ANALYSIS OF AVIAN MIGRATION USING SCHEPER'S MODEL

A Thesis

by

SEYED HOMAYOON SHOBEIRI

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Chair of Committee,	Jay R.Walton
Committee Members,	Peter Howard
	Mark Zoran
Head of Department,	Emile Straube

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ABSTRACT

Many species of birds migrate nocturnally. In nocturnally migrating birds, endogenous circadian and circannual rhythms serve as biological pacemakers (clocks). They are responsible for an individual's migratory behavioral pattern (Zugunruhe). Circannual rhythms provide the major basis for the initiation of migratory restlessness both in autumn and spring. Circadian rhythms on the other hand, play an important role in regulating the increase of nocturnal activity that is necessary for flying long distances. The significance of these endogenous factors is evident in migrating birds in the sense that, zugunruhe keeps happening even in the absence of external inputs. Plasma melatonin concentration is crucial to this phenomenon. Melatonin concentrations in most migratory birds increase at night regardless of whether individuals are day or night active. In the course of migratory seasons, many birds show restlessness and excessive activity during night. In migratory populations, night level of melatonin is lower during the migratory period, when birds show nocturnal restlessness, than before and after this period, when birds do not show nocturnal activity. The operation of this endocrine system of oscillators is far too complex to understand by intuition so we unavoidably have to resort to abstractions. Mathematical models are therefore essential in understanding the mechanism of the components of this system and to further determine the dynamics of these periodic oscillators. Scheper model is a set of delay differential equations which describes the circadian clock. This model is based on the total duration of the chain reactions and the nonlinearity between input and delayed output of the protein synthesis negative feedback loop. In this study, two systems of scheper equations were coupled to produced circannual oscillation depicting the seasonal change in the melatonin level of nocturnally migratory birds. The coupled system displayed realistic behavior of circannual rhythm with respect to period and entrainment.

To my mother and father
who are supportive every step of the way.

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1. INTRODUCTION AND LITERATURE REVIEW

Zugunruhe can be seen as a circannual oscillator switching between two circadian pacemakers: the pineal gland and the suprachiasmatic nuclei (SCN) of the hypothalamus. Most circadian oscillators are thought to exist within single cells. Therefore, transcriptional-translational feedback loops are central to understanding their mechanism. The problem is to study the dynamics of coupled circadian pacemakers and understand the interaction by which they could create an oscillator with semi-annual period.Mathematical modeling can be used for prediction. Creation of a model requires precise definitions of the variables involved in the system, the response of the system to stimuli, and interaction within components of the system. Just as the conduct and design of experiments are subject to standard procedures, so also should mathematical modeling and analysis. Therefore, our attempt is to design and investigate a mathematical model to represent the regulatory network involving circadian and circannual pacemakers by which they can regulate each other reciprocally. This model is based on Scheper's model and is a basis for investigating zugunruhe in migratory birds.The proposed mathematical model can be viewed as representational of the part of the endocrine system responsible for zugunruhe, test biological hypotheses related to migratory behavior, organize and justify the experimental data gathered in the labs, lay a foundation to conduct formal statistical analyses of parameters, and make predictions about properties of this network that are not apparent. Interactions of mathematicians with life-scientists can result in the design and execution of experiments that should produce useful results not only for the mathematical models but also for basic understanding of biology.How does this model represent the interaction between two short-period oscillators forcing one long-period pacemaker? How does the model relate to biology and endocrinology? How sensitive are the parameters? Is the model stable?

Multiple researches have demonstrated that cells extracted from the SCN in vitro generate circadian rhythm in electrical activity and other functions. Therefore, the circadian oscillation of the pacemakers does not exclusively result from the interaction between cells but each cell can produce circadian signals on their own thus, contributing to the pace of the oscillators. The basic current modeling concept is that of a negative feed-back loop (Transcription-Translation loop, or TTL), in which it is understood that the proteins (with some time lag) attenuate their own production. The first model for a biological oscillator is Goodwin's published in 1965 which includes a protein repressing the transcription of its own gene and produces the sustainable auto-oscillation. Later, this model was modified by Ruoff and Rensing where and end-product which represses the mRNA synthesis was taken into account. (Lema et al.2000) abstracted the negative feed-back loop into a mathematical model using a system of delay differential equations (DDEs). This model basically claims that the rate of change for protein content is related to the rate of gene activity. (Scheper et al. 1999) conceptualized the same model using DDEs but with 3 major differences that Lema's model. In scheper's model, messenger RNA was used instead of gene activity. Moreover, two assumptions were made: firstly, not all mRNA leads to the production of new protein but some of it decays. Secondly, nonlinearity in the protein production cascade was also taken into account as for instance when multiple mRNA molecules are the substrate in the production of protein. Many studies base on more detailed descriptions of the relevant cellular processes in the generation of circadian oscillations have been published. Per-Tim model of Leloup and Goldbeter expanded scheper's model using 4 proteins and a series of intermediate transcription-translation loos. Other studies to include the activity of every parts of the intracellular clock have been done by Forger and Peskin. In these models the delay term was replaced by the kinetics of the various components. Roenneberg and Merrow designed a network of coupled feed-back loops to study the characteristics of circadian rhythm. Nevertheless, due to stochastic variations in molecular concentrations within cells, these models tend to contribute noise

in circadian rhythms. Eberhard Gwinner (1993), has studied circannual and circadian rhythmicity involved in migration using garden warblers. In his research, he emphasized on the important role of plasma melatonin oscillation in migratory regulation of migrating avian. In their paper published in 2006, Lesie and Siegelmann analyzed the dynamics of a multistage circadian system using the scheper's model. Wu et al. 2000 , studied a coupled oscillatory model representing avian circadian regulatory system. Based on this study in sparrows pineal gland dampens more rapidly that does the SCN. As a result of this research, the importance of coupled system involving both pineal gland and SCN was brought to attentions. As illustrated above, models of circadian rhythm generation share a common element of feed-back loop. What has been missing in all of the mentioned studies is the relation between circadian and circannual pacemaker and their auto-regulatory mechanism as a verifiable mathematical model. In our model we try to establish this connection in a form of mathematical model.

1.1 BIOLOGICAL BACKGROUND

1.1.1 Biological Oscillators

Oscillations happen throughout every phenomena whereas it is physical or biological. Cells are dynamic systems which continuously change their state generating motion and intrinsic changes.Interaction between oscillations yields process of entrainment or in the other word, synchronization, which is different from a stimulus-response pattern. Oscillators are responsive to both external and internal stimuli at some times during their cycle (Gwinner 1996). If the stimulus is strong enough the oscillator can be driven meaning that it restarts its cycle every time it encounters a stimulus. Since biological oscillators are amenable to qualitative analysis, elements essential to generate the oscillation can be identified and the knowledge about the underlying mechanism can be enhanced by qualitative analysis.

Every biological oscillator has two essential components: 1) an inhibitory feedback loop, which includes one or more oscillating variables and 2) group of stimuli enforcing delay in the feedback loop causing the oscillating variables to disrupt the steady-state value before any measures to inhibit feedback is fully effective.The period of biological oscillations range from milliseconds to years whether they are based on biochemical, neuronal or physical process.

Biological oscillators are designed to be entrained (synchronized) to cycles which are either endogenous or environmental (exogenous). Most important endogenous cycles are the variations in the level of hormonal concentration over a specified course of time. Other important external cycles are molt, hyperphagia and change in reproductive state (Helm 2006). Noticeable environmental cycles that cause an entrainment among endogenous oscillators are the rotation of the earth, lunar cycle, tides, seasonal activities and migration. Biological oscillators run "free" with their endogenous period in lack of external(environmental) cues. The endogenous period is not necessarily the same as environmental period but it is usually in close proximity.

Depending on its ever-changing phase, an oscillator responds differently to a synchronization process. There are three kinds of response to any stimulus. In some phases the oscillator is advanced relative to stimulus. In some other phases the oscillator is delayed and finally, in some cases it may not even respond at all. Phase Response Curves(PRCs) demonstrate these response characteristics. Responses are determined by number of properties including: period, strength and duration of the entraining stimulus are the prime important factors. Biological oscillators as well as having a characteristic period also have a characteristic amplitude.Mathematically, considering their trajectories in the phase space, it correspond to a limit cycle. Therefore, when a perturbation is enforced they will inevitably come back to their limit cycle. Their dissipative mechanism tend to strengthen oscillations that become too small and damp oscillators that grow too large.

Biological oscillators have the following properties:

• The period of a synchronized oscillator averaged over several cycles is the same as the entraining cycle.

• An oscillator goes through several cycles before it becomes steady state oscillator.

• When the external stimuli are strong enough, the oscillator will be driven by the entraining stimuli.

• Oscillators are entrained within a "range of entrainment" which depends on period of entraining cycle, strength of entraining cycle and duration of entraining cycle.

• Any Oscillator can be entrained whether it is damped with no self-sustained period or an oscillator with sustained period.

• When oscillator is submitted to synchronized cycles of different periods its phase will be altered ranging from more advanced in long cycles to more delayed in short cycles.

1.1.2 Feedback Loops

In some processes information regarding the past or present influences the phenomena in the present and even in the future.Feedback happens between two components when they affect each other as illustrated below:

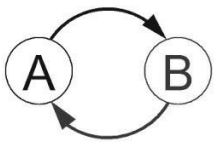

Figure 1: Simple feedback loop

Feedback divides into two different types:

• Positive feedback : This is a process in which even a small perturbation of a system include a change in the magnitude of components of the system. Considering the above figure this basically means that **A** produces more **B** which in return produces more of **A**. Positive feedback tends to cause system instability and increase in the oscillation of the system forcing the system to diverge from equilibrium. Parameters of this system accelerate towards larger values disrupting the system or forcing the system to adjust into a new stable state. Positive feedback is well studied in gene regulation specifically its association with bi-stability. In gene regulation feedback happens when a gene activates itself directly or indirectly via a double negative feedback loop. Positive feedback in gene regulation have a significant physiological consequences. As and example in the generation of nerve signals, membrane of a nerve fiber causes small leakage of sodium ions via sodium channels resulting in a change in the sodium level causing more opening of channels. Another important positive feedback mechanism in gene regulatory system is enzyme induction whereas a process in which a molecule induces the expression of an enzyme.

• Negative feedback: This mechanism is used in a system in which the results of a change act to reduce itself as to reduce changes. This idea is illustrated below:

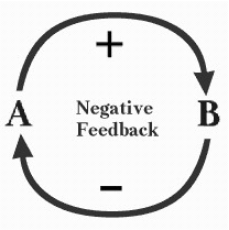

Figure 2: Negative feedback loop

Negative feedback tends to make a system self-regulating. Self-regulatory systems can produce stability and reduce the adverse effect of fluctuations. Self-regulatory mechanisms have been around since antiquity. For example, in biochemistry one set of chemicals force the system in one direction whereas other set of chemicals drives it in an opposing direction to result equilibrium. This process is called *homeostasis* in biological context.

An example for gene regulation governed by negative feedback is protein synthesis as illustrated below:

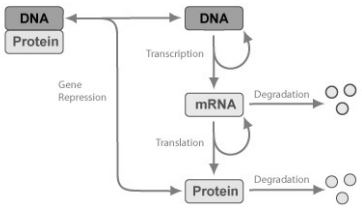

Figure 3: Protein synthesis via negative feedback

In the above diagram, DNA is transcribed in the nucleus. The resulting mRNA is transported outside the nucleus into cytoplasm and translated by ribosome into a protein. When the protein level in cytoplasm increases, the negative feedback mechanism slows the rate of DNA production and therefore slows the rate of inter-nucleus transcription and as a consequence the rate of protein synthesis is decreased.

1.1.3 Circadian Rhythms

Circadian rhythm is an almost 24 hours cycle in the physiological process of any living being. What this means is that this process is an endogenous, entrainable oscillation of about 24 hours. The word *Circadian* comes from the Latin word *circa*, meaning "approximately", and *diem*, meaning day. Circadian rhythms are endogenous meaning it is a built-in, self-sustained feature of any being. Moreover, Circadian rhythms are adjustable (entrainable) to external cues called *Zeitgeber*. Most important zeitgeber is daylight. The circadian system maintains a 24 hours rhythm that controls the functionality of many physiological processes.

As a general example, tissues throughout the body of any being exhibit circadian rhythms forming a multi-oscillatory system. Survival and quality of life of any living being is dependent on the multistage structure of circadian system which integrates external stimuli with the circadian clock to create a daily activity pattern. Circadian rhythms are endogenously created but can be adjusted by environmental stimuli. The first recorded observation of an endogenous circadian oscillation was by Jean-Jacques d'Ortans de Mairan,French scientist, in 1729. He studied the movement of leaves of the plant *Mimosa Pudica* and noticed that this 24 hours pattern continues even in the darkness.
Biological circadian rhythms have two main properties:

1. Endogenous "free-running" period lasting approximately 24 hours: The period of the circadian rhythm in constant conditions is called the "free-running" period and denoted by τ. A rhythm is not endogenous unless it is tested and persists in conditions without environmental periodic input. In daily active (diurnal) animals, τ is greater than 24 hours

whereas in nocturnal (night active) animal, τ is less that 24 hours.

2. The circadian rhythm is entrainable: Biological rhythms are adjusted when they are exposed to external stimuli such as heat and light. This process is called entrainment and these external stimuli are called zeitgeber. Circadian rhythms let organisms to anticipate and prepare for environmental changes. The rhytmicity is important in regulating and coordinating internal metabolic processes.

The simplest known circadian clock is that of the prokaryotic cyanobacteria. This clock sustains a 22 hours rhythm over several days upon the addition of ATP. A limited list of pacemakers related to circadian rhythms are:

• Melatonin secretion by the pineal gland and suprachiasmatic nucleus(SCN)

• Core body temperature

• Plasma level of cortisol

Ron Konopka and Seymour Benzer found evidence for a genetic basis of circadian rhythms in higher eukaryotes by discovering the period (*per*) locus in *Drosophila*, a species from fruitfly family, Core circadian clock genes are defined as genes whose protein products are the essential component for the generation and regulation of circadian oscillations.

Several mammalian clock genes have been identified recently. Most of their components are transcriptional activators or repressors that control protein stability and nuclear translocation. These components create two interlocking feedback loops in the first feedback loop, members of the transcription factor family, **CLOCK** and **BMAL1**, heterodimerize in the cytoplasm. After translocation to the nucleus, this complex triggers transcription of the core clock genes, *per* genes (*PER1,PER2,PER3*) and two *Cryptochrome* genes (*CRY1,CRY2*). Upon translocating back into nucleus **PER:CRY** heterodimers impose negative feedback to repress their own transcription by inhibiting **CLOCK:BMAL1** complexes.This mechanism is illustrated below:

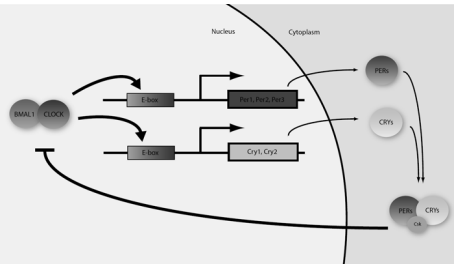

Figure 4: Mammalian circadian clock

Circadian clocks are regulated by different biochemical mechanisms. Phosphorylation, Methylation and histone accetylation and deacetylation is among the most important mechanisms. In phosphorylation a phosphate group is added to a protein which turns the protein enzyme on and off, therefore altering the protein function. During methylation, methyl group is added to a protein structure. As a consequence, gene expression is either activated or suppressed thorough changing the DNA sequence. In accetylation, adding or removal of acetyl group causes a major change in DNA expression.

1.1.4 Circannual Rhythm

Circannual rhythms are endogenously generated rhythms with a period of almost one year. Circannual rhythms are intimately involved in the seasonal organization of a behavior, providing substrate in which seasonal factors act. The actual environmental control of seasonality is exerted by light. Photoperiod is the duration of an organisms daily exposure to light. The expression of circannual rhythms depends on photoperiod. This represents permissive factor in the process of rhythm generation. The significance of endogenous factors is evident in species equipped with circannual clocks that keep running even in the absence of photoperiodic or other seasonal environmental information.

Recently, functions as diverse as molt, reproduction and migration have been confirmed to demonstrate circannual oscillations for at least twenty species of birds. In most species studied so far, circannual rhythms have been shown to free-run in constant condition with period of almost twelve months. It has been proposed that the very small changes

in day-length may be used as a zeitgeber by tropical birds. Zeitgebers other than light intensity have not yet been identified in birds (Gwinner et al. 1993). Circannual migratory programs are affected by photoperiod. The study of *zugunruhe*, the seasonally occurring restlessness during migration season, has been a major breakthrough.

These studies have shown the significance of endogenous circadian and circannual clocks for orientation in time and space. Zugunruhe shows a state of "readiness" as the expression of a birds readiness to respond to external or internal stimuli and their effect on migration (Helm 2006). Birds living in habitats where external cues such as photoperiod are poor rely merely on their endogenous clocks. Such reliance may indicate that annual timing mechanism are adaptive. The first robust avian circannual rhythm was reported in migratory behavior of the *willow warbler* by Gwinner. He studied the precise annual timing of fat deposition and the onset of spring migration of these individuals in their equitorial wintering quarters. When he studied the birds under a constant photoperiod and temperature, individuals showed persistent rhythms in migratory restlessness, suggesting the timing of spring migration may be controlled by endogenous circannual clock.

1.1.5 Melatonin and Avian Migration

Melatonin is a hormone allowing the entrainment of circadian rhythms of several biological functions (Gwinner 2003). Biological effects caused by melatonin are produced through activation of melatonin receptors. Melatonin has been identified in both plants and animals. Variation in duration of melatonin production acts as a seasonal clock in many animals. Melatonin is biosynthesized in four enzymatic steps. Melatonin is produced by trypthophan, and seratonin made at the third step.

The melatonin synthesis pathway is illustrated below:

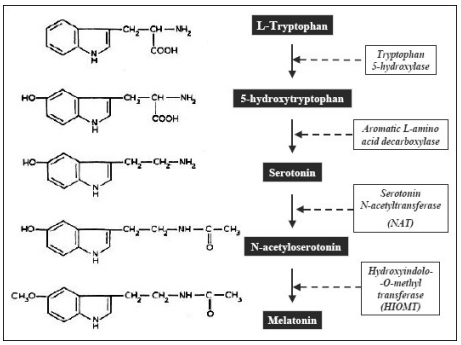

Figure 5: Melatonin synthesis pathway

Pineal gland in the brain, is the organ responsible for melatonin secretion into the blood. Melatonin is called "hormone of darkness" since its secretion mechanisms happen in both diurnal and nocturnal animals. In most animal, plasma melatonin concentrations increase at night whether the animal is day or night active. Birds that migrate during the night experience lower daily amplitude of plasma melatonin. Nocturnal migrating birds having to be active during night for migratory reasons, shows lower level of melatonin during night. In birds, the pineal gland synthesizes the hormone melatonin (5-methoxyn-N-acetyltryphtamine) and releases it into blood. So, melatonin produced in a diel fashion with high secretion at night and low secretion during the day (Gwinner 2003). This diel pattern corresponds to the light-dark cycle and is prevalent among most species irrespective of species being diurnal or nocturnal. In most species investigated so far, hight melatonin level is required for activity at night. Thus, these species migration is associated with a major switch in the circadian pattern of activity. The pineal gland and its hormone melatonin, are major components of avian circadian system. Autonomous circadian oscillation that happens in pineal gland is responsible for the rhythmic release of melatonin. The onset of night activity in nocturnal migrants is triggered by a change in the activity pattern from diurnal to nocturnal. This change is correlated with changes in the pattern of melatonin secretion. During migratory season, caged migrants shows zugunruhe. Under constant light-dark cycles, many investigated birds show a circannual rhythmicity of migratory disposition. In nocturnal migratory avians, during migratory season, the pattern of

melatonin secretion is not different form the recorded data during the other times (Gwinner 2003). However, peak night melatonin level is lower during migration times (Fusani and Gwinner 2005). Increased light input through the open eyes may induce a decrease in the circulating concentration of melatonin. Lowering melatonin release may facilitate nocturnal activity or other migration-related purposes. Eberhard Gwinner and Leonida Fusani in their article "Melatonin and nocturnal migration" , which studied a species called black cap, indicated that the decrease in melatonin during zugunruhe is related to the initiation of nocturnal migration and is not simply a result of behavior change.

In birds, the main pacemaker is located in the suprachiasmatic nucleus(SCN). The SCN receives exterior light information through the eyes, interprets it and passes it on to the pineal gland, which then secretes the hormone melatonin in response (leise and Siegelman 2006). Secretion of melatonin is an integral part of the regulation of the sleep/wake cycle in birds. This suggests that the avian biological clock is controlled by internal factors and not merely external light cues. In particular, experimental work at Texas A&M University shows that if the pineal gland is removed from a migrating bird species that is experiencing zugunruhe, the circadian clock is no longer able to exhibit zugunruhe, although interplay between other biological factors allow it to return to normal daily oscillations with a period of twenty four hours (Bentley 2001). The SCN responds to light and can be entrained by the light-dark cycle and,in turn, entrains other oscillatory tissues in the body.

1.2 MATHEMATICAL BACKGROUND

1.2.1 Oscillation

In mathematics oscillation is a measure for quantification of the amount that a function tends to move between upper and lower extremes or between multiple states of the system. Beating human heart and vibration of a string are the most tangible examples of oscillation.

There are three types of oscillators :

• Simple harmonic oscillator :

In systems where the restoring force is directly proportional to the displacement, simple harmonic oscillation happens. The simplest example is a mass attached to a spring and the only exerted forces are tension and weights. When the spring is static, the system is in equilibrium. When the system is displaced, the net restoring force bring the mass back to the equilibrium position. A simple harmonic oscillator is neither driven nor damped. The following differential equation is a mathematical representation of a simple harmonic oscillation and is called *simple harmonic oscillator equation*:

$$x''(t) + \omega^2 x(t) = 0$$

where $\omega > 0$ is a constant.

The simple harmonic oscillation equation has the following solution:

$$x(t) = a\cos(\omega t - \phi)$$

where $a > 0$ and ϕ are constants. a is called the amplitude and ω is called the angular frequency. The phase angle, ϕ, determines the times at which the oscillation attains the maximum value. The frequency (f) and the period of oscillation (T) are determined by the following formulas respectively:

$$f = \frac{\omega}{2\pi} \qquad T = \frac{2\pi}{\omega}$$

Frequency and period are determined by angular frequency whereas a and ϕ are determined by the initial condition. Following is the plot for the solution of simple harmonic oscillator equation.

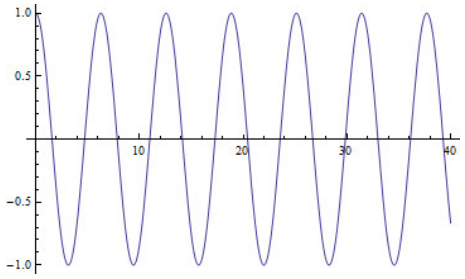

Figure 6: Solution of simple harmonic oscillator

In the above solution $\omega = 1, x'(0) = 1, x(0) = 0$. As it is illustrated above the oscillation is periodic in a sinusoidal fashion with 1 as an amplitude and $f = \frac{1}{2\pi}$ as a frequency.

• Damped harmonic oscillator:

In systems where both restoring force and friction acts on the system, damping, slows the motion of the system. The damping force opposes the motion of the system. referring back to our simple example, if the displaced mass also experience the friction, it dampens the oscillation and slows the system.

The following differential equation represents damped harmonic oscillator:

$$x''(t) + \nu x'(t) + \omega_0^2 x(t) = 0$$

where $\nu > 0, \omega_0 > 0$ are constants. The above equation is called a *damped harmonic oscillator equation*.

The solution to the above equation takes the form of :

$$x(t) = ae^{-\lambda t}\cos(\omega_1 t - \phi)$$

where $a > 0, \lambda > 0, \omega_1 > 0$ and ω are all constants. In the above solution ω_1 is a fixed angular frequency and ϕ is a phase angle.with a simple calculation, λ and ω_1 can be formulated respectively as follows:

$$\lambda = \frac{\nu}{2} \qquad\qquad \omega_1 = \sqrt{(\omega_0^2 - \frac{\nu^2}{4})}$$

ν is called *damping constant*. Damping of a simple harmonic oscillator, reduces the angular frequency and cause the amplitude to decay exponentially in time at the $\frac{\nu}{2}$ rate. Following is the plot of solution for damped harmonic oscillator equation:

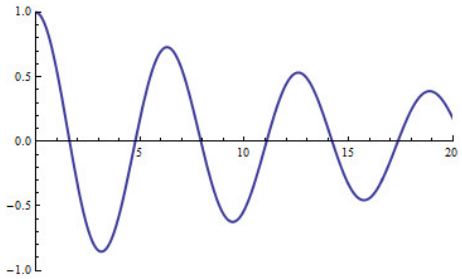

Figure 7: Solution of damped harmonic oscillator

In the above solution $\omega = 1, x(0) = 1, x'(0) = 0, \omega_0 = 1, \nu = 1$.

• Driven harmonic oscillator:

In systems in which excitation by energy transfer exerted, and compensate for the frictional force, driven oscillation happens. The following is a driven harmonic oscillator equation:

$$x''(t) + \nu x'(t) + \omega_0^2 x(t) = X(t)$$

where $\nu > 0$ is the damping constant and $\omega_0 > 0$ is the undamped oscillation frequency. Suppose that the excitation exerted by the force $X(t)$ of angular frequency $\omega > 0$ and amplitude $X_0 > 0$ in the form of simple harmonic oscillation.

The solution of a driven harmonic oscillation is :

$$Z(t) = Ae^{-\eta\omega_0 t}\sin(\sqrt{1-\eta^2}\omega_0 t + \phi)$$

The following is a plot of a solution for driven harmonic oscillator with $\sin(\lambda t)$ as a forcing function and $x(0) = 1, x'(0) = 0, \nu = 0.1, \omega_0 = 1$ and $\lambda = 0.2$

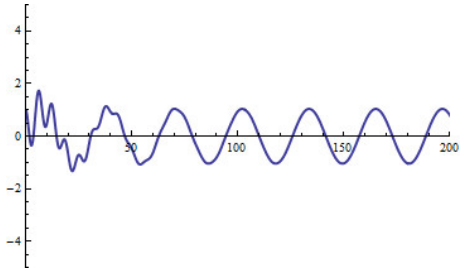

Figure 8: Solution of driven harmonic oscillator

The initial behavior of a driven oscillator is quite complex. The parameters in the above solution depend upon the initial conditions and the nature of the driving force, but deriving the detailed form is a complicated algebraic problem. The solution to the driven harmonic oscillator has a transient and a steady-state part. The transient solution is the solution to the homogeneous differential equation of motion which has been combined with the particular solution and forced to fit the physical boundary conditions of the problem at hand. The form of this transient solution is of the damped oscillator. The steady-state solution is the particular solution to the inhomogeneous differential equation of motion. It is determined by the driving force and is independent of the initial conditions of motion.

1.2.2 Delay Differential Equation (DDE)

A delay differential equation is a kind of differential equation in which the time derivative at the current time depend on the solution and possibly its derivatives at previous times. The following represents a DDE in general:

$$X'(t) = F(t, X(t), X(t-\tau_1), ..., X(t-\tau_n), X'(t-\sigma_1), ..., X'(t-\sigma_m)); t \geq t_0 \quad (1a)$$

$$X(t) = \phi(t); t < t_0 \quad (1b)$$

In DDEs, initial history function, $\phi(t)$ is needed for solubility. The quantities τ_i and σ_i are called delays. Delays may be constant, time-dependent or state-dependent. DDEs with σ (delays of derivative) are called neutral delay differential equation(NDDEs) . Since the times involved in biological processes are substantial compared with other times in most studies, it is necessary to be incorporated into mathematical models chosen for these biological studies. These process times are included as delay times in DDEs. In the application of DDEs in life-science, since high-dimensional compartment models bring out additional challenges of parameter estimation, low-dimensional delay differential models with fewer parameters are plausible alternatives. Unfortunately, the presence of such parameters often greatly complicates the systematic study of such models. I DDEs, some times, time delay are dependent on model variables(state-dependent). These models are difficult to study analytically although they may give more accurate dynamics. Recent advancements in DDEs show that they are capable of generating rich dynamics with realistic parameter values. The popular MATLAB-based **dde23** solver developed by Shampine and Thompson for DDEs is well tested. Analytically, DDEs are solved by three different methods. DDEs such as Hutchinson equation, $x'(t) = rx(t)(1 - \frac{x(t-\tau)}{k})$, is solved by method of steps. Some DDEs are infact a system of ordinary differential equations, hence by some substitution of variables they can be solved. The rest of the equations such as integro-differential equations are solved by linear chain trick method. Stability properties of linear DDEs are analyzed by studying their characteristic equations.

The pioneers of using differential equations to model biological system are Malthus, Verhulst, Lotka and Voltera. Their models proven that the simplest models can not explain the whole dynamics observed in natural systems. Here is the controversial dilemma. Either miss rich varieties of dynamics or construct larger systems and suffer form containing many parameters, signifying irrelevant parameters that can not be determined experimentally.

Trade offs for DDEs is that these models hide much of the detailed workings of the system and these details could be of interest. While numerical simulations provide the basic understanding of these systems,. Analytical results about these systems are lacking. Numerical simulation incorporates the use of parameter fitting even when the analytical results are unavailable. Delay models appearing in many branches of biological modeling from neural networks, tumor growth to circadian rhythms.

One of the most frequently employed and simple delay differential equation for demonstration purposes is the following :

$$X'(t) = X(t-1); t > 0 \tag{2a}$$

$$X(t) = 1; -1 \leq t \leq 0 \tag{2b}$$

This DDE will be solved by the method of steps. This method involves solving the equation on one interval at a time. On the first interval, $[0,1]$,

$$\int_0^t x'(s)ds = x(t) - x(0)$$

Rearranging, we have

$$x(t) = x(0) + \int_0^t x'(s)ds$$

since $x'(t) = x(t-1)$ then $x'(s) = x(s-1)$.

$$x(t) = x(0) + \int_0^t x(s-1)ds$$

since we are on the interval $[0,1]$, $0 \leq s \leq 1, so-1 \leq s-1 \leq 0$. The history function is $x(t) = 1$ for $-1 \leq t \leq 0$ so $x(s-1) = 1$ on this interval.

$$x(t) = x(0) + \int_0^t ds$$
$$x(t) = 1 + t$$

So the solution on the interval $[0,1]$ is $x(t) = 1+t$. Now on interval $[1,2]$ we use our solution from $[0,1]$.

$$\int_1^t x'(s)ds = x(t) - x(1)$$

Rearranging, we have

$$x(t) = x(1) + \int_1^t x'(s)ds$$

from our last interval we have $x(1) = 2$,

$$= 2 + \int_1^t x(s-1)ds$$

since we are on the interval $[1,2]$ so $0 \leq s-1 \leq 1$ and from our previous solution we have:

$$x(s-1) = 1 + (s-1)$$
$$= 2 + \int_1^t [1 + (s-1)]ds$$
$$= \frac{3}{2} + \frac{1}{2}t^2$$

As we can see, in every step there is added complexity involved. Below is the plot of the solution for this DDE.

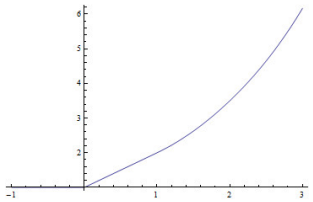

Figure 9: Solution of $x'(t) = x(t-1)$

1.2.3 Mathematical Models for Circadian Clock

Principals derived from empirical observations in chronobiology have aided scientists to devise mathematical models for circadian pacemakers (Klerman and St.Hilaire 2007). In their approach, several organisms have been covered as well as different methods. All of these models try to derive basic features from which one could form a global concept on how the system works. Circadian clocks drive biological oscillations in biochemical, molecular and physiological variables of all organisms. Therefore, the mathematical models related to circadian clocks can be divided into two separate groups.

First group is the one that describe the pacemaker core function, second group represents events based on the pacemaker activity. Creation of mathematical model requires explicit definitions of the variables involved, the response of the system to stimuli and interaction within the system. The core of the clock mechanism relies on a feedback loop of one or more molecular species affecting their own genetic expression either directly or indirectly and can be entrained to external stimuli. This biological rhythm is generated by a complex gene-protein interaction networks. Their characteristics are defined by regulatory feed back loops and non-linear dynamics which rises from Michaelis-Menten enzyme kinetics and cooperative processes.

For modeling purpose, use of limit cycle is the most popular approach. In the following, we introduce two important models related to the core clock function since our upcoming proposed model follows this approach.

• The Goodwin family

This model is considered the first model of a biological oscillator which includes a protein repressing the transcription of its own gene causing a self-sustained auto-oscillation. Through time, this model has been modified.

• Goodwin model (1965)

Nearly all the models for clock description rest on a hypothesis first studied in this model : Proteins that exert retro-inhibition on its own production.

This base model is defined by the below system of equations:

$$\begin{aligned} \frac{dx}{dt} &= k_1 \frac{1}{(k_I + z)} - k_2 x \\ \frac{dy}{dt} &= k_3 x - k_4 y \\ \frac{dz}{dt} &= k_5 y - k_6 z \end{aligned} \tag{3}$$

This model was originally proposed by Goodwin in 1965 to model oscillatory processes in enzymatic control processes. As illustrated above, this is a minimal model, based on a delayed negative feedback loop. Below is the solution plot for a specific parameters values.

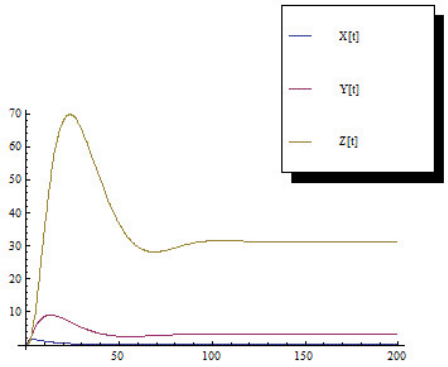

Figure 10: Solution for system of equations (3)
$k_I = 1, k_1 = k_3 = k_5 = 1, k_2 = k_4 = k_6 = 0.1$ and zero as initial history

We understand from the above plot that, no limit cycle oscillation can occur in this model due to the lack of non-linearity.

• Modified Goodwin model (Griffith , 1968)

This model is built using Hill-type enzymatic equations and addition of chemical equilibriums as well as first order kinetic terms. Addition of the above properties make the model more realistic from a biochemical point of view.

Below system represents this modification :

$$\begin{aligned} \frac{dx}{dt} &= k_1 \frac{1}{(k_I^n + z^n)} - k_2 x \\ \frac{dy}{dt} &= k_3 x - k_4 y \\ \frac{dz}{dt} &= k_5 y - k_6 z \end{aligned} \tag{4}$$

Below is the solution plot for a specific parameter values as well as a limit cycle:

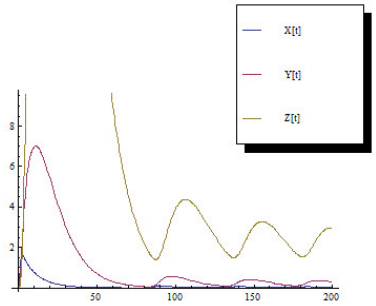

Figure 11: Solution for system of equations (4)
$k_I = 1, k_1 = k_3 = k_5 = 1, k_2 = k_4 = k_6 = 0.1, n = 10$ and zero as initial history

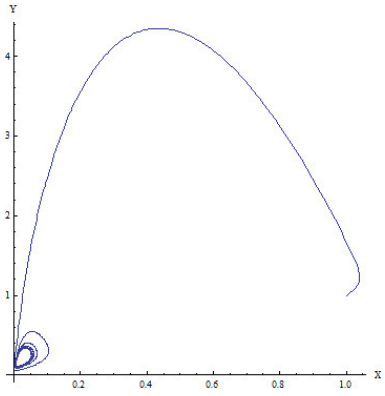

Figure 12: Limit cycle for the specified parameters

The variables x, y, z can be interpreted as the concentration of a gene mRNA, the pro-

duced protein, and a transcriptional inhibitor, respectively. Here, the hill function plays the role of repression exerted by inhibitor to the mRNA synthesis. In 1968, Griffith showed that only for $n > 8$ limit-cycle oscillations can be obtained otherwise the model displays damped oscillation. Thus, a strong non-linear repression function is needed.

• A variant of the goodwin model

In order to reduce the non-linearity in the previous model Gonze et.al. have developed a more robust system describing the behavior of a population of coupled SCN neurons. In this model, a clock gene mRNA , x , produces a clock protein , y , which activates a transcriptional inhibitor , z , . In this model, michaelian kinetics is used to model the degradation step.

Below system defines this approach:

$$\begin{aligned} \frac{dx}{dt} &= v_1 \frac{k_I^n}{(k_I^n + z^n)} - v_2 \frac{x}{k_2 + x} \\ \frac{dy}{dt} &= k_3 x - v_4 \frac{y}{k_4 + y} \\ \frac{dz}{dt} &= k_5 y - v_6 \frac{z}{k_6 + z} \end{aligned} \tag{5}$$

In this model, the variable x represents mRNA concentration of clock gene *per* or *cry*; y is the concentration of the resulting protein **PER** or **CRY**; and z is the inhibitor.

Below is the solution plot and the related limit-cycle oscillation :

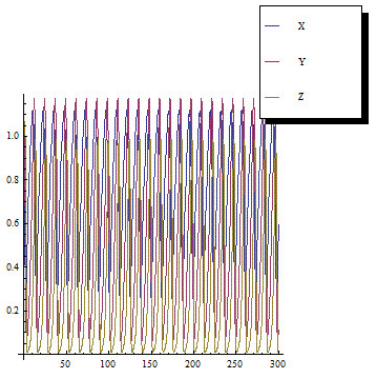

Figure 13: Solution for system of equations (5) $k_I = 1, k_1 = k_3 = k_5 = 1, k_2 = k_4 = k_6 = 0.1, n = 2, v_1 = v_2 = v_4 = v_6$

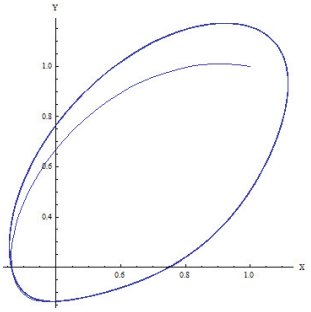

Figure 14: Limit cycle for the specified parameters

In this model the non-linearity is distributed in different degradation terms hence limit-cycle oscillations can occur in this model with low values for n. In some models the succession of steps is replaced by a delay thus constructing a delay differential equation(DDE).

• Delay version of Goodwin model

The following model disregard linear steps in biochemical aspect of circadian clocks and simply replacing them by a explicit delay. This system is presented below :

$$\begin{aligned} \frac{dx}{dt} &= k_1 \frac{1}{(k_I^n + y_{t-\tau}^n)} - k_2 x \\ \frac{dy}{dt} &= k_3 x - k_4 y \end{aligned} \tag{6}$$

The following are plots for the solution and limit cycle for this system.

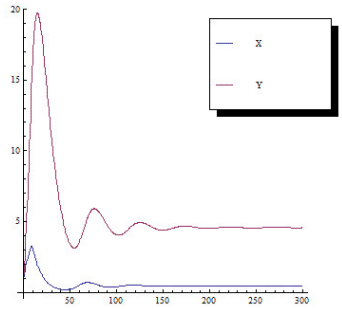

Figure 15: Solution for system of equations (6) $k_1 = k_2 = k_3 = k_4 = 1, n = 2, \tau = 8$ and with 1 as a history function

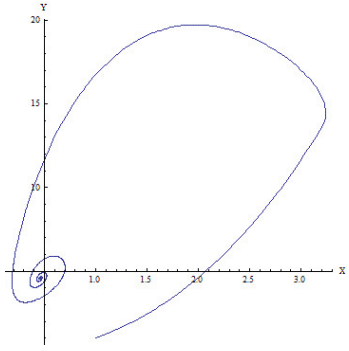

Figure 16: Limit cycle for the specified parameters

Same as the previous Goodwin models, x is represented as a gene mRNA concentration and y is a produced protein concentration. The rationale behind this model is that there are multiple factors influence the process that causing an important delay between transcription and the protein. Such factors are but not limited to transport across membrane and post-translational modification. As a summary for Goodwin models, these models produce limit-cycle(self-sustained) oscillations provided that the non-linearity is sufficient or the delay is large enough.

• Model by Lema et.al.

This model proposes a single time-delay equation (Lema et al. 2000). Also, this model takes into account the transcription of clock protein, its degradation and a delayed inhibition on protein's own expression. Therefore, facilitating analytical study of the model by reducing the number of variables and parameters. The following is the proposed model by Lema et. al. for molecular clock:

$$\frac{dE(t)}{dt} = k_e\left(\frac{1}{1+[E[t-\lambda]/k_i]^n}\right) - k_d E(t)$$

In this model, E represents the level of the mature clock protein and $E[t-\lambda]$ represents the level of the activation for the gene. k_e represents the expression rate constant and λ is a time delay. The following is the solution plot for this model. Also limit-cycle oscillation has been shown:

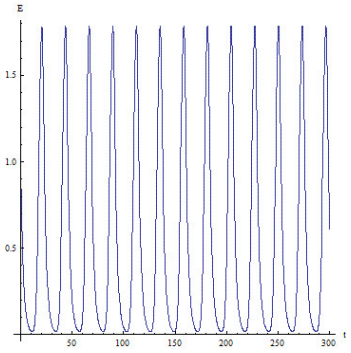

Figure 17: Solutin for Lema equation $k_1 = k_2 = k_3 = k_4 = 1, n = 2, \tau = 8$ and with 1 as a history function

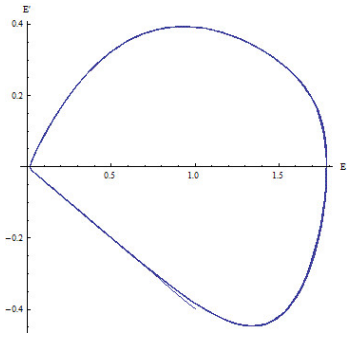

Figure 18: Limit cycle for the specified parameters

As it is illustrated above, this model shows robust and stable cycles under constant conditions with a circadian period. Also, if parameters are adjusted appropriately, 24 hours rhythm are easy to achieve.

• Other models

More models have been developed based on mRNA-Protein-Inhibitor interactions (Lema et al. 2000). Among the most popular is a modification by Ruoff and Rensing on the goodwin model. this model contains three state variables: a mRNA, the translated protein and a product produced by this protein which in turn represses the mRNA synthesis. This model describes circadian rhythms proving that the model is firmly funded on molecular evidence. Another important model based on a protein-protein interaction is *Per-Tim* model of Leloup and Goldbeter. **PER** and **TIM** proteins have a circadian oscillation and their level of expression is subjected to negative feedback between the two proteins (Leise and Moin 2007). The drawback for this model is that it looses generality and require many assumptions and guesses about the steps included. The last model is developed by Roennberg and Merrow. Their model is like two usual Goodwin oscillators that has opposite influence on each other. This model displays many feature of circadian rhythms by rising the number of model parameters (Paetkau 2006).

2. PROBLEM AND SOLUTION

2.1 Problem

In all migratory avians, biological clocks control the rhythmic processes of migratory behavior. Such behaviors are molt, hyperfagia, photoperiodicity and migratory restlessness(zugunruhe). Organs involved in these processes whether they are internal or environmental are known as oscillators. Then, oscillators interact with each other to allow entrainment of the biological clock to an almost twenty four hours cycle. The master pacemaker of avian circadian system is the suprachiasmatic(SCN) nucleus located in hypothalamus. SCN responds to light and can be entrained by the light-dark cycle (Leise and Siegelman 2006). Dawn and dusk adjust the biological clock in the SCN. Then SCN as a master pacemaker, entrains other pacemakers tissues. In the vast majority of migratory birds, migration and associated behaviors are organized based on the annual rhythmicity. In these birds circannual rhythms free-run in constant condition. But, for birds living in nature, photoperiod is the dominant circannual zeitgeber. Zeitgebers other than photoperiod and light intensity have not yet been discovered in migratory birds. Synchronized circannual rhythm, provides regulation of migration as well as cues for seasonal timing of events in several ways. First, they alter seasonal direction of migration route. Second, circannual variations affect the fat deposition characteristic of migratory birds. Third, circannual rhythm determines the time course and distance of migration. Taken together, the circannual and circadian clocksof migratory avians provide basis for their time orientation. Many results show that migratory patterns that is expressed in nocturnal activity and in a series of physiological and behavioral changes are endogenous circadian and circannual clock mechanisms. Secretion of melatonin form pineal gland which is triggered by SCN, regulates the interaction between circadian and circannual clock. The purpose of this research is to develop a model to define the interaction between circannual and circadian clocks in migratory birds.

The main interest is to model the observation that during migratory season many birds become extremely active during nights whereas they are daily active during non-migratory season. Experimental works at Texas A&M University has showed that the removal of pineal gland in migratory birds that are experiencing zugunruhe would stop the migratory restlessness. This evidence proves that pineal gland and hormone melatonin in particular are involved in migratory restlessness. When these birds were kept in controlled condition with no environmental cues, they continue to exhibit the restlessness. This gives rise to the fact that zugunruhe is mainly controlled endogenously and biological clocks are regulated by internal hormones and not merely by photoperiodicity. So, in our model we mainly focus on biochemical aspect of this mechanism. This model is predicated upon the key role of melatonin in regulating the onset of zugunruhe. Previous models for avian migratory behavior has mainly focused on the circadian aspect of migration (Beersma 2005). In this model, we will develop a base model for the interplay between circadian and circannual pacemaker.

2.1.1 Scheper Model

Scheper model is based on the biological clocks which are controlled by gene expression (Bartell and Gwinner 2005). This model neglects the constraint of specifying all the processes involved in the production of the protein. These processes are: mRNA translation, protein post-translation processing, transport across membrane and nuclear entry. This model characterizes full chain of reactions only in terms of two simple parameters. (1) the total duration and (2) the nonlinear relationship between input and delayed output of the chain. This simplification is demonstrated schematically by scheper et al. 1999. in their 1999 article " A Mathematical Model for the Intracellular Circadian Rhythm Generator". Following is the schematical comparison illustrated in the above article:

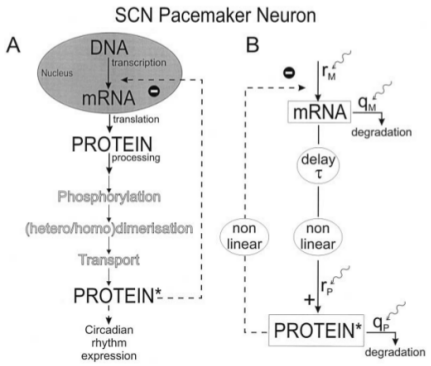

Figure 19: *A*, Elementary protein synthesis cascade of circadian rhythm generation. *B*, Model interpretation of *A*, emphasizing delay and nonlinearity.

The model is defined as follows:

$$\begin{aligned} \frac{dM}{dt} &= \frac{r_m}{1+\frac{P}{k}^n} - q_m M \\ \frac{dy}{dt} &= r_p M(t-\tau)^m - q_p P \end{aligned} \tag{7}$$

In this model M denotes concentration of mRNA and P denotes concentration of the protein.r_m and r_p represents the scaled mRNA production rate constant and the protein production rate constant respectively. q_m and q_p are the mRNA and protein degradation rate constants respectively. n is the Hill coefficient and m is the non-linearity in the protein production cascade. τ is the total duration of protein production from mRNA which is used as delay factor and k is a scaling factor. Below is the table for parameter values showing an unperturbed behavior taken from the Scheper et al. 1999.

Parameter	Values resulting in a circadian rhythm
r_m	1.0 hr^{-1}
r_p	1.0 hr^{-1}
q_m	0.21 hr^{-1}
q_p	0.21 hr^{-1}
n	2.0
m	3.0
τ	4.0 hr
k	1

Table 1: Parameters for free-running oscillation in Scheper model

Since multiple mRNA molecules are the substrate in the production os a protein, $m > 1$ implements the nonlinearity in this process. $n > 1$ could also account for any further processing steps. Because of high probability of protein-protein interactions during circadian oscillation n is given value of 2. Degradation rate constants are chosen from biological half-lives of mRNA and protein. There are two important differences between shceper model and model introduced by Lema. One is that this model deal with mRNA rather than "gene activity". The other difference is that the delay terms in these two models do not refer to exactly the same step in the process. Following is the free-running oscillation for the parameters in the Table 1 as well as limit-cycle contour.

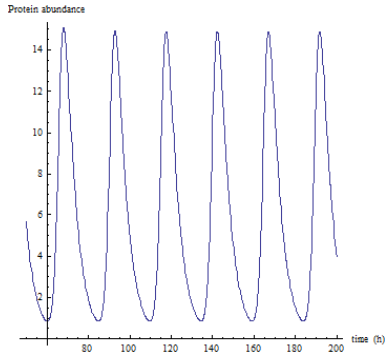

Figure 20: Circadian oscillation for parameter values in Tabel 1.

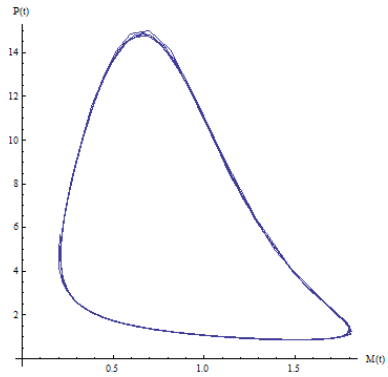

Figure 21: Corresponding limit-cycle oscillation

This model shows that nonlinearities and delay in protein synthesis negative feedback loop are essential features to have a robust circadian oscillation and realistic entrainment properties when subjected to external stimulation.

2.2 SOLUTION

2.2.1 Solution

The model presented here consists of system of nonlinear delay differential equations based of the Scheper model predicting upon the key role that melatonin regulation plays in the onset of zugunruhe. The biochemical principal governing our model is Michaelis-Menten kinetics explains how reaction rates depend on the concentration of enzyme and substrate. This model is based on the melatonin production pathway from serotonin and N-acetyl-serotonin. This pathway is illustrated below:

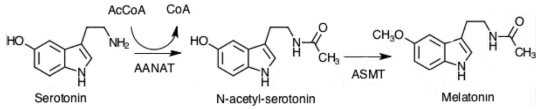

Figure 22: Melatonin production pathway

This model is based on two coupled Scheper models with a fifth equation describing the yearly oscillation of hormone melatonin. The first Scheper system describes the production of serotonin. This hormone is a precursor for production of melatonin. Second Scheper system describes the production of N-acetyl-serotonin. This a naturally occuring amino acid and a metabolic intermediatein biosynthesis of melatonin. This enzyme catalyzes the reaction of serotonin into melatonin and is involved in the day and night rhythmic production of melatonin. In this model the essential requirement for biochemical oscillators has been implemented meticulously. First, since negative feed back is inherently used as a base mechanism in Scehper model, this model also follows this mechanism since it is necessary to carry a reaction network back to the starting point of it's oscillation. Second, sufficient delay is implemented so that the reactions does not settle on a steady state. Non-linearity of the model is due to the necessity to destabilize the steady state. Fourth, the system occur on appropriate timescale that permit the network to generate oscillations.

The model is defined as follows:

$$\begin{aligned} \frac{dx}{dt} &= \frac{r_m}{k+y^n} - q_m x \\ \frac{dy}{dt} &= r_p M(t-\tau)^m - q_p y \\ \frac{dz}{dt} &= \frac{R_m}{g+w^c} - Q_m z \\ \frac{dw}{dt} &= R_p z(t-\phi)^e - Q_p w \\ \frac{dM}{dt} &= 0.1wy - 0.05M \end{aligned} \tag{8}$$

In this model, x,y denotes relative concentrations of mRNA and the effective protein of serotonin respectively. z,w represents concentrations of mRNA and the effective protein of N-acetyl-serotonin. M denotes the concentration of melatonin throughout a year. r_m and R_m are the production rate constants for serotonin and N-acetyrl-serotonin mRNA respectively. q_m and Q_m are their degradation rates. In the same fashion r_p and R_p are the production rate constants for effective proteins of serotonin and N-acetyl-serotonin respec-

tively .q_p and Q_p are their degradation rate. k and g are scaling factors. The exponents m and e implement nonlinearity in the protein production terms. The exponents n and c are Hill coefficients and were given value of 2 because of protein-protein interactions. τ is the delay in production of serotonin and ϕ is the delay in production of N-acetyl-serotonin. Our method to solve the model which is based on the delay differential equation was using the Runge-Kutta fourth-order differential integrator with step sizes of 1-24 hours. For our purpose a step size of 24 hours was most efficient. Periods were calculated using the plot. Since our parameter space is $\{r_m,k,n,q_m,r_p,\tau,m,q_p,R_m,g,c,Q_m,R_p,\phi,e,Q_p\}$ it is not feasible to explore the behavior of the system using the full 16-dimension parameter space. So, we investigated the behavior around the set point by changing only one parameter of the subspace $\{n,m,\tau,c,e,\phi\}$ at a time while keeping the rest at the set points. Therefore, we investigated more extensively the role of non-linearity and delay parameters.

Stable limit cycles and stable steady states was important in the study. Since at the boundaries between these states qualitative behavior of the system(bifurcation) happens. The effect of external stimuli was studied by changing th production and degradation rates. This is predicated on the fact that these rates are more sensitive than $\{n,m,\tau,c,e,\phi\}$ to external stimuli.

To this end the system was extended to:

$$\begin{aligned}
\frac{dx}{dt} &= \frac{r_m + S_{r_m}}{k + y^n} - (q_m + S_{q_m})x \\
\frac{dy}{dt} &= (r_p + S_{r_p})M(t-\tau)^m - (q_p + S_{q_p})y \\
\frac{dz}{dt} &= \frac{R_m + S_{R_m}}{g + w^c} - (Q_m + S_{Q_m})z \\
\frac{dw}{dt} &= (R_p + S_{R_p})z(t-\phi)^e - (Q_p + S_{Q_p})w \\
\frac{dM}{dt} &= 0.1wy - 0.05M
\end{aligned} \tag{9}$$

The perturbation terms were kept at zero in unperturbed model but assumed non-zero value during perturbation.

3. RESULT AND DISCUSSION

3.1 RESULT

For the parameter values in Table 2, free-running system will occure and a stable oscillation with a period of 375 days were obtained. Melatonin concentration fluctuates over a wide range(Fig.23). The limit cycle contour for melatonin production was obtained and parameter space was extensively studied. Apart from stable limit cycles and stable points, no other behavior was observed for this model. However, chaotic behavior can not be ruled out for some areas of parameter space.

Parameter	Values resulting in a circadian rhythm
r_m	1.0 d^{-1}
r_p	1.0 d^{-1}
q_m	0.21 d^{-1}
q_p	0.21 d^{-1}
n	2.0
m	3.0
τ	5.0 d
k	1
R_m	1.0 d^{-1}
R_p	1.0 d^{-1}
Q_m	0.21 d^{-1}
Q_p	0.21 d^{-1}
ϕ	5.5 d
e	3
c	2

Table 2: Parameters for free-running oscillation in proposed model

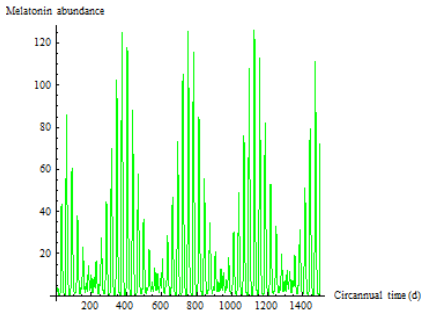

Figure 23: Circannual oscillation for parameter values in Tabel 2

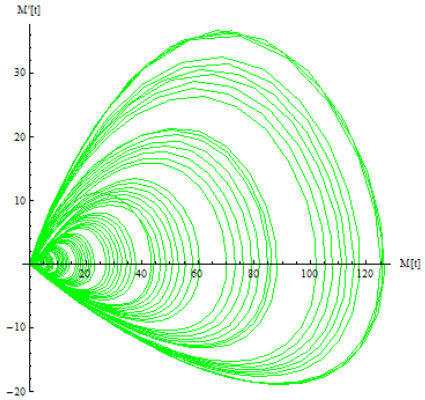

Figure 24: Melatonin limit cycle for parameter values in Tabel 2

Following figures show that limit cycle behavior and the period of free-running oscillation change when one parameter is changed with others remain constant.Because of the importance of the non-linearity and delay we only investigate the {n,m,τ} and {c,e,ϕ} subspaces. Study of {n,m,τ} showed that at {n=2,m=2.91,τ = 5}, the system always converged to stable steady state for any choice of other parameters. For {n=2,τ = 5} bifur-

cation happened at $m = 3.2$. However, limit cycles for $m > 3.2$ attained circannual periods at $m = 5.1$. For {m=2.91,τ = 5} a bifurcation happened at $n = 2.5$. Here limit cycles for $n > 2.5$ attained circannual periods at $n = 4.5$. For {n=2,m=2.91} bifurcation happened at τ = 5.5 but limit cycles for $\tau > 5.5$ attained circannual period at $\tau = 6$. Some investigation over {c,e,ϕ} shows that at {c=2,e=3,$\phi = 5.5$} the system converges to stable steady state for any choice of other parameters. For {e=3,$\phi = 5.5$} bifurcation happened at $c = 2.1$ whereas limit cycle for $c > 2.1$ attain circannual period at $c = 2.8$. For {c =2,ϕ = 5.5} bifurcation happend at $e = 3.5$ but limit cycle for $e > 3.5$ attain circannual period at $e = 5.2$. For {c = 2,e = 3} bifurcation happened at $\phi = 6$. However limit cycle for $\phi > 6$ attain circannual period at $\phi = 7$. The slop of each lines in Figures 25 and 26 shows the strong dependence of period upon the investigated parameters. Especially the delays τ and ϕ strongly influence period. The curves for c and e were the only ones with negative slopes, meaning that increase in nonlinearity of N-acetyl-serotonin production results in shorter free-running period.

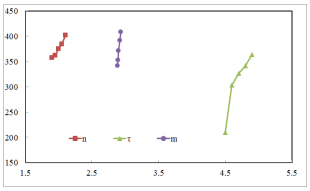

Figure 25: Period sensitivity on {n,m,τ} subspace

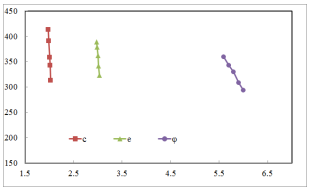

Figure 26: Period sensitivity on {c,e,ϕ} subspace

To study the entrainment, periodic external stimulation was implemented by periodically switching on for 1 day one of the parameters $\{S_{r_m}, S_{q_p}, S_{q_m}, S_{R_p}\}$ in system of equations(9). For all four parameters entrainment occurred to either shorter or longer cycles than the free-running cycle. At the onset of period stimulation, transient state happened and lasted for one or two period.

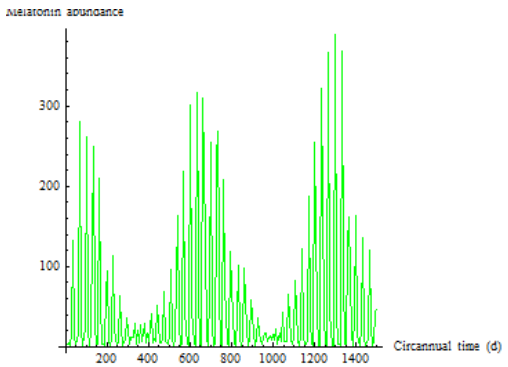

Figure 27: Entrainment of free-running system for stimulation of r_m from 1 to 2 for 1 day

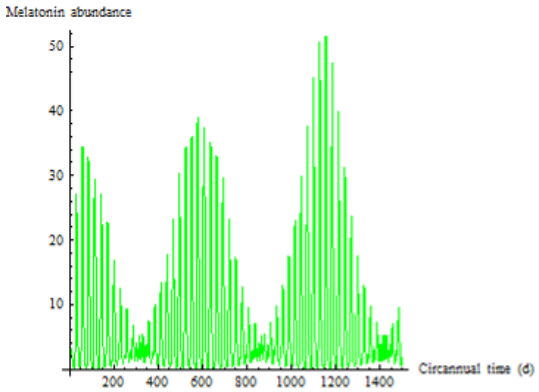

Figure 28: Entrainment of free-running system for stimulation of Q_m from 0.21 to 0.42 for 1 day

3.2 DISCUSSION

In this study a model for intracellular circannual rhythm generation was studied and its stability and robustness with realistic entrainment is investigated. In this model the complexity of reaction cascade is reduced into two specific delay and four non-linearity terms. By this mean, the necessity for a complete description of all the processes involved is avoided. At the chosen set points in parameter space, robust cycles with free-running oscillation was investigated. Limit cycle behavior was studied by changing only one parameter at a time in parameter subspaces. Eliminating non-linearities,{n=1,m=1,c=1,e=1}, results in stable steady state. Eliminating one non-linearity impose the need for other non-linearity terms to be substantially large. Also smaller delay values requires large values for non-linearity terms. Therefore, for the emergence of circannual oscillation, delay and non-linearities are essential. Strong dependence of the period of the oscillation was on delay terms and non-linearity terms of the protein synthesis cascades. Therefore, small changes in delay and non-linearity terms lead to large changes in the period. The entrainment properties was studied by periodical manipulation of production or degradation rates. This was predicated on the interference of light into the feedback loop in nature. As proven, the entrainment rate depends on the intensity of stimulus. The objective of this study was to develop a base model for circannual rhythm generation. The delay and the non-linearity are essential components in this model. They have been implemented in biochemical processes to compensate for the lack of knowledge in precise nature of migration. This model needs moderate cooperativity since all of its non-linearities are low in value.

4. CONCLUSION

In the proposed model, delay and non-linearities have been emphasized in the protein synthesis negative feedback loop as an essential components to have a robust circannual oscillation. The model displays realistic behavior of circannual rhythm with respect to period and entrainment. The most important issue investigated in this study was the coupled interaction among a set of biological pacemakers with both circadian and circannual oscillations with specific application to zugunruhe. Further studies are still needed to understand the underlying biology of the avian circannual clock. Further outcome and future investigation of this study includes analyzing dead zones for perturbation in different parameters. Also, finding the quantitative dependence of the priod of oscillation on the parameters would be an objective along advancing this model. This model will serve as a basis for interpreting experimental findings and formulating critical experiments as well as contributing to a better understanding of biochemical mechanism underlying the cricannual rhythm generation.

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