rather due to the unusual length of the light period, leading to a change in the time relation between the light profile and the oscillating components. The prolongation of the day leads to the start of protein concentration growth being shifted closer to the middle of the light period than in the unperturbed case, where it happens closer to its end. The minimum in concentration of the nuclear complex is also shifted to correspond to the start of the new night in the 16 hours shift case in contrast to the unperturbed case from figure 17 when the same minima were occurring at roughly a fourth of the dark period. The minimum of the nuclear complex concentration encourages a sharp rise in the total proteins concentration at the start of the dark period in contrast to roughly a third of the same period in the unperturbed case. This gives the protein curve more time to grow, achieving a larger maximum total protein concentration in the first part of the night instead of the late second part as in the unperturbed case. The peak in protein concentration after the prolonged day achieves 5.7 nM instead of the usual value of 5.1 nM. This elevated level of proteins leads to an elevated

level of the nuclear complex formed from them during the night after the perturbed day. This in turn strengthens the repression of gene transcription and lowers the concentration of proteins. The day after the prolonged one still exhibits a phase shift similar to the one described for the perturbed peak, however, the effect of the perturbation lessens with each successive cycle, slowly adjusting the oscillations to establish a rhythm consistent with the new light profile. Therefore, the loop adapts to the new rhythm via entrainment from light cues after a certain number of cycles. However, the adjustment is quite gradual and the system is found to fully return to a settled rhythm on the 8th day (day starting at 244 h) after the perturbed day.

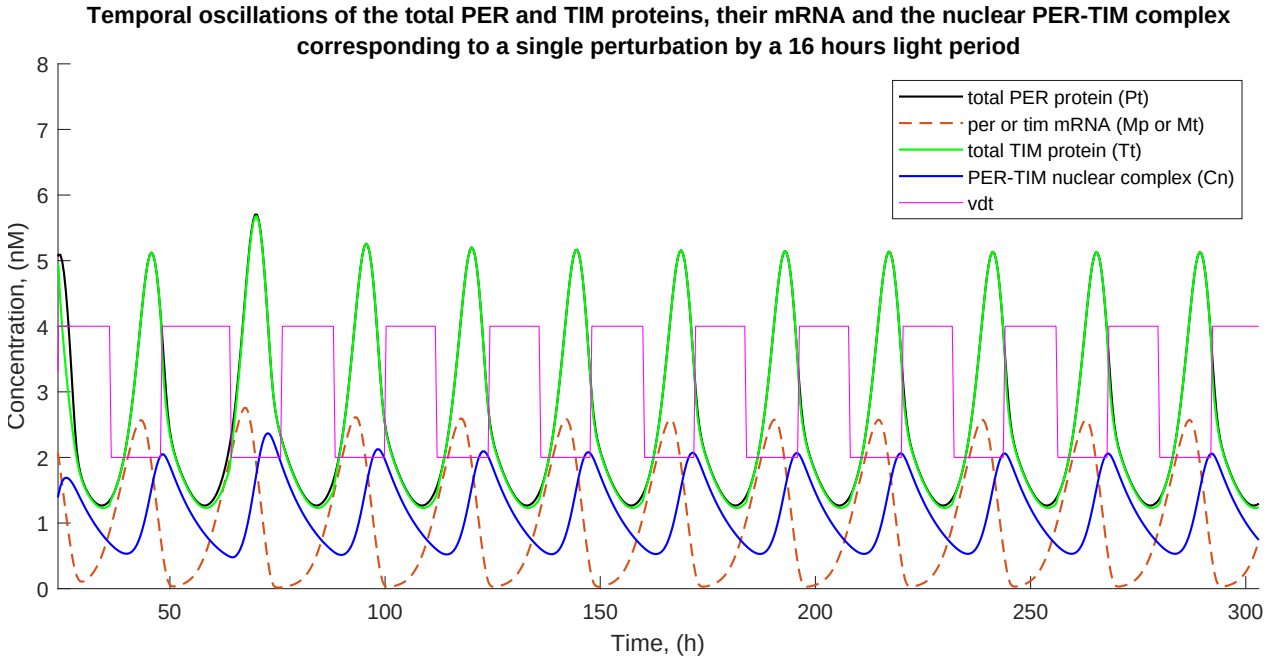

Figure 19: Temporal oscillations of the total PER and TIM proteins (P_t and T_t), their mRNA (M_p and M_t) and the nuclear PER-TIM complex (C_N) corresponding to a single perturbation by a 16 hours light period.

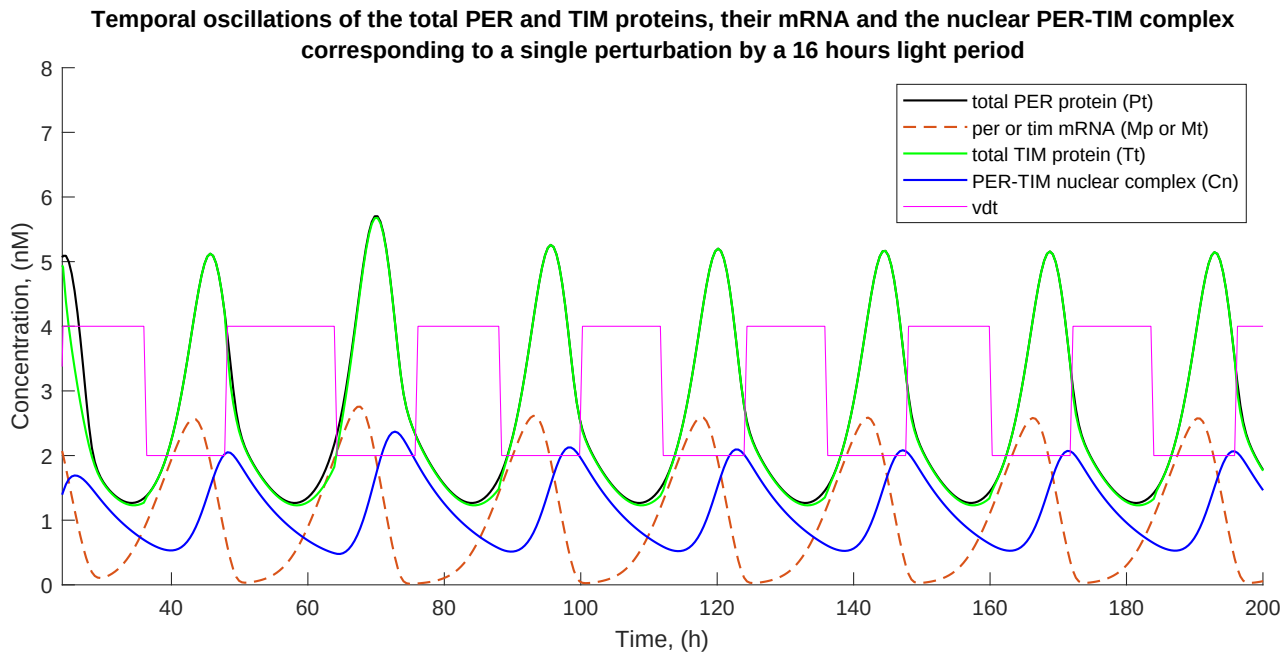

Figure 20: Magnified version of the effect of the 16 hours light period perturbation.

It is worth mentioning that the approximate day of return to normal rhythm was found by examining the graph and therefore does not carry complete accuracy due to uncertainties in the graph as well as in interpreting it. Moreover, the day of complete adjustment to the usual circadian rhythm was found by considering only entrainment with light cues, not including possible effects of additional sleep periods, meal consumption times, physical activity, and consumption of caffeinated drinks and medicaments.

Figures 21 and 22 show the temporal oscillations for the light profile and perturbation of the London (UTC) - San Francisco (UTC-7) flight and subsequent days for adaptation. The change in the light period on the day of the flight can be calculated similarly to the London - New York flight, keeping in mind that San Francisco is 7 hours behind London, and using the above-stated assumptions. The flight duration in this case is 11 hours, and if the flight departs at 0 h, upon arrival the time in San Francisco will be 11-7=4, leaving 12-4=8 more hours of light. To find the number of hours in the prolonged day, the time passed in the plane needs to be summed with the remaining hours of light upon arrival to San Francisco (11+8=19). Similarly to the previous case, the phase relationships between oscillating components and the light profile are affected by this change. Effects of the prolongation on the peaks of the analysed biological parameters are seen immediately after the perturbed day. In this case, since the light period of the day of the flight is prolonged by a further 3 hours from the London - New York case, the effects on the oscillations are similar to the effects described above for the 16 hours case but more drastic. The start of protein concentration growth corresponds to the middle of the prolonged day, and the minimum in the concentration of the nuclear complex is achieved before the start of the dark period. The protein concentration grows drastically, achieving the very elevated values of 7.13 nM for P_t and 6.73 nM

for T_2 in the first part of the night. The maxima of P_t and T_t differ significantly, due to growth in protein concentration being extended by 7 hours into the light period and hence the slower growth of T_2 during that time. The sharp rise in protein concentration leads to a maximum in nuclear complex concentration in the second part of the night, bringing the concentration of the proteins down and thus exhibiting self-regulation once again. The gradual adjustment by entrainment from light cues leads to a full settlement of the rhythm on the 15th day (day starting at 415 h) after the perturbed day. From these two cases, a conclusion can be made that for every time zone crossed, roughly two days are needed for adjustment considering only adjustment with entrainment by light cues.

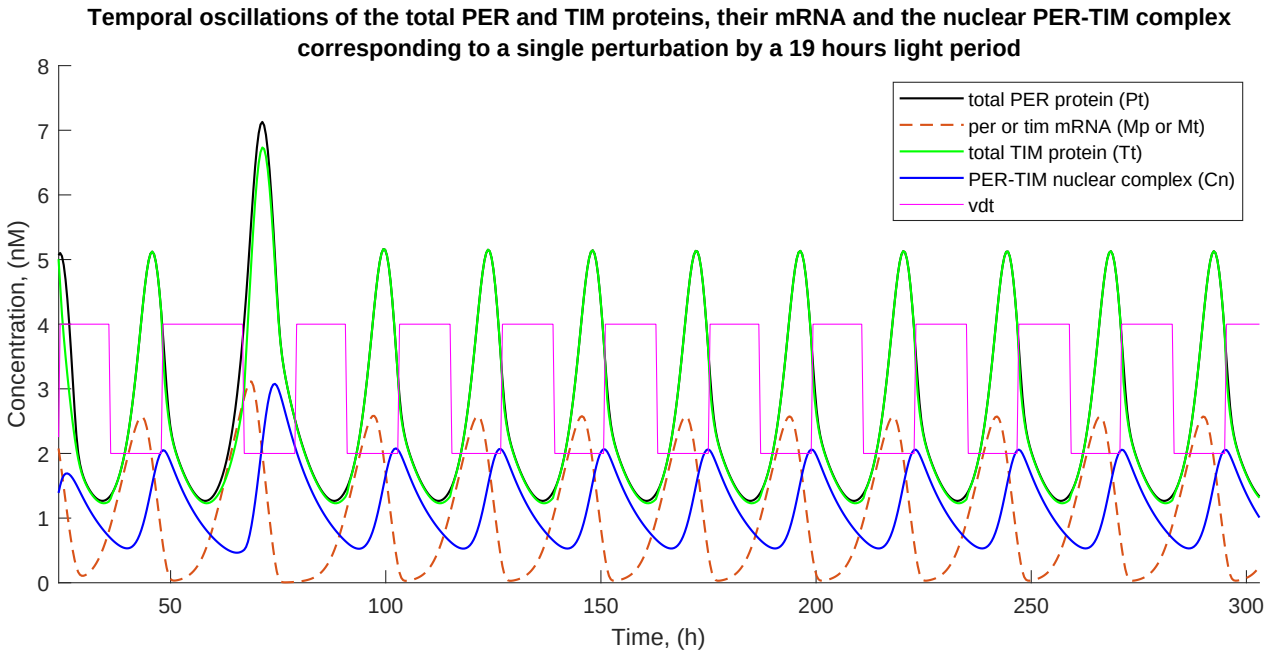

Figure 21: Temporal oscillations of the total PER and TIM proteins (P_t and T_t), their mRNA (M_p and M_t) and the nuclear PER-TIM complex (C_N) corresponding to a single perturbation by a 19 hours light period.

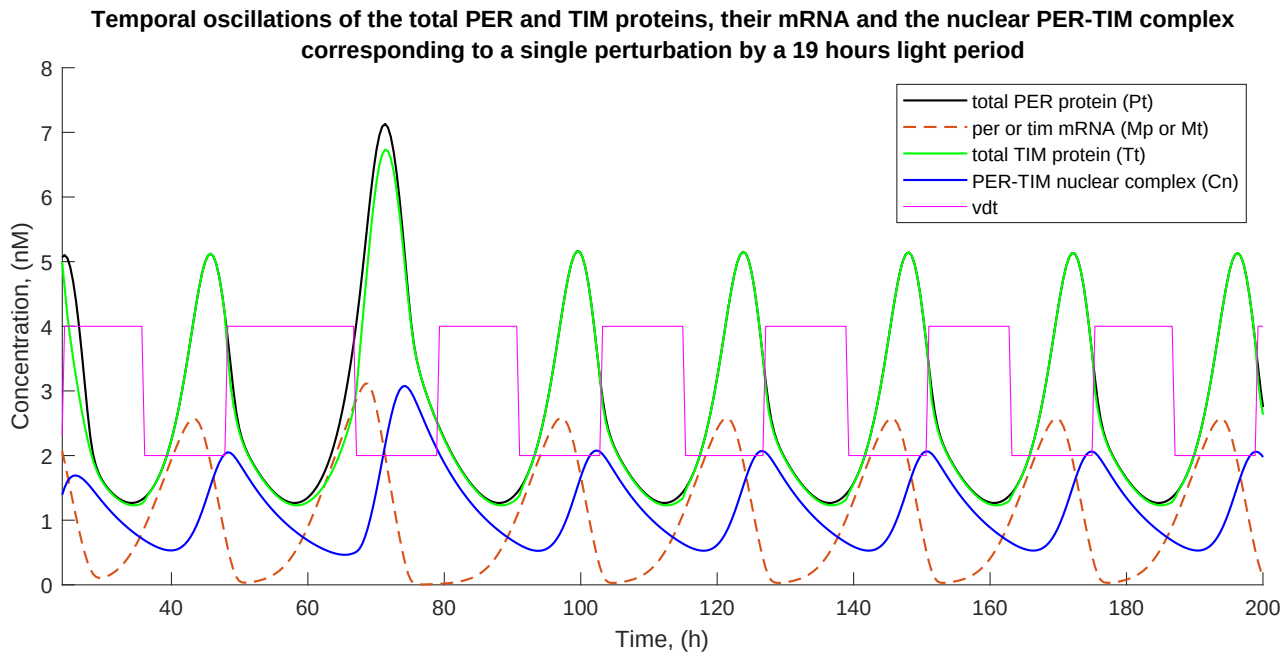

Figure 22: Magnified version of the effect of the 19 hours light period perturbation.

Graphs for perturbations from day flights can be compared to graphs of the effect of night flights to the same destinations in order to determine the best time of flight to minimise disruptions. The magnified graphs corresponding to the effect of night flights London - New York and London - San Francisco are presented in figures 23 and 24. The perturbed nights in both cases are taken to start at 60 h. The same calculations and assumptions were used for the night flights as for the day flights. Analysis of the graphs shows that the prolongation of the night period has the same effect on the circadian oscillations as the case of light period prolongation, however, the most perturbed peak occurs during the following night after the prolonged one. This is because the prolongation of the night shifts the phase of the light profile in relation to the oscillating biological parameters in the same way as the prolonged day does: for the 16 hours dark period the minimum in the nuclear complex coincides with the start of the night after the perturbed night, and for the 19 hours dark period the minimum in the nuclear complex occurs before the night after the prolonged night starts. Since the effect of the night prolongation on the relative phases of the nuclear complex and the light profile is the same as for the day prolongation, the same effect on the concentration of the total proteins is expected. Therefore, the same time is needed for adjustment considering only the effect of entrainment with light cues.

To conclude, the LD period of travelling does not affect the time needed for the entrainment of circadian oscillations to the new rhythm. However, when travelling by day, the most significant disturbance happens during the night directly following the disturbed day, and when travelling by night, the most significant disturbance happens during the night after the perturbed night. Therefore, day flights are the optimal choice to reduce the time needed for adaptation to the new time zone, as the disruption happens earlier than after a night flight.

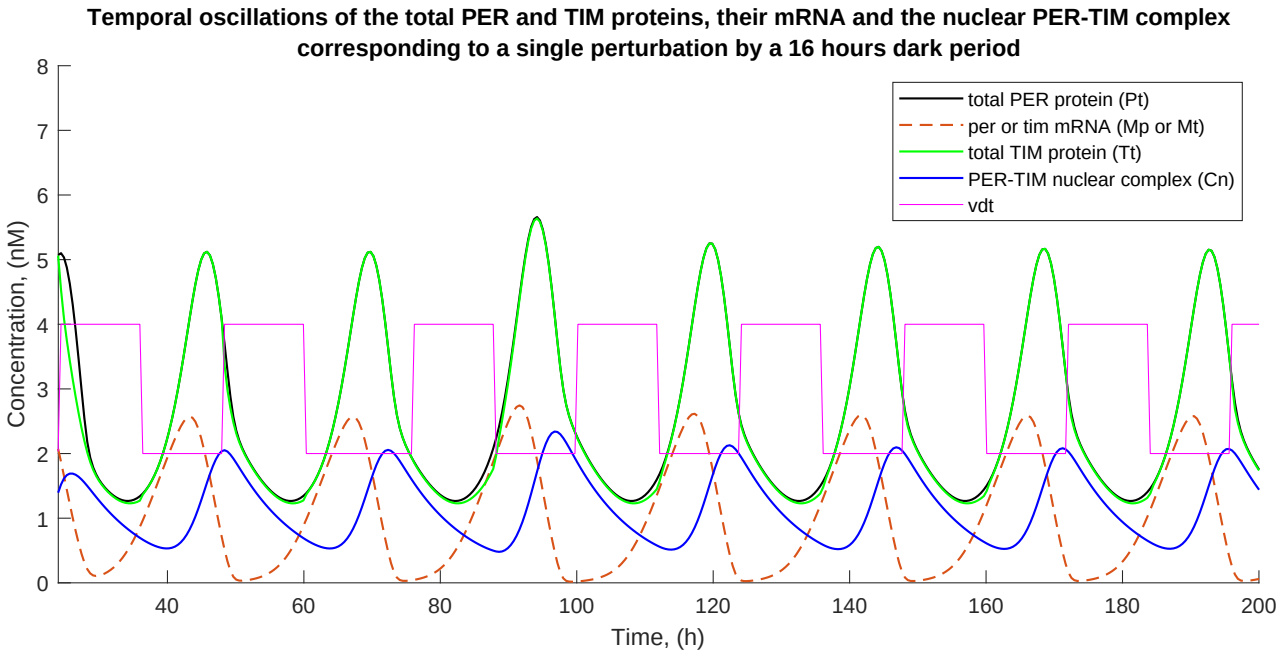

Figure 23: Magnified version of the effect of the 16 hours dark period perturbation.

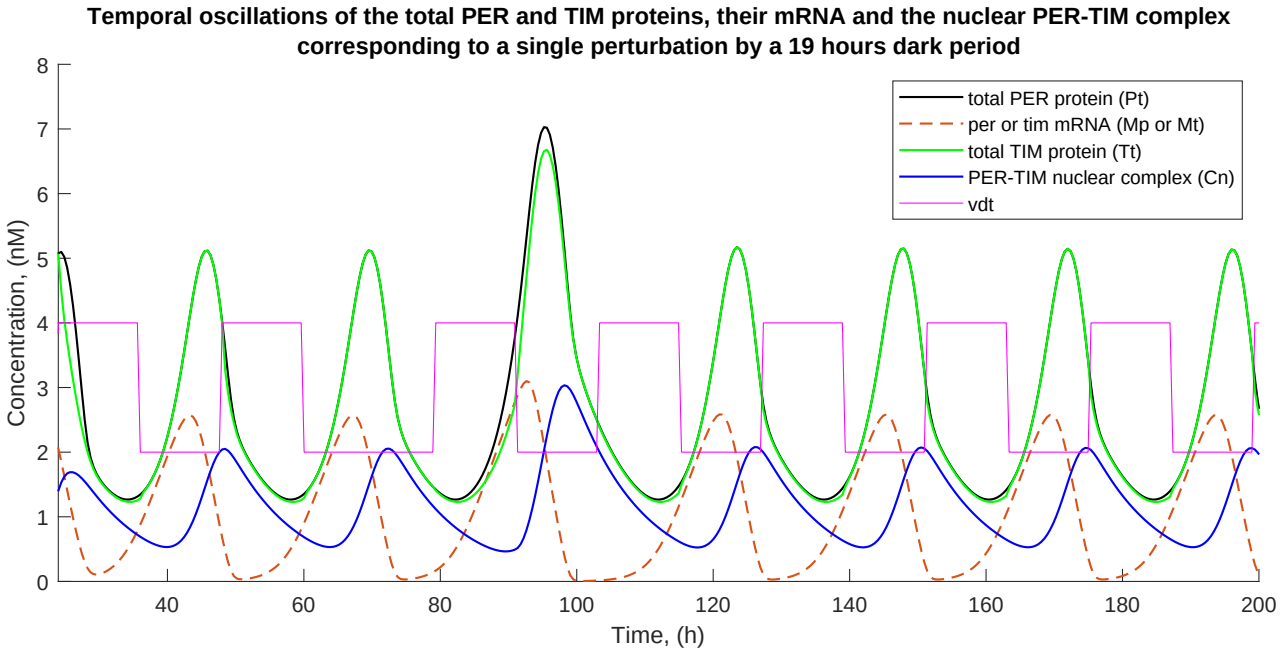

Figure 24: Magnified version of the effect of the 19 hours dark period perturbation.

5.1.2 Shift Work Simulations

The other major cause of circadian rhythm disruption mentioned in part 1.2 is shift work. This is a broad term that includes work shifts at unusual times of the day and inconsistent scheduling. In this section, the effect of a 4-day workweek for a night shift worker is simulated. For this simulation, certain assumptions were made. The subject was assumed to live by usual day hours during the rest days Friday to Sunday. During the workdays, Monday to Thursday, the sleep cycle of the subject was delayed by 10 hours and kept consistent as a 12:12 LD cycle throughout. The above assumptions can help accurately simulate for example the schedule of a worker having a night job Monday to Thursday and a day job for the remaining days, as then the worker would have to drastically convert between the two rhythms. The approximation of a 10-hour shift in the light profile for a 4-day night shift workweek was taken in accordance with the biological experiment conducted by Kervezee et al on the relation between shift work and the circadian misalignment of the human peripheral blood mononuclear cell transcriptome [54]. As before, light entrainment is the only factor for circadian rhythm adjustment considered in this simulation with acknowledgement of limitations of this approach that does not take into account effects of additional sleep periods, meal consumption times, physical activity, and consumption of caffeinated drinks and medicaments.

The graph produced in the simulation of a 4-day workweek of a night shift worker is shown in figure 25. As can be seen from the graph, transitions from rest days to workdays and vice versa cause drastic disruptions in the circadian rhythm by inducing a phase shift between the light profile and the biological oscillations during transition periods. The effects of such phase shifts and explanations are similar to those described in section 5.1.1 for jet lag, however, disruptions from night shift work are more drastic and frequent, as the disruption happens twice a week.

The prolongation of the day by 10 hours on Mondays for the transition between rest days and workdays leads to the protein growth start being shifted to the first part of the light period, and the nuclear complex concentration minimum occurring before the start of the dark period. Therefore, intense protein concentration growth happens in the second part of the light period, achieving a maximal value of 8.4 nM for P_t and 6.8 nM for T_t, in contrast to the unperturbed 5.1 nM for both.

The shortening of the night by 10 hours during the transition from workdays to rest days has the same effect of phase shift between the light profile and the biological oscillations. Due to the very short dark period, amounting to 2 hours, the light periods before and after the short night can effectively be regarded as one 26 hours long day for simplicity and correlation to previous explanations. A feature of the perturbed peak in this case is the very different maxima in P_t and T_t, 8.3 nM and 4.9 nM respectively, due to the very long light period during which T_t was forced to degrade. The graph also shows that the exceptionally drastic nature of such transition in rhythm leads to the peak of the day after the day of the transition being perturbed as well. A reasonable adjustment to the rest days rhythm happens only on the third rest day before being disrupted again by the first workday.

The above-described findings from the simple night shift work simulation suggest that the effect of

repeated adjustments from workdays, during which the subject is exposed to light at night, to rest days with conventional daytime light exposure have significant disruptive effects on the circadian rhythm, strongly affecting the rhythm of 3 days every week. Therefore, prolonged periods of such a schedule lead to continuous cycles of readjustments that require great work from the internal organs responsible for the smooth flow of the rhythm. Due to the significant effect of the circadian rhythm on the timing of many physiological functions of the organism, such prolonged drastic disruptions and consequent readjustments lead to malfunctions in the organs directly responsible for the physiological activities [55]. Such misalignments and malfunctions lead to various diseases mentioned in section 1.2, that can happen in any bodily system or affect the body as a whole [56]. Therefore, night shift workers require special work regulations to minimise the chance of being affected by serious diseases. Governments in certain countries recognise the negative effects of night shift work on working people's health. Currently, the UK has a shift work regulation that limits working hours for night workers and establishes the number of their rest breaks. It is enforced by law that night workers must not exceed an average of 8 working hours in a 24-hour period usually calculated over 17 weeks in contrast to the maximum of 9.6 for usual working hours [57]. This is an example of how results from research on the circadian rhythm can positively contribute to the wellbeing of certain groups of people. In general, to rapidly normalise the circadian rhythm after a night shift, a well-defined pattern of light and dark is needed. Thus, the subject needs to observe a regime in which periods containing light cues are followed by completely dark periods, even if this is inconsistent with the natural external environmental conditions at that time. This can be done by closing curtains or wearing a sleep mask, and by minimising exposure to blue light from devices during the new dark periods.

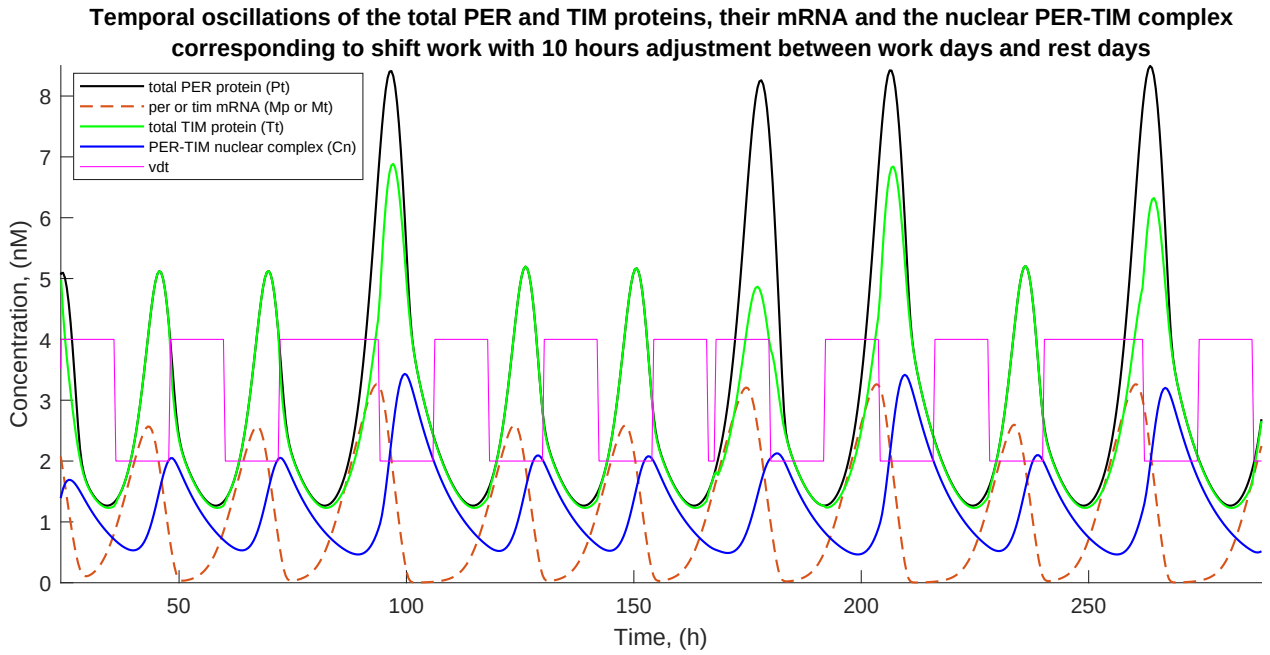

Figure 25: Temporal oscillations of the total PER and TIM proteins (P_t and T_t), their mRNA (M_p and M_t) and the nuclear PER-TIM complex (C_N) corresponding to a night shift work simulation with 10 hours adjustment between workdays and rest days.

5.2 Stochastic Light Effect simulations

As stated above, stochastic simulations offer a more realistic picture of the system's behaviour compared to deterministic simulations because they account for discreteness of events and randomness of time between reactions [58], which is especially useful for including the effect of molecular noise in the model. However, this randomness is accounted for in the condition of a constant external environment that does not change the system parameters during the time between reactions as light does. This is a limitation that needs to be addressed when stochastically simulating the behaviour of the system in a non-constant LD cycle. In this paper, a quite simple computational method useful for modelling short evolution times was used to overcome this difficulty. The principle of this method lies in breaking the overall modelled time of evolution into smaller intervals and taking the light parameter to be constant in every one of them, making the calculation with the Gillespie method possible. This approach is valid as every smaller interval involves a separate application of the Gillespie model, thus making it possible to vary the light parameter between the intervals without the need for variation during the intervals, which would go against the above-described condition of the stochastic model. In this paper, the overall time interval modelled was 120 hours, so 10 shorter time intervals each of 12 hours were used to simulate the 12:12 LD cycle stochastically. The endpoints of every time interval were taken as the initial points of the next one. This is a simplified method, however, it is useful for modelling a short time evolution of the system using already existing computational methods. Results obtained using the stochastic model can be compared to deterministic predictions.

Due to stochasticity, every run of the code produces a slightly different line in the graph. Figures 26-29 show *per* or *tim* mRNA, total PER and TIM protein, and nuclear PER-TIM complex oscillations against time for the stochastic and deterministic cases for system size Ω=600. The stochastic model results are seen to closely follow the shape of the deterministic prediction, however, they exhibit perturbations of the line due to the effect of molecular noise. The clear presence of the previously-mentioned phase drift of stochastic results in relation to deterministic results can also be noted.

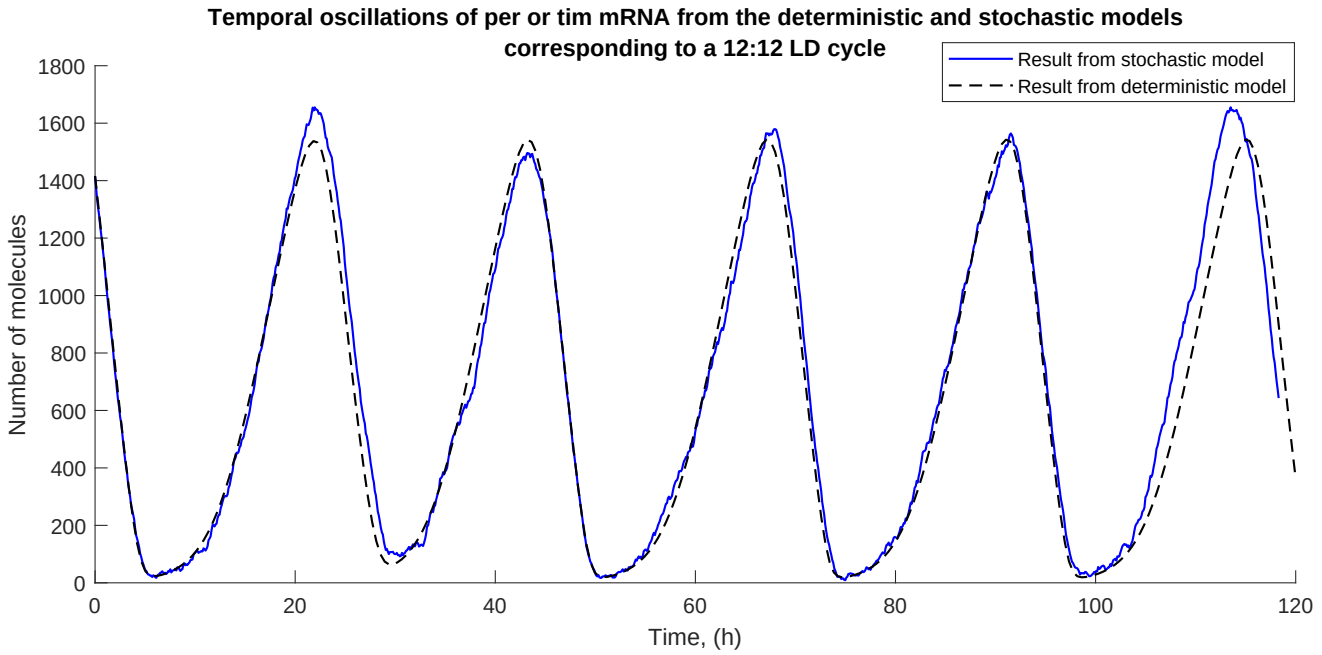

Figure 26: Temporal oscillations of the *per* or *tim* mRNAs (M_p and M_t) from the deterministic and stochastic models corresponding to a 12:12 LD cycle for system size Ω=600.

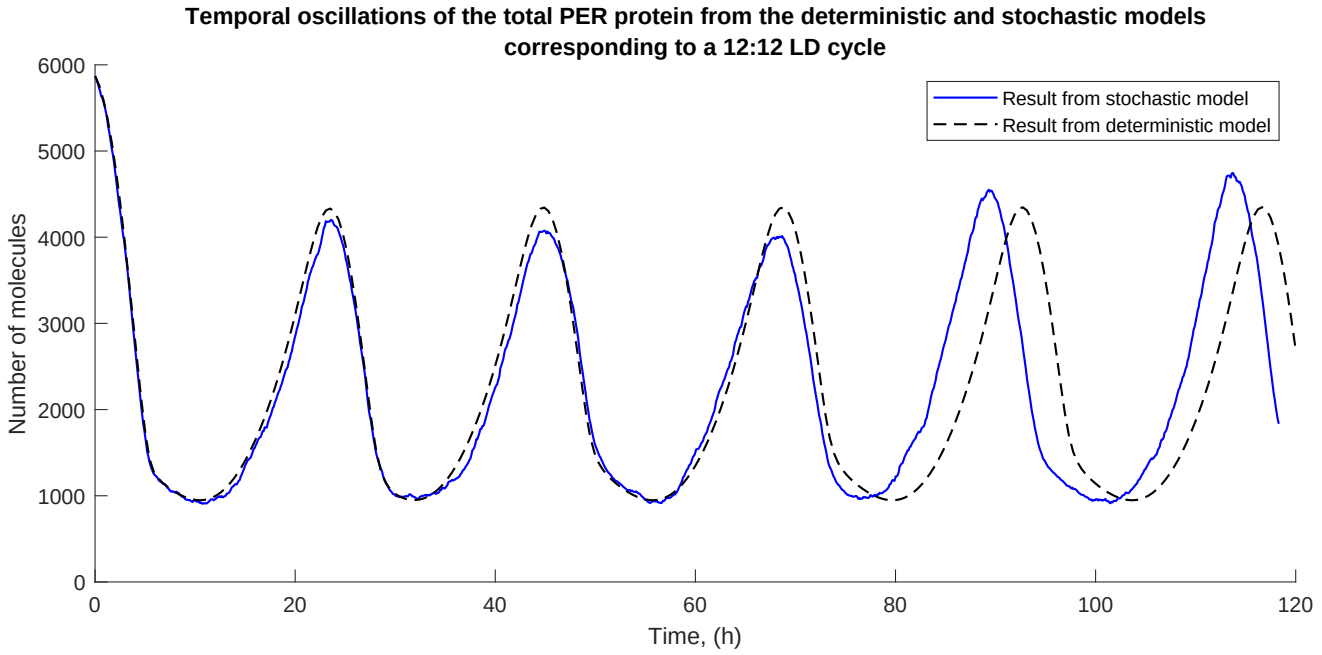

Figure 27: Temporal oscillations of the total PER proteins from the deterministic and stochastic models corresponding to a 12:12 LD cycle for system size Ω=600.

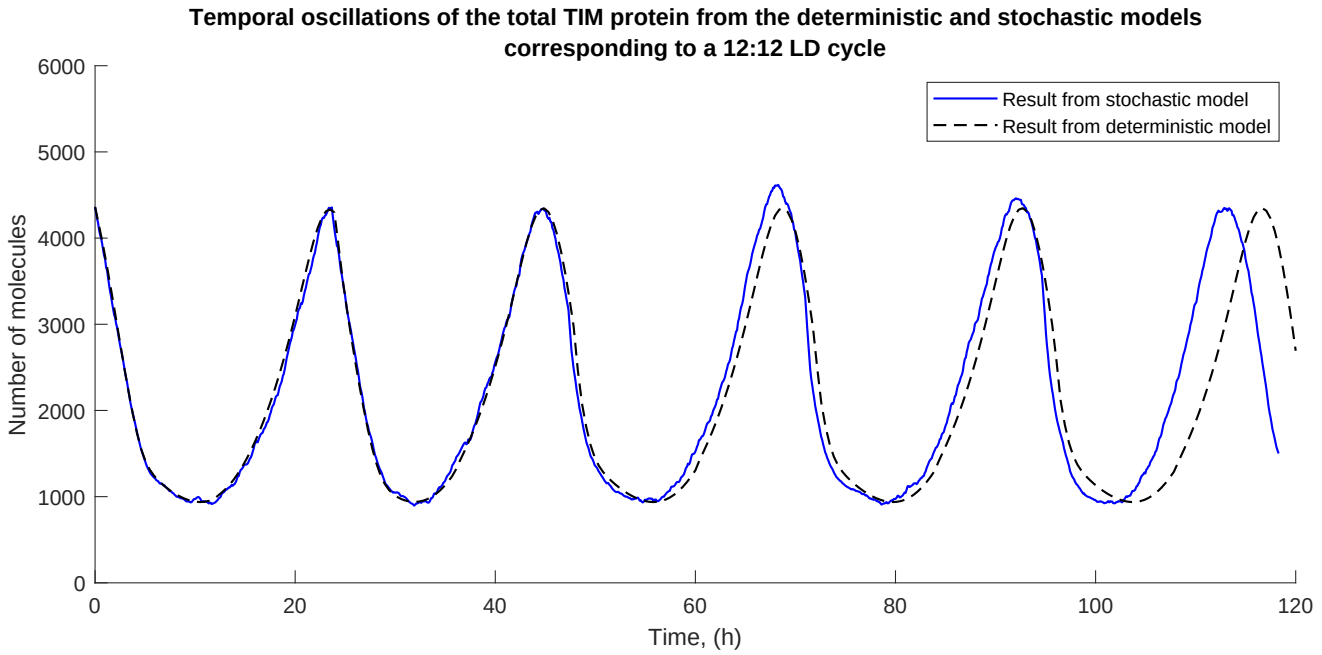

Figure 28: Temporal oscillations of the total TIM proteins from the deterministic and stochastic models corresponding to a 12:12 LD cycle for system size Ω=600.

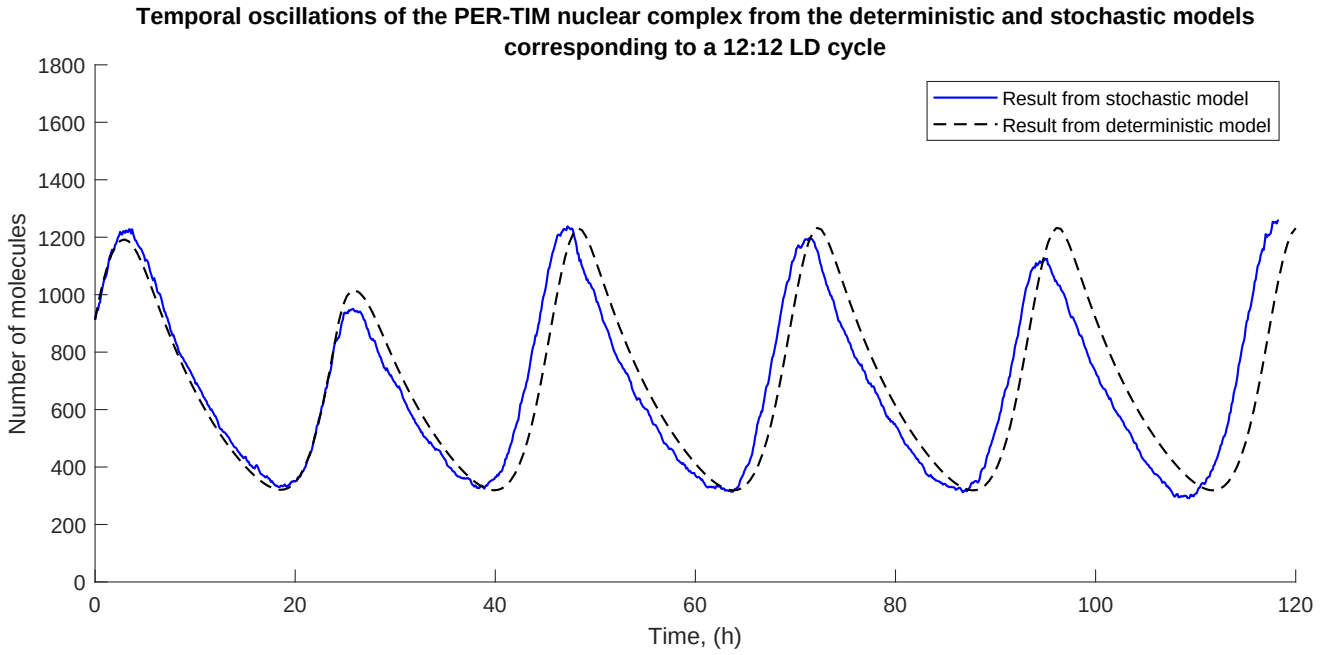

Figure 29: Temporal oscillations of the PER-TIM nuclear complex from the deterministic and stochastic models corresponding to a 12:12 LD cycle for system size Ω=600.

The method used in this paper for stochastic simulations is not claimed to yield perfectly accurate results accounting for all sources of molecular noise and perturbations on the system but is rather a starting point or a foundation for more complicated and more realistic methods. The *Slow Input Approximation* method (SIA) and the step-by-step integration method will be further explored in section 6.1 along with a new method called *Extrande* that is claimed to overcome limitations of currently employed methods [59].

6 Conclusion

To conclude, the paper examined the methods currently employed in research for building mathematical models of the Drosophila circadian rhythm.

To do so, the first part was focused on defining biological rhythms in general along with their significance in adaptation to the environment, needed for survival. The difference between endogenous and exogenous rhythms was presented, in order to state that the circadian rhythm is endogenous with a period of roughly 24 hours and can be entrained by external light cues. Research results showing the strong correlation of the circadian rhythm to daily physiological functions of living organisms were included, demonstrating that misalignment between external and internal rhythms can be dangerous for health, emphasising the importance of research on this topic.

The second part was focused on providing a base for the mathematical model by outlining a simplified structure of its functioning, as well as detailing biological processes involved in the system. The main molecular processes related to the regulation of circadian oscillations along with the main clock genes *per* and *tim* were then presented through an extensive description of the negative feedback loop of the Drosophila. A possible way of extension of these simple biological principles to the more complicated mammalian system was also provided in order to show the close correlation between the two, providing a framework for future research on the mammalian system that can build on the principles presented in this paper.

The parts explaining biological processes provided sufficient information to progress to the mathematical description of the circadian rhythm. This was started by providing a clear distinction between the deterministic and stochastic models that can be implemented to simulate the behaviour of this system. The main mathematical features of both models were presented in subsequent parts concerning the Goodwin model expanded by a Michaelis-Menten term, and the stochastic chemical kinetics equations. Using the MATLAB code for the deterministic model, a graphical representation of the limit cycle of the repression mechanism between the nuclear PER-TIM complex and the production of mRNAs from genes was obtained. This was then compared to results from the stochastic model for three different values of the system size (Ω= 200, 600, 1000) that simulate the effect of molecular noise on the system. A comparison of results from the two models showed that the general shape of the limit cycle is unaltered, however, depending on the system size, graphs can exhibit different levels of perturbation with smaller values of the system size leading to a stronger effect of the perturbation.

The subsequent part of the paper focused on simulating the effect of light on the Drosophila circadian rhythm by means of the maximum T_2 degradation rate parameter v_{dT}. This was taken to be 2 for darkness and 4 for light. The effect of this parameter on circadian oscillations provided a framework for constructing simulations of real-life situations. By using a square-wave light profile with equal light and dark lengths, daily oscillations of the proteins, mRNAs, and the nuclear complex were simulated and found to be consistent with results from biological research, confirming the accuracy of the mathematical model even with simplifying assumptions. The light profile framework was then used to investigate the properties of the two main sources of disruptions mentioned in this paper: jet lag and shift work. The simulations were constructed by using several simplifying assumptions, such as considering only entrainment with light cues, not including possible effects of additional sleep periods, meal consumption times, physical activity, and consumption of caffeinated drinks and medicaments. By simulating day and night flights to two destinations with different time zones and analysing graphical results, a conclusion was made that for every time zone crossed, roughly two days are needed for adaptation to the new rhythm. Moreover, the conclusion was made that day flights are the best choice to reduce the time needed for adaptation to the new time zone, as the disruption happens earlier than after a night flight. The simulation of the effect of night shift work on the circadian rhythm confirmed the negative effect of this type of employment by analysing the disruptions caused by transitions between workdays, when the person is exposed to light at night, and rest days, when the subject is exposed to light at usual times. A possible way to quickly normalise the cycles after disruption by night shift work was suggested, which involved observing a regime of alternating light and dark periods by creating them artificially if such cycle is inconsistent with the external LD cycle.

The final part of the paper was focused on methods for stochastic simulation of the effect of light on the system. Graphs were obtained by varying the light parameter and running the code for small time intervals, which is a simple method appropriate for modelling a short time evolution of the system. The stochastic model results were compared to the corresponding deterministic results, which showed that the two methods produce the same line shape but can have a phase drift between them.

Overall, the paper presented a detailed explanation and analysis of methods for simulation of the Drosophila circadian rhythm using the deterministic and stochastic models. The graphical representations of the oscillations in concentration of the elements involved in the feedback loop in different conditions obtained from the models can be useful for biological and medical research, paving the way for experiments involving extreme or unusual conditions which cannot be easily conducted in a laboratory environment. The framework introducing the effect of light into the model was used to see the changes in concentration of the elements in constant darkness, a 12:12 LD cycle, and when exposed to light pulses, as well as to model jet lag and night shift work. A major finding of the paper involves the time of circadian rhythm disruption after a flight. Simulations suggest that long day flights induce a disruption during the night directly following the prolonged day rather than during the next one as happens with night flights, which can serve as a reason to pick day flights when travelling long distances. Another major finding lies in the visual confirmation of the negative effect of night shift work on the circadian rhythm, which can potentially serve as an additional reason to support night shift workers with special work regulations. The framework

introducing the effect of light presented in the paper can be extended to simulate any shape of the LD cycle without the need to perform harmful biological experiments, thus expanding the possibilities of scientific research in the field.

6.1 Further Work

The methods used for modelling the Drosophila circadian rhythm from the present paper can serve as a foundation that can be extended to simulations of the mammalian circadian rhythm, from which results directly concerning humans can be obtained. This can be done by modifying the Drosophila PER-TIM loop into a PER-CRY loop, as described in section 2.4. The asymmetrical loop case can be explored by changing the relative parameters of the two parts of the loop, which would bring the model closer to reality. Moreover, further work on the effect of light on the circadian system can be conducted by relaxing the assumption made in part 5.1. This would increase the complexity of computations required for modelling the oscillations while bringing the simulations closer to reality. Effects of additional sleep cycles, timed meal consumption, caffeine, and medications can be incorporated into the model as well, to see the full picture of the dependence of the oscillations on external cues.

Finally, methods for advanced stochastic simulations mentioned in 5.2, can be implemented in the model to minimise errors. Currently, there are two main methods used for such advanced simulations. The first one, called *Slow Input Approximation* method, involves making the approximation that the input parameter remains piecewise constant over the time between any two reactions [59]. This is a good approach if the dynamic inputs are changing over a long timescale, so that over two close time points they are almost constant. This method has a major limitation in the fact that it keeps track of the past values of inputs without doing it for the present instantaneous value. This goes against the principles of Markovian processes lying at the core of accurate stochastic simulations, in which the probability of new events depends only on the value obtained in the previous event [60]. This leads to large errors of the SIA method. In the second method, the evolution of the system is found by numerical step-by-step integration of the master equation until the integral reaches a predefined value. This method generally gives accurate results and can have time-varying inputs, however, it poses computational difficulties in its execution.

A new method published in 2016 called *Extra Reaction Algorithm for Networks in Dynamic Environments* or simply *Extrande* was claimed to have the potential to overcome the limitations of previously used methods, bringing simulations even closer to reality. The principle of operation of this method lies in the introduction of an additional reaction channel into the system, which is not present biologically and thus does not change the number of molecules. The propensity function introduced in section 3.4 is taken to vary over time in this case, giving a constant value between events of the overall system when summing the propensities of all the reaction channels. This summed propensity value must correspond to the upper bound of the possible propensity in a certain time interval of the original system. In order to perform these calculations, the assumption needs to be made that while the input affects the behaviour of the network, the effect

of the network on the input is negligible. This makes it possible for Extrande to find the upper bound of the total propensity for a specified time interval by considering the future trajectory of the input parameter [59]. Therefore, it can be seen that the process of finding optimal methods for approaching reality as closely as possible using mathematical modelling methods is still ongoing and therefore is an opportunity for further scientific work.

Appendix 1

Ten kinetic equations used in the deterministic model for the Drosophila circadian rhythm:

$$\frac{dM_P}{dt} = v_{sP}\frac{K_{IP}^n}{K_{IP}^n + C_N^n} - v_{mP}\frac{M_P}{K_{mP} + M_P} - k_d M_P$$

$$\frac{dP_0}{dt} = k_{sP}M_P - V_{1P}\frac{P_0}{K_{1P} + P_0} + V_{2P}\frac{P_1}{K_{2P} + P_1} - k_d P_0$$

$$\frac{dP_1}{dt} = V_{1P}\frac{P_0}{K_{1P} + P_0} - V_{2P}\frac{P_1}{K_{2P} + P_1} - V_{3P}\frac{P_1}{K_{3P} + P_1} + V_{4P}\frac{P_2}{K_{4P} + P_2} - k_d P_1$$

$$\frac{dP_2}{dt} = V_{3P}\frac{P_1}{K_{3P} + P_1} - V_{4P}\frac{P_2}{K_{4P} + P_2} - k_3 P_2 T_2 + k_4 C - v_{dP}\frac{P_2}{K_{dP} + P_2} - k_d P_2$$

$$\frac{dM_T}{dt} = v_{sT}\frac{K_{IT}^n}{K_{IT}^n + C_N^n} - v_{mT}\frac{M_T}{K_{mT} + M_T} - k_d M_T$$

$$\frac{dT_0}{dt} = k_{sT}M_T - V_{1T}\frac{T_0}{K_{1T} + T_0} + V_{2T}\frac{T_1}{K_{2T} + T_1} - k_d T_0$$

$$\frac{dT_1}{dt} = V_{1T}\frac{T_0}{K_{1T} + T_0} - V_{2T}\frac{T_1}{K_{2T} + T_1} - V_{3T}\frac{T_1}{K_{3T} + T_1} + V_{4T}\frac{T_2}{K_{4T} + T_2} - k_d T_1$$

$$\frac{dT_2}{dt} = V_{3T}\frac{T_1}{K_{3T} + T_1} - V_{4T}\frac{T_2}{K_{4T} + T_2} - k_3 P_2 T_2 + k_4 C - v_{dT}\frac{T_2}{K_{dT} + T_2} - k_d T_2$$

$$\frac{dC}{dt} = k_3 P_2 T_2 - k_4 C - k_1 C + k_2 C_N - k_{dC} C$$

$$\frac{dC_N}{dt} = k_1 C - k_2 C_N - k_{dN} C_N.$$

Definitions:
M_P and M_T - *per* and *tim* mRNAs,
P_0, P_1, P_2 - three forms of phosphorylation of the PER protein,
T_0, T_1, T_2 - three forms of phosphorylation of the TIM protein,
C - PER-TIM cytosolic complex,
C_N - PER-TIM nuclear complex.

Parameter values used in the paper:
$v_{sP} = v_{sT} = 1nMh^{-1}, v_{mP} = v_{mT} = 0.7nMh^{-1}, K_{mP} = K_{mT} = 0.2nM, k_{sP} = k_{sT} = 0.9h^{-1}, v_{dP} = v_{dT} = 2nMh^{-1}, k_1 = 0.6h^{-1}, k_2 = 0.2h^{-1}, k_3 = 1.2nM^{-1}h^{-1}, k_4 = 0.6h^{-1}, K_{IP} = K_{IT} = 1nM, K_{dP} = K_{dT} = 0.2nM, n = 4, K_{1P} = K_{1T} = K_{2P} = K_{2T} = K_{3P} = K_{3T} = K_{4P} = K_{4T} = 2nM, k_d = k_{dC} = k_{dN} = 0.01h^{-1}, V_{1P} = V_{1T} = 8nMh^{-1}, V_{2P} = V_{2T} = 1nMh^{-1}, V_{3P} = V_{3T} = 8nMh^{-1}, V_{4P} = V_{4T} = 1nMh^{-1}$.

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