

Modeling the circadian dynamics of morning and evening oscillators and the role of circadian rhythm in learning and memory



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Certificate

This is to certify that the thesis titled “**Modeling the circadian dynamics of morning and evening oscillators and the role of circadian rhythm in learning and memory**” being submitted by **Shiju S** to the Indraprastha Institute of Information Technology Delhi, for the award of the degree of Doctor of Philosophy, is an original research work carried out by him under my supervision. In my opinion, the thesis has reached the standards fulfilling the requirements of the regulations relating to the degree. The results contained in this thesis have not been submitted in part or full to any other university or institute for the award of any degree/diploma.

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Abstract

Circadian rhythms (CR) are 24h endogenous oscillations seen in all organisms that control different biological processes including sleep-wake cycles, body temperature, cell division cycle, heartbeat, and metabolic rates. Light, the prime zeitgeber, accounts for the day-night changes and seasonal variation of the environment, and it modifies the endogenous (free running) period of the circadian rhythm, known as entrainment. In mammals, suprachiasmatic nucleus (SCN) is the main brain region that acts as a circadian master oscillator, which controls all the other peripheral oscillators. The circadian pacemaker in SCN plays an important role in encoding day length and seasonal changes. Identifying the molecular mechanism responsible for this encoding is an interesting problem that has attracted attention for a considerable period of time. A set of hypothesis has been proposed by Daan and his colleagues that the encoding is done by the circadian pacemaker that consists of two distinct oscillators of which one is the morning (M) oscillator that locks on to dawn and controls the morning activities, while the other is an evening (E) oscillator that locks on to the dusk and controls the evening activities. So far Daan's hypotheses have neither been confirmed nor verified. The other problems that have attracted attention recently are the experimental generation of daily variation in firing patterns and the circadian modulation of synaptic plasticity, learning, and memory. However, molecular mechanisms responsible for the generation of daily varying firing patterns and the role of circadian rhythms in modulating learning and memory are not clearly delineated. Understanding of these problems requires careful construction of mathematical models with different time scales based on molecular mechanism of gene regulatory and signaling pathways.

In this thesis, the following aspects of circadian rhythms are modelled and analyzed.

(i) Construct and analyze the mathematical model of the circadian gene regulatory network (GRN) to verify the Daan's hypothesis about ME oscillators. Modeling capture the differences in the variations in the period, phase response curves, entrainment in ME oscillators, and splitting. Our model largely supports the presence of ME oscillators in SCN.

(ii) Construct and analyze a multi-scale model to determine the role of both slow varying mammalian circadian oscillations (in hrs) and Gaussian noise in modulating the rapidly varying firing patterns (in milliseconds) exhibited by SCN neurons under constant dark conditions.

(iii) Finally, Construct and analyze a multi-scale model to infer the role of SCN circadian rhythm in modulating learning and memory at hippocampus and amygdala. Particularly, model is constructs based on the molecular mechanism that controls the circadian variation of long term potentiation/depression (LTP/LTD), and fear conditioning paradigm protocols. The models are validate with the experimental data that provides considerable insight about how the time-of-the-day affects the cognitive functions.

(iv) Besides, we develop a Hilbert transform (HT) based numerical tool to extract important characteristic features of circadian rhythms like phase response curves, entrainment, period variations, and phase variations from both the model and experimental circadian time series.

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Chapter 1

Introduction

The cells and tissues in many organisms exhibit rhythmic oscillations that varies from seconds to hours to years. Some of the examples of rhythmic behaviors are sleep and wake cycle, changes in the body temperature, cell division cycles, metabolic rates and the variations in the periodic heart beat. Biological processes responsible for these behavior have been studied in detail to understand the etiology of the rhythmic processes. One of the rhythmic behavior that has been extensively studied over the years is the circadian (Latin *circa*, meaning "about", *dian*, meaning "day") oscillators that has a free running period of approximately 24h, entrains to external environmental stimuli, and the period of the oscillator do not vary significantly under varying temperature conditions. The circadian oscillations can persist even in the absence of external cues like light-dark (LD) cycles and temperatures. *Drosophila*, *Neurospora*, and algae displays circadian behavior and entrains in response to external stimuli like daily or seasonal changes.

In mammalian system, the rhythmic behavior of the circadian clock emanates from Suprachiasmatic nuclei (SCN), which is the endogenous master oscillator with a period close to 24h [1]. The anatomy of the SCN is extremely complex, and it consist of approximately 20000 circadian pacemaker neurons. Importantly, in SCN, the individual neurons are capable of exhibiting self sustained oscillations and the cells are coupled to each other through hormonal peptides to work together as one pacemaker [2]. SCN is therefore refered to as master oscillator which controls the amplitude, and phase of the peripheral slave oscillators that are present almost all throughout the body that includes hippocampus, amygdala, heart, liver, kidney etc. Therefore, SCN maintains a constant phase relationship not only with the external cues to keep the circadian clock synchronized with the local time, but also with the internal cues to maintain certain phase relationship with the peripheral oscillators. As a

result, circadian clocks in SCN are flexible, adaptable, and adjust its speed to the external zeitgeber. SCN can be broadly divided into two anatomical and functional subdivisions [3]; core (ventrolateral) and shell (dorsomedial) as shown in Fig.1.1. Core neurons relay light information from the retinohypothalamic tract (RHT) to the rest of the SCN through gamma-aminobutyric acid (GABA) and vasoactive intestinal peptide (VIP). Neurons in the shell region communicate with other cell population through GABA and arginine vasopressin (AVP). Subparaventricular zone (sPVZ) and the dorsal medial hypothalamus (DMH) receives the output from the SCN neurons. It has been shown that the ablation of SCN results in the complete loss of circadian function that includes elimination of behavioral activities, loss of oscillations, and desynchronization of phases in peripheral oscillators controlled by SCN [4].

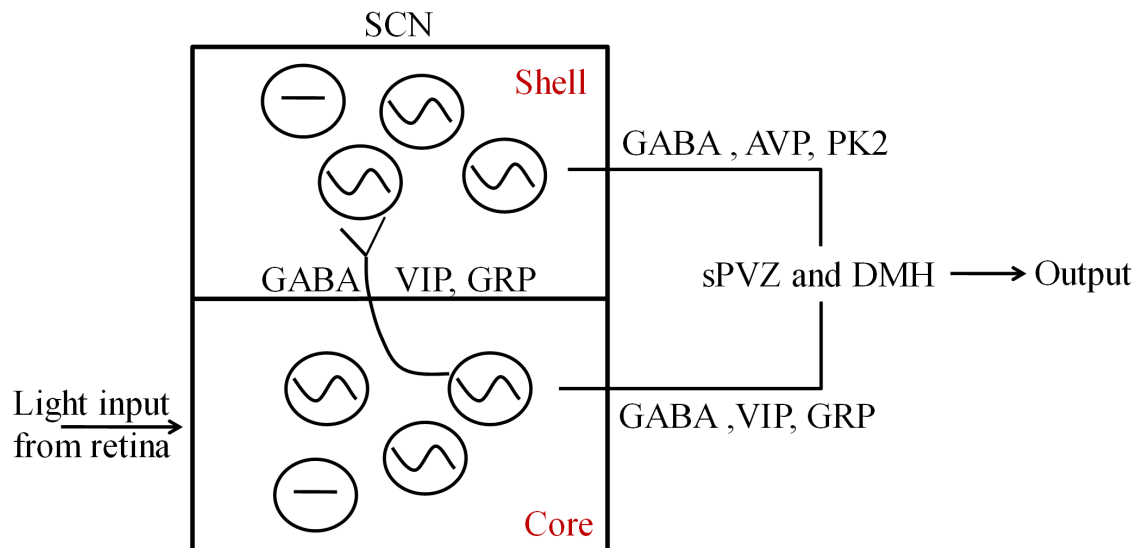


Fig. 1.1 Anatomy Of SCN. SCN divided into two- part: core and shell. Core receive light input from RHT and relay it to the other part of SCN. Both core and shell communicate with other cell population.

The cell autonomous circadian oscillator is present not only in unicellular organisms, but also in multicellular organisms [6]. With the advent of new genetic techniques, molecular mechanisms of gene expressions and the regulations responsible for the generation of circadian oscillations are well characterized. In particular, the time-of-day effects to the external cues are well studied in the models of fungal species *Neurospora*, fruit fly *Drosophila*, plant *Arabidopsis thaliana*, and mammalian mice models [7–9]. Without any exceptions, the cell autonomous circadian rhythms of all the species are tightly regulated by the interlinked multiple negative and positive feedback loops that guide circadian pacemakers to function under various conditions [10]. There are three different controls that happen at gene regulatory level in circadian rhythms [11]. At RNA level control happens by alternate splices.

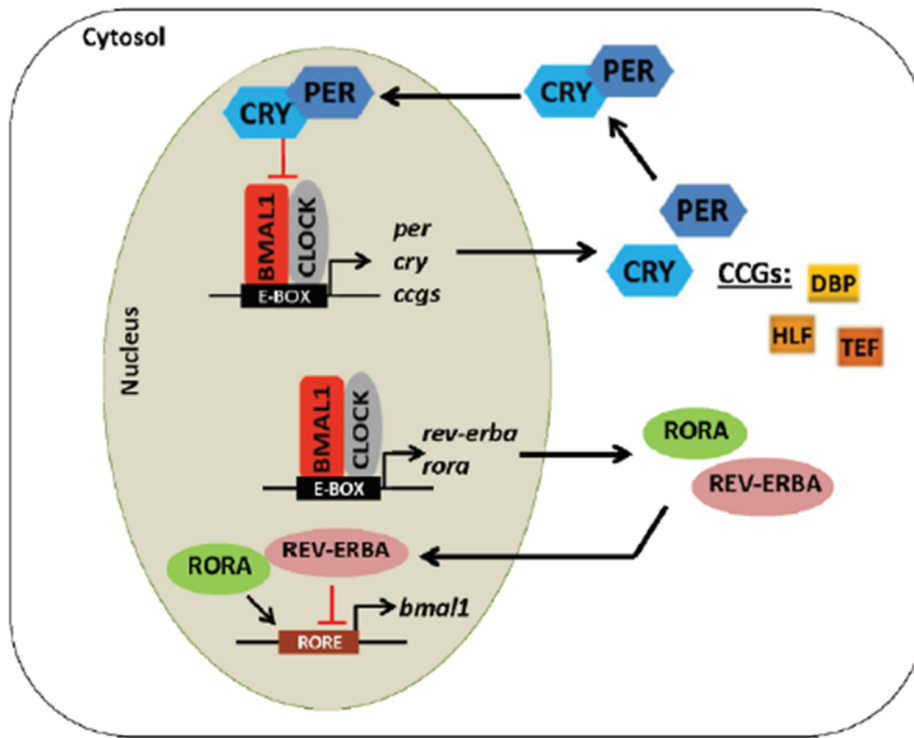


Fig. 1.2 Transcriptional-translational feedback loops of SCN. *per* and *cry* gene's transcription process is activated by transcription factor (TF) CLOCK-BMAL1, while the different PER and CRY protein heterodimeric complex bind directly to the TF, reduce the transcription of *per* and *cry* genes. ROR α activates, whereas REV-ERB α represses the transcription of the *clock* and *Bmal1* gene. The *rev-erb α* gene is also activated by the TF, and hence BMAL1 indirectly inhibits its own gene transcription. Figure is taken from [5].

At translational level the clock proteins are phosphorylated for stability and degradation. Also, these controls provide the delay in the negative feedback loop for oscillation. Finally, degradation reactions at mRNA and protein level maintain the period and amplitude of the oscillation.

To be specific, in the mammalian system, the transcriptional-translation network of the circadian oscillator consists of positive and negative limbs with *Bmal1*, *Clock* genes and their proteins. BMAL1 and CLOCK constitute the positive limbs [12]. The CLOCK-BMAL1 heterodimer binds together to form a complex that positively regulates the negative limb genes *per1/2/3*, and *cry1/2*, and their protein products PER 1/2/3 and CRY1/2 [13] by binding to their E-box promoter regions [14]. The cytoplasmic proteins PER and CRY form a heterodimer PER-CRY that translocates to the nucleus to repress their own transcription by binding to the CLOCK-BMAL1 complex and thus establishing the negative feedback loop [15]. The orphan receptors REV-ERB α , and ROR α repress and activates the transcription of *Bmal1* respec-

tively [16, 17]. There is also a positive feedback loop by which PER2 protein regulates *Bmal1* positively [13, 18] by negatively regulating Rev-erb α [16] and this creates an asymmetry between *per1* and *per2* negative limbs. A network of transcriptional-translational feedback loops is shown Fig. 1.2.

Time delay between transcription and nuclear accumulation is necessary for circadian rhythm generation. Posttranslational processes including phosphorylation, complex formation, nuclear relocation, and degradation introduce sufficient delay for oscillation [19]. Phosphorylation reaction activates or inactivates the clock proteins, and also initiate protein degradation [20]. Phosphorylation reactions are important to convey 24h time constant to the circadian gene regulatory network (GRN) [13]. It affects the stability and nuclear transport of clock proteins. In SCN, nuclear location of *per1* and *per2* mRNA depends on their phosphorylation state [21]. In the absence of *cry* level rising, phosphorylation destabilizes *per1*, which deny its accumulation in cell culture [13]. Finally phosphorylation of cAMP response element binding protein (CREB) induces transcription of *per1* and *per2* gene via calcium and cAMP response element (CRE) [22].

Though the basic guiding principle in the circadian oscillators are the presence of auto-regulatory feedback loops, there are also other differences in circadian organization in diverse species, and new information is accumulated through careful experimentation to unravel the circadian function that may be different due to these differences. For example, one interesting observation about mammalian SCN is its entrainment to light, whereas the peripheral oscillators are not entraining. Similarly, it has been clearly shown that peripheral oscillators are phase delayed by 4-12 hours in comparison to circadian oscillations of SCN indicating the time delayed entrainment of peripheral oscillators [23]. Further, it is still not known how the master SCN oscillator controls the peripheral oscillators and the molecular mechanism responsible for this control is yet to be elucidated.

1.1 Circadian rhythm and health

Circadian rhythm not only affects the functions of individual cells in tissues, but also organs. Over the years enormous evidence has accumulated that connects the circadian disruptions to many clinical disorders like metabolic syndrome, obesity, hypertension and cardiovascular diseases [24, 25]. Abnormal circadian rhythm leads to sleep disorders, seasonal affective disorders, mental health disorders (depression), obesity, diabetes and importantly, it is shown to adversely affect the cognitive functions like learning and memory [26]. One of the major disrupting factor of circadian rhythms is the artificial electrical lighting, that disrupts the

sleep-wake cycles. It has been documented that the consequence of exposure to unnatural light exposure that results in significant alteration is circadian rhythms [27]. Circadian rhythm controls the production of melatonin hormone which passes the light information from the eyes to the brain through optic nerves, which ultimately help to control our sleep-wake cycle. Jet lag is another common cause of mismatch in synchronization between external light-dark cycle and circadian rhythms when people fly to different time zones. Parkinson disease is associated with disruption of circadian sleep-wake cycle, and other type of sleep disorders are also observed in those patients [28]. Understanding the working of biological clock and its molecular mechanisms may lead to the discovery of new therapeutics with an enhanced efficacy for the treatment of many circadian disorders.

Another area that has recently attracted significant attention is the cognitive impairment due to circadian disruptions. The hippocampus is a region of the brain which is the center for learning, memory formation and storage, where clock gene expression and synaptic plasticity shows circadian rhythmicity. The SCN connects to hippocampus, which successively mediates hippocampal activation [29, 30]. Experimental results showed that the circadian rhythm has a significant influence on learning and memory, but the exact mechanism is not yet clear. Under LD condition mice showed peak memory in the early daytime, and under constant darkness, memory shows the same peaking time, which is independent of the time of training [31]. Another evidence indicates memory formation depends upon training time [32]. In rats, melatonin regulates memory [33] and it inhibits long-term potentiation (LTP) in the hippocampus [34]. The melatonin release and accumulation are controlled by the circadian clock, which raises the possibility that learning and memory can be modified by the circadian clock through hormonal signaling. Ruby et.al [26] showed that circadian regulation of learning might be exerted via cyclic GABA output and the circadian system is involved in memory function. They showed that arrhythmic hamsters fail to perform a simple learning task, which indicates that the circadian system has a significant role in learning and memory. Previously mentioned evidence along with other experimental results stipulate a strong relationship between the circadian system and learning and memory in the hippocampus. This is another area whether new technologies are developed to understand the role of circadian modulation on learning and memory.

In the next section, definitions and terminologies that have been used extensively in the area of circadian biology are provided. These definitions are used extensively in the later chapters.

1.2 Definitions

1.2.1 Nocturnal and diurnal organism

Nocturnal organisms are active during the night and sleep during the day (e.g., Mice). Its reverse in the diurnal organisms which is active during the day and sleeps at night (e.g.: *Drosophila*, human beings).

1.2.2 Free running rhythm

Circadian rhythms (CR) are self-sustained oscillations, and they free run in the constant conditions, which are independent of the environment. Free running period of CR depends on the species. For example, free running period of *Drosophila*, *Neurospora*, and mice are 24.4h, 21.5h, and 23.7h, respectively.

1.2.3 Zeitgeber

Circadian rhythms respond to external cues such as light, temperature, and food availability, which are referred as zeitgeber ("time giver"). Light is the prime zeitgeber for circadian rhythm which is known as photic zeitgeber that accounts for the day-night changes and seasonal variation of the environment. Temperature cycle, exercise, and social interactions also can entrain circadian rhythm, which are known as nonphotic zeitgeber.

1.2.4 Circadian time (CT) and subjective day/night

If the organism kept under DD condition, it free runs with a period which is not equal to 24h. In this free running condition, time is expressed in circadian time (CT), where one circadian cycle is equally divided into 24 circadian unit. Each unit is defined as the $1/24^{th}$ of the free-running period. For example, a free running period of mice under DD condition is 23.7h, 1 CT is equal to 59.25mins (23.7/24 h). Since the mice is a nocturnal animal, they are active at night time and rest at daytime. Therefore, CT0 indicate the beginning of the rest phase of the mice which is defined as the subjective day, CT12 is the beginning of the active phase, which is referred to as subjective night.

1.2.5 Zeitgeber time (ZT)

In the presence of LD cycle, time of the circadian system is defined in terms of zeitgeber, known as zeitgeber time (ZT). ZT0 is defined as the onset of the light phase, and ZT12 is the onset of the dark phase.

1.2.6 Internal time and external time (ExT and InT)

Two different time conventions are discussed in [35], internal time (InT) for DD condition and external time (ExT) for LD condition. According to their protocol, internal time is given as $[CT0 - 10]_{mod24}$ and external time is defined as $[ZT + \text{half of the duration of dark phase}]_{mod24}$.

1.2.7 Constant conditions and light-dark conditions

Depending on the availability of light in a day, the experimental environmental condition can be divided into three cases:

Constant darkness (DD): No light is available throughout the day. Mammals experience constant darkness during hibernation.

Constant light (LL): Light is available throughout the day.

Light-Dark condition (LD): Light and Dark is available in the specific duration of the day. It is the experimental condition for normal day-night variation. Duration of the light and dark may vary depending on the seasonal variation.

1.2.8 Photoperiod (duty cycle)

Duration of the light phase in a single LD cycle is commonly expressed in percentage. This known as photoperiod or duty cycle

$$Photoperiod(\%) = \frac{L}{L+D} 100 \quad (1.1)$$

Where L is the duration of the light phase, and D is the duration of the dark phase. $photoperiod = 0$ referred as DD condition, and $photoperiod = 100$ referred as LL condition.

1.2.9 Entrainment

Zeitgeber modifies endogenous (free running) period of the circadian rhythm, which is known as entrainment. It synchronizes the internal circadian rhythm to external time cues. Entrainment can affect the overall timing of sleep and wakefulness. For example, length of the day and night may vary according to seasonal changes, where long nights during winter and long days during summer. SCN adapts to these seasonal changes in the natural light-dark cycle. The peripheral clocks are also entrained by the SCN and show circadian oscillation during physiological events. Endogenous signals including glucocorticoids and cortisol may be responsible for the circadian entrainment of peripheral oscillators.

1.2.10 Aschoff's rule

Aschoff's rule describes the difference between the period of oscillation of nocturnal and diurnal organism under constant light conditions [36]. According to this rule, for a nocturnal organism, with increase in the light intensity, the period of the oscillation increases and activity time decreases. Whereas, for a diurnal organism, with increase in the light intensity, the period of the oscillation decreases and activity time increases.

In the subsequent subsections, definitions and terminologies that has been used extensively in the area of cognition that relates to learning and memory are provided. These are used extensively in the later chapters.

1.2.11 Memory

Memory is an electrically encoded representation of an event. There are different types of memory; all involve changes happening due to learning. Based on the experience, individual improve the future behavior. Memory is broadly divided into two categories, declarative and non-declarative memory:

Declarative memory: Also known as explicit memory, it is the memory of facts and events. It can be easy to acquire and easy to forget and can be consciously recalled. Declarative memory is further divided into episodic memory and semantic memory.

Episodic memory: It refers to the memory of experience associated with time, events or place.

Semantic memory: Memory that refers to the knowledge that we acquire throughout the life.

Nondeclarative memory: Also known as implicit memory, which is associated with the skills, habit, and behaviors, which required repetition and practice. There is less chance to forgotten once learned.

1.2.12 Long term potentiation (LTP)/long term depression (LTD), and spike time dependent plasticity

LTP/LTD refers to the strengthening/weakening of synaptic connection between the neurons, which are considered as the principal mechanism underlying learning and memory. The synaptic changes (LTP/LTD) plotted as a function of the relative timing of pre and post action potentials is called spike time dependent plasticity (STDP). Maximal synaptic modification occurs when the temporal difference of pre and postsynaptic action potentials are small, while no plasticity occurs if the temporal difference is large.

1.3 Feedback loops

In the input/output system (I/O), a feedback loop is described as the one in which some component of the output of the system routed back as the input. In Fig. 1.3 A, the output of the system *A* is routed back to its input through a feedback system *B*. A feedback loop can amplify or inhibit the overall output of the system. A feedback loop can be explained better in terms of molecular network motif (Fig. 1.3 B, C). A feedback loop is positive or negative depending on the signs of the edges (Fig. 1.3 D).

Feedback loops are a rule rather than exception in biological oscillators. Analyzing the oscillations of biological system through models is an important aspect that provide insights into the workings of the molecular mechanism under normal and pathological conditions where the dynamics changes. Many models of circadian oscillators that are analyzed and explained in the later chapter to explain the dynamics captured by the experimental data, involves feedback loops that modulates the amplitude and period of the circadian oscillations. To capture the different types of feedback loops that operate in the molecular networks of circadian biology, a summary with examples of different types of feedback loops and their dynamics are explained in the following subsections.

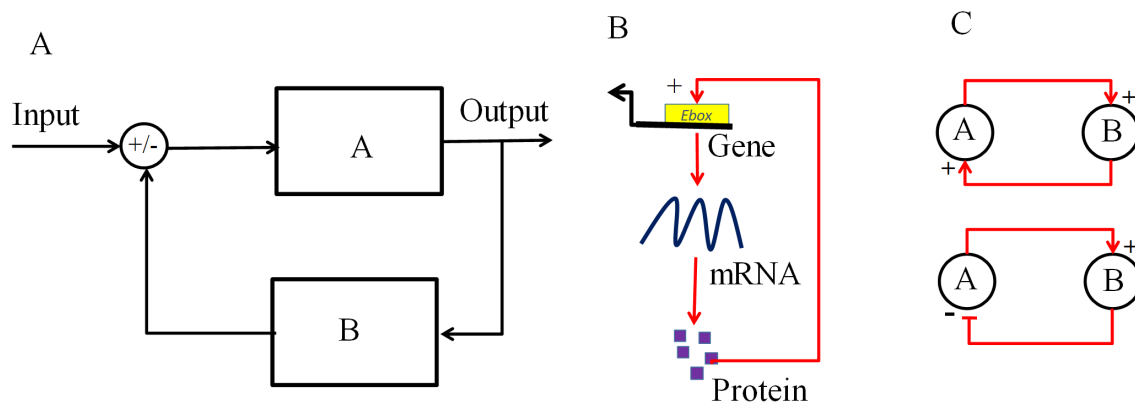


Fig. 1.3 Feedback loop. (A) Block diagram of feedback loop. Output of the system A is given to the feedback system B and it is then routed to the input of system A. (B) A gene regulatory transcriptional-translational positive feedback loops. (C) Abstract form of positive and negative feedback loops where A and B are the nodes and the arrow denote edge with sign. The sign determine the nature of the feedback loop.

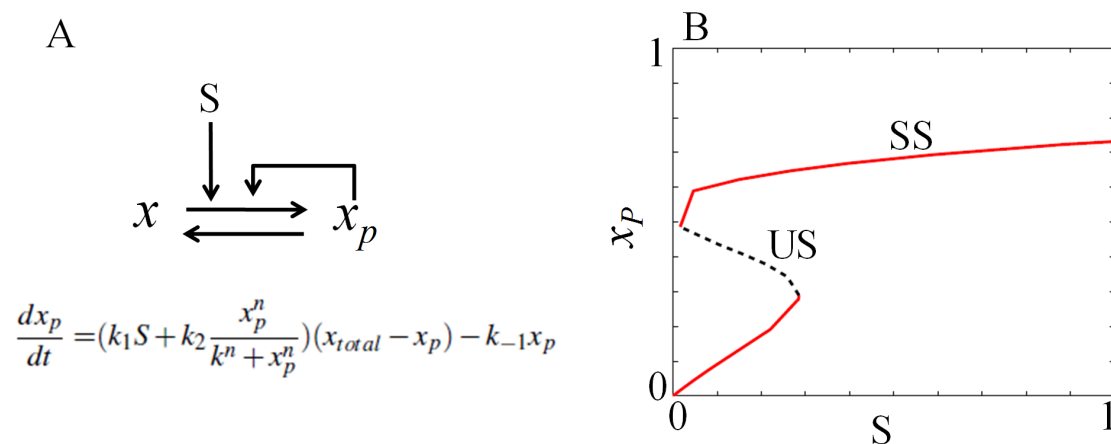


Fig. 1.4 Positive feedback and bistability. (A) Schematic view of simple positive feedback loop and its dynamics. A phosphorylation-dephosphorylation reaction, where phosphorylated product (x_p) promote its own production from x . In the equation, S is the signal, second term capture the positive feedback regulation by Hill's equation with $n > 1$, and last term is the reversible reaction. (B) Codimension-1 bifurcation diagram with S as bifurcation parameter. System has two stable steady states (red lines, SS), which are separated by one unstable steady state (black broken line, US). ODE for simulation is adapted from [37], and the parameters are $k_1 = 1$, $k_2 = 2$, $k = 0.5$, $x_{total} = 1$, $k_{-1} = 1$, $n = 5$.

1.3.1 Positive feedback loop

In a positive feedback loop, increment in one of the variable or process causes an increase in the response of another variable or process. A simple positive feedback loop that is commonly encountered in molecular biology is the phosphorylation-dephosphorylation reaction as shown in Fig 1.4 A, where phosphorylated product (x_p) promote its own production from x . Positive feedback loop can create three different types of responses depending on the network model and the choice of parameters. They are (a) a graded or hyperbolic response, (b) an ultrasensitive or switch-like response and (c) an ultrasensitive or switch-like response with hysteresis. Hysteresis creates memory, where it depends on the choice of the initial conditions. For example, hysteresis creates bistability, where two stable steady states are separated by an unstable steady state (Fig 1.4 B). Depending on the strength of the input signal, and if the input signal strength is below a certain threshold value, the system will be in one stable steady state, when it exceeds the threshold value, system switch to another steady state. The point where the stable and unstable steady state meets is called a bifurcation point. In the case of bistable system, the dynamics of the stable steady state is a node, whereas the dynamics of the unstable steady state is a saddle. The point where unstable saddle collides with stable node is called the saddle-node bifurcation. In Fig 1.4 B, a codimension-1 bifurcation diagram that exhibits saddle-node bifurcation is constructed from the model shown on the left for the bifurcation parameter 'S'.

1.3.2 Negative feedback loop

In a negative feedback loop, the increment in one variable or process causes a decrease in the response of another variable or process. In the homeostatic process, negative feedback stabilize the system, to a level that is relatively constant. Negative feedback loop can also generate oscillatory responses. A negative feedback loop with two components doesn't exhibit oscillations (Fig 1.5 A, B), and for sustained oscillation, at least 3 component is required. The third component introduces sufficient delay in the feedback loop, which induced the system periodically to overshoot and undershoot its steady state (Fig 1.5 C, D).

1.3.3 Mixed feedback loop

If a system containing both positive and negative feedback loop, then it promotes oscillations [39]. Positive feedback loop generate bistability and the negative feedback loop operate the system to and fro between the two stable states. Fig 1.6 shows that in a three component

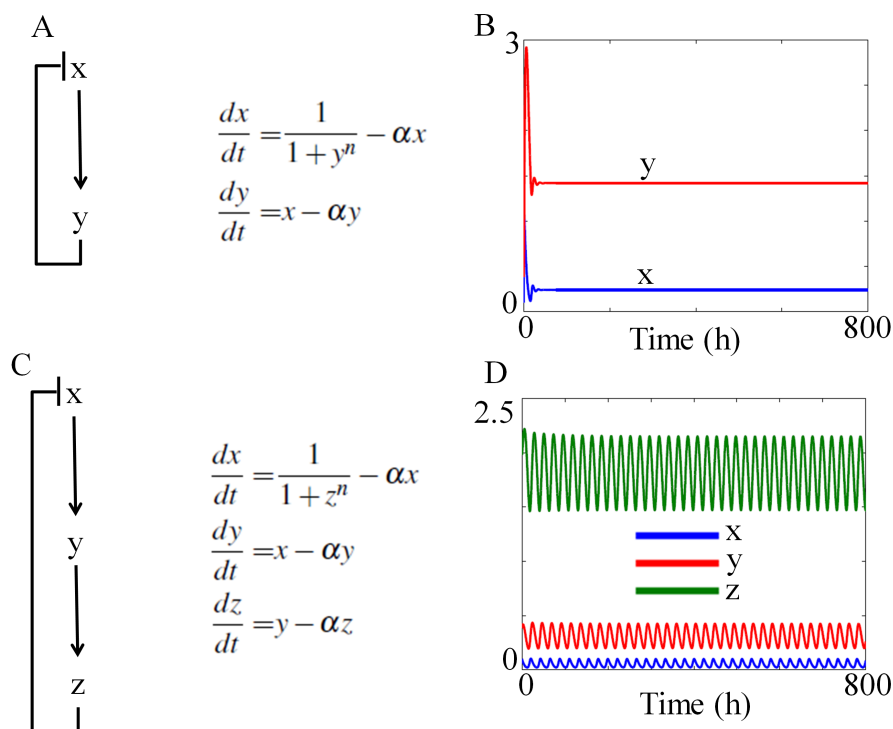


Fig. 1.5 Negative Feedback. (A) Negative feedback with two component (x, y), which doesn't produces oscillations (B). (C) Negative feedback with three component (x, y, z) produces oscillations (D) [38]. $\alpha = 0.17$ and $n = 9$ are used for simulation.

negative feedback loop oscillations are not possible at lower degree of cooperativity ($n < 9$), however, positive feedback connection promote oscillation at lower degree of cooperativity ($n = 7$). Tyson et al. proposed a circadian model of *Drosophila* with a double negative and positive feedback between monomer and dimer of PER protein. The equilibrium between monomer and dimer results in the protection of dimers from the DBT degradation and thereby increases the total concentration of monomer and dimer and its accumulation. Detailed dynamics of this model will discuss in chapter-3. Oscillations are also possible in a two component model with interlocked positive and negative feedback loop [40]. In Fig 1.6D, x activates y , and y inhibit x . Additionally, x activate its own production (autoregulation). This extra positive feedback brings sufficient time delay for oscillation. Fig 1.6E shows the time series of oscillations, that are not smooth, rising phase is slow and falling phase is fast, which is termed a relaxation oscillator. Circadian system consists of interlocked positive and negative feedback loops, which generate sustained oscillations. Interlocked molecular feedback loops of *Neurospora* and *Drosophila* circadian systems are shown in Fig 1.6 F, G.

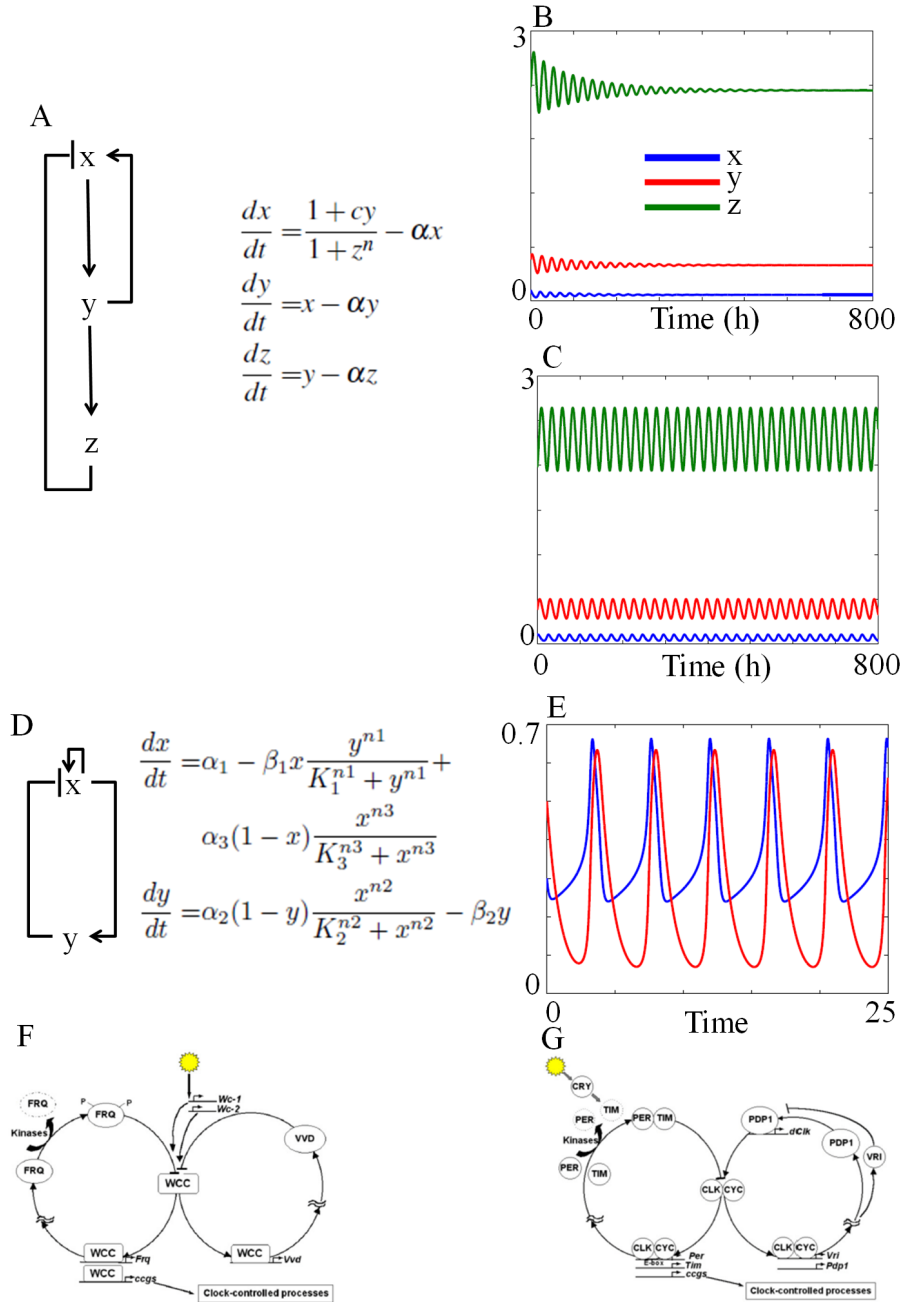


Fig. 1.6 Mixed Feedback. (A) z inhibit x , and y promote x , forming a negative-positive feedback loop. (B) Damped oscillations for low cooperativity ($n = 7$), when the strength of positive feedback loop is low ($c = 0.1$). (C) Sustained oscillation for low cooperativity ($n = 7$), when the strength of positive feedback loop is high ($c = 5$). $\alpha = 0.17$ is used for simulation. (D) Interlocked negative-positive feedback loop with two component. x activates the production of y , and x is inhibited by y . Also, x activates its own production. (E) Two component model produces relaxation oscillations. Model equations are taken from [40]. $\alpha_1 = 0.02$, $\alpha_2 = 3$, $\alpha_3 = 3$, $\beta_1 = 3$, $\beta_2 = 1$, $K_1 = 0.5$, $K_2 = 0.5$, $K_3 = 0.5$, $n_1 = 8$, $n_2 = 8$, $n_3 = 8$ are used for simulation. Interlocked molecular feedback loops of *Neurospora* (F) and *Drosophila* (G). Figures (F, G) are taken from [45].

1.4 Biological background of the problem

One of the interesting problems that have attracted attention for a considerable period of time is to identify the molecular mechanisms responsible for encoding day length in the circadian pacemaker. It has long been thought that circadian pacemaker consists of two mutually coupled identical, but functionally distinct oscillators named morning (M) and evening (E) oscillators [41–43], and these are responsible for encoding day length and seasonal variations. This hypothesis was based on the observation that in hamster constant light exposure causes splitting of locomotor activity rhythms[44], where single daily bout of wheel-running activity dissociate into two components.

Splitting was first explained mathematically by Pittendrigh and Daan’s dual oscillator model [41], and inspired by their works, Daan et al. [43] cogently put together the criteria for morning and evening oscillators in terms of the molecular mechanisms of circadian gene expression. They supported their hypothesis based on the experimental data obtained from both wild and mutant mice. However, due to various reasons, the existence of two different types of oscillators in SCN in circadian system is not accepted [46]. In review on ME oscillators based on experimental data, specifically from mice and *Drosophila*, Helfrich-Förster et al. [47] were unable to arrive at a consensus model of dual oscillator to describe ME activities due to various factors like number of neurons, its anatomical location, differential light responses, the role of different neurotransmitters in different species, and importantly, the complex wiring of the molecular network. Therefore, in-silico modeling based on gene regulatory network may be a way to resolve the role of ME circadian oscillators in SCN that may help to validate the hypothesis proposed by various groups.

Another problem that has attracted the attention recently is the generation of circadian firing patterns and the circadian modulation of synaptic plasticity, learning and memory, which show daily variations. It has been shown that the mean value of conductance of SCN neurons and the frequency of the spontaneous firing rate (SFR) vary in a circadian manner [3]. Similarly, the role of circadian rhythms in influencing learning and memory has been studied for a long time. For example, Chaudhury et al. [48] found that long term potentiation (LTP) in the CA1 region of mice showed diurnal variation, where LTP during night is higher than day. Under constant dark (DD) condition, LTP shows higher magnitude during subjective night indicating the endogenous circadian rhythm modulation of the synaptic plasticity [48]. Circadian rhythm modulates acquisition, recall, and extinction in fear-conditioned mice [31], however, molecular mechanisms and network dynamics responsible for the modulation are not well known. Construction of multi-scale in-silico model that has slow varying GRN and fast varying electrophysiological model may provide insight, validation, and prediction about

the role of circadian rhythm in modulating firing patterns and on the time-of-the day effects of learning and memory.

Understanding the role of morning and evening oscillators in circadian rhythms requires careful construction of gene regulatory networks based on molecular mechanism of gene expression and to understand the circadian modulation of learning and memory requires building of multi-scale models that couples fast varying electrophysiological conductance dynamics and slow varying molecular network models. Many mathematical models for gene regulatory networks of circadian rhythms have been proposed for *Neurospora*, *Drosophila*, and mammals [49] to understand the workings of circadian pacemaker under various conditions. Specifically, previous models of ME oscillators are phenomenological in nature and are not based on the molecular basis of circadian oscillations and these models mostly accounted only for the splitting behavior [42, 50]. Till this date, there are hardly any attempts to understand the origin of ME dual oscillators from the point of view of GRN models. Similarly, to investigate the mechanism of synaptic plasticity, different mathematical models of LTP/LTD/STDP were previously developed, and a detailed review of the models of synaptic plasticity and their characteristics properties were discussed in [51]. Computational models for fear conditioning at amygdala were also previously developed [52–56] and explained the mechanism and dynamics behind learning, recall, and extinction. However, these models for synaptic plasticity, learning, and memory have not included the daily variations in the cognitive functions. To the best of our knowledge, a multi-scale model that includes circadian GRN and electrophysiology coupled by neurotransmitters are not currently available to explain the importance of circadian variation of LTP/LTD, learning and memory.

1.5 Present work: statement of the problems

In this thesis, we investigate three different problems on circadian rhythms that are provided below.

- (i) To construct and analyze mathematical models of the circadian GRN using the tools of dynamical systems to verify the Daan's hypothesis for ME oscillator.
- (ii) To construct and analyze a multi-scale model to determine the role of slow varying mammalian circadian oscillations (in *hrs*) and Gaussian noise in modulating the rapidly varying firing patterns (in *milliseconds*) exhibited by the ionic channels of SCN under DD conditions.

(iii) Finally, the aim is to construct and analyze the multi-scale model to captures the role of circadian rhythm in modulating learning and memory. Particularly the model is built based on the molecular mechanism that controls the circadian variation of LTP/LTD, and fear conditioning, which provides insight about how the day-night and seasonal variations affect the cognitive functions.

Besides, we develop a technique based on Hilbert transform (HT) to extract important characteristic features of circadian rhythm like phase response curves, entrainment, period variations and phase variations from model and experimental circadian time series.

1.5.1 Thesis organization

This thesis is organized as follows: In chapter-2, various existing dynamical models, tools and techniques used in analyzing these models are described. A Hilbert transform (HT) based numerical method to analyze the time series of the circadian rhythms are introduced in chapter-3. A single cell dual oscillator model of mammalian circadian rhythms to explain ME oscillator is explained in detail in chapter-4. In chapter-5, a multi-scale model which incorporates both GRN and electrophysiological level of SCN is described in detail to explain the role of mammalian circadian oscillations and Gaussian noise in modulating the firing patterns. In chapter-6, construction of multi-scale model and its numerical simulation is explained in detail to describe the circadian modulation of learning and memory. In chapter-7, conclusion, and an outlook of the future work is given.

Chapter 2

Dynamical models and techniques

2.1 Introduction

The advances in genetic and recombinant technologies have provided insight about the functions of the living cells to a very minute detail. Molecular biologists are highly successful in dissecting every details of the molecular interactions responsible for the different behavior of the cells under various conditions. The molecular interactions obtained from these experiments are neatly visualized in the form of graphs that has nodes and directed edges. These directed graphs with nodes connected by edges with positive or negative signs represent molecular circuits or regulatory networks. This directed network is further converted into sequence of biochemical kinetic steps that represent the gene-protein and protein-protein interactions so that a precise mathematical models can be developed based on mass action kinetic laws. The dynamical properties of the regulatory networks from the analysis of this model provides a good understanding of why the biological systems behave in the way they do. Excellent review on molecular network, mathematical models, and biological interpretation is provided in [57]. To understand circadian rhythms from modeling perspective, different types of models have been proposed and its broadly summarized in [58]. Presently, the detailed molecular interactions that gives rise to circadian oscillations are clearly elucidated and the mathematical models are built based on the molecular mechanism of circadian gene expression. The commonly built mathematical model for circadian rhythms is based on the nonlinear differential equations, which can be either based on the laws of mass action kinetics [59, 60] or based on both mass action and enzyme kinetics [61]. These models are amenable to perform stochastic simulations to account for the effect of noise in circadian systems [62], and can also be suitably converted to delay models [63–65]. Recently, summary

of all the available models for mammalian circadian rhythms are provided in [66]. However, there are groups which still persists with mass-spring type of nonlinear oscillator models to effectively simulate the circadian rhythm effect on sleep and wake cycles [67].

2.2 Mathematical models of circadian rhythms

An overview on various types of mathematical models used to simulate circadian rhythms and the type of dynamical analysis carried out on these models are presented in the following sections. Some of the well known mathematical models, ranging from phenomenological to detailed molecular-based models employed to simulate various properties of circadian rhythms are explained in detail in this section. One of the earliest and simplest model used to simulate circadian rhythm is the simple harmonic oscillator [58], and it is mathematically represented as

$$\frac{d^2x}{dt^2} + \alpha^2 x = 0 \quad (2.1)$$

where x is the position coordinate and α is a scalar which controls the period of oscillation. Equation 2.1 provides the behavior of pendulum that oscillates forever in the absence of friction. This can be reduced to a normal form and its given as:

$$\frac{dx}{dt} = y \quad (2.2)$$

$$\frac{dy}{dt} = -\alpha^2 x \quad (2.3)$$

The amplitude of this linear harmonic oscillator depends on the initial conditions (Fig. 2.1 A, B), and exhibits metastable oscillations. However, It is assumed that the circadian oscillations are of limit cycle type oscillators, the equations governing are of nonlinear in nature, and it is independent of initial conditions. A classic example of the limit cycle oscillator model is the van der Pol oscillator [68, 58], which is an electric circuit based oscillator that generates self-sustained oscillations. This can be broadly considered as a variation of simple harmonic oscillator with a nonlinear damping term, and a forcing term required to overcome the damping [58]. It is a second order nonlinear differential equation that is given as follows:

$$\frac{d^2x}{dt^2} = \mu(1 - x^2) \frac{dx}{dt} - \alpha^2 x \quad (2.4)$$

Where x is the position coordinate and μ is a scalar which controls the strength of damping. This can be reduced to normal form as follows:

$$\frac{dx}{dt} = y \quad (2.5)$$

$$\frac{dy}{dt} = \mu(1 - x^2)y - \alpha^2 x \quad (2.6)$$

Van der Pol oscillator produces limit cycle oscillations (Fig. 2.1 C, D), where for all initial conditions the model generates oscillation with the same amplitude for the choice of parameters. In the phase plane analysis of vander Pol oscillator, it is shown that oscillations return to the same distorted circle for different initial conditions (Fig. 2.1 D). This is a prototype oscillator that is used extensively for a long time, prior to elucidating the molecular mechanism of circadian rhythms.

To get circadian period and light effects, Pittendrigh [69] modified the van der Pol oscillator and developed phenomenological circadian oscillators. Kronauer [67] used a limit cycle van der Pol oscillator (amplitude is normalized to 1) to show the effect of light on the human circadian system. These models add a zeitgeber term, $z(t)$ to the van der Pol oscillator to explain the effect of external forcing signal on circadian oscillation as follows:

$$\frac{d^2x}{dt^2} = \mu(1 - x^2)\frac{dx}{dt} - \alpha^2 x + z(t) \quad (2.7)$$

Later, different modified versions of the van der Pol oscillator were used for describing different properties of circadian rhythm [70–74]. However, all these models are phenomenological in nature and the molecular mechanisms responsible for generating various functions are not considered.

When the role of negative feedback in gene regulatory network of circadian system gained importance and prominence the shift from mass-spring phenomenological oscillator model to molecular mechanism based models occurred. Goldbeter first proposed the realistic model for *Drosophila* that accounted for various properties like oscillations under continuous darkness or light, phase response curves, entrainment to light-dark cycles, and transient suppression of oscillations under constant light [75, 76]. Subsequently, many molecular mechanism based mathematical models for gene regulatory networks of circadian rhythms have been proposed for *Neurospora* [77], *Drosophila* [77, 78] and mammals [61, 59, 79–81, 11] to understand the workings of circadian pacemaker under various conditions. Four important mammalian

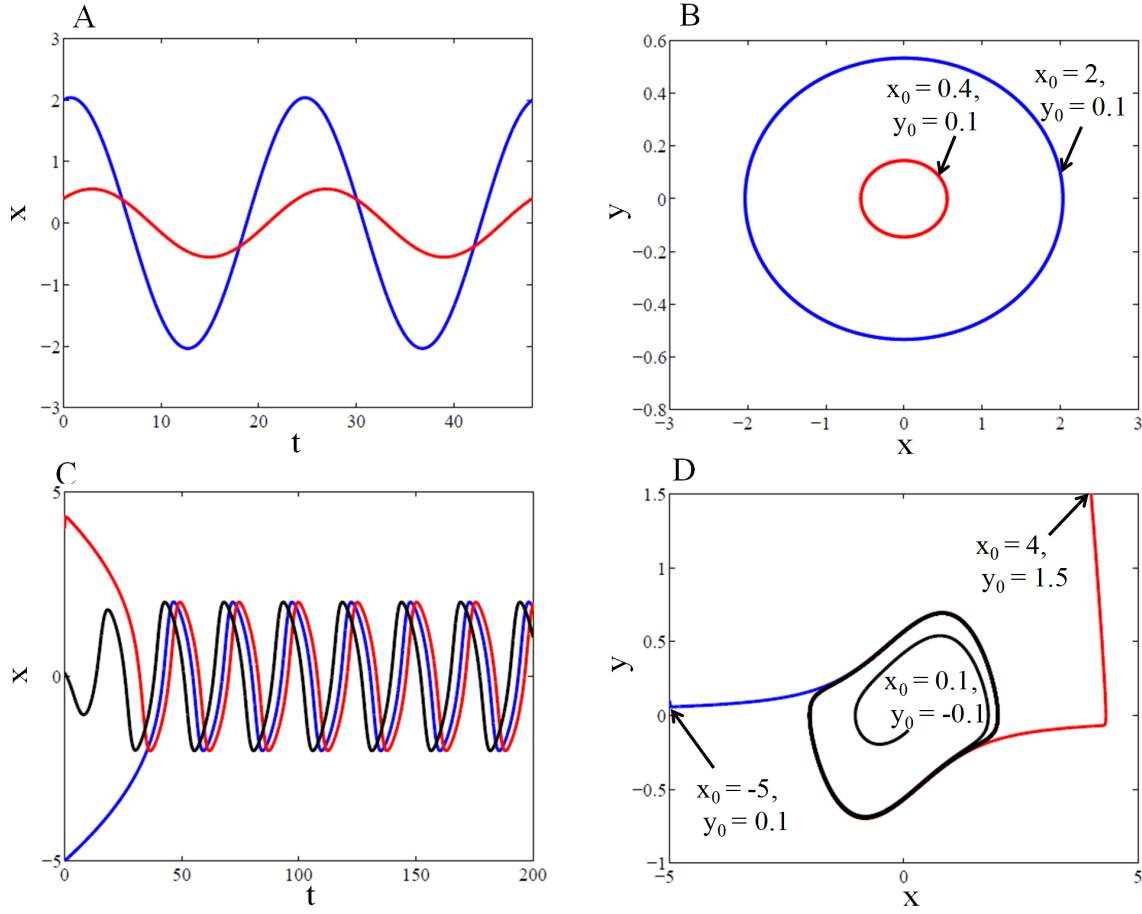


Fig. 2.1 Harmonic and limit cycle oscillators. (A) Time series are obtained by integrating the equations(2.2, 2.3) with different initial conditions. Blue line is the time evolution of the variable x with an initial condition of $x_0 = 0.4$ and $y_0 = 0.1$, where as red line is the time evolution of the variable x with an initial condition of $x_0 = 2$ and $y_0 = 0.1$. Amplitude of the oscillations are depends on the initial conditions, which is a characteristics of Harmonic oscillations. (B) Phase plane representation of harmonic oscillation. Different initial condition produces different circle. (C) Time series are obtained by integrating the equations(2.5, 2.6) with different initial conditions. Blue line is the time evolution of the variable x with an initial condition of $x_0 = -0.5$ and $y_0 = 0.1$, red line is the time evolution of the variable x with an initial condition of $x_0 = 4$ and $y_0 = 1.5$, and black line is the time evolution of the variable x with an initial condition of $x_0 = 0.1$ and $y_0 = -0.1$. (D) Phase plane representation of van der Pol oscillator. For any initial condition, system will returns to a single limit cycle.

models of circadian rhythms are considered in this work for comparison [61, 59, 79, 80] and these models are provided in the table 2.1. These models are considered based on their ability to capture important characteristic features of the circadian rhythms like entrainment, phase

response curves, period length variations of normal and mutant species, and period length variations with increasing constant light intensity to account for Aschoff's rule. Importantly, they differ in the way they model the same circadian characteristic features of mammalian system. For example, Forger's model is solely built based on mass action kinetic laws, whereas rest of the other three models are based on both mass action and Hill's kinetic formalism. Therefore, the number of variables and parameters in these models differ widely. The type of clock proteins considered in the simulation also differed in these models. The Becker-Weiman model considered only PER2 protein, but not PER1. Also, not all the model captured the PRC as observed in the experiments, but when the model was suitably modified, it was possible to capture the PRC [82]. Therefore, these examples suggest that the circadian models are very specific to the nature of the problem and the model was built based on the information available during the time of the construction of the model.

Table 2.1 Comparison of previous mammalian circadian model predictions with experimental data.

Oscillator characteristics			Experiments	Forger and Peskin, 2003 [59]	Mirsky et al., 2009 [80]	Becker-Weimann et al., 2004 [79]	Leloup and Goldbeter 2003 [61]
Number of Variable / parameter				73/36	21/132	7/24	16/55
Period length under DD	Wild type		< 24	< 24	< 24	< 24	< 24
	<i>per1</i> mutant		< 24	< 24*	Arrhythmic	NA	Arrhythmic
	<i>per2</i> mutant		Arrhythmic	Arrhythmic	Arrhythmic	Arrhythmic	NA
Period length under LL	Wild type		> 24	< 24*	NA	< 24*	> 24*
	<i>per1</i> mutant		> 24	< 24*	NA	NA	Arrhythmic
	<i>per2</i> mutant		< 24/ Arrhythmic	Arrhythmic*	NA	arrhythmic	NA
Period variation with increasing light intensity (Aschoff's rule)	Wild type		Period increases	Period decreases*	NA	Period decreases*	Period increases*
	<i>per1</i> mutant		Period increases	Period decreases*	NA	NA	Arrhythmic*
	<i>per2</i> mutant		Period decreases	Arrhythmic*	NA	arrhythmic*	NA
Entrainment	Wild	<i>per1</i> peak-ing time	Light phase	Light phase*	NA	NA	Light phase
		<i>per2</i> peak-ing time	close to dusk	Light phase*	NA	Light phase*	NA
	<i>per1</i> mu-tant	<i>per2</i> peak-ing time	Closer to dusk	Light phase*	NA	NA	NA
	<i>per2</i> mu-tant	<i>per1</i> peak-ing time	Light phase	Arrhythmic*	NA	Arrhythmic*	NA
PRC				Not matched	NA	Not matched	matched

* Results were not shown in the literature, but we obtained from simulation. NA: Not applicable

It is also important to mention that Goodwin model and its variations are used extensively to simulate many circadian properties successfully [83]. Goodwin proposed a very interesting generic molecular model for biological oscillators [38] that consists of three variables (X, Y, Z). The model has a negative feedback loop where the output of last variable inhibits the production of the first variable. The equation that governs the oscillatory dynamics is given as:

$$\frac{dX}{dt} = v_1 \frac{K_1^n}{K_1^n + Z^n} - K_2 X \quad (2.8)$$

$$\frac{dY}{dt} = K_3 X - K_4 Y \quad (2.9)$$

$$\frac{dZ}{dt} = K_5 Y - K_6 Z \quad (2.10)$$

In the model Hill's equations was included for the negative feedback, which brings nonlinearity in the model. Several years later Ruoff et al. [84] replaced the variable X, Y, and Z component by mRNA, protein, and a transcriptional inhibitor respectively. Different modified versions of Goodwin model is still used by researchers to explain the various properties of circadian oscillators. Using Goodwin model, Rouff et al. [85] simulated the PRC of *Neurospora*, Gonze et al. used coupled Goodwin model to simulate synchronization properties of the circadian oscillators [86] and also the effect of molecular noise on the circadian rhythm [87]. Diekman et al. [88] used Goodwin model to build a multi-scale model to simulate the effect of transcription activation on the voltage gated channels at SCN.

2.3 Mathematical models of spiking neurons in SCN

Though models based on molecular mechanisms of gene expression in the SCN neurons are studied extensively, the contributions by the voltage-gated channels are not modeled in detailed. In recent times however, the contribution from the voltage-gated channels for the important functioning of circadian rhythms have been modeled to understand the mechanisms that drive the rhythmic firing patterns that occur in SCN [89, 88, 90, 91]. The major problem that persists in this area is that the molecular interactions between the gene regulatory network, signaling network and the voltage-gated channels that drives the circadian firing patterns is not clearly known. Presently it is known that the SCN neuronal populations fire during the day and silent during the night and the set of currents that is responsible for this firing patterns are Na^+ , Ca^{2+} , calcium activated potassium currents, and certain rectifier currents [3]. Recently, there are few examples of voltage-gated mathematical models coupled to gene regulatory network that have been proposed to simulate the circadian firing pattern in SCN [92]. These are derived primarily from the Hodgkin-Huxley model [93] that generates action potentials or spikes. Generation of action potential is due to the opening and closing of ionic channels. Neuron integrates input (synaptic current) from the other neurons, and when it exceeds a threshold value, they fire an action potential, which have different shape,

frequency and amplitude. Some of the well known models that generates action potential are shown in Fig 2.2 and these mathematical models of spiking neurons ranges from abstract to biophysical models.

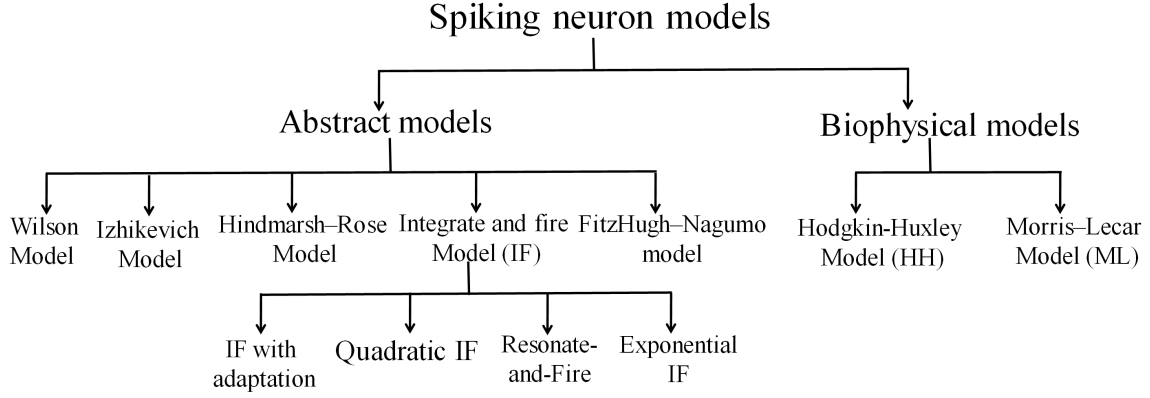


Fig. 2.2 Spiking neuron models.

Integrated and fire (IF) model is the earliest and most widely used spiking neuron model and it was first introduced by Louis Lapicque [94]. It was an electric circuit based model which consisting of a parallel resistor (R) and the capacitor (C), which represent the cell membrane leakage resistance and capacitance respectively. The dynamic of membrane potential (v) is given as:

$$C \frac{dv}{dt} = I - \frac{v}{R}, \quad \text{if } v \geq v_{th} \text{ then } v = v_{reset} \quad (2.11)$$

When the current (I) flowing into the circuit, capacitor charge to certain threshold potential (v_{th}), firing an action potential and discharge to a resetting value (v_{reset}). Various modified versions of IF model were introduced to reproduce the neuronal behavior. It is observed that in many neurons firing rate due to the application of constant current decreases throughout the spike-train. In order to include this behavior, a new version of IF model with adaptation is introduced by incorporating an adaptive conductance which depends on neuronal firing [95]. A nonlinear integrate and fire model (quadratic integrate and fire model) was also proposed [96], where the current equation is a quadratic function of membrane voltage. Another nonlinear integrate and fire model, exponential IF was proposed [97] that incorporate a current which is an exponential function of membrane voltage. A resonate and fire model was proposed [98], which demonstrate how subthreshold oscillations affects the firing dynamics of neurons. The models of the above type, particularly the IF model was coupled to the gene regulatory network to study the relationship between the firing frequency and the gene expression

amplitude of circadian rhythm [99]. The model incorporates slow time-scale circadian gene regulatory process that involves important clock components, signaling dynamics with VIP and intracellular Ca^{2+} as the important secondary messenger that take the role of intermediate time scale dynamics, and to capture the fast-scale electrophysiological dynamics, IF model was introduced. Among the three models introduced in their work, IF model is not very realistic as it is not based on molecular mechanism of voltage dynamics. However, their complex model, first of its nature, captures many interesting dynamics that are observed experimentally including the firing pattern variations that is circadian in nature.

2.4 Mathematical modeling of noise in circadian rhythms

In biological system, noise refers to the variability (fluctuations) in behavioral, neuronal, and gene expression level. Experimentally observed noise that affects biological system is usually classified into three categories; intrinsic, extrinsic, and external noise. Intrinsic noise arises from the probabilistic characteristics of biochemical reactions, and it is defined as the randomness of biochemical interactions of molecules in biological system. This noise is predominant when the number of reaction molecule is low (e.g. gene networks). Extrinsic noise generated due to the stochasticity in biological process, and cell to cell differences (e.g. different in size or number of ribosomes). Noise that arise due to the random variations in the environmental parameters such as temperature, PH, kinetic parameters, etc. are termed as external noise.

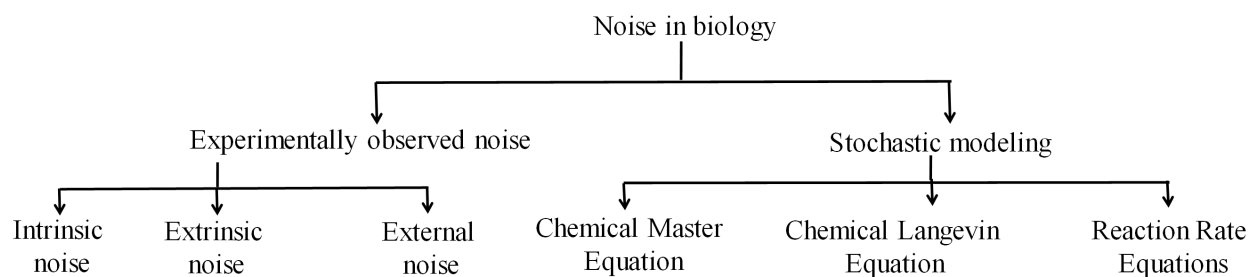


Fig. 2.3 Different noise that observed experimentally in biological systems and different noise modeling approaches

Different mathematical approaches are adopted for accounting the effect of noise in the mathematical models of biological process. All methods are capitalized on the idea that fluctuations in the process decrease as the number of molecules increases. Chemical master equation (CME) is a set of linear, autonomous ordinary differential equations (ODEs) which

is used to describe the elementary reactions. Each ODE represents the possible state of the system. CME describe the time evolution of the probability of a chemical system being in a particular state. Finding the solution of CME is very difficult when the state space of the reaction species are large and the transition has different time scale [100, 101]. Chemical Langevin equation (CLE) is a set of nonlinear, autonomous stochastic differential equations (SDEs), solution of each SDE is time-dependent real-valued random variable, which represent the level of chemical species. By making modeling assumptions, CLE can be derived from CME and computational complexity for simulating CLE is much lesser than CME. CLE is more suitable when system tend to steady state where an additive white noise terms are included in the deterministic equation. Reaction rate equation (RRE) is a set of nonlinear, autonomous ODE's, its solution is a real number which represents the concentration of species at time t . Many realistic and powerful deterministic gene regulatory mathematical models of circadian rhythms are proposed by various groups to simulate autonomous oscillations close to 24h [62, 102, 103, 87]. All these realistic models are also shown to be robust to molecular noise. Molecular noise refers to the presence of fewer molecules in the cell that is involved in regulating the biological processes. In circadian system, for example, the number of clock proteins involved in regulation may range from 10 to 100 molecules that gives rise to molecular noise, yet it maintains a constant circadian period. This indicates the robustness of circadian rhythms to noise, so that clock runs accurately and activates/deactivates other clock proteins at appropriate time of the day. The temperature is also compensated in these models [104–107].

Table 2.2 Stochastic version of Goodwin model (Equations 2.8-2.10). Deterministic to stochastic conversion is adapted from [87]

Number	Probability of reaction	Transition
1	$w_1 = (v_1 \Omega) \left(\frac{K_1 \Omega^n}{K_1 \Omega^n + Z^n} \right)$	$X \rightarrow X + 1$
2	$w_2 = K_2 X$	$X \rightarrow X - 1$
3	$w_3 = K_3 X$	$Y \rightarrow Y + 1$
4	$w_4 = K_4 Y$	$Y \rightarrow Y - 1$
5	$w_5 = K_5 Y$	$Z \rightarrow Z + 1$
6	$w_6 = K_6 Z$	$Z \rightarrow Z - 1$

Monte-carlo algorithm that has been used extensively to simulate noisy biological systems at mesoscopic level is the Gillespie's method [108]. Gillespie algorithm or the stochastic simulation algorithm (SSA) can simulate the intrinsic fluctuation of biological system. Gillespie method, firstly, associates a probability to each of the reaction steps. Then at each time step, the algorithm randomly determines the occurrence of a particular reaction as well

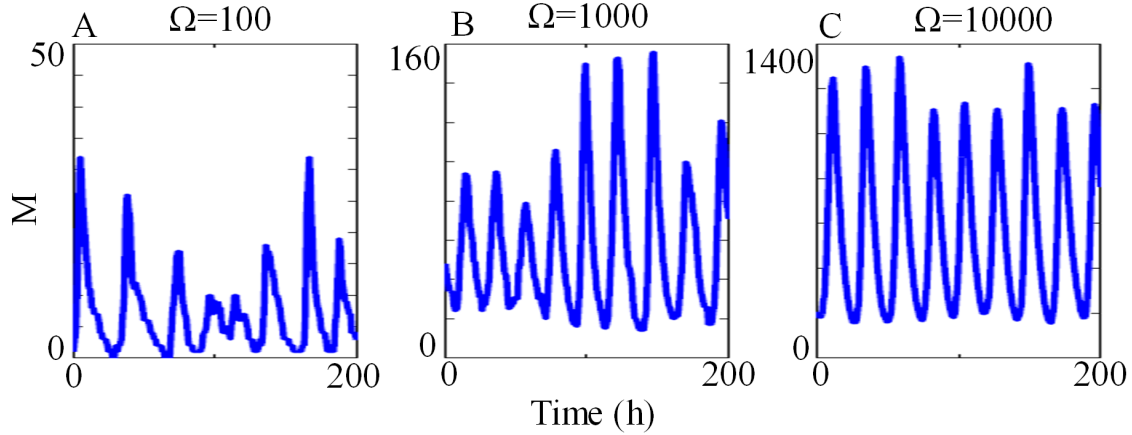


Fig. 2.4 Stochastic simulation via Gillespie algorithm. Stochastic time series of the variable X under different values of Ω (A) $\Omega = 100$, (B) $\Omega = 1000$, (C) $\Omega = 10000$. Effect of noise is higher when number of molecules are less.

as the time interval to the next reaction step. The numbers of molecules of the different reacting species as well as the probabilities are updated at each time step. A parameter denoted Ω permits the modulation of the number of molecules present in the system. A prototype example of Gillespie's algorithm applied to Goodwin model is shown in Fig. 2.4. The stochastic version of the Goodwin model (Eqs 2.8-2.10) is given in the Table-2.2, and the time series of stochastic simulation via Gillespie algorithm is shown Fig. 2.4.

2.5 Techniques

2.5.1 Dynamical systems theory applied to circadian rhythm models

Different tools in dynamical systems theory has been used to analyze the nonlinear differential equation models of circadian rhythms to obtain specific types of solutions, namely the oscillatory dynamics. The nonlinear differential equations are complex and consists of many variables and huge number of parameters. The following description is based on the Tyson et al. review article [109], and we modified to explain the circadian rhythm. The nonlinear differential equations of the circadian rhythms can be generally expressed as

$$\frac{dx_i}{dt} = f_i(x_1, \dots, x_n; p_1, \dots, p_m), \quad i = 1, \dots, n \quad (2.12)$$

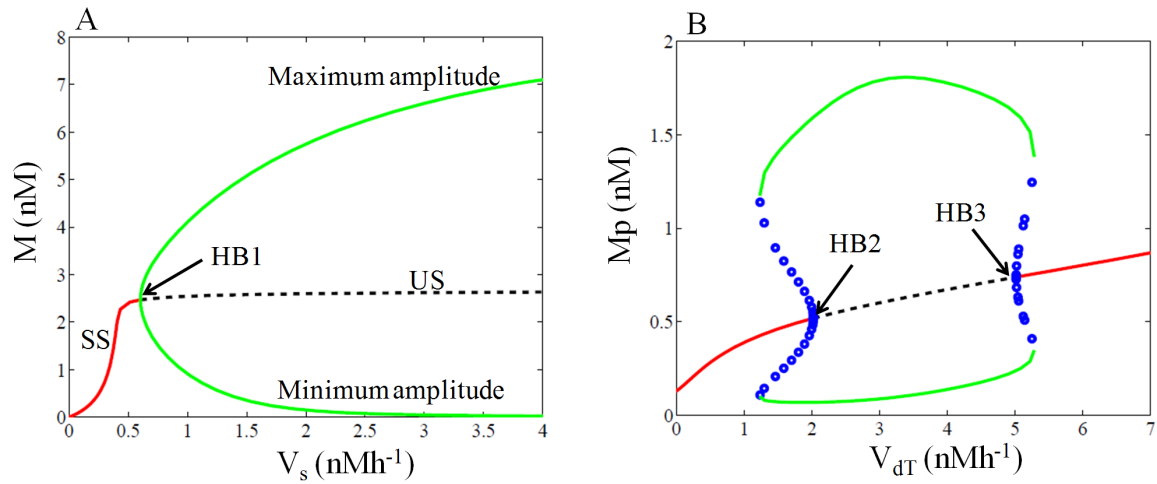
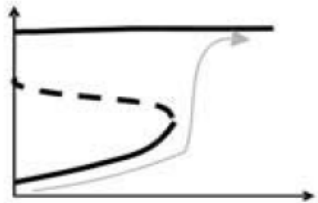
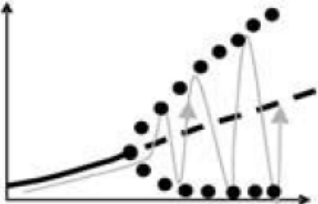
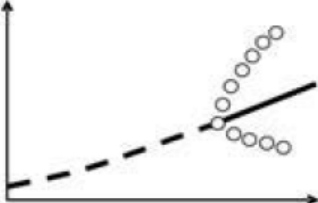
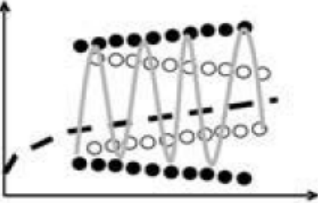
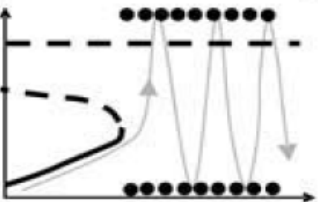
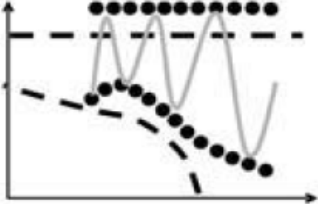


Fig. 2.5 Hopf bifurcation. (A) Supercritical Hopf bifurcation: a stable steady state become unstable when a stable limit cycle is born. Diagram represents stable steady state (red line, SS), unstable steady state (black dashed line, US), maximum and minimum oscillatory amplitude (green line) of *frq* mRNA (M) as a function of the light controlled parameter v_s . Bifurcation diagram generated in xppaut using the *Neurospora* model equations and parameters provided in [77]. (B) Subcritical bifurcation: Unstable steady state becomes stable when an unstable limit cycle is born. Diagram represents the *per* mRNA (Mp) as a function of light controlled parameter V_{dT} . Bifurcation diagram generated using the *Drosophila* model equations and parameters provided in [77].

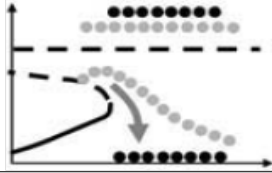
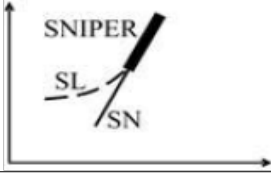
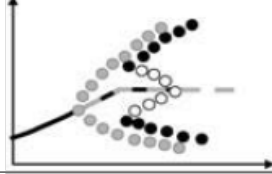
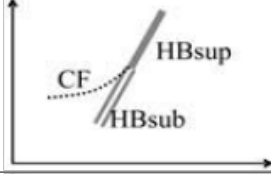
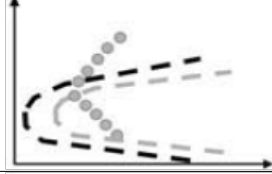
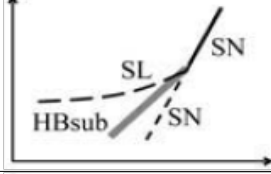
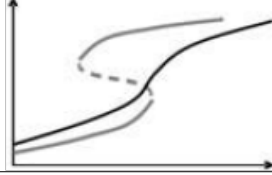
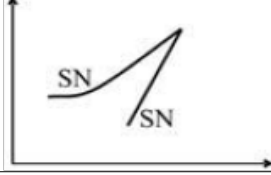
The x_i 's are different clock proteins that play a major role in the oscillatory dynamics, p_i 's are the parameters that may be Hill's constant, Michaelis constant, kinetic constants of different order, activation or inhibition constant or binding constants. One of the parameters, say p_1 , can be considered as light dependent that enhances the production of the clock proteins. These parameters when varied, it changes the dynamics of the system from steady state to oscillatory state. All the function f_i 's will be having one or many or all of the following terms: synthesis, activation, inactivation, complexation, and degradation. These terms can be either based on the laws of the mass action kinetics or Hill's kinetics or the combination of both. The nonlinear differential equations are of the initial value problem (IVP) type and the set of initial conditions $x_1(0), \dots, x_n(0)$ are provided to simulate the dynamics over period of time $[0: t_{end}]$. The type of solution that one is interested in circadian model is the limit cycle oscillations. A limit cycle is an isolated closed trajectory that attracts or repels all the other nearby trajectories, and it is possible only in the nonlinear system [114, 115]. The nature of oscillatory solutions depends on the parameter in the model. For example, when the light dependent parameter p_1 is changed, the solution of the nonlinear ODE may go either from stable limit cycle to stable steady state or to unstable limit cycle. The

Table 2.3 Definition and example of codimension one bifurcations (adapted from [110])

Bifurcation	Nature of the solution before and after the bifurcation	1D example	Ref
Saddle-node (SN)	3 steady state to one steady state		[111]
Supercritical Hopf (HBsup)	1 stable steady state to unstable steady state and small amplitude stable limit cycle		[77]
Subcritical Hopf (HBsub)	1 unstable steady state to stable steady state and small amplitude unstable limit cycle		[77]
Cyclic fold (CF)	No oscillatory solutions to 1 stable oscillation and 1 unstable oscillation		[112]
Saddle-node infinite-period (SNIPER)	3 steady states to unstable steady state and large amplitude oscillation		[112]
Saddle-loop (SL)	Unstable steady state (saddle) to unstable steady state and large amplitude oscillation		[113]

qualitative changes in the solution is called bifurcation and the exact parameter values that brought about these changes are called as bifurcation points. The bifurcation point where the birth of stable limit cycle is called as Hopf bifurcation that is supercritical in nature. The characterization of bifurcation is done by changing one parameter p_1 , and keeping all the parameters constant and plotting the variation in the steady state of one dynamical variable,

Table 2.4 Definition and example of codimension-two bifurcations (adapted from [110])

Bifurcation	Nature of the solution before and after the bifurcation	1D example	2D example	Ref
Saddle-node loop (SNL)	SN and SL to SNIPER			[113]
Degenerate Hopf (dHB)	HBsup to HBsub and CF			[113]
Takens-Bogdanov (TB)	SN, HB and SL to SN			[113]
CUSP	Bistability (2 SN) to Monostability			[113]

say x_1^* , that is representative of all the other dynamical variable is called as codimension-1 bifurcation diagram. If the number of parameters varied is two, then it is called codimension-2 bifurcation. Besides, Hopf bifurcation, different types of bifurcations observed in the biological systems are listed in the Tables-2.3, 2.4, which are adapted from [110], and as of now, except of Hopf bifurcations, other bifurcations are rarely observed in the nonlinear models of circadian rhythms.

The one-parameter Hopf bifurcation diagram constructed by many groups to explain the influence of light on circadian rhythms [77, 61, 79]. Bifurcation diagram of the circadian model of *Neurospora* and *Drosophila* [77] as a function of the light-controlled parameter are shown in Fig 2.5. At a supercritical Hopf bifurcation (HB1), one stable equilibrium becomes unstable and limit cycle that is born is stable (Fig 2.5A). At subcritical Hopf bifurcation (HB2, HB3), an unstable equilibrium become stable and limit cycle born is unstable (Fig 2.5B).

The other circadian features that provide valuable information about the behavior of the species like phase response curves, entrainment curves, and the construction of actogram

from the molecular model to compare with actogram of activity plot is explained in the following sections.

2.5.2 Phase response curve (PRC)

A plot of perturbed phase against the phase shift for one circadian cycle due to the application of pulse stimulus provides the phase response curves (PRC) [116]. These curves explain the relationship between a small duration of the stimulus, such as light that is applied at different phases in one cycle, and the corresponding phase shifts. PRC provide vital information about the range of entrainment of a circadian system, which in turn helps to understand the adaptation to external seasonal variations like long and short day cycles. PRCs are also categorized as Type-1 and Type-0 depending on the jumps in the phase shifts from delay to advance or vice-versa (Fig.2.6). Type-1 PRC has a continuous transition between phase advances and delays (phase shifts are small), whereas Type-0 PRC display discontinuity between advance and delays (large phase shifts).

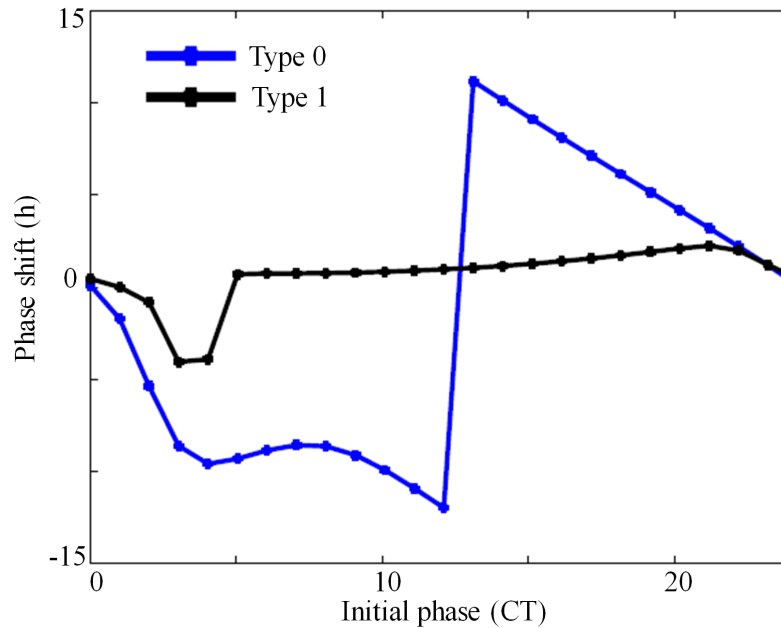


Fig. 2.6 PRC. Type-0 and type-1 PRCs. Type-0 PRC has larger phase shifts (blue lines), whereas Type-1 has smaller phase shifts (black lines). PRC has been obtained by integrating the model equations in [111].

2.5.3 Actogram

Recording wheel running activity is the best possible way to track circadian locomotor activity. Circadian locomotor activity rhythms are normally represented as a graph called actogram (Fig 2.7), where horizontal line represents days and thick black bars plotted on horizontal line represents the activity. There are two different ways to represent daily activity using actogram: single-plotted actogram (Fig 2.7A) and double plotted actogram (Fig 2.7B). In single-plotted actogram x -axis represents one day (24h) and in double plotted actogram x -axis represents two days (48 h). Both actogram plot days sequentially from top to bottom, while in double-plotted actogram two days are plotted on each horizontal line and it shows the second day on the far right of each line, as well as at the beginning of the second horizontal line, and so on.

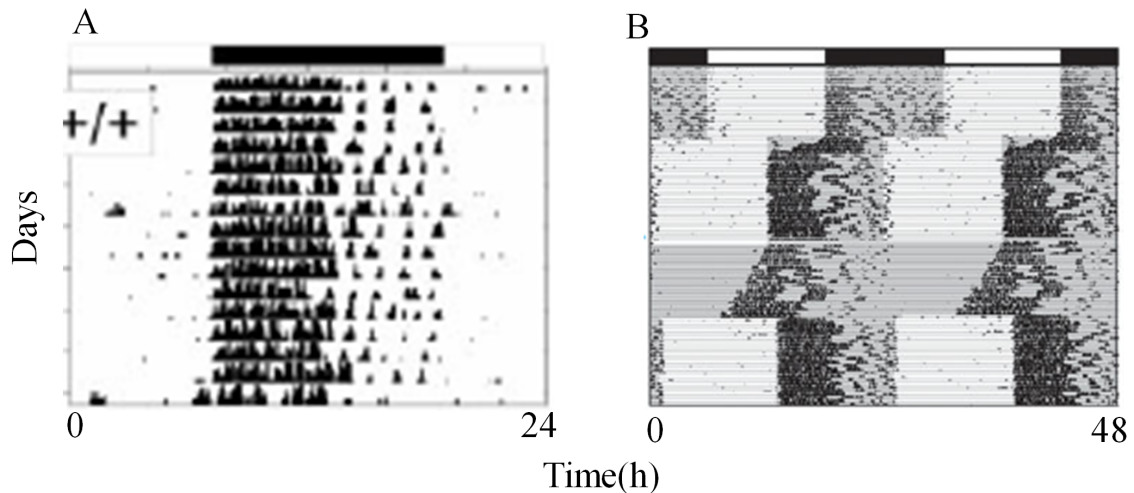


Fig. 2.7 Actogram. (A) Single-plotted actogram [117], x axis represent one day. (B) Double-plotted actogram [118], x axis represents two days. White and Black bar on the top of the diagram represent day and night cycle respectively.

For a mathematical model, the analog of wheel running activity can be constructed using computer program. In this work, a Matlab program for generating actogram is developed. In that program, the analog of wheel running activity occurs whenever a particular dynamical variable in the model is above some threshold value. Fig 2.8 explains the procedure that is used to construct the actogram from Goodwin model (Equation 2.8-2.10). The reference time series (Normalized value of the variable X) is given in the Fig 2.8A, where the black broken line is the threshold value defined in the program. This value is user defined (for example, mean value of the time series or $3/2$ of the maximum value of the time series). Here the threshold value as 1.6 times the mean value of the reference time series. Whenever

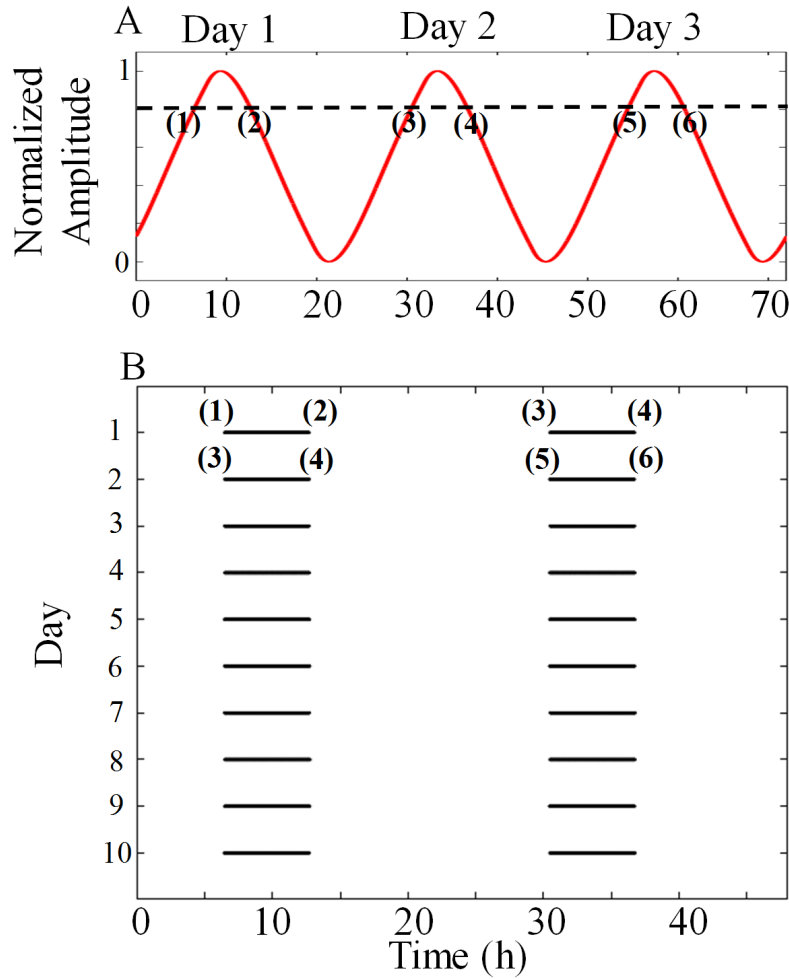


Fig. 2.8 Construction of actogram using Matlab (A) Reference time series for actogram. Time series of the variable X normalized in such a way that maximum value is 1 and minimum value is 0. Dotted lines show the threshold value for defining wheel-running activity. The numbers in the brackets show the points at which the time series crosses the threshold. (B) Double-plotted actogram of the Goodwin model. Black horizontal bars represent the wheel running activity. The numbers given in the brackets represent the starting and ending time of the activity which is the *modulo* – 48 of time corresponding to the same number indicated in the time series

the time series amplitude reached above the threshold value, a horizontal line is drawn on actogram for the corresponding day, which represents the free-running activity. The number indicated on the time series is the time at which the reference time series crosses the threshold. The *modulo*(48) (*modulo*(24) for single plotted actogram) of this time is used to define the starting and ending time of the activity on actogram (Fig 2.8B).

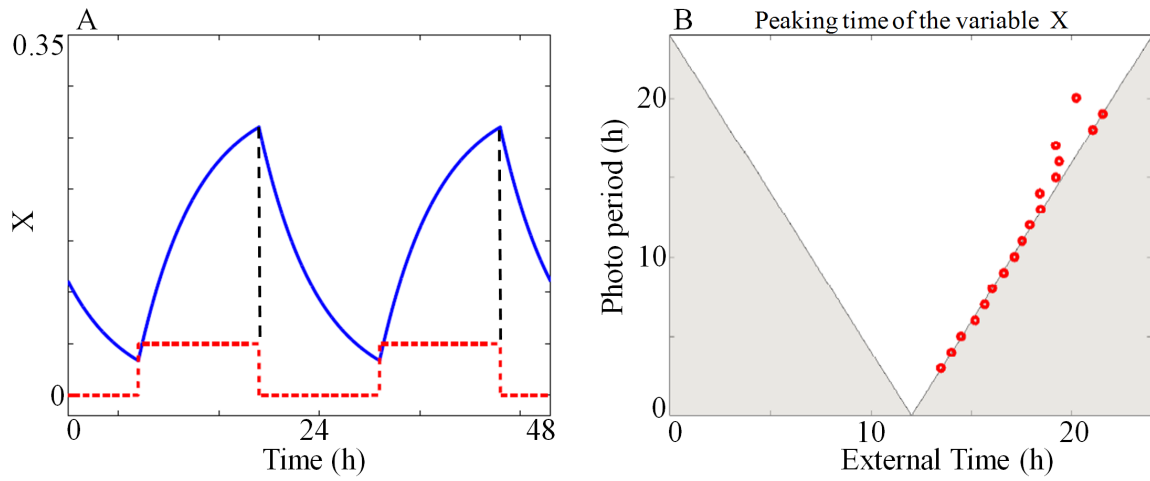


Fig. 2.9 Entrainment-V curve. (A) Entrainment of the Goodwin model (Eqs 2.8-2.10). Model is forced by the external LD cycle (red broken lines) with a period of 24h and photo period 12h. Effect of LD is included in the model by adding a time dependent parameter $L(t)$ on the right hand side of the equation (2.8), and it is square wave with maximum value is 0.05 (light) and minimum value 0 (dark). (B) Entrainment under whole range of photoperiod. Grey shading indicate dark phase and white shading represents the light phase. Red circles are the peaking time of the variable X under different photoperiod. In most of the photoperiod, X variable peaking near the transition between Light and dark phase.

2.5.4 Entrainment-V diagram

In the field of circadian rhythm research, time of day is commonly represented in circadian time (CT) or zeitgeber time (ZT). Circadian time is usually used when there is no external cues (DD), where CT0 is the starting of subjective day and CT12 is the starting of subjective night. Zeitgeber time is used when there is an external signal (zeitgeber), common zeitgeber is light-dark (LD) cycle, where ZT0 is taken as the start of the light phase. Two different time conventions are discussed in [35], internal time (InT) for DD condition and external time (ExT) for LD condition. According to their protocol internal time given as $[CT0 - 10]_{mod24}$ and external time is defined as $[ZT + \text{half of the duration of dark phase}]_{mod24}$. By using the external time convention, we constructed an entrainment-V diagram which defined the external time of the whole range of the different photoperiods (Fig 2.9), and plot the peaking time of the variable X in the Goodwin model (Eqs. 2.8-2.10) under different photoperiod. According to this diagram, mid of the light phase is ExT12 for all photoperiods. This entrainment diagram is helpful for analyzing entrainment property of the circadian model and it is previously employed in [82].

In the final section, a brief description of the software tools that are used for simulation of mathematical models and to construct the bifurcation diagram is provided.

2.6 Software tools

Software tools are very important at different stages of computational modeling of biological system, which consists of numerical simulations, model analysis, data handling, parameter estimation, and model validation. Various programming languages such as python, R, Matlab, xppaut, C, java, COPASI etc. are available for analyzing and simulating complex computational models. Specifically, to analyze mathematical models of circadian systems that exhibits oscillatory solutions, xppaut and Matlab are widely used. Xppaut is a freely available standalone program written by Bard Ermentrout [119] that allows the user to explore solutions of ordinary differential equations (ODE) system. It is a conventional numerical tool for solving, simulating and analyzing dynamical systems that is accepted by the scientific community. The dynamical system can be modeled as deterministic, stochastic, integral-differential equations, and partial differential equations. Xppaut is very useful to analyze the varieties of equations numerically and provides multiple options for integrating stiff equations and non-stiff equations. Further, in xppaut, null-cline analysis, plotting of directional fields and bifurcation analysis can be quickly and efficiently carried out even for the models that has huge sets of variables and parameters. In this work, xppaut is used for constructing all the bifurcation diagrams of the dynamical systems. Matlab[®] routines (Matlab 8.1, The MathWorks, Natick, MA) is a standard programming tool that is widely used in mathematical modeling of biological systems. In Matlab routines, user can choose different ODE solvers depending on the nature of the differential equations. Importantly, Matlab routines has many powerful and efficient inbuilt functions such as *fminsearch*, *GA* for estimating kinetic parameter of the nonlinear ODE systems. In this thesis, all the simulation and analysis are done by using xppaut and Matlab routines.

Chapter 3

Hilbert transform based time series analysis of the circadian gene regulatory network

Preamble

Parts of this chapter have been published in:

Shiju, S., and Sriram, K. (2019). Hilbert transform-based time-series analysis of the circadian gene regulatory network, *IET System Biology*, ISSN : 1751-8849 ([Link](#))

3.1 Introduction

Signal processing tools have been used extensively to understand the properties of circadian rhythms. For example, wavelet transform was applied on the circadian experimental time series to extract period variations in SCN [120, 121] and to study the interaction of ultradian oscillation with circadian rhythm [122]. While Forger applied Fourier transform to the circadian model with biochemical feedback to understand the effect of feedback loops to variations in period [123], Escalante et al. [124] employed Laplace transform to model circadian rhythms. Laplace transform, though suitable to analyze stationary data, cannot apply to realistic non-stationary biological signals. Fourier transform can detect the frequency components in the signal, but, doesn't provide information about the instantaneous frequency. Wavelet transform can estimate instantaneous frequency with better resolution in time,

but depends strongly on the selection of proper wavelets. Moreover, wavelet analysis is computationally intensive, and have to take care of harmonics and edge effects [121].

Mathematical models of circadian rhythms are highly complex, nonlinear, high dimensional and cannot be subjected to linear signal processing tools like Fourier, Laplace, and wavelet transforms directly because of the problems mentioned earlier. All the circadian properties from the model are obtained through numerical simulations under various conditions, since analytical solutions are difficult to obtain for coupled nonlinear ODE models. Circadian properties that are obtained through numerical simulations include fitting model to the experimental time series to get the period and amplitude, to get the PRC under pulse perturbations, and subjecting the model to various light-dark (LD) cycles to study entrainment properties. Therefore, analysis of simulated time series from the model through proper tools is highly important to get insight into various properties of the circadian systems. Presently, to obtain the period of stochastic and deterministic time series from the model, various techniques like mean crossing method [62], Fourier [123], and wavelet transforms [120, 121, 125] were employed. Similarly, to construct phase response curves, numerical methods like isochron and adjoint methods were used [126]. This indicates that diverse tools are used to extract different properties of circadian rhythms.

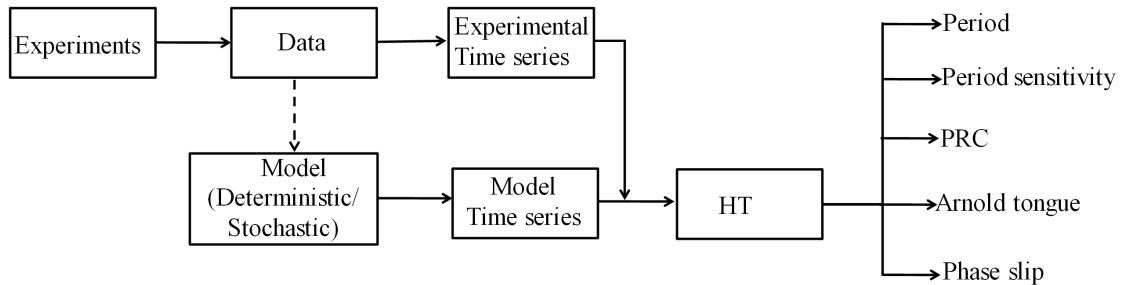


Fig. 3.1 Outline of the HT analysis. Mathematical models are developed based on the experimental data, this deterministic/stochastic model generate circadian time series. Simulated or experimentally collected time series is then processed using Hilbert transform to analyse the different properties of circadian rhythm.

Interestingly, most of the important characteristic properties of the circadian rhythms like period, phase response curves, entrainment properties, and robustness to molecular noise can be obtained from the instantaneous phases of the oscillations. Instead of using different techniques to obtain different properties of the circadian rhythms, in this chapter, we show that HT can be used to extract most of the important circadian properties from the time series got from the circadian models. HT has been widely applied to diverse areas to analyze the time series of mechanical vibrations [127], to analyze data from geophysics [128],

mass flow [129], and in bio-medical research [130]. HT was also employed to construct PRC [131] and to extract phase information [132, 122, 133] from circadian data. Moreover, different groups used HT based methods to construct numerical PRC [134], to examine entrainment [135], and to study the synchronization properties [136] of oscillatory system. Besides circadian rhythms, HT was also used by Wang et. al to study memory performance on visual and auditory data [137]. In this chapter, we specifically show how HT can be used to determine period variations, construct PRCs, identify period sensitivity of the parameters to perturbations, and to get Arnold tongues to map the entrainment regions (Fig.3.1). We also compare our results of PRC, period sensitivity, and entrainment with the existing methods to show that our method is robust and versatile. We also propose a novel method (both model and numerics) in combination with HT to determine whether the circadian time series under forcing period conditions entrains completely (phase locking), partially (phase slips) or neither. We show that our method works well for both deterministic and stochastic time series under forcing conditions. We believe that this simple method will be useful to the experimentalists to quickly identify regions of entrainment to the external forcing period signals. To illustrate the use of HT in analyzing circadian time series, we take as a test case the time series from the fruit fly model of circadian rhythms [111] and the time series from the experimental data [138] to demonstrate entrainment to different forcing cycles.

3.2 Materials and method

We use Xppaut [119] and Matlab for numerical simulation, and the programs along with the link to the figures are printed in the Appendix-D.1.

3.2.1 Circadian time series from the two variable model of fruit fly

We summarize first the Tyson et al. [111] model of fruit fly followed by the definition of HT. The gene regulatory model of fruit fly is based on the production of PER and TIM protein by *per* and *tim* mRNA respectively. These proteins are transported to the cytoplasm that may either undergo proteolysis or forms a stable product by heterodimerization. The heterodimers in turn feedbacks and inhibits the *per/tim* transcription. However, Tyson et al. [111] proposed that the equilibrium between monomer and dimer results in the protection of dimers from the *double-time gene* (DBT) degradation and thereby increases the total concentration of monomer, dimer and its accumulation. This results in a double negative, positive feedback between monomer and dimer. Without considering PER and TIM separately, a three-variable

model was initially proposed with M , P_1 and P_2 that respectively are the mRNA, protein monomer and dimer. Then based on the assumption that the dimerization reaction is fast, the model was reduced to two-dimensional nonlinear ordinary differential equations that are reproduced below.

$$\frac{dM}{dt} = \frac{v_m}{1 + (P_t(1 - q)/2P_{crit})^2} - k_m M \quad (3.1)$$

$$\frac{dP_t}{dt} = v_p M - \frac{k_{p1}P_t q + k_{p2}P_t}{J_p + P_t} - k_{p3}P_t \quad (3.2)$$

$$q = \frac{2}{1 + \sqrt{1 + 8K_{eq}P_t}} \quad (3.3)$$

In the equation, M is the mRNA and P_t is the total protein, where, $P_t = P_1 + 2P_2$. All the variables and parameters of the model are the same as in [111] unless specified otherwise. The parameter values are also provided in the caption of Fig.3.2. Codimension-1 bifurcation diagram for the mRNA (M) as a function of K_{eq} is shown in the Fig.3.2 A. For lower values of K_{eq} , the system is in the stable steady state. As K_{eq} increases, the stable steady state becomes unstable through Hopf bifurcation (HB1) and generate oscillations. Further, increase in K_{eq} leads to a loss of periodic orbits through supercritical Hopf bifurcation (HB2) that has an unstable steady state surrounded by a stable limit cycle. We also show the variations of the period to the change in K_{eq} . As shown in Fig.3.2B, we identify three regimes in the bifurcation diagram. In regime-I (R1), for low values of K_{eq} (between 0 and 100 approx.), there is a rapid variation in the period that goes from 5h to 50h, and this is the highly sensitive regime. In regime-II (R2), between 100 and 450 of K_{eq} values, the period is 24.2h which is close to circadian. This wide regime is the most stable regime with period being insensitive to parameter variations. In regime-III (R3), (K_{eq} between 450 and 580) the period is modestly sensitive to parameter variations. In the present work, regime-II (R2) is the region of our interest, and all the properties are studied for $K_{eq}=200$. Using HT, we also perform period sensitivity analysis for the other parameters in regime-II (R2) and regime-I (R1), and we discuss this in detail in section 3.4.

In the absence of negative feedback loop, the model exhibits bistability due to the presence of monomer-dimer positive feedback loop of PER protein. The $\frac{dM}{dt}$ in the absence of negative feedback loop takes the form $v_m - k_m M$, and $\frac{dP}{dt}$ retains the original form. Phase plane analysis of this 2D model exhibits mono and bistability for the choice of K_{eq} parameter. Codimension-1 bifurcation diagram exhibits saddle-node bifurcation (Fig. 3.2 C, D). The bistable character of the model explains the mutant behavior that exhibits no endogenous rhythms, yet responds

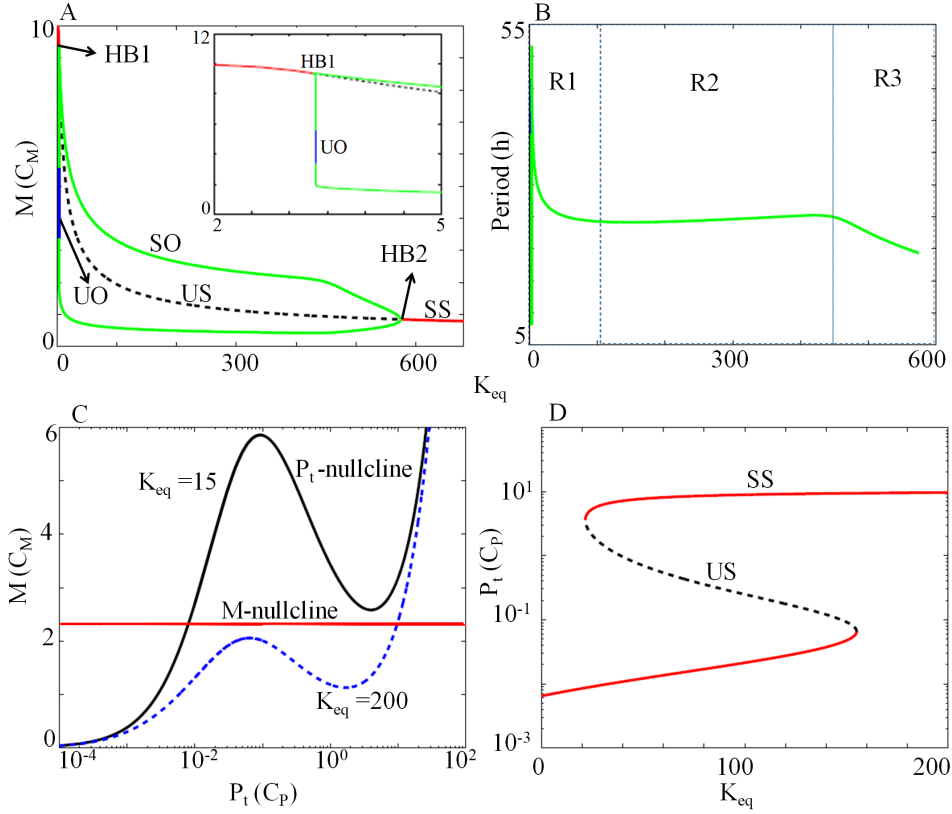


Fig. 3.2 Codimension-1 bifurcation diagram and bistability. (A) For lower values of K_{eq} , the system is in the stable steady state (Red lines, SS). As K_{eq} increases oscillations appear via Hopf bifurcation (HB1). Black broken lines are the unstable steady state (US), blue line are unstable oscillation amplitude (UO), and green lines are stable oscillations amplitude (SO). Sustained oscillations disappeared via supercritical Hopf bifurcation (HB2) and the system enters the stable steady state. Inset shows the enlarged view of bifurcation diagram for lower values of K_{eq} . Xppaut [119] is used for simulating the bifurcation diagram. (B) Period of oscillations as a function of K_{eq} . R1 and R3 are the regions where period is highly sensitive to K_{eq} , whereas R2 is the region where period is almost insensitive to K_{eq} . Simulation results are obtained by integrating the equations (3.1) and (3.2) with parameter values $v_m = 1 \text{ C}_m \text{ h}^{-1}$, $k_m = 0.1 \text{ h}^{-1}$, $v_p = 0.5 \text{ h}^{-1}$, $k_{p1} = 10 \text{ C}_p \text{ h}^{-1}$, $k_{p2} = 0.03 \text{ C}_p \text{ h}^{-1}$, $k_{p3} = 0.1 \text{ h}^{-1}$, $P_{crit} = 0.1 \text{ C}_p$, $J_p = 0.05 \text{ C}_p$. (C) Nullcline analysis of the model without negative feedback on transcription, where $\frac{dM}{dt} = v_m - k_m M$. Even though the system not producing any oscillations, it switch between two stable steady states depending on the value of K_{eq} . During day time ($K_{eq} = 15$), system is in a stable steady state with $P_t \approx 0.008$, where as during night time ($K_{eq} = 200$), system is in a stable steady state with $P_t \approx 10$. (D) Steady states of P_t as a function of K_{eq} , where the system exhibit a hysteresis behavior. For simulating (C) and (D) we use $v_m = 0.23 \text{ C}_m \text{ h}^{-1}$ and rest of the parameter values are same as that of in (A). As used in [111], C_p and C_m are the characteristics concentrations for protein (P_t) and mRNA (M), respectively.

to light, and this is the only model in circadian rhythm where saddle-node bifurcation is related to mutant behavior that exhibits no endogenous rhythms.

To assess the robustness of the model to molecular noise, we also perform a stochastic simulation of the two variable model. The deterministic model cannot describe the molecular fluctuations at the population levels, and therefore, we use the stochastic simulation algorithm (SSA) to determine the robustness of the model to noise through HT method. We performed stochastic simulations according to Gillespie's method [108], and the results are discussed in the section 3.3. The transition probabilities of the reaction and the transitions are given in Table 3.1. The scaling parameter Ω is the system size (See [100, 62]) that can use to modulate the molecular noise; higher the Ω , less the molecular noise, and less the period variations. There are five reaction steps, each of them is associated with a probability, which depends on the deterministic kinetic parameters.

Table 3.1 Stochastic version of Tyson et al. model [111]. Deterministic to stochastic conversion is adapted from [87]

Number	Probability of reaction	Transition
1	$w_1 = \frac{vm\Omega}{1+(P_t(1-q)/2P_{crit}\Omega)^2}$	$M \rightarrow M + 1$
2	$w_2 = k_m M$	$M \rightarrow M - 1$
3	$w_3 = v_p M$	$p_t \rightarrow P_t + 1$
4	$w_4 = \frac{P_t \Omega (k_{p1} q + k_{p2})}{J_p \Omega + P_t}$	$P_t \rightarrow P_t - 1$
5	$w_5 = k_{p3}$	$P_t \rightarrow P_t - 1$
6	$q = \frac{2}{1 + \sqrt{1 + 8 \frac{K_{eq}}{\Omega} P_t}}$	

The above 2D fruit fly model is also different from the existing gene regulatory circadian models of *Drosophila*, *Neurospora*, and the mammals; (i) The model is two dimensional and is based on the positive feedback loop due to the dimerization of PER protein. (ii) The model time series exhibits a sort of relaxation oscillations rather than smooth sinusoidal oscillations that are seen in all the existing models of circadian rhythms. (iii) The model exhibits both bistability and Hopf bifurcation for appropriate choice of parameters to explain the effect of light on mutants and normal phenotypes respectively, whereas existing circadian models explains the behavior of normal and mutant phenotypes only through Hopf bifurcations. (iv) The 2D model exhibits both Type-I and II phase response curves and the only simple gene regulatory model which we know of is the Goodwin's 3D model for *Neurospora* proposed by Ruoff et al. [85]. Finally, the model is also robust to molecular noise. This we show by performing stochastic simulation by Gillespie's method [108] and by HT we find that

the period distribution (variance) is close to the period (mean) of the fruit fly circadian oscillations (20-25h).

3.2.2 Hilbert transform

HT converts the time domain signal $x(t)$ to an another time domain signal $y(t)$ [139, 140]. HT of a signal $x(t)$ can be considered as the convolution of signal $x(t)$ and $1/\pi t$ [139].

$$y(t) = \frac{1}{\pi} \int_{-\infty}^{\infty} \frac{x(\tau)}{t - \tau} d\tau \quad (3.4)$$

Simply, HT performs a $-\pi/2$ phase shift for every spectral component of $x(t)$. A signal $x(t)$ and its Hilbert transform $y(t)$ are mutually orthogonal and have same autocorrelation and energy density spectrum [139, 140]. A complex time signal $z(t)$ can be constructed using $x(t)$ and $y(t)$ which is called analytic signal.

$$z(t) = x(t) + iy(t) \quad (3.5)$$

and in polar coordinates, $z(t)$ is given as

$$z(t) = a(t)e^{i\theta(t)} \quad (3.6)$$

with

$$a(t) = [x^2(t) + y^2(t)]^{(1/2)}, \quad \theta(t) = \tan^{-1}\left(\frac{y(t)}{x(t)}\right) \quad (3.7)$$

$a(t)$ is the envelope or amplitude of the analytic signal, $\theta(t)$ is the phase, and the time derivative of $\theta(t)$ provides instantaneous frequency given as

$$\text{Instantaneous frequency, } f_i(t) = \frac{1}{2\pi} \frac{d\theta(t)}{dt} \quad (3.8)$$

Instantaneous frequency is a positive function of time that varies with respect to the period of oscillation. Time average of the instantaneous frequency provides the average frequency in a signal spectrum [141]. HT is more suitable to analyze both stationary and non-stationary data because of the following reasons:

- (1) HT of a signal has the same frequency and amplitude of the original signal, which makes it a powerful tool for signal analysis.
- (2) HT is nonlocal in time, hence it is suitable for the computation of instantaneous character-

istics of the signal, whereas, Fourier transform and Laplace transform are local in time.

(3) Unlike wavelet transform, HT is versatile, which is adaptable to different type of signals. However, in wavelet transform analysis depends strongly on the proper choice of wavelet function that in turn depends on specific signal.

3.3 HT method to determine the period of circadian oscillator

One of the important characteristic features of circadian oscillator is its period. The period of oscillator determines the endogenous rhythm that is unique to the given species. Further, determining period from the time series is an important aspect to understand and compare the dynamics of wild and mutant phenotypes behaviors of the circadian systems. However, oscillations observed in the experiments are noisy and extracting the period from a few, noisy, and some time damping cycle is difficult. To capture this noisy oscillation, and its period as seen in the experiments, both deterministic and stochastic simulations are carried out for the 2D model. Existing methods to calculate the period of noisy time series takes into account the time difference between the two consecutive peaks (trough), but these are not very reliable method because many local maxima and minima will occur that lead to an inaccurate estimation of the period. To overcome this problem, Goldbeter et al., calculated the time difference between two consecutive upward crossing of mean levels of a suitable oscillatory variable [62]. Another common method to determine the period is from the power spectrum of time series of any oscillatory variable. Though this gives the time-invariant amplitude and frequency, it will not provide instantaneous frequency [142]. Therefore, we use HT method to obtain the instantaneous frequency of an oscillator from the time series. Before we apply HT, we generate time series by integrating the 2D model for the choice of kinetic parameters. We simulate both the deterministic (equations 3.1–3.2) and stochastic versions (Table-3.1) of the model. For all the numerical analysis, we use the time series of the dynamical variable $M(t)$, detrend it from its average, and convert it to an analytic signal of the form given in equation (3.5) by HT, from which we obtain the instantaneous phase ($\theta(t)$) as in equation (3.6) and (3.7).

We propose two different ways to determine the period numerically from the instantaneous phase of the time series. In the first method, we plot the instantaneous phase that changes from $-\pi$ to π , i.e., the total phase change is 2π . Then we determine the time points where the absolute phase difference between two consecutive instantaneous phases, $|\theta(t) - \theta(t + \Delta t)| \geq$

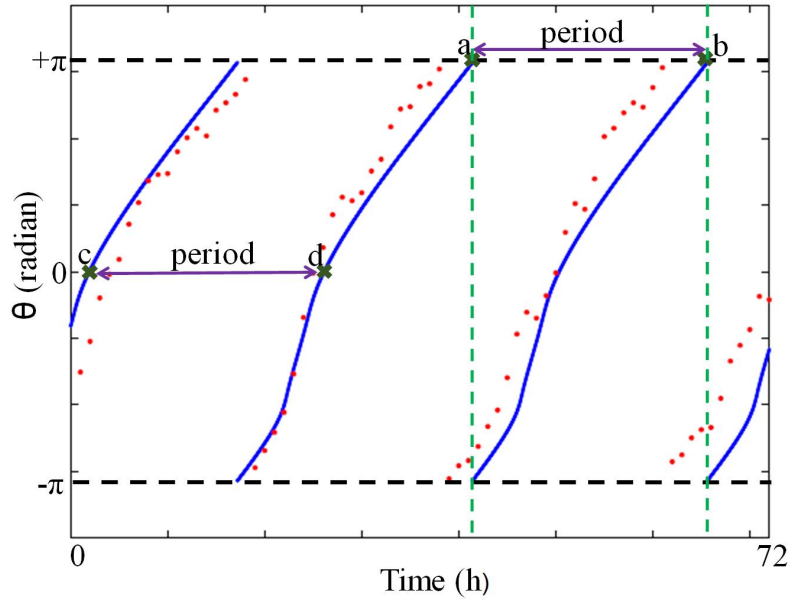


Fig. 3.3 Two different methods to determine the period from HT. Blue lines are the instantaneous phase of the deterministic model, and red dots are the instantaneous phase of the stochastic model. In the first method, points *a* and *b* are the two consecutive phase switching points, where the phase changes from π to $-\pi$. Note that at the phase switching points, instantaneous phase of the stochastic model does not always reach to π or $-\pi$. In the second method, points *c* and *d* are the two consecutive zero crossing of the instantaneous phase.

1.5π (Fig.3.3, points *a* and *b*). This time point indicates phase switch from $-\pi$ to π and it provides the measure of one complete cycle of the oscillation (we choose 1.5π because in stochastic simulation, it is not necessarily that the phase switch happens exactly from $-\pi$ to π , see Fig.3.3, red dots. Therefore, we arbitrarily took it as 1.5π , and this can be varied according to the noisy system). We repeat this for the whole time series and the time differences between two consecutive phase switching points (Fig.3.3) give the period for one cycle. A similar approach has been employed previously for understanding the dynamics of brain signals [143]. In the second method, we extract the points where there is a positive zero crossing of the instantaneous phase (Fig.3.3, points *c*, *d*). We then calculate the period by taking the time difference between two consecutive zero crossing. However, we use the phase-switching method to determine the period in all our calculations.

The time series obtained from the deterministic model is shown in Fig.3.4A and the instantaneous period and the period distribution is shown in Fig.3.4B and C. HT is an infinite impulse response (IIR) filter, which require computation on an infinite timescale. However, software package like Matlab implements finite impulse response (FIR) HT by applying window method [144]. This window method may distort the beginning and end of the data, which is

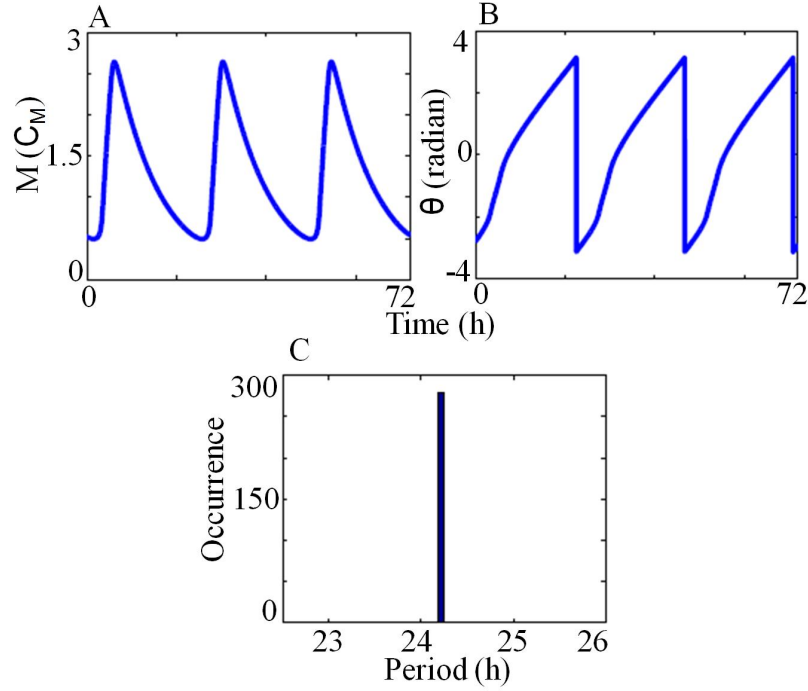


Fig. 3.4 Period distribution of the deterministic time series. (A) Time series of the variable M . (B) Instantaneous phase of the time series calculated via HT method. (C) Period distribution of the model. Endogenous period is close to 24.2h as reported in the literature [111]. Simulation results are obtained by integrating the equations (3.1) and (3.2) with $K_{eq} = 200 C_p^{-1}$. Rest of the parameter values are same as in the Fig.3.2. As used in [111], C_p and C_m are the characteristics concentrations for protein (P_t) and mRNA (M), respectively.

called as the windowing effect [134]. To reduce the windowing effect, and to calculate the mean period, we drop the five oscillation cycles at the beginning and the end of the simulated time series. As reported in the literature [111], the mean period of the fruit fly oscillator obtained by HT method is 24.2h. We then proceed to compute the period of the stochastic time series using the Gillespie method (Fig.3.5). We use three different Ω values for stochastic simulation: $\Omega = 10$, $\Omega = 100$, and $\Omega = 10000$. It is clear that the effect of molecular noise is more when $\Omega = 10$ and shows very large fluctuations in oscillations (Fig.3.5A). The time series also shows a large number of local maxima and minima in a single cycle, and the amplitude values take a very wide range. We perform stochastic simulation for 2500h to calculate the period distribution via HT method for $\Omega = 10$ (Fig.3.5D), $\Omega = 100$ (Fig.3.5H) and $\Omega = 10000$ (Fig.3.5L). Our results are on par with mean crossing method [62] shown in Figs.3.5C, G and K. As in the case of any stochastic circadian model, the simulation of this 2D model also show robust circadian oscillations even when fewer number of molecules are

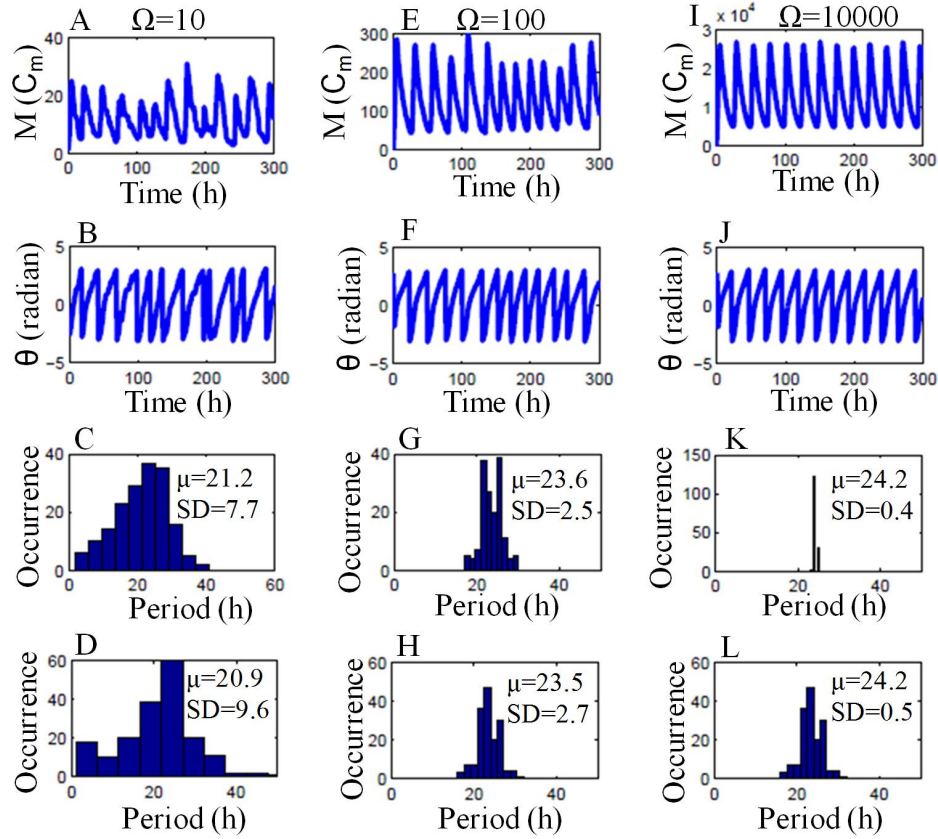


Fig. 3.5 Stochastic Simulation, determination of instantaneous phase and period distribution of Tyson et al. [111] model using HT method. (A) Time series of the variable M obtained from the stochastic simulation for $\Omega = 10$, (B) instantaneous phase (C) period histogram calculated via mean crossing method [62]. (D) Histogram of the period obtained via HT method. The distribution of the circadian period is over the range 0-50h. In all cases, the mean period and standard deviations for a single run are provided in the figure itself, and we calculated the period for the time series simulated for 2500h. The average period is 19.13h for ten simulations (not shown), and each simulation is carried over for 2500h. (E) Time series for $\Omega = 100$, (F) instantaneous phase, (G) period histogram calculated via mean crossing method, (H) Period histogram calculated via HT. The distribution of the period is over the range of 18-30h. The average period is 23.23h for ten simulations (not shown), each over 2500h. (I) Time series for the stochastic simulation for $\Omega = 10000$, (J) instantaneous phase, (K) period histogram calculated via mean crossing method, (L) period histogram calculated via HT. The average period is 24.14h for ten trials, each trail simulated for over 2500h. As Ω increases, mean period obtained by stochastic simulation approaches to a deterministic value (24.2h) and the standard deviation of the period distribution is very less. As used in [111], C_m is the characteristics concentrations for mRNA (M).

present. For $\Omega = 10000$, the model show robust circadian oscillations, the period histogram is much narrower, and the mean period is close to the deterministic value.

In subsequent sections, we take both the deterministic and stochastic time series to study the characteristic properties of circadian rhythms using HT. We also use this time series to validate the novel method that we propose in the later section to determine the phase variations of forced circadian oscillator under different external forcing period conditions.

3.4 Influence of kinetic parameters on the period: Period sensitivity from HT

A robust circadian system has to maintain the amplitude, period, and phase relationship between the molecular components regardless of the environmental variations and fluctuations. Besides robustness to molecular noise, another measure that provides information about the robustness/sensitivity of the model is the variations in the amplitude or period in response to parameter perturbations. The period of oscillations (τ) depends on the reaction rate parameters, and a small perturbation (Δp) in these parameters (p_j) may cause a large change in the period. Understanding period sensitivity provides insight into the functioning of the circadian system, and hence, helps to identify period sensitive parameters to modulate the period of the oscillator to simulate various phenotypes. Previous works have developed different methods to measure the period sensitivity of the circadian models [126, 145]. In [126], period sensitivity was determined from the rate of phase difference accumulated around each cycle and we call this as the phase accumulation method. This method requires to solve the adjoint equation through backward integration and this leads to instability and computational difficulty. To overcome this difficulty, we use HT to calculate the period sensitivity based on the finite difference method that is given below as:

$$\frac{\partial \tau}{\partial p_j} \approx \frac{\tau(p + \Delta p) - \tau(p)}{\Delta p} \quad (3.9)$$

We calculate the period of unperturbed ($\tau(p)$) and perturbed ($\tau(p + \Delta p)$) system by HT method. We rank-order the parameters for wild-type (WT) according to the absolute magnitude of sensitivity (Fig.3.6A), and the ranking matches with the reported results [126]. This ranking of the model parameter perhaps may help to redesign the whole model or may help to reduce the number of dynamical variables in the model that are insensitive. Top-ranked sensitivity parameter, k_m is the exponential degradation constant of mRNA (M). Our sensitivity analysis reveals that the model shows period insensitivity to two crucial parameters, K_{eq} and k_{p1} , and agrees with the reported result [111]. However, the sensitive

parameters are present mostly in mRNA (M) equation. We also perform period sensitivity analysis when the K_{eq} is low. As shown in Fig. 3.2B, there are three regimes where R2 is the regime of wild-type and R1 is the regime, where the period is high for a very low K_{eq} values and this regime is taken as the mutant regime. We took K_{eq} as 15 to simulate the period sensitivity of the mutant (Fig. 3.6B). We find that for a very low K_{eq} value, most of the parameters involved are in the production, degradation and positive feedback loop of total protein P_t (J_p , v_p , and k_{p1}) are more sensitive to variations in period than in the wild type. The period sensitivity of K_{eq} is also higher (Fig. 3.6B, inset), and this result agrees with the bifurcation diagram in Fig. 3.2B that period change is high for the mutant (R1) than in the wild-type (R2). This indicates that positive feedback may be playing a strong role in influencing the period in R1 regime. Thus HT method captures the period sensitivity to variations in parameters of both wild and mutant types effectively.

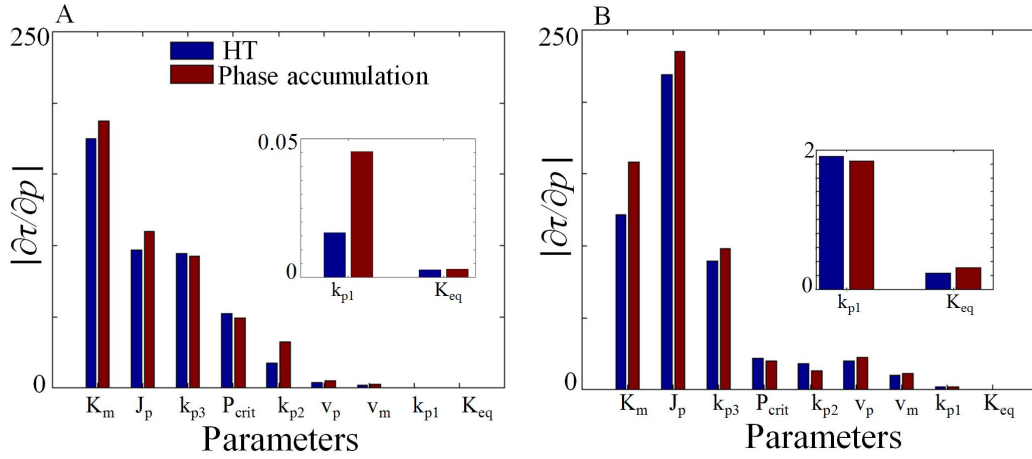


Fig. 3.6 Comparison of HT based period sensitivity with phase accumulation method. (A) Period sensitivity for WT ($K_{eq} = 200$) calculated via HT method (blue) and the period sensitivity is calculated via phase accumulation method as described in [126] (brown). k_{p1} , K_{eq} are almost insensitive to the period of oscillations (inset), and this agrees with the published results [111, 126]. (B) Blue indicates the period sensitivity calculated via HT for mutant ($K_{eq} = 15$) (R1 in Fig.3.2B), and brown represents the period sensitivity calculated via phase accumulation method. Period sensitivity of K_{eq} is higher for mutant than that of the wild type (inset).

3.5 Phase response curves

PRC for various circadian systems under different conditions is well documented (for example, *Drosophila* [146] and mammals [147]). To determine the goodness of the mathematical

models of circadian rhythms, PRC is generated by light pulse stimulation and its effect is compared with the experimental data. Different numerical methods have been previously developed to calculate the PRC [119, 126] and one of them use adjoint method to construct PRC. In this section, we propose HT based numerical method to the construct PRC of the 2D model of the fruit fly and compare our results with PRC got from the adjoint method [126]. For this, we first consider the circadian ODE model of the form

$$x'(t, p) = f(x(t, p)) \quad (3.10)$$

Integrating the above equation gives the vector of state variables X :

$$X(t, p) = \{x_1, x_2, \dots, x_i, \dots, x_n\} \quad (3.11)$$

and p is the vector of parameters given as

$$p = \{p_1, p_2, \dots, p_j, \dots, p_m\} \quad (3.12)$$

τ_0 is the free-running period of the system. Discretizing the time over one period gives

$$t_{dt} = \{t_1, t_2, \dots, t_k, \dots, t_q\} \quad (3.13)$$

Where t_q be the time at which phase is circadian time 24 (CT24).

Let S be the stimulus applied to perturb the system and it is given as:

$$S_j = \begin{cases} p_j + \Delta p_j, & \text{for } t \in [t_k, t_k + d] \\ p_j, & \text{for } t \notin [t_k, t_k + d] \end{cases} \quad (3.14)$$

Where Δp_j is a pulse given at time t_k for the time duration d . We compute the PRC for the parameter p_j

$$PRC_j = \{\Delta\theta_{j1}, \Delta\theta_{j2}, \dots, \Delta\theta_{jk}, \dots, \Delta\theta_{jq}\} \quad (3.15)$$

where $\Delta\theta^{jk}$ is the average value of phase differences between perturbed and unperturbed phase of the state variable given below

$$\Delta\theta^{jk} = \frac{1}{N} \sum \theta_{perturbed}^{jk} - \theta_{unperturbed} \quad (3.16)$$

Here, N is the total number of discrete points for which we calculate the Hilbert phases. $\theta_{perturbed}^{jk}$ is the phase of perturbed system, where parameter p_j is perturbed at time t_k for the time duration d while $\theta_{unperturbed}$ is the phase of unperturbed system. Positive and negative phase difference implies respectively phase lead and phase lag. We also describe below how to calculate the phase difference between the perturbed and unperturbed limit cycle oscillation using HT.

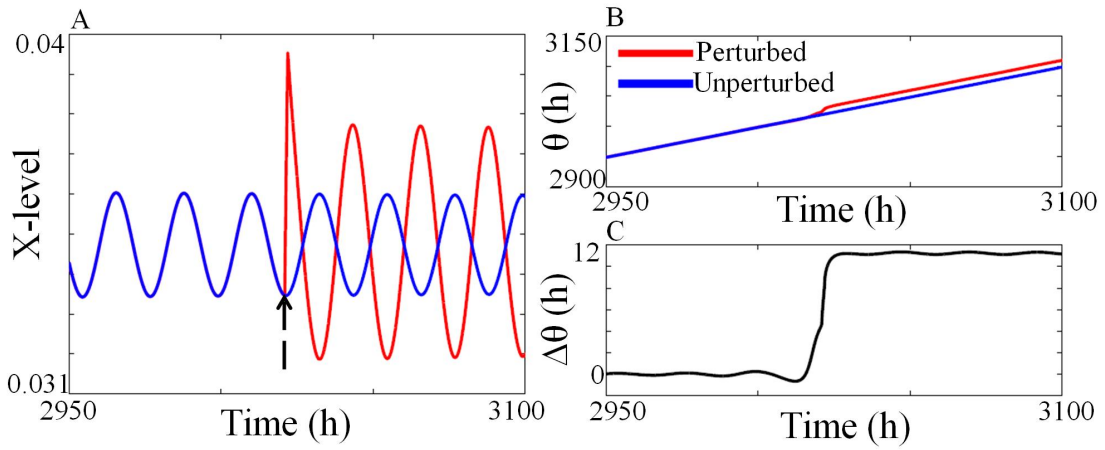


Fig. 3.7 Illustration of phase difference obtained from the perturbed and unperturbed oscillator via HT method. (A) Unperturbed time series is shown as in blue line and perturbed time series as in red line. Black broken arrow indicates the phase at which the perturbation is applied. (B) Instantaneous phases of perturbed and unperturbed time series calculated via HT. (C) The phase difference between the perturbed and unperturbed time series. Time series is obtained by integrating the ODE-provided in [85], where the variable X represent the mRNA.

$$\theta_{perturbed}^{jk} = \tan^{-1}\left(\frac{X_{pH}^{jk}}{X_p^{jk}}\right) \quad (3.17)$$

$$\theta_{unperturbed} = \tan^{-1}\left(\frac{X_{Hu}}{X_u}\right) \quad (3.18)$$

where X_{pH}^{jk} and X_{Hu} are the HT of perturbed (X_p^{jk}) and unperturbed (X_u) state variable respectively. Thus,

$$\Delta\theta^{jk} = \frac{1}{N} \sum \tan^{-1} \left[\frac{X_{pH}^{jk} X_u - X_{Hu} X_p^{jk}}{X_p^{jk} X_u + X_{pH}^{jk} X_{Hu}} \right] \quad (3.19)$$

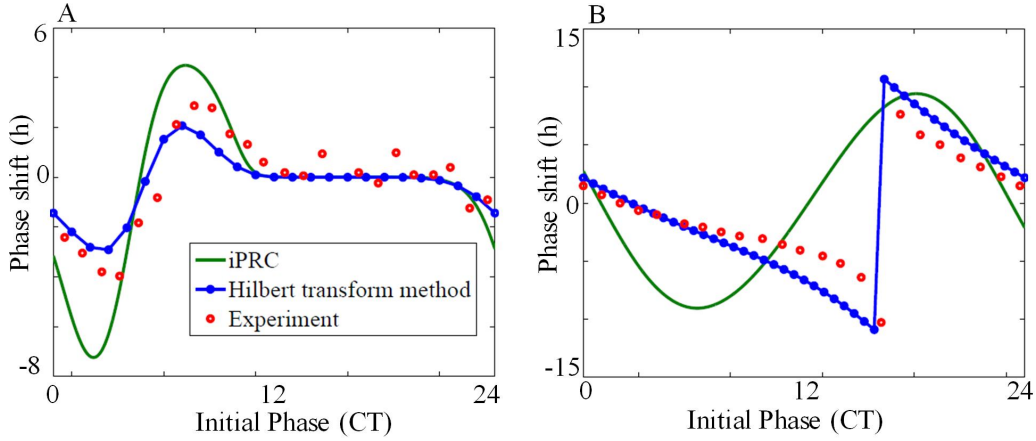


Fig. 3.8 Comparison of PRCs by HT and iPRC methods for the *Drosophila* and *Neurospora* circadian models. (A) PRC of the *Drosophila* is constructed from the circadian model described by the equations (1a)-(1j) in [77]. The experimental data of PRC was extracted from [147]. To simulate PRC, we set CT6 as the maximum of *per* mRNA. The light perturbation here takes the form of a 3h long two-fold increase in the parameter V_{dT} . PRC obtained via both iPRC (green line) and HT methods (blue line) are projected along with the experimental data (red circles). The x axis is converted into circadian time (1CT(h) = 59.5 min). (B) PRC is constructed from the *Neurospora* circadian model described in [85] and the experimental data of PRC is also extracted from [85]. To simulate PRC, maximum of X , the mRNA variable is set as CT4. PRC calculated via HT (blue line) and iPRC methods (green line) are projected along with the experimental data (red dots). The perturbation here takes the form of a 1h long two-fold increase in the parameter k_1 in the Goodwin model of *Neurospora*. The x axis again converted to the circadian time (1CT(h) = 55.75 min). PRC's of both *Neurospora crassa* and *Drosophila* via HT method matches well with the experimental data in comparison to iPRC method.

To determine how well HT method performs in simulating PRC of various species, besides getting the PRC for the fruit fly model [111], we also generate PRC of other well known circadian models of *Drosophila* [77], *Neurospora* [85], and compare the results with the experimental PRC. Fig.3.7 shows how the parameter perturbation affect the time series (Fig.3.7A) and their phase (Fig.3.7B). The phase difference between the Hilbert phases of the perturbed and unperturbed time series is shown in Fig.3.7C. We specifically compare our

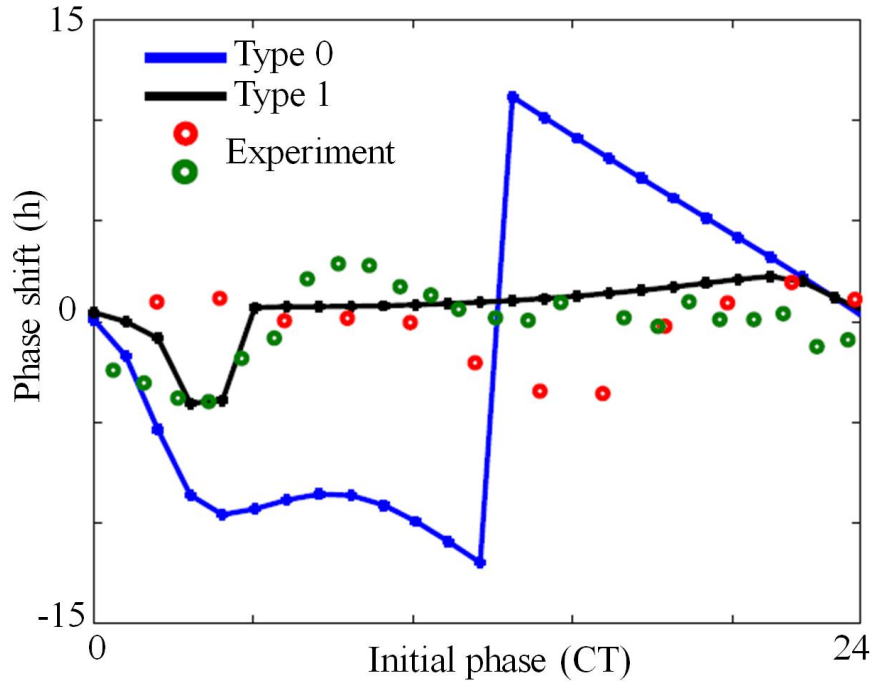


Fig. 3.9 Type-0 and type-1 PRC of Tyson et al. model [111]. PRC obtained via HT method. For small amplitude perturbations, model shows type-1 PRC and for high amplitude perturbation, type-0 PRC is seen. Calculated PRC is matched with the published result (Figure 5A in [111]). For type-1 PRC, K_{eq} is changed from 200 to 15 for 0.7h, and for type-0 PRC, K_{eq} is changed from 200 to 2 for 2h. Experimental PRC is extracted from [146] (red circle) and [148] (green circle). Note the model published by Tyson et al., the PRC did not match well with the experimental PRC. So the simulated PRC from HT method also did not match with the experimental PRC, but matched exactly with the model PRC simulated [111].

method with the infinitesimal PRC (iPRC) method to capture the PRC's of *Drosophila* [77] and *Neurospora* [85] (See Fig.3.8). The iPRC method requires to solve adjoint equations that are unstable and to get the solution requires backward integration that is difficult to program. Further, PRC of *Drosophila* model obtained from the iPRC method is qualitatively very similar (Fig.3.8A, green line) but quantitatively incorrect. This method also works well only if the perturbation is very small [145], and for large perturbation, this method fails to predict PRC accurately. This method also fails to capture Type-0 PRC of *Neurospora* model (Fig.3.8B). HT based method, on the other hand, could reproduce quantitatively and qualitatively both Type-0 and Type-1 PRC's. Our method is efficient and accurate in comparison to the iPRC method, and this is because our algorithm computes the instantaneous phase of the oscillation for a large number of cycles and take its average. We also construct Type-0 and Type-1 PRC's of the 2D fruit fly model (Fig.3.9), and it agrees well with the

simulated results [111]. However, the model in [111] fails to reproduce the experimentally observed PRC [146, 148].

3.6 Mapping the entrainment region–Arnold tongue

Entrainment happens when the frequency of a circadian oscillator is relatively close to the frequency of external forcing signal. The ratio of the period of the forced oscillator and the period of forcing signal is called period number (rotation number). The plot of period number as a function of forcing signal frequency shows a staircase structure, known as devil's staircase [149, 150]. We construct the devil's staircase from the model described in the equations (3.1, 3.2), and it is shown in Fig. 3.10A. Some regions of devil's staircase show constant period number for a range of frequency, which is the entrainment regions. Entrainment states can also be represented by Arnold tongues, which are the regions of period locking, where external forcing signal and the resulting forced oscillations of the circadian model have their periods in a specific ratio. In the entrainment region, the model can show $n : m$ period locking. Here m and n are the numbers of forcing signal cycles and forced oscillation cycle respectively. In Tyson et al. model [111], the forcing signal is Light-Dark (LD) cycle. In LD cycle simulation we use $K_{eq} = 200(1 - a)$ for light phase and $K_{eq} = 200$ for dark phase. Here a is proportional to the intensity of illumination [111], and we take it as the forcing signal amplitude. To simulate Arnold's tongue, we use the LD cycle with 50% photoperiod (duty cycle), which denotes a 24h forcing period with 12h light phase and 12h dark phase.

We construct the entrainment zone by first choosing appropriate forcing signal and model parameters. We then integrate the two variable ODE's and calculate the period of oscillation (τ_{oscr}) by applying HT to the time series of the M variable. We compare this period with the external forcing period (τ_E) given by $|m - \frac{\tau_{oscr}}{\tau_E}| \leq \varepsilon$, where ε is a small value to account for integration error. If the above inequality is satisfied, then it is said to be $1 : m$ entrainment, where m is the number of forcing cycles. Similarly, we also determine the $n : m$ entrainment, where we calculate n number of oscillations in m forcing cycle. The Arnold tongue for the 2D model (Fig.3.10B) is in good agreement with the reported one (Fig.5B in [111]). From the figure, it is clear that if the strength (amplitude) of the forcing signal is increased/decreased, then the entrainment region also expands/shrinks, a common characteristic of limit cycle oscillators. The entrainment region provides the parameter a for which 1:1 phase locking of entrainment and phase slips are possible. This information is used in the next section to validate the new method we propose in combination with HT for phase entrainments.

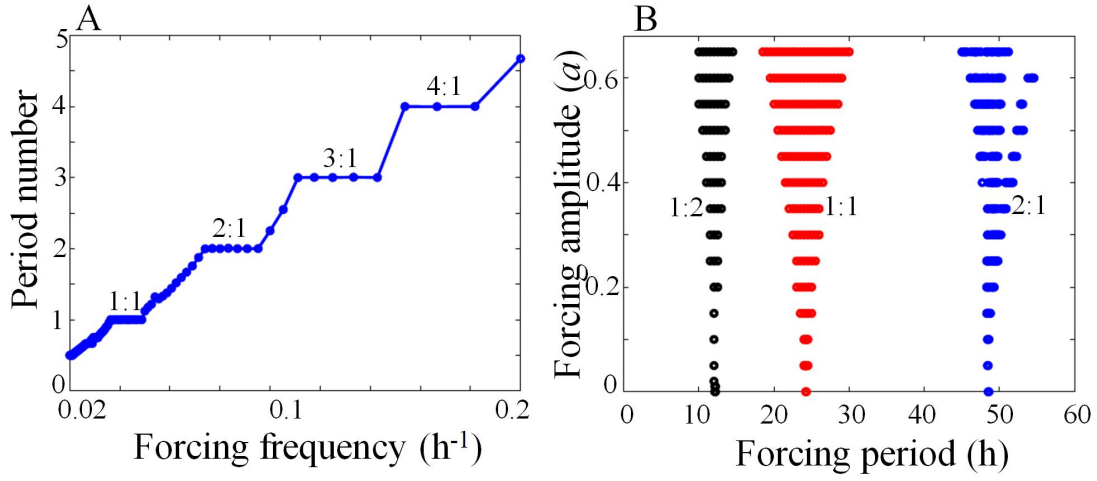


Fig. 3.10 Devil's staircase and Arnold's tongue. (A) Devil's staircase. Period number as a function of forcing frequency. Some regions show a constant period number for a range of frequency. (B) Arnold's tongue. When the oscillator period is close to forcing signal period, entrainment occurs (1:1). Outside the entrainment region, the quasi-periodic behavior is observed. In 1:2 region, we observe for every one cycle, there is a two-cycle forcing period, whereas in 2:1 region, for every two cycles, there is one cycle of forcing period. We obtain the Devil's staircase and Arnold's tongue by integrating the equations (3.1) and (3.2). For LD cycle simulation, we use $K_{eq} = 200(1 - a)$ in the light phase, and $K_{eq} = 200$ at dark phase, where a is proportional to the intensity of illumination [111]. To simulate Devil's staircase, we use $a = 0.5$ with a duty cycle of 50% for the LD cycle. The period of the oscillator is calculated using HT method. Arnold's tongue matches with the reported result (Figure 5(B) in [111]).

3.7 Phase equations

Phase equation describes the dynamics of phase difference between free running oscillator subjected to an external forcing signal. Phase locking happens during entrainment, where the phase difference between forcing signal and forced oscillator phases should maintain a stable value. Phase equation can be utilized to determine phase locking, and hence entrainment and phase slips. In the subsequent part of this section we explain this in detail.

3.7.1 Derivation of the scaled phase equation

Entrainment for the deterministic and stochastic model of circadian rhythms have been studied and discussed in [151], and they studied the effect of molecular noise on phase

locking and phase slip phenomena in detail. We provide below the equations that we use to determine the nature of entrainments like phase locking and phase slips from the time series data for a given forcing period.

We consider θ_{ext} and θ_{inst} respectively the instantaneous phases in radians of the external (forcing) and forced oscillations that governs the circadian oscillators. The phase difference is

$$\Psi = \theta_{ext} - \theta_{inst} \quad (3.20)$$

and the rate of change of this phase difference (radians/h) is given as

$$\frac{d\Psi}{dt} = \frac{d\theta_{ext}}{dt} - \frac{d\theta_{inst}}{dt} \quad (3.21)$$

$$\frac{d\Psi}{dt} = \omega_{ext} - \omega_{inst} \quad (3.22)$$

Note that we have taken the time in hours (h). To get the period, we take $\omega_{ext} = \frac{2\pi}{T_{ext}}$, $\omega_{inst} = \frac{2\pi}{T_{inst}}$ and we substitute this in the above equation to get

$$\begin{aligned} \frac{d\Psi}{dt} &= \frac{2\pi}{T_{ext}} - \frac{2\pi}{T_{inst}}. \\ \frac{d\Psi}{dt} &= 2\pi \frac{T_{inst} - T_{ext}}{T_{ext} T_{inst}}. \end{aligned} \quad (3.23)$$

Where, T_{ext} and T_{inst} are the period of forcing signal and forced oscillator respectively. T_{ext} have a constant value over the period of time, whereas, T_{inst} is varied over time, and the procedure for calculating $T_{inst}(t)$ will be discussed in the section 3.7.2. We scale the equation (3.23) and reduce to dimensionless by taking $\psi = \frac{\Psi}{\Psi_0}$ and $\tau = \frac{t}{T_{ext}}$ and the scaled equation is

$$\frac{\Psi_0}{T_{ext}} \frac{d\psi}{d\tau} = 2\pi \frac{T_{inst} - T_{ext}}{T_{ext} T_{inst}} \quad (3.24)$$

$$\frac{\Psi_0}{2\pi} \frac{d\psi}{d\tau} = \frac{T_{inst} - T_{ext}}{T_{inst}} \quad (3.25)$$

If we take the constant Ψ_0 to be 2π radians then the above equation becomes

$$\frac{d\psi}{d\tau} = \frac{T_{inst}(\tau) - T_{ext}}{T_{inst}(\tau)} \quad (3.26)$$

This is the dimensionless phase equation we use to calculate the phase slip. To calculate the rate of phase slip per day ($\frac{d\psi_d}{dt}$), we modify the above equation as:

$$\frac{d\psi_d}{d\tau} = \left(\frac{T_{avg} - T_{ext}}{T_{avg}} \right) \times 24 \quad (3.27)$$

Where, T_{avg} is the average period of the forced oscillator, and its is given as $\frac{1}{n} \sum (T_{inst})$ with n being the number of cycles. Note the difference in the equations (3.26) and (3.27) and we use this two equations in the next section to calculate the region of phase locking and phase slips for the given parameter as well as the time series for the fruit fly model.

3.7.2 Numerics of the phase equation: phase locking and phase slip

To calculate phase slip, we compute the period for each cycle using HT and assume that the period is constant for one complete cycle of oscillation, we take this period as instantaneous period (T_{inst}) for one complete cycle as shown in Fig.3.11. We calculate phase slip by integrating the equation (3.26) by Euler's method with an integrating step of $\Delta\tau$, which is same as the sampling time of the time series. We calculate the phase slip from the time series we obtain from the fruit fly model model [111] and the experimental data we got from the mice SCN culture [138] to illustrate the effectiveness of our novel method.

Phase slip calculated for the time series obtained from fruit fly model

We use two forcing periods 23 and 25h to illustrate phase locking and slips in the fruit fly model [111]. When forcing amplitude $a = 0$, the model oscillates with a free running period of 24.2h. A Phase slip of 1.2h is obtained if the forcing period T_{ext} is 23h and according to the equation (3.27), the rate of phase slip per day (24h) is 1.19h. We also calculate the rate of phase slip per day for various values of a with a period forcing of 23h and plot of $\frac{d\psi_d}{dt}$ vs a is shown in Fig.3.12 A. There is a critical value of a below which phase slip occurs, and above which forced oscillator is completely phase locked to the applied external signal. We also repeat this to the forcing signal with a period of 25h. When $a = 0$, phase slip occurs, and it

is -0.8h and the rate of phase slip per day is -0.79h. We again obtain the rate of phase slip per day for different values a with forcing period 25h and the plot of $\frac{d\psi_d}{dt}$ vs a is shown in Fig.3.12C.

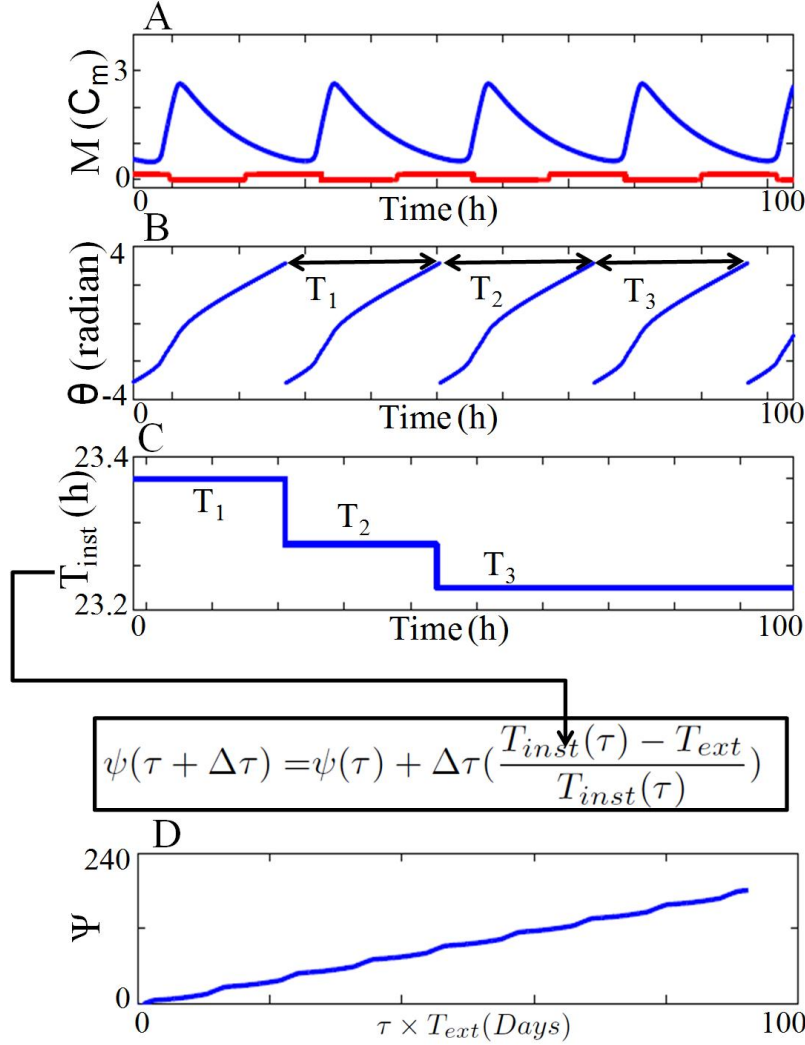


Fig. 3.11 Calculation of phase slip. (A) Time series is obtained from the model. (B) Instantaneous phase is calculated via HT. The period of each cycle is calculated as discussed in section 3.3, and this period assigned as the instantaneous period ($T_{inst}(t)$) for one cycle (C). (D) This instantaneous period obtained is used for calculating the phase slip by routing it to the equation (3.26). This equation is numerically integrated via the Euler's method. As used in [111], C_m is the characteristics concentrations for mRNA (M).

We also numerically integrate the equation (3.26) to calculate the phase slip and locking for different a values with two different forcing signals of period 23 and 25hrs. The results are shown in Fig.3.12E and 3.12F respectively for various a values. We specifically select

four different a values, three from the non-entrainment region, and one from the entrainment region as shown in Fig.3.12B (red '+' and green 'x'). In Fig.3.12E, period of the forcing signal is 23h, when $a = 0$ (without forcing signal), slip progress at constant rate (blue line). When $a \neq 0$, the system exhibits periodic tendency for locking (black and green line). When $a = 0.2$, the system is completely phase locked ($\frac{d\psi}{dt} = 0$, red line). Corresponding time series for $a = 0.15$ are shown in Fig.3.12 D. We observe similar results for the forcing period of 25h (Fig.3.12 F, G).

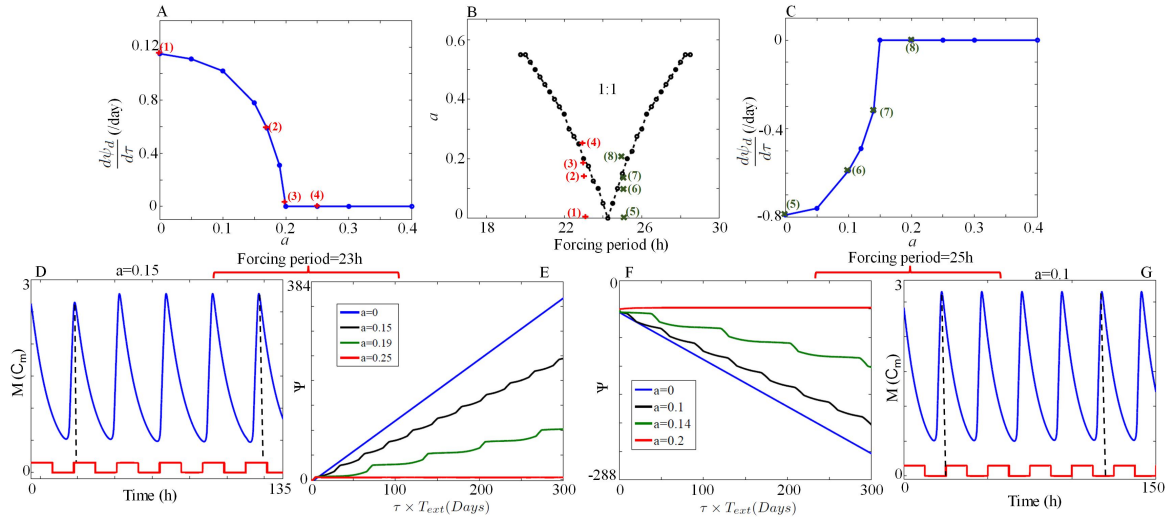


Fig. 3.12 Entrainment diagrams. $\frac{d\psi}{dt}$ versus a for the Tyson et al. [111] model with a forcing signal period of 23h (A) and 25h (C). Calculated points are showing in the blue dots. Red '+' and green 'x' are the corresponding points from the 1:1 Arnold's Tongue (B). (D) Time series of the model with a forcing signal period of 23 h and amplitude value $a = 0.15$. The model is not completely locked, and in each cycle, it shows different peaking time with respect to external forcing signal. Black dotted line shows the peaking time of the variable M . (E) Phase slip calculated for a forcing signal with period 23 h and photoperiod 11.5h for 300 days. When $a = 0$, no locking occurs, and for non zero values of a , it shows the periodic tendency for locking. (F) Phase slip calculated for a forcing signal with period 25 h and photoperiod 12.5 h. (G) Time series of the model with a forcing signal period of 25h and amplitude value $a = 0.1$. As used in [111], C_m is the characteristics concentrations for mRNA (M).

To see the effect of molecular noise on entrainment, we use the stochastic time series of the Tyson et al. model with a forcing period of 23h and calculate the phase slip using the equation (3.26). The results are shown in Fig 3.13. When $\Omega = 1000$ and $\Omega = 10000$, entrainment is seen for the higher values of a (Fig.3.13A, B). For $\Omega = 10000$ (Fig.3.13B), the phase slip that we obtained from the stochastic simulations matches well with the deterministic simulation (Fig.3.12E). This indicates that the model is robust to noise and its entrainment property to

the external forcing signal is similar to that of the deterministic model provided that Ω is large enough.

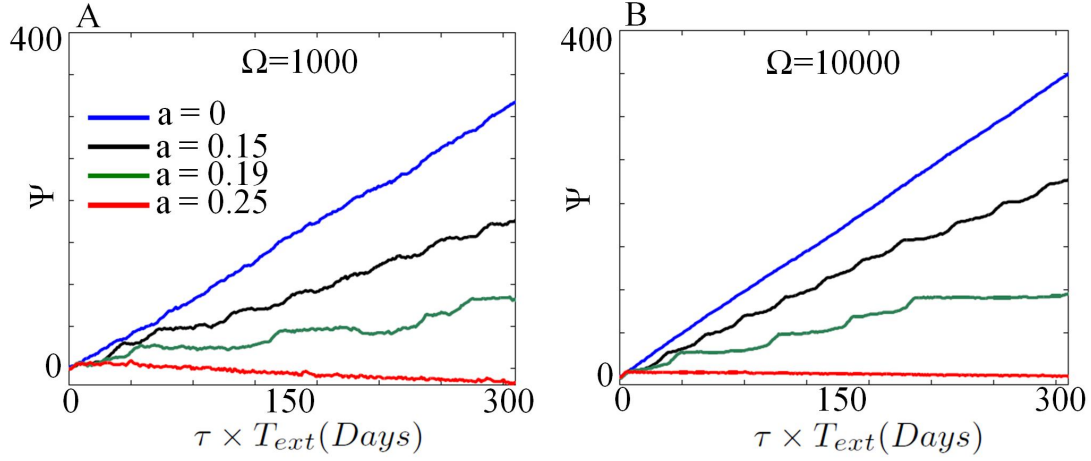


Fig. 3.13 Effect of molecular noise on entrainment. The effect of molecular noise on the stochastic version of Tyson et al. model with (A) $\Omega = 1000$ and (B) $\Omega = 10000$. In all simulation, the period of forcing signal is 23h.

3.8 Validation of the phase equation: Identification of phase-locked regime in the entrained experimental time series of SCN under different forced temperature cycles

In this section, we validate HT based analysis of entrainment property to experimentally collected circadian data. Bordygov et al. [138] investigated the entrainment properties of mouse SCN slice time series data to the externally forced temperature cycles. They recorded the time series of bioluminescence signal from PER2::LUC mouse SCN slice under different period of temperature cycle (forcing signal). Here, we analyze this SCN experimental data (Fig. 3.14A, E, I) with our phase equation model to determine the entrainment of PER2 bioluminescence to the temperature cycle. For this, we first calculate the Hilbert phase of the detrended data ((Fig. 3.14B, F, J), and using this phase information, we calculate the instantaneous period (T_{inst}) as discussed in the section (3.7.2). The results are shown Fig. 3.14C, G, K. The T_{inst} obtained is routed to the equation (3.26) to calculate the phase locking regime (Fig. 3.14D, H, L). From HT based numerics it is clear that PER2 entrains to the temperature cycles, and in the entrained region, temperature cycle and the SCN PER2 data

have the same period (Fig. 3.14C, G, K). Also, during the entrainment period, phase slip calculated through the equation (3.26) is constant (Fig. 3.14D, H, L). These results are in good agreement with the findings that SCN entrains to the temperature cycle for different periods of 22h, 24h, and 26h, given the temperature variation is strong enough (6°C to 8°C temperature variation) [138].

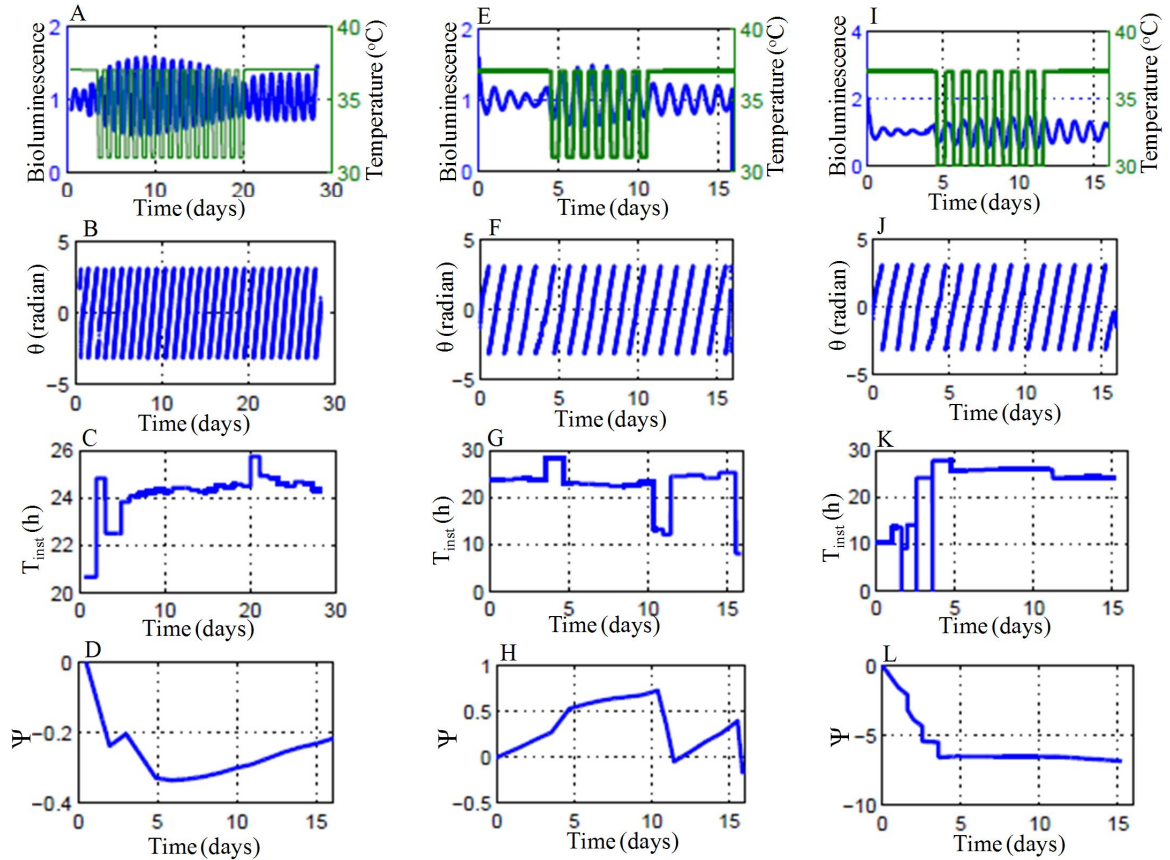


Fig. 3.14 Entrainment of experimental SCN circadian time series to different forced external temperature cycles. (A, E, I) Time series (blue line) of the bioluminescence signal from PER2::LUC mouse SCN slice [138]. Green lines indicate the various temperature level during the days. Initially, the temperature kept at a constant level, and after a few days it drops to some lower level periodically with a forcing period (T_{ext}) of (A) 24h, (E) 22h and (I) 26h. B, J, and F represents the instantaneous Hilbert phase of the time series shown in A, E, and I respectively. (C, G, K) T_{inst} is calculated as described in Fig. 3.11. Calculated phase slip according to the equation (3.26) for the time series with respect to the forcing signal as shown in A, E, and I are provided in D, H, and L respectively.

3.9 Discussion and conclusion

In this chapter, we introduced HT based method to analyze the time series of circadian models. HT performs a $-\pi/2$ phase shift for every spectral component of the time domain signal $x(t)$ and give instantaneous phase via the construction of the analytical signal $z(t)$. We showed that the period distribution of noisy time series, PRC's, Arnold tongue, and phase slip phenomena can be effectively computed from the instantaneous phase information. In the presence of noise, obtaining frequency information from the simulated (stochastic) data is more challenging and hence it is difficult to calculate the period. However, we showed in section 3.3 that HT can be utilized to calculate the period distribution of noisy time series obtained from stochastic simulation, and the estimated periods matched well with the published results. Further, period of the oscillations depends on the model parameters, and the period sensitivity analysis helps to identify the parametric dependencies on the oscillatory period of the model. We showed in section 3.4 that the period sensitivity using HT method provides excellent results that are in agreement with the published results [126, 111].

PRC is a powerful tool to analyze the performance of the circadian model. Previously, experimental PRC of the SCN clock cell under temperature perturbation were constructed using HT [131]. Also, HT has been applied to construct numerical PRC of oscillatory system [134], however amplitude information was utilized to calculate the phase difference. Unlike previous methods, with a detailed mathematical explanation, we directly utilized the phase information for constructing PRC in section 3.5. Further, based on our method we constructed both Type-0 and Type-1 PRC's of various circadian model under light perturbation that agreed very well with the published results [111, 77, 85].

Entrainment property tells the ability of a circadian model to adapt to the day length variation of the environment. We applied HT method to determine the period mismatch between circadian model and the external forcing signal by constructing Arnold's tongue in section 3.6. We successfully reproduced the wedge-like shaped region for 1:1, 1:2, and 2:1 entrainment regions for *Drosophila* model [111]. Phase slip phenomena is another method to examine the entrainment property. Previously, in [135] the amount of entrainment is quantified using HT method. However, their method doesn't provide information about the time instant when entrainment (phase locking) or nonentrainment (phase slip) occur. In this work, we proposed a simple model and numeric in section 3.7 to determine the entrainment properties of the system under the external light-dark forcing periods. Importantly, we showed that phase locking and phase slips can be obtained from the time series data using the HT method. We specifically compared the results obtained from HT method with the experimental data [138] and the results were found to agree very well. Importantly, we believe that this method can

be used directly to the forced experimental data with known forcing periods to determine the occurrence of entrainment or not. In summary, we showed here that HT based time series analysis of circadian data can be used to determine most of the circadian properties like period distribution, period sensitivity, phase response curves, and entrainment.

Chapter 4

Hypothesis driven single cell dual oscillator mathematical model of circadian rhythms

Preamble

Parts of this chapter have been published in:

Shiju, S., and Sriram, K. (2017). Hypothesis driven single cell dual oscillator mathematical model of circadian rhythms. *PLoS ONE*, 12(5), e0177197 ([Link](#))

Shiju S and K Sriram, (2016, March). Mathematical modelling of circadian oscillator that explain the role of *per1* and *per2* in morning and evening oscillator. Presented at *ICMMDESCA-2016, Kanpur, India : Indian institute of Technology Kanpur*.

4.1 Introduction

The circadian pacemaker in SCN plays an important role in encoding day length and seasonal changes, identify the molecular mechanism responsible for this encoding is an interesting problem that has attracted attention for significant period of time. It has been proposed that the circadian pacemaker consists of two distinct oscillators named morning (M) and evening (E) oscillator [41–43], and these are responsible for encoding day length variation and seasonal changes in SCN. The morning (M) oscillator locks on to dawn and controls the morning activities, while the evening (E) oscillator locks on to the dusk and controls

the evening activities. The idea of ME oscillators was proposed a long time back when the hamster's locomotor activity under constant light splits into two different components [44] and the lesion in SCN annihilate the two separate components into a single locomotor activity [152]. Even though splitting has lead to the concept ME oscillator, its appearance is not due to the ME oscillator [46].

Inspired by the previous works of Pittendrigh and Daan's dual oscillator model [41], Daan et al. [43] convincingly put together the basis for morning and evening oscillators in terms of the molecular mechanisms of circadian gene expression. We interpret here the genes accountable for the dual oscillator in single cell are *per1/cry1* and *per2/cry2*, even though they impact each other, they can oscillate and operate separately, and we assume *per1/cry1* act as M oscillator and *per2/cry2* act as an E oscillator. We present below the abstract of Daan's hypotheses (H1–H5), which we validate by constructing coupled set of nonlinear differential equation model based on the molecular mechanisms of circadian gene expression.

(H1) The maxima of *per1* gene occurs around early subjective day (CT3-6) and *per2* around the late subjective day (CT10) with *per1* leading *per2*. The light accelerates (phase advances) *per1/cry1* and therefore they constitute M oscillator while *per2/cry2* decelerates (phase delays) and thus constitutes the E oscillator. The peaking time of *per1* and phase advances in the presence of light stipulate that *per1* may be the candidate for morning (M) oscillator, while the peaking time of *per2* during the late subjective day and light influenced phase delays designates *per2* may be the candidate for an evening (E) oscillator.

(H2) Under DD conditions, in comparison to wild type, The intrinsic period of both M and E oscillators under DD condition is shorter than that of wild type, which means that both *per1* and *per2* mutants under DD conditions should have a shorter period than the wild type. In the case of *per1-per2* double mutant, ME oscillator is dysfunctional and hence oscillations are not possible.

(H3) Circadian pacemaker shows bidirectional phase response curve (PRC) under light pulse perturbation with *per1* being primarily responsible for phase advances while *per2* is responsible for phase delays. In other words, suppression of phase advance is expected to occur in *per1* mutation while in *per2* mutation suppression of phase delay is expected to happen.

(H4) Under different light-dark (LD) conditions, the phase difference between M and E oscillator increases with increase in the photoperiod with M oscillator locks onto dawn and the E oscillator locks onto the dusk.

(H5) Under constant light conditions (LL), the period of circadian oscillation is expected to increase in *per1* mutant mice (*per2* limb intact) while in *per2* mutant mice (*per1* limb intact), the period is expected to decrease.

In the above stated hypothesis, we have not considered the after effects of light. Besides these five hypotheses, we also explain the appearance of splitting phenomena through coupling of the dual oscillator models with neuropeptides as coupling agents. In free running wheel activity, during long days, phase separation between coupled oscillators increases and at certain time, the single rhythmic activity "splits" into two different bouts with the period of each bout is less than one circadian period. We explain these splitting phenomena in terms of molecular mechanisms of circadian gene expression.

Daan et al. [43] supported their hypothesis based on experimental data obtained from both wild and mutant types mice and these data are collated from different sources that includes phase response curves (PRC) [153], phase angle differences [18, 154], and entrainment curves under different light-dark conditions [155]. Hence, the primary aim of this work is to build a mathematical model of circadian GRN with the assumption that *per1* and *per2* will be taken as a candidate genes for morning and evening oscillators respectively and to verify whether the dynamics of *per1/2* genes and PER1/2 proteins satisfy Daan's hypothesis H1-H5.

4.2 Materials and methods

We use Xppaut [119] and Matlab for numerical simulation, and the programs along with the link to the figures are printed in the Appendix-D.2.

4.2.1 Model

Mathematical models for gene regulatory networks of circadian rhythms have been proposed for *Neurospora* [85, 77], *Drosophila* [77, 78, 104] and mammals [61, 59, 79, 80] to understand the workings of circadian pacemaker under various conditions. However, till this date, there are hardly any attempts to understand the origin of ME dual oscillators from the point of view of GRN models. Previous models of ME oscillators [42, 50] are phenomenological in nature and are not based on the molecular basis of circadian oscillations and these models mostly accounted only for the splitting behavior. Existing models cannot be directly used because of various problems (see the Table 4.6 on the pros and cons of existing models). So we develop a consensus model that is similar to most of the well known existing GRN models [61, 59, 79, 80] but in addition, we also include detailed *per2* negative limb and add an explicit direct positive feedback loop between PER2 and *Bmal1* in the network

[13, 18]. We estimate the parameters of the model from the experimental data obtained from different sources.

In developing the mathematical model for circadian rhythms to explain Daan's hypothesis for the dual oscillator model, we made the following assumptions: We do not consider (i) *cry* genes separately because Okamura et al. [154] has shown that *cry1/2* do not have distinguishable peaking rhythms as that of *per1/2* (ii) the detailed phosphorylation reactions so that the number of variables are kept to a minimum (iii) the *Rorc* gene, as it is not a part of core circadian oscillators that explains dual oscillator hypothesis (iv) the *clk* genes in the model since the CLK protein levels are constant throughout the day [155]. Finally, we consider the direct influence of light on *per1* and *per2* expression and this is different from the customary introduction of light in the model through parameters that affects the transcription rates of *per* circadian genes [61].

The present mathematical model consists of (i) Hill's equation to describe the positive and negative regulations (ii) Michaelis-Menten equation to describe the degradation of mRNA's and proteins (iii) mass action kinetics to describe both the complexation and first order degradation reactions. In the model, we also consider the nuclear and cytosolic PER1/2 and BMAL1 proteins separately. The terms that are used in the present model equations are similar to those of models [61, 79, 80], but it's different from the detailed mass action kinetics model described in [59]. The variables M_{p1} , M_{p2} , P_{1c} , P_{1n} , P_{2c} , P_{2n} , M_b , B_c , B_N , M_R , R , P_{B1} , and P_{B2} are *per1* mRNA, *per2* mRNA, PER1 protein (cytosol), PER1 protein (nucleus), PER2 protein (cytosol), PER2 protein (nucleus), *Bmal1* mRNA, BMAL1 protein (cytosol), BMAL1 protein (nucleus), *Rev-erb α* mRNA, REV-ERB α protein, PER1-BMAL1 complex, and PER2-BMAL1 complex, respectively. Transcription rates are denoted by v_{si} 's ($i = 1, 2, \dots$), Michaelis constants by k_{ei} 's, degradation constants by k_{di} 's, production and complexation rates by k_{pi} 's, m and w are Hill's coefficient to denote co-operativity, k_{ai} 's denote activation constants, k_{li} denote inhibition constant and L is the light parameter (under DD condition, $L = 0$).

There are overall 13 equations with 60 parameters. We estimate all the parameters for the wild type by genetic algorithms to fit the mRNA's and proteins got from the experiments. We also include the positive regulation of *Bmal1* by PER2 protein that gives rise to the positive feedback loop [13, 18]. This assumption is based on the experimental evidence performed in vitro and in vivo in NIH3T3 fibroblasts that PER2 coregulates *Bmal1* by binding with the nuclear receptor *PPAR α* [156]. However, presently there is no evidence that this happens in SCN and we only speculate this coregulation. This speculation is added as a new feedback in the model that was not hitherto considered in the earlier models of

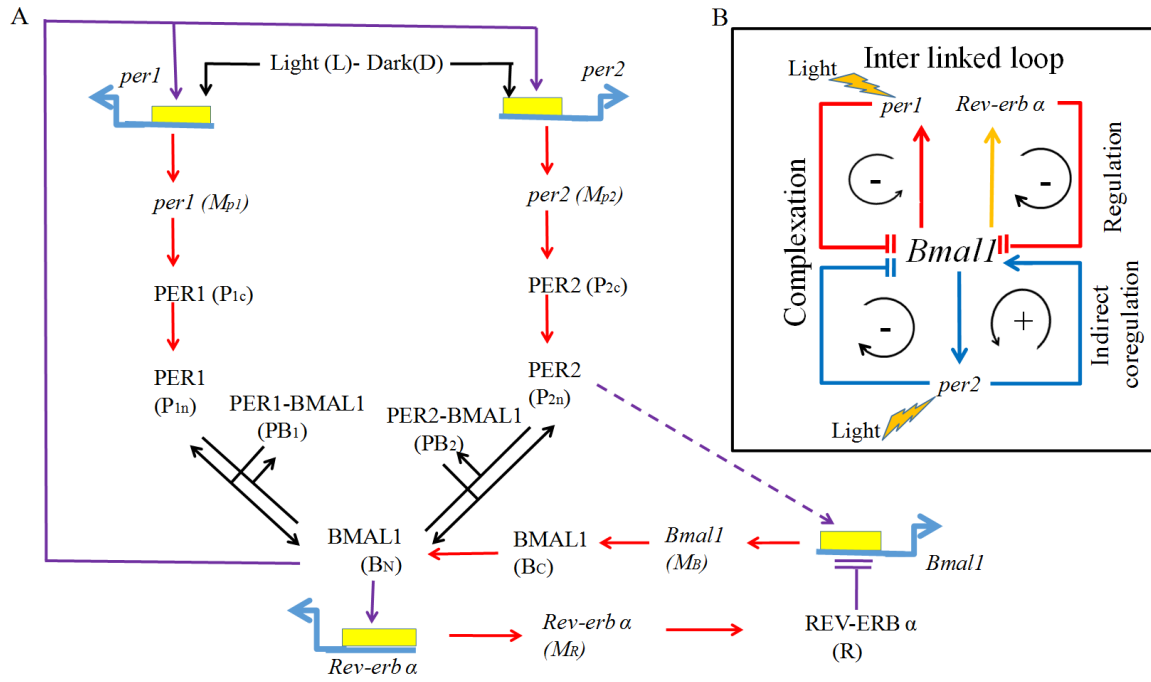


Fig. 4.1 The gene regulatory network of circadian rhythms with feedback loops. (A) The genes and RNA's are shown in italics, while the proteins and protein complexes are shown in capitals. The dynamical variables that are used in the model equations are given in the brackets. The protein BMAL1 positively regulates *per1*, *per2*, and *Rev-erb α*. *per1* and *per2* produce the corresponding proteins PER1 and PER2 and these proteins interact separately with BMAL1 to form a complex PER1-BMAL1 and PER2-BMAL1 to complete the negative feedback loop. *Rev-erb α* negatively regulates *Bmal1* transcription to complete the second negative feedback loop. PER2 coregulates *Bmal1* positively [156] and closes the only indirect positive feedback loop. Light is the external zeitgeber. (B) Summary of the complete network shown in (A) that captures three negative and one positive feedback loops.

mammalian circadian rhythms. In summary, we assume that there is an additional indirect positive feedback between *Bmal1* and PER2, where PER2 coactivates through a hitherto unknown nuclear receptor. We do not consider the indirect positive feedback of PER2 that regulates *Rev-erb α*, which in turn regulates *Bmal1* through its protein REV-ERB α in the model. The biological network with interlinked feedback loops is shown in Fig 4.1.

The ode's are

$$\frac{dM_{p1}}{dt} = v_{s1} \frac{B_N^m}{k_{a1}^m + B_N^m} - v_1 \frac{M_{p1}}{k_{e1} - k_{d1}M_{p1}} + L \quad (4.1)$$

$$\frac{P_{1c}}{dt} = k_1 M_{p1} - v_2 \frac{P_{1c}}{k_{e2} + P_{1c}} - k_{d2} P_{1c} \quad (4.2)$$

$$\frac{P_{1n}}{dt} = k_2 P_{1c} - v_3 \frac{P_{1n}}{k_{e3} + P_{1n}} + k_{p1} P B_1 - k_{p2} P_{1n} B_N - k_{d2} P_{1n} \quad (4.3)$$

$$\frac{dM_{p2}}{dt} = v_{s2} \frac{B_N^m}{k_{a2}^m + B_N^m} - v_4 \frac{M_{p2}}{k_{e4} - k_{d4}M_{p2}} + L \quad (4.4)$$

$$\frac{P_{2c}}{dt} = k_3 M_{p2} - v_5 \frac{P_{2c}}{k_{e5} + P_{2c}} - k_{d5} P_{2c} \quad (4.5)$$

$$\frac{P_{2n}}{dt} = k_4 P_{1c} - v_6 \frac{P_{2n}}{k_{e6} + P_{2n}} + k_{p3} P B_2 - k_{p4} P_{2n} B_N - k_{d6} P_{2n} \quad (4.6)$$

$$\frac{M_B}{dt} = v_{s3} \frac{k_{I1}^2}{k_{I1}^2 + R^2 + (\frac{R}{k_x})} - v_7 \frac{M_B}{k_{e6} + M_B} - k_{d7} M_B \quad (4.7)$$

$$\frac{B_c}{dt} = k_5 M_B - v_8 \frac{B_c}{k_{e8} + B_c} - k_{e8} B_c \quad (4.8)$$

$$\begin{aligned} \frac{B_N}{dt} = & k_6 B_c - v_9 \frac{B_N}{k_{e9} + B_N} + k_{p1} P B_1 - k_{p2} P_{1n} B_N + \\ & k_{p3} P B_2 - k_{p4} P_{2n} B_N - k_{d9} B_N \end{aligned} \quad (4.9)$$

$$\frac{M_R}{dt} = v_{s5} \frac{B_N^s}{k_{a4}^s + B_N^s} - v_{10} \frac{M_R}{k_{e10} + M_R} - k_{d10} M_R \quad (4.10)$$

$$\frac{R}{dt} = k_7 M_R - v_{11} \frac{R}{k_{e11} + R} - k_{d11} R \quad (4.11)$$

$$\frac{P B_1}{dt} = -k_{p1} P B_1 + k_{p2} P_{1n} B_N - k_{d12} P B_1 \quad (4.12)$$

$$\frac{P B_2}{dt} = -k_{p3} P B_2 + k_{p4} P_{2n} B_N - k_{d13} P B_2 \quad (4.13)$$

In the present work, CT is the circadian time with CT0 taken as the start of the subjective day under DD condition. ZT is the zeitgeber time with ZT0 taken as the start of the light phase under LD conditions. According to the convention proposed by Daan and Merrow [35], *InT* is the internal time given as $[CT-18] \bmod 24$ and *ExT* is the external time given as $[ZT + \text{half of the duration of the dark phase}] \bmod 24$.

4.2.2 Parameter estimation via Genetic algorithm(GA)

Parameter estimation for our model is performed using the genetic algorithm (GA) in MATLAB which repeatedly modifies the population of an individual solution [157]. At each step, genetic algorithm randomly selects 2 of the parent parameter sets and they are randomly crossed and mutated to produce a new parameter set. Each parameter set was assigned a cost function based on certain criteria that are described below. Over successive generations, the parameter set evolves toward the best solution (Low value of cost function).

Cost function

For selecting the best solution with minimum error, the algorithm evaluates a cost function (fitness function) which determines the magnitude of the prediction error. In this work, the cost function $C_i(x(t, p))$ is defined by the squares of the error between the outputs from the model and the experimental data. Using this cost function we reproduced the experimentally observed circadian oscillations. Constraints for the parameter estimation in the order of their relevance were:

- (1) Cost function $C_1(x(t, p))$ for free running period of oscillation close to 23.7h

$$C_1(x(t, p)) = (23.7 - \tau_{DD})^2 \quad (4.14)$$

where x is the solution of the differential equations, p is the parameter vector, and τ_{DD} is the free running period of the model.

- (2) Cost function $C_2(x(t, p))$ for exact peaking time of the molecular components of the circadian clock.

$$C_2(x(t, p)) = \sum_{i=1}^n \left(\frac{T_{pi}}{24} - \frac{t_{pi}}{\tau_{DD}} \right)^2 \quad (4.15)$$

Where t_{pi} is the peaking time of the variable x_i and T_{pi} is the experimentally observed peaking time range of available molecular components.

- (3) Cost function $C_3(x(t, p))$ for the exact phase relationship between the molecular components of the circadian clock.

$$C_3(x(t, p)) = \sum_{ij} \left(\frac{T_{ij}}{24} - \frac{t_{ij}}{\tau_{DD}} \right)^2 \quad (4.16)$$

Where t_{ij} is the phase difference between the variable x_i and x_j , T_{ij} is the experimentally observed phase difference range of available molecular components.

(4) Cost function $C_4(x(t, p))$ for decent trough/peak ratios of the molecular components.

$$C_4(x(t, p)) = \sum_i \left(z_i - \frac{\min(x_i)}{\max(x_i)} \right)^2 \quad (4.17)$$

Where z_i is the experimentally observed trough/peak ratio range of available molecular components.

Thus, the final cumulative cost function is $C(x(t, p)) = C_1 + C_2 + C_3 + C_4$.

4.3 Results

4.3.1 Peaking times and phase relations of mRNA *per*'s, *Bmal1*, and *Rev-erbα* under DD conditions, and the effects of light on late and early subjective night: Support of hypothesis-1 (H1)

Numerical simulations of the nonlinear model equations (Eqs 4.1- 4.13) with estimated parameters are shown in Figs 4.2 A-F. The period of free-running oscillations is 23.75 h, which is typical of mammalian circadian oscillator under constant darkness (DD) [41]. We take the light parameter $L = 0$ in the model to be the DD condition. In Table 4.1, we provide the simulated and experimental peaking times of all the clock components and they are in the expected range. To compare the phase relationships among the individual clock components, peaking time of *per1* mRNA at CT6 is taken as the reference under DD conditions. The mRNAs *per1* and *per2* show a reasonable phase difference in their peaking time as seen in the experiments [13]. PER1 and PER2 proteins also maintain a right phase delay from their respective *per1* and *per2* mRNA's and their peaking time are also in good agreement with the reported experimental results [158]. In summary, our model fits the data well by maintaining the right temporal phase relationships among different clock components.

We also simulate the effects of light at CT14 (early subjective night) and at CT22 (late night). Albrecht et al. [159] applied light pulse at CT14 and CT22, and it induced phase delay and advance respectively. Similarly, in simulations, the light pulse applied at early subjective night (CT14) induces phase delay while at late subjective night (CT22) induces phase advance (Fig 4.2 G). The model simulation captures the temporal dynamics and show proper phase differences between *per1/2* mRNA's and their proteins PER1/2. Further, the model captures the effect of light on phase acceleration and deceleration of *per* genes at different circadian time. This points to the fact that *per1* may be the candidate gene for M oscillator and *per2* for the E oscillator.

To consider further effects of light, we construct codimension-1 bifurcation diagram with light L as the bifurcation parameter (Fig 4.3A). For a very high value of L , the system remains in a stable steady state. As L decreases, the stable steady state becomes unstable through supercritical Hopf bifurcation, where the unstable steady state is surrounded by the stable limit cycle. Further decrease in L results in stable oscillations. However, the range of L for which the oscillations occur is very small. Unlike in many well-known models, we do not consider in our model the effects of light regulation through the parameters that affect the transcriptional production rate of *per1*, but as a stand-alone individual parameter that affects directly the rates of *per1* and *per2*. With L as the bifurcation parameter, we obtain an inverted supercritical Hopf bifurcation (Fig 4.3A). We take $L = 0$ as the DD condition that oscillates with a period of 23.75h and is taken as the reference period. For high L values, as observed in many circadian experiments [41, 160], the circadian oscillations are arrhythmic and this is observed in our simulations. For the range of L values that we obtain oscillations, the period of the oscillator varies marginally from 23.75 to 23.8h (Fig 4.3 B).

Table 4.1 Comparison of different peaking time of different clock variables in CT.

Rhythmic output	Model	Experiment	Reference
<i>per1</i> mRNA	CT 6	CT 4-6	[7, 34]
<i>per2</i> mRNA	CT 9.3	CT 6-12	[7-34]
<i>Rev - ErbA</i> mRNA	CT 6.5	CT 2-6	[35, 36, 37]
<i>Bmal1</i> mRNA	CT 19.4	CT 15-21	[7,34]
PER1 protein	CT8	CT 9-14	[7, 34]
PER2 protein	CT 12.3	CT 10-14	[7,34]

We also study the effect of positive feedback between PER2 and *Bmal1* with parameter v_{s4} that contributes to the strength of feedback loop. We determine the bifurcation range and period variations with L as the bifurcation parameter for different v_{s4} values (Fig 4.4A). The

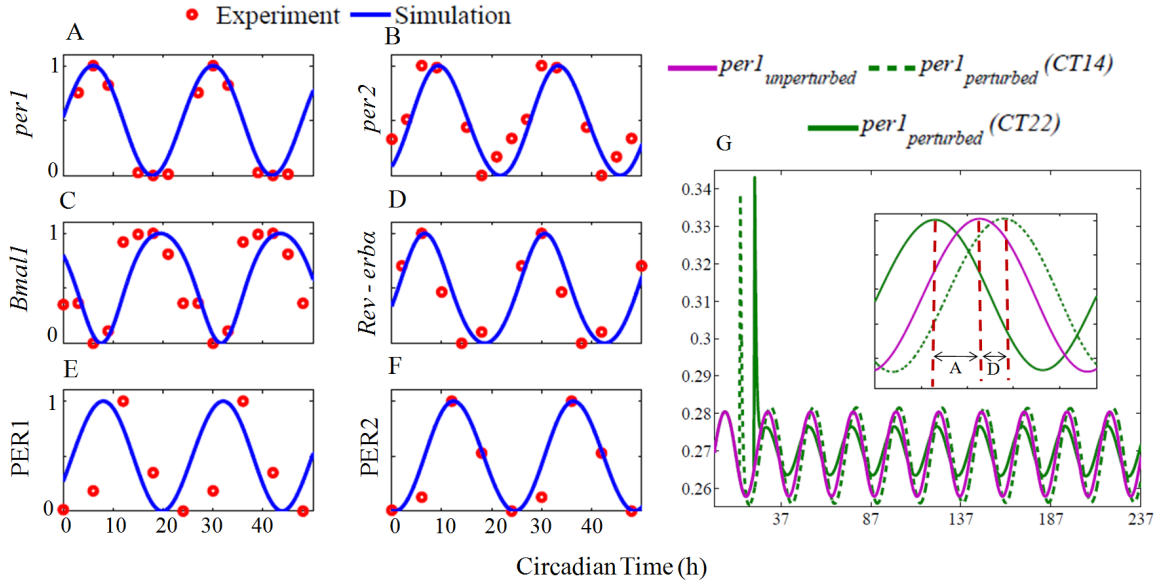


Fig. 4.2 Free-running circadian oscillations under constant darkness and the effect of light pulse at certain circadian time. Blue curves are from simulation, and red circles are the experimental data points. Simulation results were obtained by integrating the model equations (Eqs 4.1- 4.13) with the estimated parameters, which are given in the Table A.1 (parameters used for DD, LD). For comparison, the individual time series were normalized to maximum 1 and the minimum 0. Experimental data points of *per1*, *per2*, and *Bmal1* mRNA were extracted from [13], *Rev – erba* from [161] and *PER1*, *PER2* protein from [158]. (G) *per* expression in response to light. A 30 min light pulse with amplitude of 0.2 is applied at CT14 (early night), It shows a phase delay (green broken line). The same light pulse applied at CT22 (late night), induce phase advance (green solid line), and agrees with the experimental results [159]. Inset shows perturbed time series came back into the original limit cycle with a delay ('D') or with an advance ('A') with respect to unperturbed time series.

increase in parameter v_{s4} increases the Hopf bifurcation regime and thereby also increases the period range with the increase of the light parameter in wild-type (Fig 4.4B).

We also simulate the sensitivity of period to the variations of all the parameters and found that v_{s4} is highly sensitive as observed in simulations (Fig 4.5). We calculated the period sensitivity as the phase accumulated around steady state orbit after one cycle of revolution [164] and its given as:

$$\frac{\partial \tau}{\partial p_j} = \frac{\partial \theta(t, \tau)}{\partial p_j} - \frac{\partial \theta(t)}{\partial p_j} \quad (4.18)$$

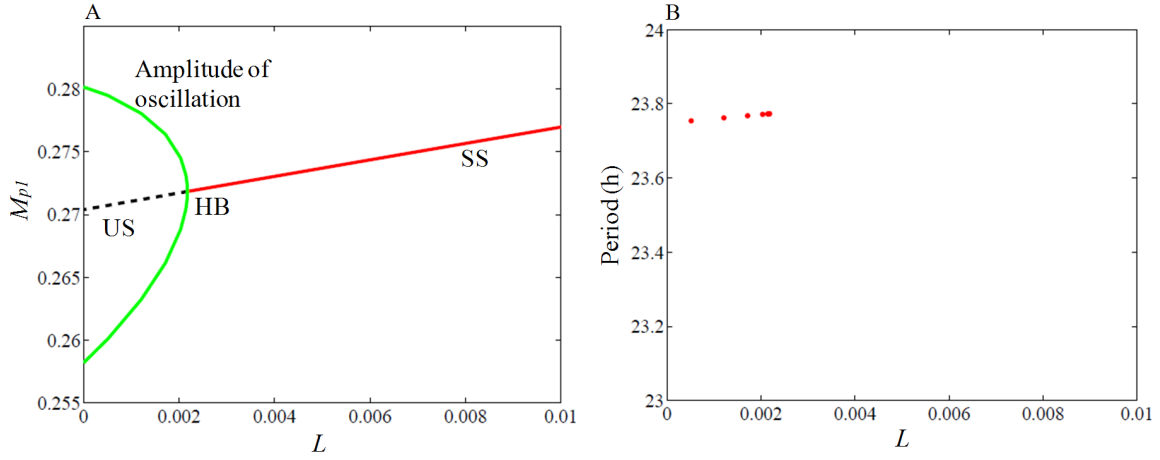


Fig. 4.3 Bifurcation analysis with light L as the parameter. (A) Oscillation amplitude is shown in green with $L = 0$ represents the DD conditions for the variable M_{p1} (*per1* mRNA). Black broken lines are unstable steady states. As light intensity increases, sustained oscillation disappeared via supercritical Hopf bifurcation (HB) and the system enters the stable steady state (red lines). (B) Period variation as a function of light intensity. Period increases very modestly with the increase in light intensity. We used Xppaut [119] for simulating bifurcation diagrams. Simulations are obtained by integrating the model equations (Eqs 4.1- 4.13) with the estimated parameters, which are given in the Table A.1 (parameters used for DD, LD simulation, WT).

t should be sufficiently large to avoid the transient effect. Where τ is the period of the oscillation, p_j is the parameter, $\frac{\partial \theta(t)}{\partial p_j}$ is the phase response with respect to variation in parameter at time t , and $\frac{\partial \theta(t, \tau)}{\partial p_j}$ is the phase response with respect to variation in parameter after one cycle. The period sensitivity analysis clearly indicates parameters associated with *per2* loop are more sensitive (Fig 4.5) and this is also seen in various mammalian circadian models [59, 165].

4.3.2 Dynamics of *per1* and *per2* mutants and *per1-per2* double mutants (Hypothesis -H2)

In experiments, under DD conditions, the period of *per1* mutant mice is shorter than the wild type [158, 160, 162], and in simulation we fit the experimental data [162] to capture this effect (Figs 4.6 A-C). We find that the *per1* mutant for which *per2* is functional, exhibits shorter period. In the case of *per2* mutant, the dynamics of two different mutant phenotypes, namely *per2^{Brdm1}* and *per2^{ldc}* are available in the literature and their dynamics are different. Under DD conditions, the oscillations are absent in *per2^{Brdm1}* mutant [18, 158], whereas

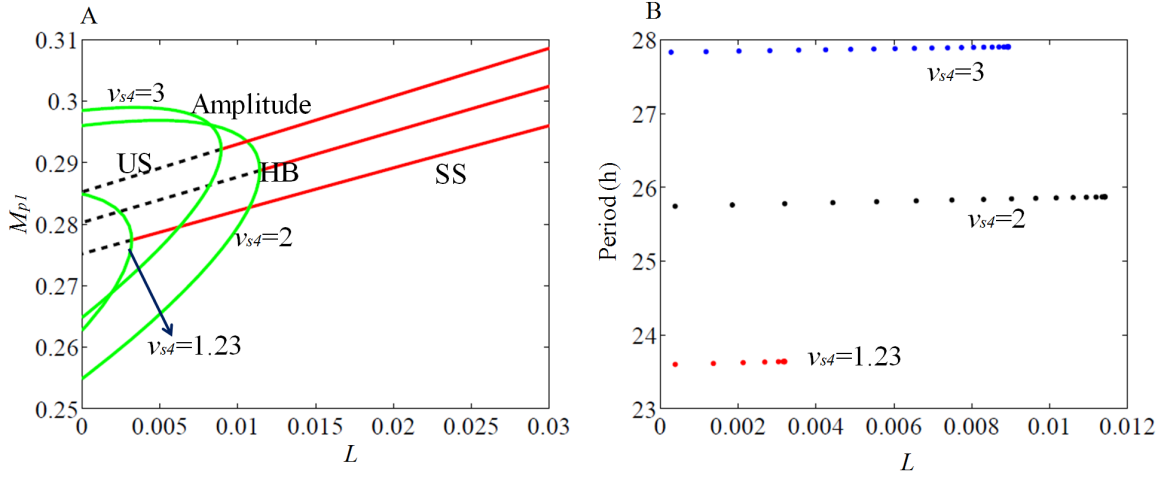


Fig. 4.4 Bifurcation analysis of the effect of *Bmal1*- PER2 positive feedback loop. (A) Hopf bifurcation obtained for the range of v_{s4} that modulates the positive feedback loop. (B) The period of oscillation is shown to increase with the increase in v_{s4} . *per2* mutant mice showed period decrement in LL condition [160], here we observed that the positive regulation of *Bmal1* mRNA by PER2 protein enhance the period of oscillation of the system under LL condition. Simulations are obtained by integrating the model equations (Eqs 4.1- 4.13) with the estimated parameters, which are given in the Table A.1 (parameters used for DD, LD simulation, WT).

in the *per2^{ldc}* mutant, rhythmic oscillations are observed [158, 162, 147]. We simulate the behavior of both *per2^{ldc}* mutants (Figs 4.6) and *per2^{Brdm1}* (Fig 4.7), but to account for hypothesis H2, we consider only *per2^{ldc}* mutant that exhibits oscillations. The period of the *per2^{ldc}* mutant in the simulation is 21.75 h and is shorter than the wild type (23.75 h), and this agrees well with the experiments [158, 162]. We also simulate *per1* – *per2* double mutant by silencing the transcription rates of *per1* and *per2* mRNA ($v_{s1} = 0$, $v_{s2} = 0$). In experiments, *per1* – *per2* mutant mice were found to be arrhythmic [162] and this behavior is accurately captured by our model (4.7 D). According to hypothesis H2, this indicates that both morning and evening oscillators are dysfunctional. Besides *per1/2* knockouts, our model also correctly captures the arrhythmicity in *Bmal1* mutation (4.7 B, C)) as seen in the experiments in [163]. In Table 4.2, we provide the summary of period changes seen in both simulations and experiments for wild type (WT) and the mutants. We also provide in Table 4.3, the peaking time of different molecular components in the mutants seen in both simulations and experiments.

In summary, our model accounts for most of the knockout phenotypes by capturing the rhythmic and the arrhythmic behaviors. The shorter period in both *per1* and *per2* mutants under DD conditions and arrhythmicity in *per1* – *per2* double mutant indicates that the

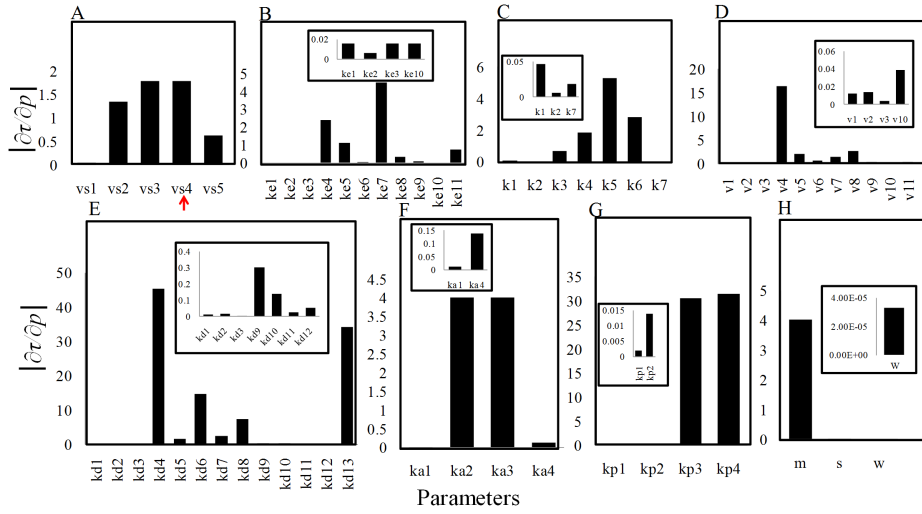


Fig. 4.5 Period sensitivity of the WT-DD-model parameters. Period sensitivity of transcription rate (A), Michaelis constant (B), protein synthesis (C), enzymatic degradation (D), exponential degradation (E), activation constant (F), complex formation and dissociation (G), and Hill's coefficient (H). While considering transcription rate, *Bmal1* loop shows higher sensitivity (v_{s3}, v_{s4}), and *per2* loop slightly lesser (v_{s2}). For all the other constants, the parameters of the *per2* loop showed higher sensitivity ($v_4, k_5, k_{d4}, k_{p4}, k_{p3}, k_{a2}, m$). Red arrows indicate the parameters related to the positive feedback loop between *Bmal1* and PER2 (v_{s4}), where the period is shown to be highly sensitive to changes in the parameter.

Table 4.2 Period of molecular phenotype of mutants in mammalian SCN

Mutants	Experimental period	Simulated period from model (h)	Model parameters changed from wild type	References
<i>per1</i>	21.6 -23.8	23.44	$v_{s1} = 0nMh^{-1}, v_4 = 0.43nMh^{-1}, k_{p4} = 0.19nM^{-1}h^{-1}$	[35]
<i>per2^{ldc}/per2^{Brdm1}</i>	21.7-22.5/Arrhythmic	21.75/Arrhythmic	$v_{s1} = 0.7nMh^{-1}, v_{s2} = 0nMh^{-1}, v_{s5} = 0.5nMh^{-1}, v_1 = 0.44nMh^{-1}, v_2 = 1.38nMh^{-1}, v_3 = 1.67nMh^{-1}, k_{d3} = 0.08h^{-1}, k_1 = 1.44h^{-1}, k_{p1} = 0.11nM^{-1}h^{-1}, k_{p2} = 0.18nM^{-1}h^{-1}$ (parameters for <i>per2^{ldc}</i> rhythmic) $v_{s2} = 0nMh^{-1}$ (for <i>per2^{Brdm1}</i> arrhythmic)	[12,34, 40]
<i>per1 - per2</i> double mutant	Arrhythmic	Arrhythmic	$v_{s1} = 0nMh^{-1}, v_{s2} = 0nMh^{-1}$	[34]
<i>Bmal1</i>	Arrhythmic	Arrhythmic	$v_{s3} = 0nMh^{-1}, v_{s4} = 0nMh^{-1}$	[42]
<i>Rev - erba</i>	Rhythmic	Rhythmic	$v_{s5} = 0nMh^{-1}$	[10]

model simulations support hypothesis H2. This also indicates *per1* and *per2* as the plausible candidates for M and E oscillator respectively.

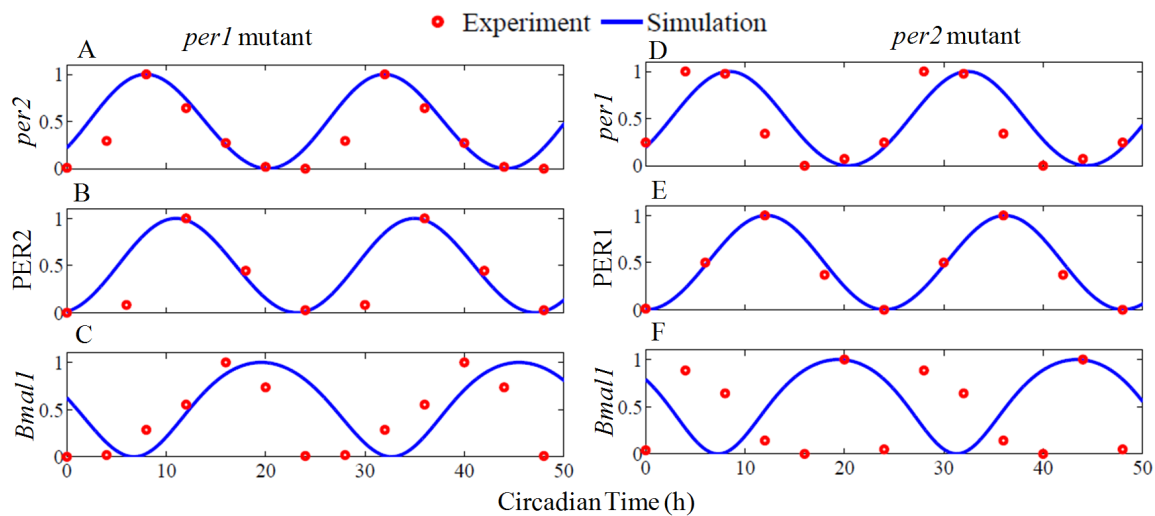


Fig. 4.6 Simulation of the rhythmic behavior of *per1* and *per2* mutant mice. (A-C) Simulations of *per1* mutant. Sustained oscillation of *per2* mRNA, PER2 protein, and *Bmal1* mRNA are shown in blue lines. The simulated results are in good agreement with the experimental data (red circle) that are observed for *per1^{ldc}* mutant mice [158]. (D-F) Simulation for *per2* mutant. Similar to *per1* mutant, sustained oscillations of *per1* mRNA, PER1 protein, and *Bmal1* mRNA are observed in *per2* mutant and are in good agreement with the experimental results that observed in *per2^{ldc}* mutant mice [158]. For comparison, each time series were normalized between 0 and 1. For *per1* mutant, parameter values adjusted are $v_{s1} = 0nMh^{-1}$, $v_4 = 0.43nMh^{-1}$, and $k_{p4} = 0.19nMh^{-1}$. For *per2* mutant, parameter values varied are $v_{s1} = 0.7nMh^{-1}$, $v_{s2} = 0nMh^{-1}$, $v_{s3} = 4.5nMh^{-1}$, $v_{s5} = 0.5nMh^{-1}$, $v_1 = 0.44nMh^{-1}$, $v_2 = 1.38nMh^{-1}$, $v_3 = 1.67nMh^{-1}$, $k_1 = 1.44h^{-1}$, $k_{d3} = 0.08h^{-1}$, $k_{p1} = 0.11nM^{-1}h^{-1}$, and $k_{p2} = 0.18nM^{-1}h^{-1}$. Remaining parameters are same as that of in Fig 4.2. *per2* mRNA that peaks at CT8 is taken as the reference point in the case of *per1* mutant, and PER1 protein that peaks at CT12 is taken as the reference point for *per2* mutant.

Table 4.3 Peaking time of different oscillatory components in mutants given in CT.

Mutants	Peaking time of <i>per1</i>		Peaking time of <i>per2</i>		Peaking time of <i>Bmal1</i>		Peaking time of PER1		Peaking time of PER2		Ref
	Exp	Model	Exp	model	Exp	model	Exp	model	Exp	model	
<i>per1</i>	NA	NA	8	8	16	19.33	NA	NA	12	11	[34]
<i>per2</i>	5-8	8.4	NA	NA	20	19.4	12	12	NA	NA	[34]

EXP = Experiments, Refs = References, NA = Not applicable

4.3.3 Phase response curves of wild and mutant types (Hypothesis-H3)

The circadian pacemaker in SCN responds differentially to light pulse at different phases of one circadian cycle. Application of light pulse during subjective day results mostly in dead zones, during early and middle of subjective night results in phase delay and during the late subjective night till the start of subjective day results in phase advance [147]. This selective bidirectional response of circadian pacemaker to light is mediated by multiple

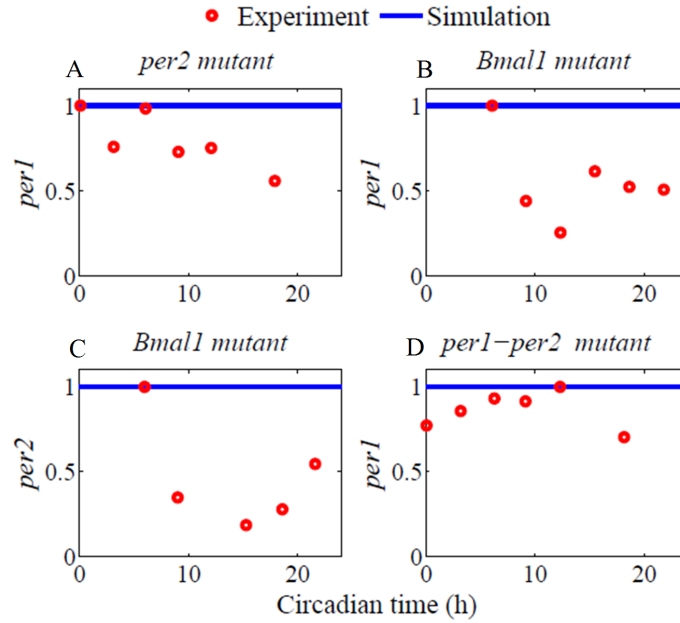


Fig. 4.7 Validation of the model against the arrhythmic behavior of the mutants. (A) Blue lines are the simulated *per1* mRNA for the *per2* arrhythmic mutant model ($v_{s2} = 0 \text{ nMh}^{-1}$). Red circles are the experimental data of *per1* mRNA in *per2*^{Brdm} mice [162]. (B) Blue lines are simulated *per1* mRNA for the *Bmal1* mutant model ($v_{s3} = 0, v_{s4} = 0$). Red circles are the experimental data of *per1* mRNA in the *Bmal1* mutant mice [163]. (C) Blue lines are simulated *per2* mRNA for the *Bmal1* mutant model. Red circles are the experimental data of *per2* mRNA in *Bmal1* mutant mice [162]. (D) Blue lines are the simulated *per1* mRNA for the *per1* – *per2* double mutant model ($v_{s1} = 0 \text{ nMh}^{-1}, v_{s2} = 0 \text{ nMh}^{-1}$). Red circles are the experimental data of *per1* mRNA in the *per1* – *per2* double mutant mice [162]. For mutants, their transcription rate constants are made zero. Time series are normalized in such a way that maximum value is 1. Simulation results were obtained by integrating the model Eqs 4.1-4.13. Parameters values, except transcription rate constants are same as in Fig 4.2

signaling pathways and it signifies that PRC depends on the time-of-day input and gated by different signaling pathways. Different scenarios on the effect of light on circadian rhythms are proposed: (i) Pittendrigh and Daan (1976) [41] postulated that the PRC of E oscillator has only phase delays while that of M oscillator exhibits only phase advances. (ii) On the other hand, Daan et al. [43] hypothesized that the PRC can be bidirectional in response to light and large phase delays will occur in the E-only oscillator, while large phase advance will occur in the M-only oscillator. These predictions are based on the observations made by Albrecht et al. [159] that in the *per1* mutant, at ZT22 light pulse suppressed phase advance, whereas in the *per2* mutant at ZT14, light pulse eliminated phase delay. (iii) Daan et al. [43] also proposed that under light pulse perturbation, phase advances of both *per1* and *per2* are suppressed during subjective day, but not during the early subjective day (see Fig 3A

in [43]). Strong phase delays are possible only during the subjective night. Therefore, in this scenario, in comparison to WT, phase delays in *per1* and *per2* mutants are much larger and smaller respectively and this also encapsulates both scenarios (i) and (ii). We expect our model simulation to capture PRC of scenario (iii).

To simulate scenario (iii), we construct three PRC's, one for wild type, and the other two for *per1* and *per2* mutants separately. We apply light pulse for duration of 30 min at different phases of one circadian cycle. In the case of *per2* mutant, we specifically consider *per2^{ldc}* mutant that oscillates under DD conditions and not the arrhythmic *per2^{Brdm1}* mutant. We also project the PRC simulation curves on to the PRC of the experimental data constructed from the free-running activity of mice under normal and mutant conditions [147]. The experimental PRC curves of WT and mutants exhibits phase delays during the late subjective night, advances during the early subjective day. A large phase delay is seen between CT 15-19 (Fig 4.8 E-G). The only fact that distinguishes PRC of mutants from wild type is that in comparison to WT, phase delays are lesser in the *per2* mutant, while in the *per1* mutant, phase delays are larger. This is also expected in Daan's hypothesis (Fig 3A in [43]).

Our model simulates bidirectional PRC as seen in the experiments, but WT and *per2* mutant PRC show large phase delay than in the experiments. The maximum delay in the *per1* mutant simulation is phase shifted by 2h from the experiments. To ameliorate this problem, we introduce two gating variables, one each for the light activation of *per1* and *per2*. The idea of gating variables was previously used in the mammalian circadian models based on molecular mechanisms of gene expression [82, 166], and taking cue from there, we also similarly framed the gating expressions that act via the light variable *L* in *per1* and *per2* equations. The experimental results [167] show that photic inputs are phase gated that modifies the light responses at different phases of the oscillation. In our model, light *L* induces *per1* and *per2*, and therefore we modulate this induction through the gating variables. In order to match with experimental data, we scaled down the light parameter *L* for *per1* induction, and for *per2*, we made light parameter reach high during the early subjective night and low during both late subjective night and day. The mathematical expressions for gating variables are given below.

$$G_{per1}(t) = \frac{L_{max}}{2} \quad (4.19)$$

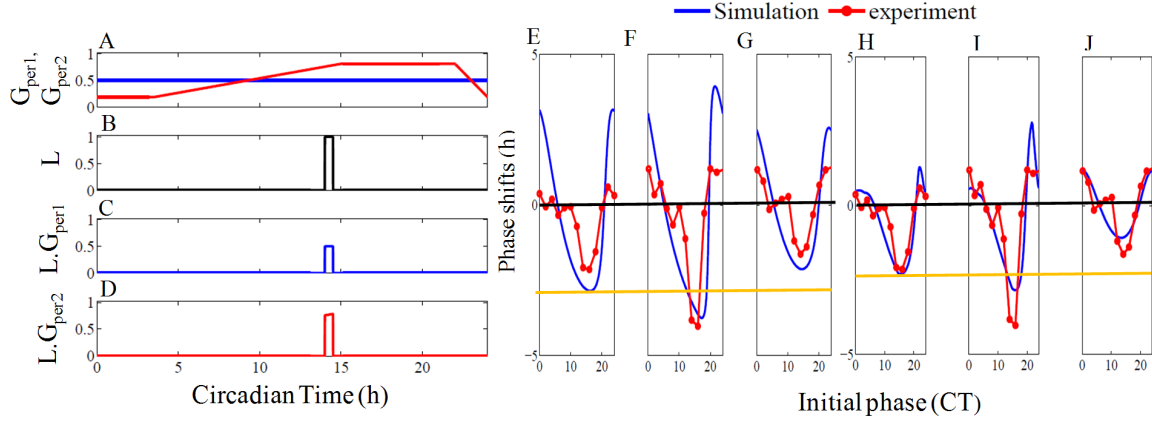


Fig. 4.8 Gating variables, phase response curves of wild type, *per1*, and *per2* mutants with and without a gating variable. In the left of the figure is shown the gating variable changes the light intensity at different phases. (A) Constant and varying gating variables are used for *per1* (blue) and *per2* (red) respectively. Gating variable for *per1* is constant throughout the whole circadian period, whereas gating variable for *per2* is changed with circadian time, and reaches the maximum value between CT15 and CT20. It is at this maximum value of gating variable, a maximum delay in PRC is observed experimentally [61]. (B) An example of single light pulse with unit amplitude applied at CT14 for 30 min duration is shown. (C) The light pulse for *per1* and *per2* after multiplying with the gating variable. Light pulse for *per1* is scale down to half and the light pulse for *per2* have different values at CT14. Simulated phase response curves without the gating variable for (E) wildtype (F) *per1* mutant and (G) for *per2* mutant. Experimental data points were extracted from [147] and they are shown in red circles and a continuous line was drawn for readability. The blue lines are simulated PRC curves. To simulate PRC, light pulse L in the model was applied for duration of 30 mins with an amplitude value 0.35, and phase difference is measured after 10 cycles. For WT, *per1* mRNA (M_{p1}) peaking at CT6 is taken as the reference point. *per2* mRNA peaks at CT 8 is taken as the reference point when simulating *per1* mutant, and PER1 protein (P_{1c}) that peaks at CT 12 is taken as the reference point when simulating *per2* mutant. For clarity, two horizontal lines are drawn; one to show the zero phases (in black) and the other magenta line in the bottom indicates the maximum phase delay of wild type. In all the experimental PRC, CT15 is the phase at which maximum delay occurs. (F) *per1* mutant PRC for which the maximum phase shift occurs at CT 15 and it is much higher than the wild type. (G) *per2* mutant PRC for which, the maximum phase shift occurs also at CT15, but it is lower than the wild type. Simulated PRC with the gating variable for (H) wild- type (I) *per1* mutant and (J) for *per2* mutant. Compared to the PRC without a gating variable, *per2* mutant shows suppressed phase delay. Parameters for WT are as in (Fig 4.2), and parameters for *per1* mutants and *per2* mutant are as in Fig 4.6.

$$G_{per2}(t) = \begin{cases} y_1, & \text{mod}(t, \tau) \leq t_1 \\ L_{max}(\frac{1.3\text{mod}(t, \tau)}{\tau}), & t_1 > \text{mod}(t, \tau) \leq t_2 \\ y_2, & t_2 > \text{mod}(t, \tau) \leq t_3 \\ (y_1 - y_2)(\frac{\text{mod}(t, \tau) - t_3}{t_4 - t_3}) + y_2, & t_3 > \text{mod}(t, \tau) \leq t_4 \\ y_1, & \text{otherwise} \end{cases} \quad (4.20)$$

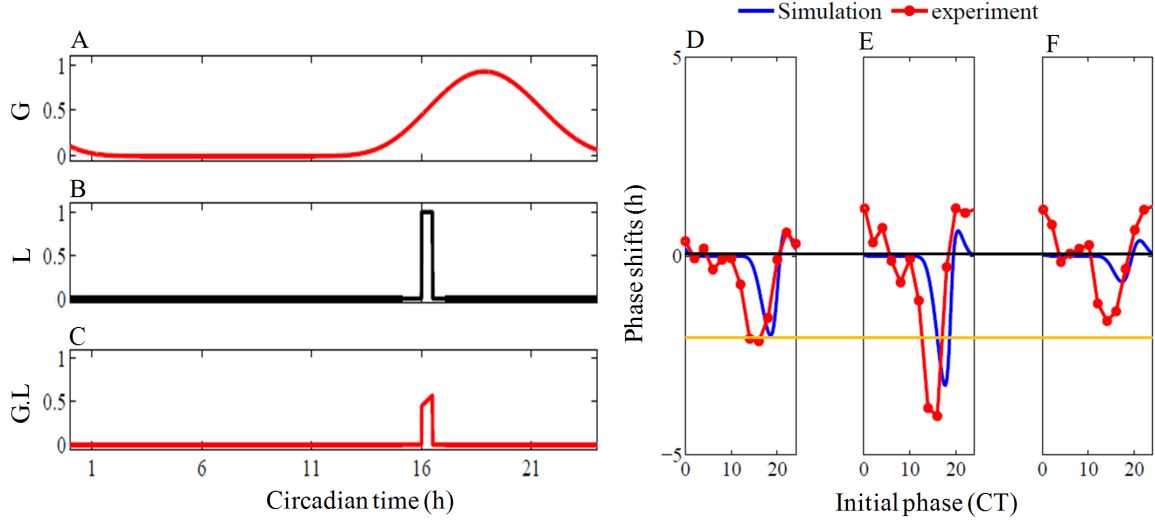


Fig. 4.9 (A-C) gating variable common to both *per1* and *per2*. Light input is regulated by a suitable clock variable, *Bmal1* mRNA (M_B). Simulated phase response curves with the gating for (D) wild- type (E) *per1* mutant and (F) for *per2* mutant. A dead zone is observed between CT3 to CT10 for WT, CT0 to CT10 for *per1* mutant and CT2 to CT10 for *per2* mutant. To simulate the PRC, light pulse L in the model was applied for a duration of 30 min with an amplitude value 0.2, and phase difference is measured after 10 cycle. The reference points for different phenotypes are similar to that of the previous PRC in the main text. Experimental data points extracted from [61] are shown in red circles and a continuous line was drawn for readability. The blue lines are simulated PRC curves from the model.

where $L_{max} = 1$, $t_1 = \frac{3.5\tau}{24}$, $t_3 = \frac{22\tau}{24}$, $t_4 = \tau$, $y_1 = L_{max}(\frac{1.3t_1}{\tau})$, $y_2 = L_{max}(\frac{1.3t_2}{\tau})$

In DD conditions, τ is the free-running period and in LD conditions it takes the entraining period of 24h. The temporal dynamics of gating variables are shown in (Fig 4.8). The gated PRC's are in good agreement with the experimental PRC's, but again the dead zones are not observed during subjective day (Fig 4.8 H, J). We modified again the gating variables and could simulate the dead zones during the subjective day besides the phase advances during the late subjective night and strong phase delays during subjective night. To capture the dead zone in our model PRC, we introduced a second gating variable as described below.

$$G = (M_{B_{normalized}})^{10} M_{B_{max}} \quad (4.21)$$

Where, $M_{B_{normalized}} = \frac{M_B - M_{B_{min}}}{M_{B_{max}} - M_{B_{min}}}$, and $M_{B_{max}}$ is the maximum value of M_B and $M_{B_{min}}$ is the minimum value of M_B . Simulated PRC with the new gating variables are shown in the Fig 4.9.

Table 4.4 Summary of PRC.

Mutant	Hypothesis [15]	Experiments [41]	Simulation
<i>per1</i>	Suppressed advance and enhanced delay	Delay is enhanced but advance is not suppressed	Delay is enhanced but advance is not suppressed
<i>per2</i>	Suppressed delay and enhanced advance	Suppressed delay and enhanced advance	Delay is suppressed but advance is not enhanced

These results are in agreement with Daan et al. [43] as well as Pittendrigh and Daan's [41] predictions, where the dead zones, phase advances, and delays occurred at the appropriate circadian time for all the cases and we could capture the differential time-of-day effects for light pulse perturbations in the simulation. The hypothesis regarding PRC, experimental and simulated PRC results are summarized in Table 4.4, which strongly suggests *per1* and *per2* acts like M and E oscillator respectively.

4.3.4 Entrainment of wild type, *per1* and *per2* mutants to different photoperiods (Hypothesis -H4)

Entrainment of the endogenous circadian pacemaker to different external LD cycles (photoperiods) provides information about the encoding of day length. Specifically, external LD cycles of different ratios capture long or short days and endogenous circadian network should perceive and map this external cues. Therefore, circadian pacemakers should adapt and be plastic to entrain and encode variations in LD cycles. To evaluate the performance of our model, we consider different photoperiods for which we expect *per1* tracks only dawn by peaking during or close to the light phase and *per2* tracks dusk by peaking during the transition from light to dark phase.

To simulate different LD cycles, we modulate the light parameter L in the model as a square-wave function that goes from $L = 0$ during the dark phase to $L = 0.1$ during the light phase. Experimental results indicate that in wild-type, *per1* and *per2* mRNA levels in mammals rise up during the light phase and falls down during the dark phase [79, 169, 170, 168]. However, the peaking time of *per1* and *per2* mRNA differs significantly under different photoperiods and their phase differences increase with increase in the photo periods [79, 169]. The simulation results of both wild and mutant types are shown in Figs 4.10- 4.13 for different

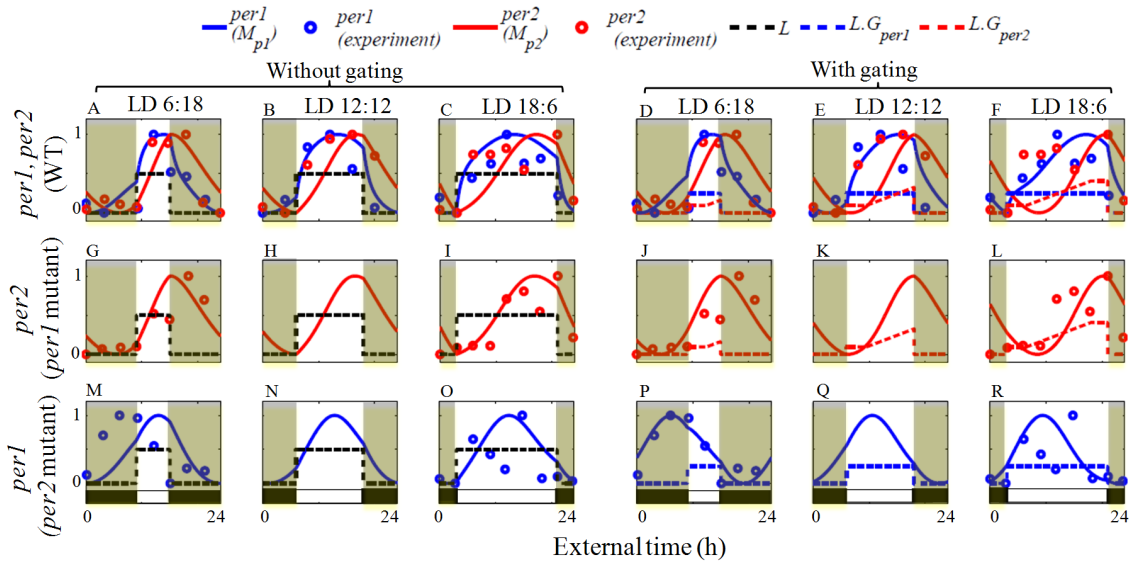


Fig. 4.10 Wild type and mutant entrainment to different LD cycles with and without the gating variable. (A-C) The model entrains to various external LD cycle without the gating variable, and (D-F) with gating variable (equations 4.19, 4.20) for WT. Blue and red lines are the simulated *per1* and *per2* mRNA, whereas blue and red circles are the experimental *per1*, *per2* mRNA respectively. Since an external cue forcing the oscillation, we consider the x axis as external time rather than circadian time. External time is defined as the middle of the light phase which in the present case is time 12. Model simulations of wild type show a maximal *per1* expression in the light phase whereas *per2* gene peaks close to dusk (off set of the light phase) during the entrainment. In (G-L) the *per1* mutant that expresses only *per2*, peaks following the dusk, and in (M-R), *per2* mutant, *per1* peak follow the dawn (on set of light phase). Under long photoperiods, *per1* mutant with gating variable displays more delay in *per2* peaking (L) than in without gating variable (I). Compared with WT, In *per2* mutant, *per1* peaks advanced both in without gating variable (M-O) and with gating variable (P-R), the magnitude of advance is more in with gating variable. Time series are normalized so that maximum value is 1 and minimum value is 0. L values changed in a square wave manner, during the light phase the value of L is 0.1 and in dark phase the value of L is 0. Experimental data points for LD 12:12 were extracted from [168], and all other data points were extracted from [79]. The black bar in (M-R) is the dark phase, while the unfilled white bar is the light phase. This bar is also common for the entire figure and for clarity it is shown only in the last row. Parameters for WT are as in (Fig 4.2), and parameters for *per1* mutant and *per2* mutant are as in (Fig 4.6).

photoperiods. In wild-type simulations (Fig 4.10A-C), the rising portion of both *per1* and *per2* mRNA occurs during the light phase and starts to decrease during the dark phase as seen in the experiments [82, 169, 170, 168]. Under short photoperiods, *per1* peaking occurs near the light offset (Fig 4.10), but as photoperiod increases, peaking occurs near the middle of the light phase (Fig 4.10 A and 4.10B). On the other hand, peaking time of *per2* mRNA,

as observed in the experiments [79, 169], is always close to the transition between light and dark phases (dusk) (Fig 4.10 A-C). We also observe that, when photoperiod increases, our model show broadening of the maxima of both *per1*, *per2* oscillations (Fig 4.10A-C). In WT, under different photoperiods, the phase difference between *per1* and *per2* never went beyond 4 h (Table 4.5).

To assess whether different photoperiods affects *per1* and *per2* mutants, we simulate mutants under different LD cycles. We observe that for different LD cycles, peaking time of *per2* expression in *per1* mutant show no significant variations in comparison to WT (Fig 4.10 G-I), and it peaks always at the transition between the light and dark phase. Similarly, for the *per2* mutant, the peaking always occurs during the light phase, but we see phase advance of *per1* gene expression in the *per2* mutant, compared with WT. We also observe an increase in the phase difference between *per1* and *per2* peaking time with the increase in photoperiod (Table 4.5), therefore an uncoupling of *per1* and *per2* gene expression occurred in mutants.

We also simulate the entrainment curves with gating variable that is described in the previous section (equations 4.19, 4.20). We observe that in WT with gating variable, *per1* and *per2* peaking time is delayed under long days (Fig 4.10 D-F) than without the gating variable (Fig 4.10 A-C). Peaking time of *per2* expression in the *per1* mutant (Fig 4.10 J-L) did not show much variations in comparison to WT (Fig 4.10 D-F), but the peaking time of *per1* expression in the *per2* mutant is more advanced (Fig 4.10 P-R).

In the case of proteins, phase difference between the peaking time of PER1 and PER2 in the Siberian hamsters [155] is very small under different LD conditions (Fig 4.11, red and blue circles). However, peak duration of PER protein abundance increases with increase in the photoperiod. Based on these observations, Hastings et al. [46] indicated that Daan's hypothesis of *per1/per2* may not be the right candidate for ME oscillators. However, we firmly believe that *per1/per2* mRNA's and not their proteins PER1/PER2 may be the right candidate to look into as ME oscillators, because these proteins undergo various post-translational modifications as well as involve in diffusion process from cytoplasm to nucleus for regulation. So PER proteins may not be the right choice to explain ME oscillators. Further, Hastings et al. [46] have come to this conclusion from one case of photoperiod experiments [155]. We believe that PER protein peaking time may be different for different photoperiods and to verify this result, we simulate and plot the nuclear PER1(P_{1n}) and PER2(P_{2n}) proteins time series for two different LD cycles (8:16 and 16:8) (Fig 4.11) and compare it with the experimental data points of Siberian hamsters [155]. Although the duration of PER1 peak increases with increase in photoperiods, our simulation results did not follow the experimental results. Besides LD cycle of 8:16 and 16:8, we also simulate for different

Table 4.5 Peaking time of *per1* and *per2* mRNA under different photoperiods.

	WT			<i>per1</i> mutant	<i>per2</i> mutant	
	<i>per1</i> peak-ing time (I)	<i>per2</i> peak-ing time (II)	Absolute phase difference between <i>per1</i> and <i>per2</i> peaking time II-III(h)	<i>per2</i> peaking time(III)	<i>per1</i> peaking time(IV)	Absolute phase difference between <i>per1</i> and <i>per2</i> peaking time III-IV(h)
DD	CT 6	CT 9.3	3.3	CT 8	CT 8.5	0.5
without gating						
LD 6:18	ExT 13.84	ExT 15.26	1.42	ExT 15	ExT 13	0.2
LD 12:12	ExT 13.49	ExT 16.69	3.2	ExT 16.3	ExT 12.86	3.4
LD 18:6	ExT 13.37	ExT 17.3	3.9	ExT 16.88	ExT 12.37	4.5
with gating						
LD 6:18	ExT 13.52	ExT 15.83	2.3	ExT 15	ExT 6.1	8.9
LD 12:12	ExT 15.21	ExT 18	2.79	ExT 17.79	ExT 10.51	7.2
LD 18:6	ExT 17	ExT 20.77	3.77	ExT 20.3	ExT 9.42	10.88

ExT = external time. CT = circadian time.

photoperiods and found that the phase difference between PER1 and PER2 increases with increase in the photoperiods (Fig 4.12). Our model simulation gives PER1 peaking time close to dusk and PER2 peaking close to midnight. There is a considerable phase difference in the peaking time of PER1 and PER2 in our simulation and this is different from what Hastings et al. [46] have proposed. Therefore, based on both mRNA and protein peaking time of *per*'s our model supports Daan's hypothesis.

We also simulate the entrainment curves of the *per* mRNAs for a range of photoperiod (Fig 4.13). *per1* peaking time in WT occur in the middle of the light phase under long photoperiod (Fig 4.13A), but for very short photoperiods our model fails to peak in the middle of light phase. In *per2* mutant, *per1* always follow the middle of the light phase (Fig 4.13C). In WT model, gating variable shifts the peaking time of *per1* near to light offset, however it still peaks during the day time. However, in *per2* mutant (Fig 4.13 G), *per1* peaking time follows the light onset rather than light offset. On the other hand, peaking time of *per2* is near the light offset in both WT (Fig 4.13B) and in *per1* mutant (Fig 4.13D). By using the gating variable, peaking time of *per2* is close to dusk (Fig 4.13 F and H). These simulation results clearly shows that *per1* behaves like and M oscillator, and controlled by light onset (dawn), and *per2* behaves more like an E oscillator controlled by the light offset (dusk).

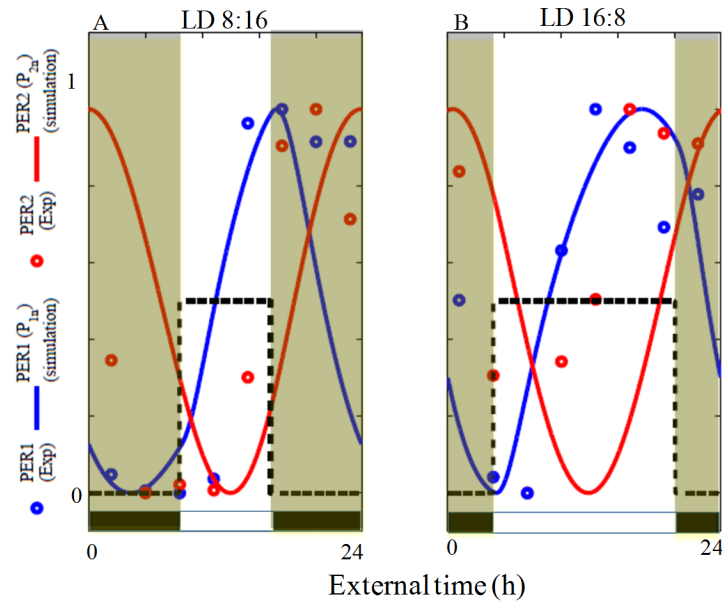


Fig. 4.11 Photoperiodic variation of the PER protein in WT. Experimental and simulated nuclear PER1 and PER2 protein under LD 8:16 (A), and LD 16:8 (B). Compared to the experimental data, phase difference between simulated PER1 and PER2 protein is higher. Experimental data points are extracted from [155]. Time series are normalized so that the maximum value is 1 and minimum value is 0. Parameters for simulation are as in Fig 4.2.

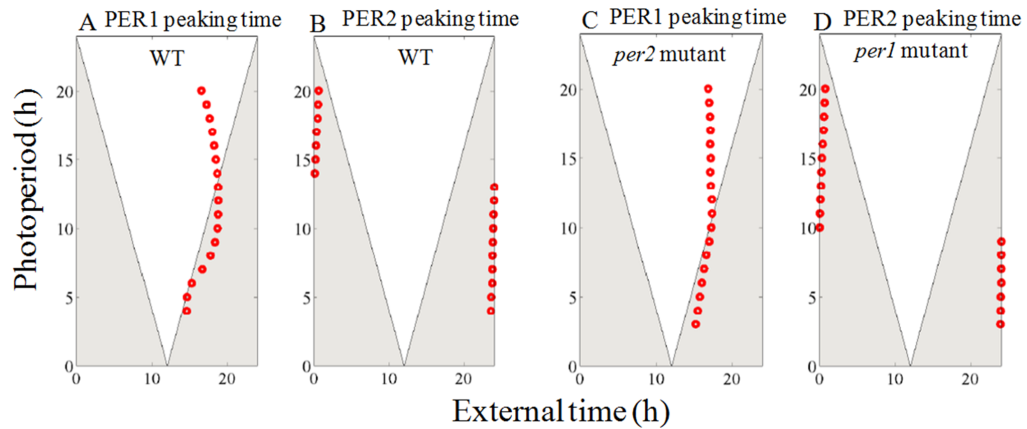


Fig. 4.12 Peaking time of PER1 and PER2 protein under different photoperiods. PER1 peaking time in WT (A) and *per2* mutant (C) is near the light offset, as photo period increases it moves towards light phase. PER2 peaks near the midnight in both WT (B) and *per1* mutant (D). The phase difference between PER1 and PER2 increases with increase in photo period.

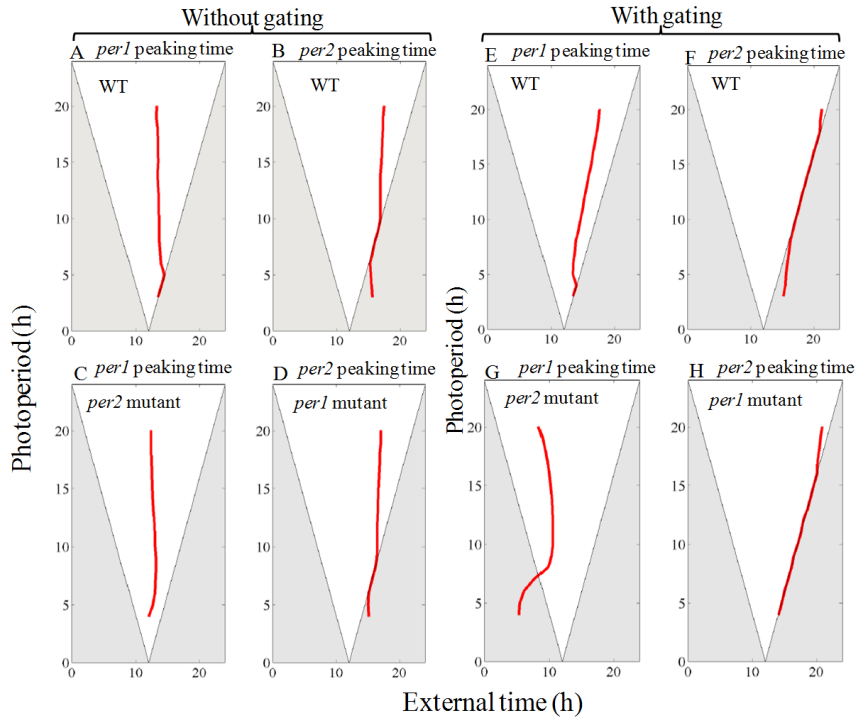


Fig. 4.13 Peaking time of *per1* and *per2* mRNA under different photoperiods. (A-D) Simulation without a gating variable. (A) In WT, under short photoperiod, *per1* peaks near the dusk, photo periods increases its peaking time, shift towards the middle of the light phase. (B) *per2* peaks near the light offset, follow the dawn. (C) In *per2* mutant, *per1* always peak near the middle of the light phase. (D) In *per1* mutant, *per2* follows dusk, as in the WT. (E-H) Simulation with gating variable (equations 4.19, 4.20). (E) In WT, *per1* peaking time is shifted from middle of the light phase. (F) *per2* peaks exactly follows the dusk. (G) In *per2* mutant, *per1* peaking time always follows the dawn or late night. (H) In *per1* mutant, *per2* peaking time follows the dusk. Taking together, it is clear that *per1* is the part of M oscillator, follows the dawn and *per2* is the part of E oscillator, follows the dusk.

4.3.5 Period length variations of wild type and mutants at constant light (Hypothesis-H5)

According to Aschoff's rule, for nocturnal animals, the period increases as a function of increasing light intensity, but the circadian activity decreases. The hypothesis is that under constant light conditions (LL) longer period is observed in the E oscillator than in the M oscillator [41, 42]. In *per1* mutant mice, where *per2* (E oscillator) is operational, it is known that the period increases with increase in light intensity [43]. In the case of *per2* mutant mice, where *per1* is operational (M oscillator), period decreases with increase in light intensity in comparison to DD [43]. The effect of light intensity on the period length under the constant

light condition is shown in Fig 4.14 A for the parameters that are used to fit the experimental data for DD and LD condition as in Fig 4.10 A.

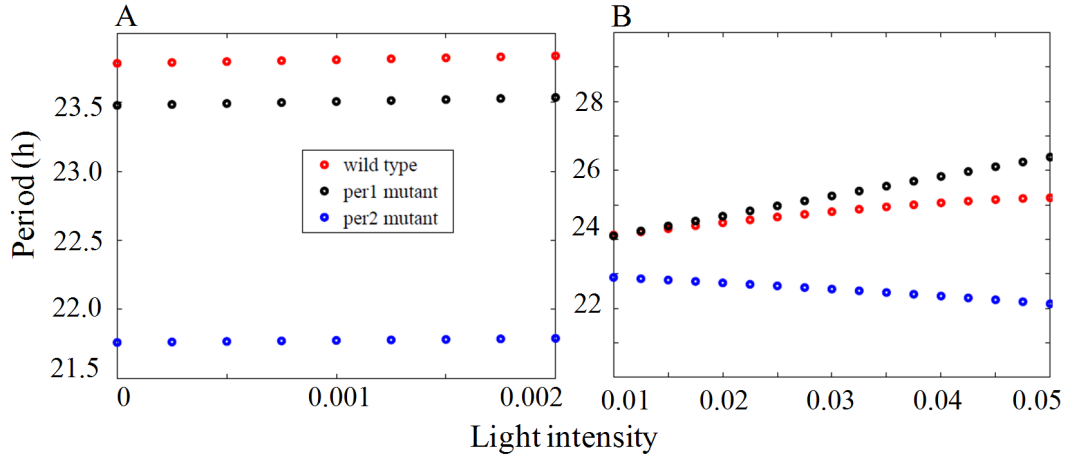


Fig. 4.14 Period length as a function of light intensity. (A) Simulation results are shown for the period length variation with respect to light intensity under constant light condition for wild type (red dots), *per1* mutant (black dots) and *per2* mutants (green dots) with normal set of parameters (as in Fig 4.10). Oscillations are found only for the small range of values of L with the corresponding period variation also being very small. (B) Simulation results of period length variation with respect to light intensity under constant light condition with the modified set of parameters (given in the Table A.1, parameters used for LL condition). In wild type and *per1* mutant, period increases with increase in light intensity and in *per2* mutant, period decreases with increase in the light intensity, which agrees with the experimental results [79].

The range of L values for which the period varies is very small and we didn't observe the trend seen in the experiments [79] in our simulations. To account for this effect in our model, we adjust the model parameters for both WT and mutants and the parameters are given in the Table A.1 (parameters used for LL condition). Most of the parameters that we change to simulate the effects of constant light are the production and degradation rates of *Bmal1*, *per1/2*, and *PER1/2*. After the parameters are adjusted, the period increases with increase in light intensity in both wild-type and *per1* mutant, and this follow Aschoff's rule [36]. In the case of *per2* mutant, we observe a decrease in the period with the increase in light intensity (Fig 4.14 B), and this did not follow the Aschoff's rule [43, 79, 36]. These results are in good agreement with the experimental results [79]. Taken together, we conclude that, under constant light condition, light accelerates *per1* and hence period decreases (frequency increases) and in the case of *per2*, light decelerates and hence the period increases (frequency decreases).

decreases). This simulation supports both hypothesis H1 and H5 for the effects of light and this points to the fact that *per1* acts like an M oscillator, while *per2* acts like an E oscillator.

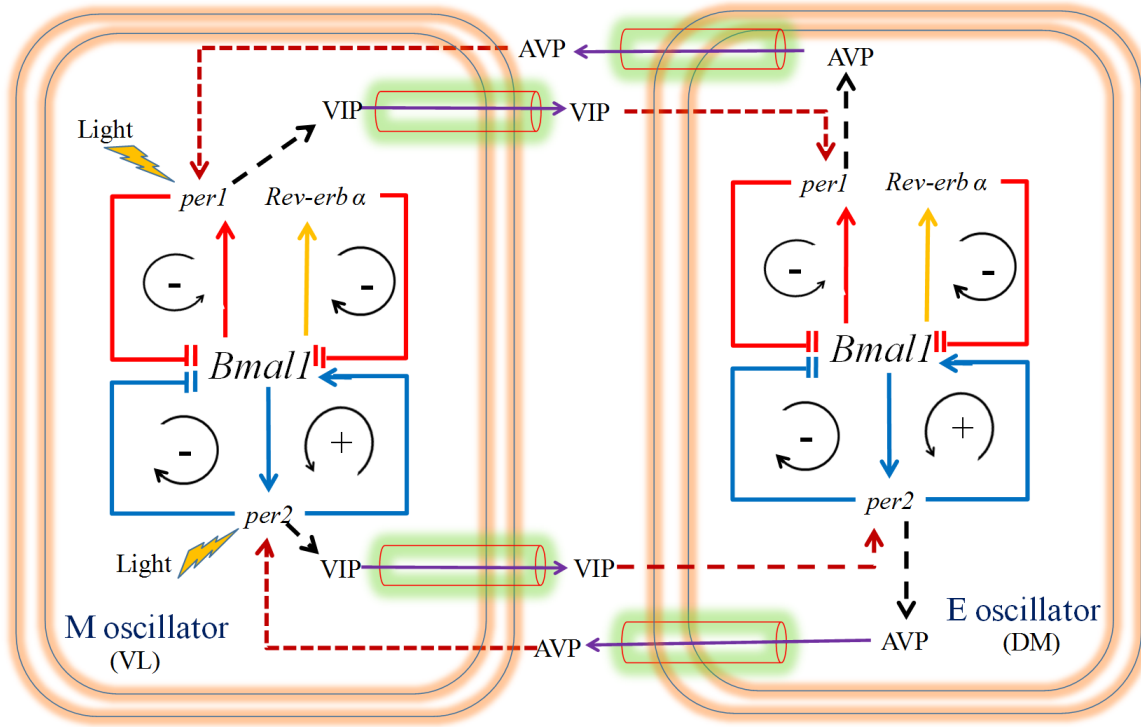


Fig. 4.15 Molecular network of coupled oscillator. Coupled oscillator is constructed from two single cell dual oscillators. M oscillator is present in VL region that guides morning activities and E oscillator in DM region guides evening activities. These two oscillators respond to light differentially. Light accelerates M oscillator while light decelerates E oscillator. The two oscillators are coupled to each other by the neuropeptides AVP and VIP that acts as coupling agents for M and E oscillators. We assume here that VIP and AVP are controlled by PER1/2 in VL and DM regions respectively. Further, we assume that AVP induces *per1* and *per2* expression in the M oscillator, and VIP induces *per1* and *per2* expression in the E oscillator.

4.4 Coupled oscillator model for splitting: Role of neuropeptides as coupling agents and construction of molecular actogram

Splitting in circadian rhythms are observed under constant light when a single daily bout of locomotor activity exhibits two components, one during subjective day and the other during

subjective night. Even though Daan's hypothesis include splitting behavior, it's argued that to explain ME concept splitting need not be invoked (See [46] for detailed discussion). There are different experimental evidences and hypothesis are exist for explaining the mechanism behind splitting. It has been shown in hamsters that during splitting PER1 rhythms in core and shell regions of SCN are antiphase to each other [171]. Horacio et al. [172] claim that antiphase oscillations in the left and right side of the SCN could be the molecular mechanism behind the 12h split rhythm of locomotor activity. Evans et al. [173] proposed that under normal days SCN neurons synchronized through a phase dependent coupling mediated by neuropeptides, where clock components show similar peaking time. However, under long days, these clock components peak 6-12h apart. Further, different regions of SCN that show distinct photoperiodic responses are identified [174] and they clearly classified an M oscillator region, which drives activity onset, and an E oscillator region, which drives the activity offset. Schwartz et al. [175] link *per1* and *per2* with dawn and dusk respectively and proposed that M and E oscillator may be entrained by distinct molecular mechanisms. These findings suggest that no conclusive evidence is obtained for explaining the exact mechanism behind splitting. Hence, In our model, we assume that splitting of daily bout of locomotor activity is due to the large phase difference between circadian rhythm at DM and VL region of SCN rather than splitting of circadian rhythm at left and right hemisphere of SCN.

We therefore explain splitting through coupled oscillator model within our framework of dual oscillator model by making assumptions that cluster of M oscillators are present in ventrolateral (VL, core) region, while cluster of E oscillators are present in dorsomedial (DM, shell) regions and they are coupled by neuropeptides that controls the phase differences between the M and E oscillators. Many coupled oscillator models [42, 50, 176, 177, 136] have been proposed to explain splitting phenomena, but they are all phenomenological in nature. For the single cell dual oscillator model that we described in earlier sections, the phase differences observed between *per1* and *per2* in WT under constant light condition (LL) was found to be very small and splitting was not seen in the simulations. Splitting appears to be a network property rather than that of a single oscillator. Therefore, taking cue from earlier works [42, 50, 176, 177, 136], we built a coupled oscillator model with neuropeptides VIP and AVP as the coupling agents. In our model, VIP is expressed in the neurons localized mostly in the VL part of SCN [178], where light is directly received [179]. This light information is transferred to the dorsomedial part [179], where AVP neurons are located [179]. VIP is arrhythmic under DD condition but displays sustained circadian oscillations under LD conditions [179]. AVP oscillates under both DD and LD conditions [180]. The network of each single cell individual oscillator has the same structure described in the earlier sections except that the parameters are different in these two oscillators. We make a strong assumption

that VL region has M oscillator that guides morning activities, since it is exposed to light while DM region harbors E oscillator that is not directly exposed to light and therefore guides evening activities. For M oscillator in VL region, we choose parameters for which *per1* loop dominates based on hypothesis (H5), and here the period of the *per1* oscillator decreases with increase in light intensity [43]. Again based on the hypothesis (H5), E oscillator in DM region, we choose parameters for which *per2* loop dominates, and here the period increases with increase in light intensity [43]. Further, we assume that VIP and AVP are controlled by PER1/2 in VL and DM regions respectively and VIP induces the transcription of *per1/2* mRNA in DM region while AVP induces *per1/2* mRNA's in the VL region. The coupled oscillator model with full parameters are provided in the Appendix B and Table B.1.

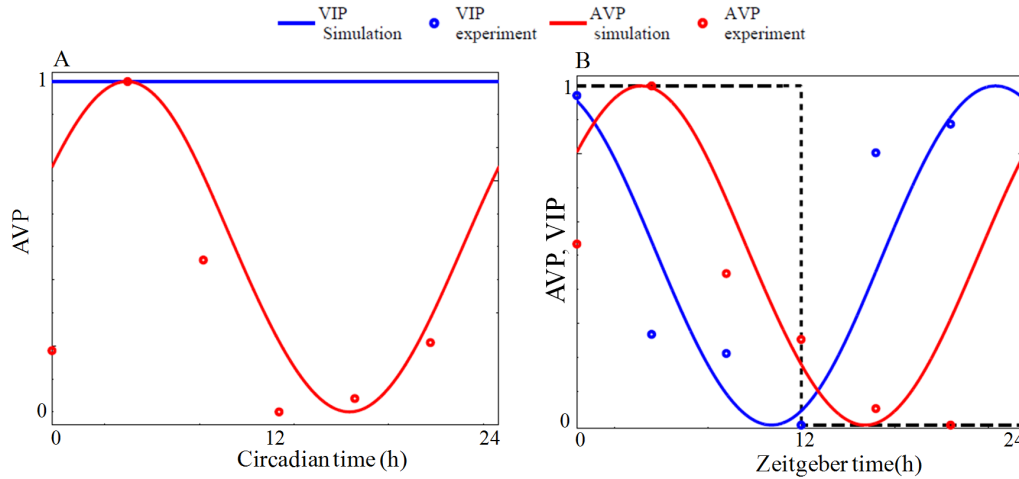


Fig. 4.16 Model fitting of AVP and VIP experimental data. (A) Under DD condition only AVP shows circadian oscillation and VIP is arrhythmic. Red line indicates AVP simulation and red circle indicates AVP data from experiments [180]. (B) Under LD condition, shown in black dotted line, both VIP and AVP show sustained oscillations. Red and blue lines indicate AVP and VIP simulation respectively, while red and blue circles are the data from experiments AVP from [180] and VIP from [181] respectively. Time series are normalized with maximum and minimum as 1 and 0 respectively. Under DD condition, AVP mRNA that peaks at CT4 is taken as the reference point. Simulation results are obtained by integrating the equations given in the Appendix B with parameter set given in the Table B.1.

To simulate splitting, we choose parameters for M (VL region) and E (DM region) oscillators that cycles with a period of $23.3h$ and $23.4h$ respectively under DD conditions. As it is known that period and phase of individual neurons in SCN maintain different period and phases [182], for simulation the periods of oscillators in VL and DM regions are taken differently. Light directly affects the VL region, and this light information is transferred to DM region through VIP. When VL and DM oscillators are coupled by the neuropeptides

AVP and VIP, they synchronize to a common period of 23.6 h under DD conditions (Initial condition of VIP is considered as $0.1nM$, and $\beta = 0.01nM$).

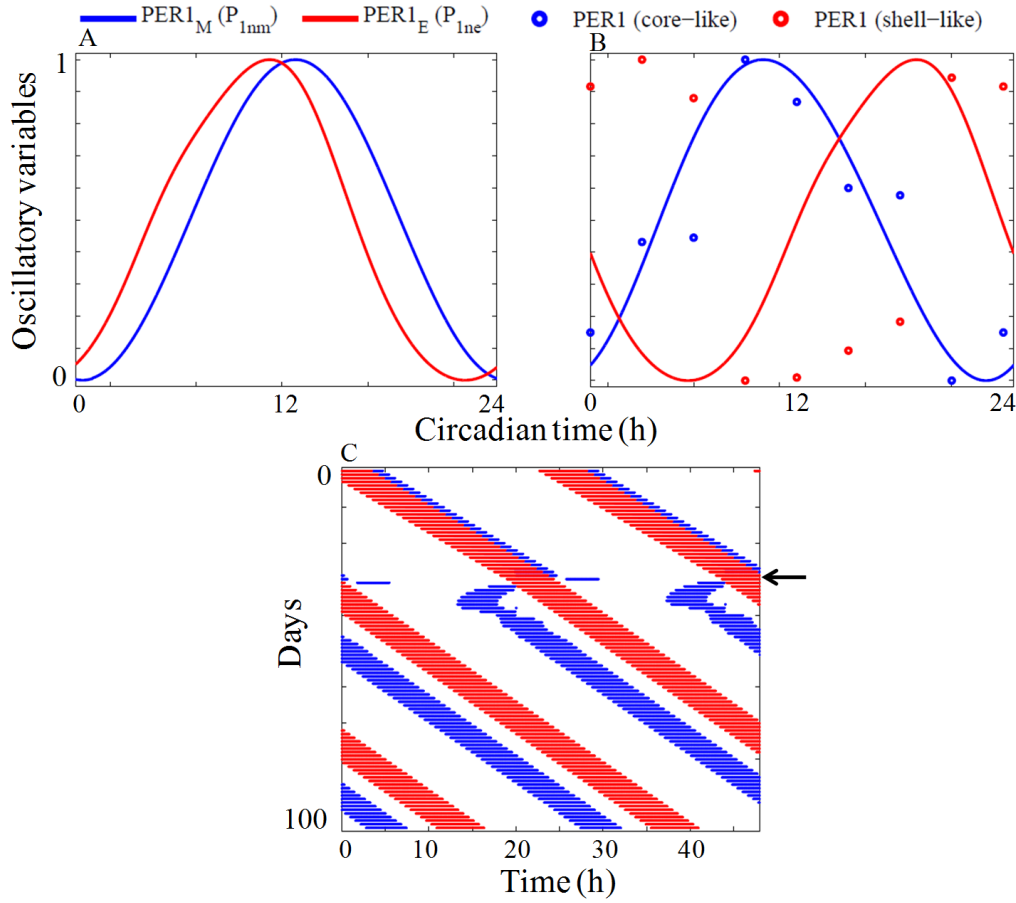


Fig. 4.17 Stable split under LL condition. (A) The time series of PER1 protein in VL region (P_{1nm}) and DM region (P_{1ne}) are shown in blue and red lines respectively under normal condition. Simulation results are obtained by integrating the equations given in the Appendix B. Initially we set VIP and AVP coupling parameters to $v_{cm1} = 0.35nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, and the AVP and VIP production rate to $k_{vs1} = 0.001h^{-1}$, $k_{vs2} = 0.015h^{-1}$ for unsplit condition. Rest of the parameters is given in the Table B.1. The phase difference between proteins PER1, P_{1nm} of morning oscillator and P_{1ne} of the evening oscillator, is very small (≈ 30 min). After 30 days, we vary the coupling parameters to $v_{cm1} = 0.28nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, and the production rates AVP and VIP to $k_{vs1} = 0.001h^{-1}$, $k_{vs2} = 0.009h^{-1}$. When parameters are varied, there is an increase in phase difference between P_{1nm} (blue line) and P_{1ne} (red line). (B) Blue circles are experimentally observed PER1 protein at the core region of SCN and red circles are experimentally observed PER1 protein at the shell region of SCN, at the time of splitting [171]. (C) Simulated actogram of the coupled oscillator model. In simulations, actogram is constructed when the normalized value of the variables P_{1nm} or P_{1ne} are above certain threshold value. The threshold we define here is 1.3 times the mean value of the normalized value of P_{1nm} or P_{1ne} . Blue horizontal lines represent M activities and red horizontal lines the E activities. Arrow on the right indicates the time at which parameter is varied. At the beginning of splitting, oscillator in VL region (M oscillator) has a shorter period than oscillator in DM region (E oscillator). After 30 days when parameters are varied, exchange of phase occurs between M and E components as seen in the experimental actogram (Fig 2C in [41]). Finally both the components maintained a stable phase relationship and oscillate with same period. Time series are normalized so that maximum value is 1 and minimum value is 0. We choose $L = 0.02$ for the simulation.

We estimate the AVP and VIP model parameters by fitting to the experimental data of AVP and VIP [181, 180] under both DD and LD conditions (Fig 4.16). Like previous models [50, 176], we generate different splitting patterns by varying the coupling strength under constant LL conditions with the assumption that the long exposure to light will gradually change both the coupling strengths and the expressions of both VIP and AVP neuropeptides. To construct the actogram based on molecular network simulation, we assume that PER1 protein from the VL region(P_{1nm}) determines morning activities whereas the PER1 protein of the DM region(P_{1ne}) determines the evening activities. We took PER1 protein in both VL and DM region to explain splitting because behaviorally split hamsters showed antiphase oscillations of PER1 expression in each side (core and shell) of the SCN [171]. It should be noted that presently there is no evidence to indicate a direct influence of *per*/PER on free running activities and this is only an assumption.

To plot the actogram of molecular network model that is analogous to the actogram of free wheel running activity, we assume the normalized value of the variables P_{1nm} or P_{1ne} are above certain threshold value and these values are plotted as activities. The threshold value we define is 1.3 times the mean value of the normalized value of P_{1nm} or P_{1ne} .

We reproduce many experimentally observed splitting patterns by changing the select parameters of the coupled model and one typical splitting pattern is shown in (Fig 4.17C) and the rest are shown in the Fig 4.18. In simulation, initially P_{1ne} , the PER1 of evening oscillator (DM) phase leads over P_{1nm} , the PER1 of the morning oscillator (VL) and the phase difference between PER1 of morning and evening oscillator is small (Fig 4.17A). After 30 days, when we decrease the coupling strength (v_{cm1}) and production rate (k_{vs2}) of AVP, we observe a big change in the phase difference and phase exchange takes place between P_{1ne} and P_{1nm} (Fig 4.17B). The model also fits the experimental data of PER1 [171] present in the core (VL) and shell (DM) regions fairly well. In experimentally observed actogram (Fig 2C in [41]), initially M and E activity components are in a fused state with E component leading over M component. After few days, when exposed to light, the two components split, and exchange of phase takes place with M component phase leads over E component. In our simulation, we capture this effect by changing the coupling parameters of the neuropeptides under constant light conditions. At the beginning of splitting, M oscillator has a shorter period than the E oscillator, after which both components maintains a stable phase relationship and oscillates with a same period. The simulated actogram (Fig 4.17C) is very much agrees well with the experimentally observed splitting pattern (Fig 2C in [41]). We also simulate different split and re-fused molecular actogram patterns that are observed in the free running wheel activity experiments by varying the coupling parameters and these patterns are shown in Fig 4.18.

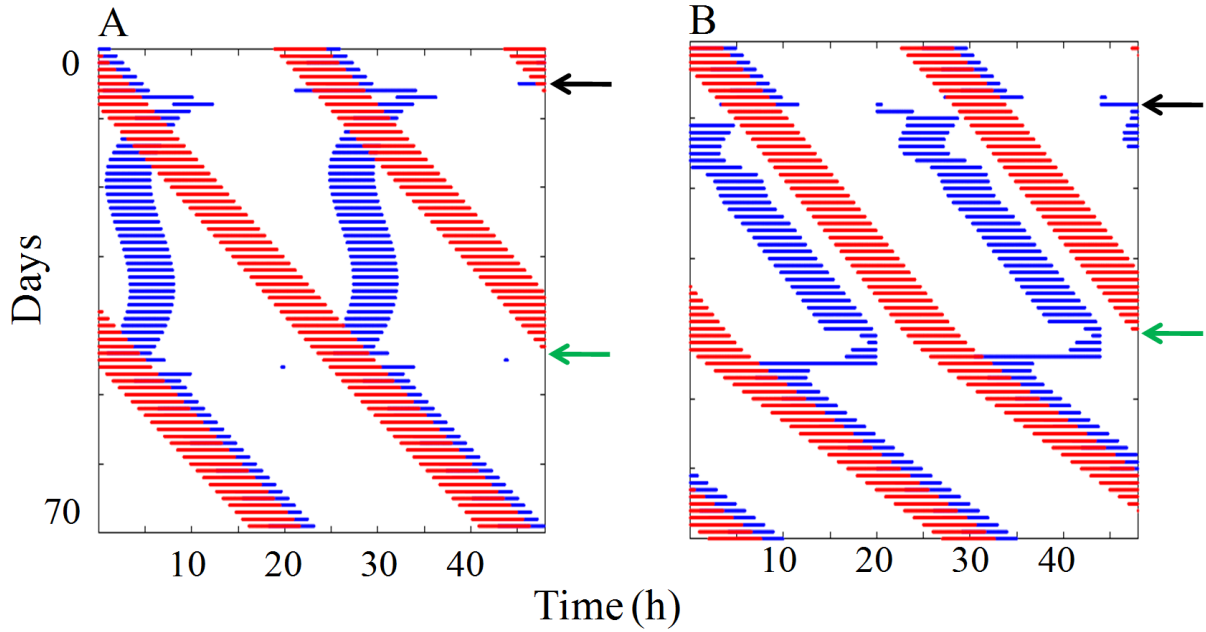


Fig. 4.18 Split and re-fuse under LL. (A) Simulated actogram of the model. Arrows indicate the point at which the parameter change occur. Initially set the parameters as $v_{cm1} = 0.35nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, $k_{vs1} = 0.001h^{-1}$, $k_{vs2} = 0.015h^{-1}$ for unsplit condition, then changed the parameters (day 6-black arrow) to $v_{cm1} = 0.01nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, $k_{vs1} = 0.001h^{-1}$, $k_{vs2} = 0.01h^{-1}$ for splitting. When splitting occurs M oscillate with a period lesser than E and finally M regains its original phase. Then we change the parameters to initial value for unsplit condition (day 44-green arrow). The simulated actogram is very much agrees well with the experimentally observed splitting pattern (Fig 2B in [41]). Here we proposed that splitting may be occur due to the transient change in the coupling strength between M and E oscillators, after the transient, the system will retains its original strength and re-fuse the splitting component. (B) Initially set the parameters as $v_{cm1} = 0.35nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, $k_{vs1} = 0.001h^{-1}$, $k_{vs2} = 0.015h^{-1}$ for unsplit condition, then changed the parameters (day 8-black arrow) to $v_{cm1} = 0.28nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, $k_{vs1} = 0.001h^{-1}$, $k_{vs2} = 0.009h^{-1}$ for splitting and again change the parameter (day 41- green arrow) to $v_{cm1} = 0.35nMh^{-1}$, $v_{cm2} = 0.25nMh^{-1}$, $k_{vs1} = 0.004h^{-1}$, $k_{vs2} = 0.025h^{-1}$ for re-fuse. The simulated actogram is very much agrees well with the experimentally observed splitting pattern (Fig 2D in [41]).

In summary, we have shown that the splitting phenomena can be captured by coupling two individual ME dual oscillators. We believe that the splitting is caused by ME oscillators, but these oscillators are highly regiospecific, where in one that is present in VL region with *per1*/PER1 dominant has a signature of M oscillator while the other present in DM region with *per2*/PER2 dominant has a signature of E oscillator. As a proof of concept, we coupled these oscillators through neuropeptides that modulates these oscillators and for

certain coupling parameters, splitting under constant LL conditions is achieved and anti-phase relationship is maintained between the two oscillators. We also showed that different splitting patterns can be obtained when the neuropeptides are modulated and these neuropeptides are themselves induced by constant light. Therefore, through simulations, we provided a speculative molecular interpretation for splitting. However, presently, we have taken a simplistic view of taking VIP and AVP as coupling agents to generate splitting patterns, and the contribution from other neurotransmitters like GABA, NMDA, and AMPAR are yet to be studied.

Table 4.6 Comparison of New model predictions with experimental data and previous model predictions.

ME oscillator characteristics			Experiments	Forger and Peskin, 2003 [59]	Mirsky et al., 2009 [80]	Becker-Weimann et al., 2004 [79]	Leloup and Goldbeter 2003 [61]	New model
Number of Variable / parameter				73/36	21/132	7/24	16/55	13/60
Period length under DD	Wild type		< 24	< 24	< 24	< 24	< 24	< 24
	<i>per1</i> mutant		< 24	< 24*	Arrhythmic	NA	Arrhythmic	< 24
	<i>per2</i> mutant		Arrhythmic	Arrhythmic	Arrhythmic	Arrhythmic	NA	Arrhythmic
Period length under LL	Wild type		> 24	< 24*	NA	< 24*	> 24*	> 24
	<i>per1</i> mutant		> 24	< 24*	NA	NA	Arrhythmic	> 24
	<i>per2</i> mutant		< 24/ Arrhythmic	Arrhythmic*	NA	arrhythmic	NA	< 24/ Arrhythmic
Period length variation with increasing light intensity in LL(Aschoff's rule)	Wild type		Period increases	Period decreases*	NA	Period decreases*	Period increases*	Period increase
	<i>per1</i> mutant		Period increases	Period decreases*	NA	NA	Arrhythmic*	Period increases
	<i>per2</i> mutant		Period decreases	Arrhythmic*	NA	arrhythmic*	NA	Period decrease
Entrainment	Wild	<i>per1</i> peak-ing time	Light phase	Light phase*	NA	NA	Light phase	Light phase
		<i>per2</i> peak-ing time	close to dusk	Light phase*	NA	Light phase*	NA	Closer to dusk
	<i>per1</i> mutant	<i>per2</i> peak-ing time	Closer to dusk	Light phase*	NA	NA	NA	Closer to dusk
		<i>per1</i> peak-ing time	Light phase	Arrhythmic*	NA	Arrhythmic*	NA	Light phase
PRC (Whether it matched with experimental results or not)	Without gating variable			Not matched	NA	Not matched	matched	matched
	With gating variable ⁺			matched	NA	matched	NA	matched

* Results were not shown in the literature, but we obtained from simulation. + Different Gating variables were used for different models. NA: Not applicable

4.5 Discussion

In this chapter, we simulated the single cell dual oscillator model of mammalian circadian rhythms based on the hypothesis proposed by Daan et al. [43]. The hypothesis is strongly based on the collation of various experimental data that includes *per1* and *per2* peaking times, their PRC's, entrainment to different LD conditions, and importantly we also simulate the splitting behavior observed in the locomotor activities. The dual oscillator we considered in our work is that in a single cell, *per1/cry1* and *per2/cry2* are the two oscillators that

Table 4.7 Comparison of ME oscillator in mammals and *Drosophila*.

Properties	Mammals in SCN	<i>Drosophila</i>	Present mathematical model for ME oscillator	Ref
Number of neurons	20000 clock neurons	150 clock neurons in brain	Single cell model is constructed to illustrate ME oscillator/ coupling of two single cell ME oscillator is used to explain splitting patterns	[47, 183]
Anatomical locations of ME cells	M and E cells exist in the SCN, but no clear evidence for the exact division of the area that contains M or E cells. The cells are distributed in the caudal, rostral and midrostral part of SCN	M oscillator is present in small ventral lateral region (s-LNv and half in LNd). E oscillator is present in 5 th dorsal lateral region (s-LNv and other LNd)	In SCN, M oscillator is taken to be present in ventrolateral regions, and E oscillator is taken to be present in dorso-medial	[47, 183–185]
Molecular components responsible for ME oscillator	Redundant genes are present and they are 3 <i>per</i> (<i>per1/2/3</i>) and 2 <i>cry</i> (<i>cry1/2</i>) genes	There are no redundant genes and only one <i>per/Tim/Cry</i> genes are present	<i>per1</i> and <i>per2</i> genes and their proteins are considered. <i>Cry</i> genes are not considered	[47, 183–185, 160]
Response of WT to LL	Oscillations are preserved. Period increases with increase in the light intensity and follows Aschoff's rule	Arrhythmic when light intensity is greater than 5 lux	Period increases with increase in the light intensity (Aschoff's rule)	[47, 183–185, 160]
Response of molecular component/activity pattern to different LD cycle (Entrainment)	<i>per1</i> mRNA follows dawn and <i>per2</i> mRNA follows dusk for various LD cycles. It encodes day length variations	Presently, genes that code for dawn and dusk are not identified. However, the wheel running activity shows an M peak in the dawn and E peak in the dusk	<i>per1</i> mRNA follows dawn and <i>per2</i> mRNA follows dusk for different LD cycles	[47, 183–185, 160]
The response of M and E oscillator to LL	As light intensity increases, period decreases in the M oscillator, and period increases in E oscillator	Only in <i>Cry</i> mutants it is observed that as light intensity increases, the period in M oscillator decreases and the period in E oscillator increases (Aschoff's rule)	As light intensity increases, the period in M oscillator decreases and the period in E oscillator increases	[47, 183–185, 160]
Neurotransmitters as coupling agents that may play a role in ME oscillator	Neuropeptides like VIP, AVP, GABA may play a role in ME oscillator, but their roles are not clear yet	The neurotransmitters are PDF, NPF, sNPF, Cha, ITP, and here PDF+ neurons are responsible for M activity and PDF- and DN1 neurons are responsible for E activity	VIP and AVP are used as neurotransmitters that couple ventrolateral and dorsomedial part of SCN	[47, 183–185, 160]
Splitting pattern	Activity pattern split into two separate bouts at LL	Bimodal activity rhythm is seen for LL at very low light intensity (0.01 lux) as well as in LD	Different Splitting patterns are observed in the coupled oscillator at constant LL for varying strength of neuropeptides AVP and VIP	[47, 184, 185, 160, 44]

can function independently, yet they influence each other. We also added a direct positive feedback from PER2 to *Bmal1* based on the experimental evidence in NIH 3T3 cells and liver

tissue [156] that PER2 coregulates *Bmal1* through the nuclear receptor PPAR α . However, in SCN this evidence is yet to be found. Due to lack of information, we added directly the *Bmal1* regulation by PER2 and as we get new information, we need to refine this model. We have not included the indirect regulation of PER2 on *Bmal1* through Rev-erb α , even though REV-ERB α regulation of *Bmal1* is included. We are particularly interested in knowing the role of *per1* and *per2* in encoding day length. In the case of splitting, the coupled oscillator model that we considered is the two single cell oscillators that interact through neuropeptides like VIP and AVP. The important contribution of this work is that we explained the entire hypothesis H1-H5 through the construction of realistic single cell mathematical model based on the known molecular mechanisms of mammalian circadian gene and protein regulatory networks.

Religio et al. [81] developed a mathematical model of mammalian circadian rhythm with two-feedback loop which consists of 19 variable and 71 parameters. This model reproduced the exact peaking time and phase relationship between mRNAs and proteins. They also simulated experimentally observed knockout phenotypes and period variations. However, this model considered *per* as a single gene rather than two separate genes (*per1/per2*). Also, they didn't include the effect of light on the circadian rhythms. These two features are the important characteristic features for examining the ME oscillator. The present single cell mathematical model does satisfy many of the Daan's hypotheses, and a comparison of our model with other well-known models are listed in the Table 4.6. Based on our simulations, we find that *per1* acts as and M oscillator, whereas *per2* as the E oscillator. However, our simulations are based on single cell model and have not considered the network properties of SCN that harbors more than 10000 neurons on each side of SCN. We have also not considered the role of other important genes like *cry1/2* and *Rorc* in influencing *per1/2* dynamics. We have also not simulated the aftereffects of light. In spite of these drawbacks, simulations of our minimal model construct points strongly that *per1* and *per2* act as ME oscillator and encodes day length.

It's also interesting to draw a comparison between mouse and *Drosophila* experimental works for ME oscillators since many of the works on dual oscillators recently has been in *Drosophila* where clearly two distinct peaks in activity rhythm have been observed [47, 186, 184, 185]. Helfrich-Förster et al. [47] have reviewed and compared dual oscillator model of mice and *Drosophila*, and in Table 4.7 we summarize their work and compare with our present mathematical model. Based on Helfrich-Förster et al. [47] work, it appears that presently it's too early to arrive at a consensus model of dual oscillator to describe ME activities atleast in mice and *Drosophila*, and it's due to various factors like number of neurons and its anatomical location, differential light responses, the role of different neurotransmitters

in different species, the wiring of molecular network etc. It will be interesting to model the molecular networks in *Drosophila* for ME oscillator and compare with the mouse models for differences and similarities and the advantages/disadvantages that confer on *Drosophila* with a fewer number of ME cells.

We also find that different splitting patterns cannot be obtained from single cell model and could simulate all the patterns only through the construction of coupled oscillator model with suitable changes in the parameters. Existing network models based on molecular interactions explain synchrony [86], and the encoding of seasonal variations [187], but these models have not separately considered the role of *per1* and *per2* in their network. It will be interesting to know whether the existing network models can explain the hypothesis if they are properly modified to include *per1* and *per2* separately. Presently it can only be speculated based on our simulations that *per1* and *per2* in a single cell act as an ME oscillator that tracks dawn and dusk respectively, but to counteract the seasonal and external variations, and noise, inter and intra cellular interactions of the population of oscillators in SCN may be necessary. In future, we intend to carry out simulations at a network level through coupling of multiple single-cell circadian oscillator models to verify Daan's hypothesis.

Chapter 5

Multiscale model of SCN to explore the role of noise on circadian variation of firing pattern

Preamble

Parts of this chapter have been published in:

Shiju S and K sriram (2018, March). A multiscale model explains the circadian phase dependent firing pattern variations in Suprachiasmatic nuclei and the occurrence of stochastic resonance, *10th International Conference on Bioinformatics and Computational Biology - BICOB-2018, USA* (Link)

5.1 Introduction

While in the last chapter, mathematical model of ME oscillator and splitting behavior were discussed, here the focus is set on the circadian variation of firing patterns at SCN in the presence of noise. Output from the circadian gene regulatory network controls behavioral and electrophysiological activities such as sleep wake cycle, hormone regulation, and neuronal firing rates. Understanding the electrophysiology of SCN neuron is a significant problem in computational biology. In electrophysiology, action potential or spike is an electrical signal that transport information from one neuron to other. SCN neurons produce spontaneous action potential due to the opening and closing of ionic channel but with varying firing

frequency during 24h circadian cycle. It is conventional that ionic channels control the electrophysiological properties of neuron, which in turn rely on the conductance of ionic channel. The average value of channel conductance fluctuates in a circadian manner [188]. Daily variation of SCN firing rate was previously identified [189, 190], where firing rate peaking during subjective day (3-10Hz) and reaches the minimum during subjective night (0-3 Hz) under DD condition [191].

Even though the GRN that generate circadian rhythm in SCN is well studied, less is known about the mechanism responsible for circadian modulation of firing rate variations and how firing frequency correlates with circadian gene expression. However, in mammalian system a direct association between modulation of firing rate rhythm and circadian gene rhythm have been reported [191], where it provides the evidence that *per1* plays an important role in modulating the spike frequency in SCN neuron. Therefore, we aspire to inspect the following questions through multiscale model simulations; (i) How the fast varying firing patterns (0 -10Hz) of SCN neurons are modulated by slow varying GRN of circadian oscillation of 24h (ii) What is the role of noise in regulating the firing patterns in SCN and (iii) How the interaction of noise, slow varying GRN and fast varying ionic channels lead to varying firing patterns during the subjective day and night in circadian systems.

In this work, an effort has been made to give reasonable explanation for the above questions by building a multiscale model. We also investigate the role of noise in providing optimal firing response in a circadian time dependent manner by invoking the idea of stochastic resonance (SR), a noise-induced phenomenon which has a wide range of application in different fields of science, and in particular neuronal system. The response of a nonlinear system to noise reaches a maximum for an optimum value of noise intensity is the typical characteristics of stochastic resonance. Benzi et al. [192] first introduce SR phenomenon in a dynamical system that subjects to both periodic and random perturbation, however, noisy nonlinear systems can display SR even without an external forcing signal [193, 194], which is called coherence resonance (CR). In the neuronal system, SR is an important mechanism that neurons use to detect subthreshold signals. Neurons converting incoming signals into a train of action potentials that reaches to central processing centers in the brain. The addition of noise enhances the subthreshold signals to cross the firing threshold and produce a spike train.

Further, we also show that the simulated firing patterns of multiscale model of SCN closely follow the experimental results [191] and importantly, for a particular circadian time exhibits stochastic resonance. Interestingly, this is the first instance in which the interaction of different natural signals with two varying time scales emerge from a single neuron in the

presence of noise that appears due to random opening and closing of voltage gated channels are used to explain the modulation of firing patterns.

5.2 Materials and method

We use Xppaut [119] and Matlab for numerical simulation, and the programs along with the link to the figures are printed in the Appendix-D.3.

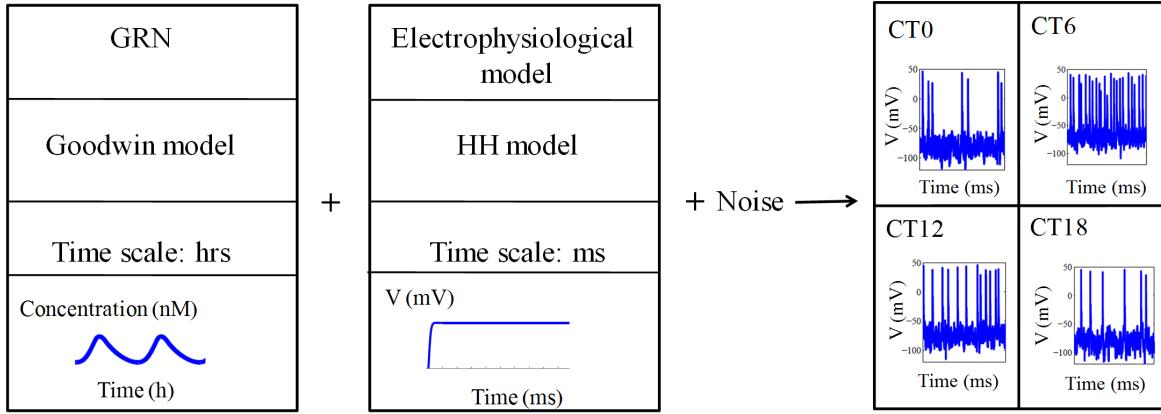


Fig. 5.1 Schema of multiscale SCN model. GRN is based on the Goodwin oscillator model, where the clock concentration varies in circadian manner with a period close to 24 h. The slow varying GRN (in the left column) drives the fast varying electrophysiological HH model, which is below the threshold and in steady state (in the middle column), generates action potential in the presence of noise with a period close to millisecond scale (in the last right column). Note that the firing pattern is highly circadian phase dependent and it is shown here for CT0, 6, 12 and 18 hrs.

5.2.1 Multiscale model

We choose two models that have different time scales in SCN: (i) A variant of Hodgkin-Huxley (HH) model developed by Diekmann et al. [88] precisely for SCN neurons and we use this model to reproduce the dynamics of firing rates in SCN (Fig 5.1). This is the model for a slow-fast system. The voltage has a slow timescale while the gating variables have a fast timescale. (ii) The Goodwin type model for gene regulatory network captures the dynamics of mRNA and proteins and has a time scale in the order of hours. We couple both these models of disparate timescale unidirectionally with *perl* mRNA regulating specific ion channels in the HH model. This is the forcing term of our model. We also add delta-correlated

Gaussian noise to the HH model and determine the noise intensity for which signal to noise ratio (SNR) is maximum. We describe below in detail the two models, simulations, and validation with the experimental data.

5.2.2 Electrophysiological model of SCN

The current balance equation of SCN by Diekmann et al. [88] is provided below, and to this we add Gaussian noise.

$$\begin{aligned}
 C \frac{dV}{dt} &= I_{app} - I_{Na} - I_{CaL} - I_{CaNonL} - I_{KCa} - I_{K-leak} - I_{Na-leak} + D * w \\
 &= I_{app} - g_{Na} m^3 h (V - E_{Na}) - g_K n^4 (V - E_K) - g_{CaL} r_L f_L (V - E_{Ca}) \\
 &\quad - g_{CaNonL} r_{NonL} f_{NonL} (V - E_{Ca}) - g_{KCa} s^2 (V - E_K) \\
 &\quad - g_{K-leak} (V - E_K) - g_{Na-leak} (V - E_{Na}) + D * w
 \end{aligned} \tag{5.1}$$

$$\frac{dq}{dt} = \frac{q_\infty - q}{\tau_q} \tag{5.2}$$

where $q = m, h, n, r_L, r_{NonL}, f_{NonL}$, and s

$$\begin{aligned}
 m_\infty &= \frac{1}{1 + \exp(-(V + 35.2)/8.1)}, & h_\infty &= \frac{1}{1 + \exp((V + 62)/2)}, \\
 n_\infty &= \frac{1}{(1 + \exp((V - 14)/-17))^{0.025}}, \\
 r_{L,\infty} &= \frac{1}{1 + \exp(-(V + 36)/5.1)}, \\
 r_{NonL,\infty} &= \frac{1}{1 + \exp(-(V + 21.6)/6.7)}, \\
 f_{NonL,\infty} &= \frac{1}{1 + \exp((V + 260)/65)}, & s_\infty &= \frac{(10^7)(Ca_s^2)}{(10^7)(Ca_s^2) + 5.6}, \\
 \tau_m &= \exp(-(V + 286)/160), \\
 \tau_h &= 0.51 + \exp(-(V + 26.6)/7.1), \\
 \tau_n &= \exp(-(V - 67)/68), & \tau_{rL} &= \tau_{rNonL} = 3.1, \\
 \tau_{fL} &= \tau_{fNonL} = \exp(-(V - 44)/220),
 \end{aligned}$$

$$\tau_s = 500 / ((10^7)(Ca_s^2) + 5.6), \quad f_L = \frac{K_1}{K_2 + Ca_s},$$

$$\frac{dCa_s}{dt} = -k_x(I_{CaL} + I_{CaNonL}) - Ca_s/\tau_{shell} + b_s$$

where w is the white noise with zero mean and unit variance. D is the intensity of the noise, which has the dimension of current. Here we assume that w is meant to constitute the combined effect of the stochastic activity of ion channels on the voltage dynamics of the neuron. Parameter values used for simulations are same as that in original model [88] except $g_{Na-leak} = 0.052nS$, $g_{KCa} = 180nS$, $g_{K-leak} = 0.15nS$ and $\tau_{shell} = 0.01ms$.

5.2.3 GRN model of SCN

Our GRN model is based on Goodwin oscillator [38] which consist of mRNA (M_P), protein (P) and phosphorylated protein (P_p). This model incorporates mRNA and protein production and degradation and a negative feedback of phosphorylated protein on the transcription rate of its gene which is highly nonlinear in nature. The dynamics of the GRN is:

$$\frac{dM_P}{dt} = a \left(\frac{0.001^{n_c}}{0.001^{n_c} + P_p^{n_c}} - M_P \right) \quad (5.3)$$

$$\frac{dP}{dt} = a(M_P - P) \quad (5.4)$$

$$\frac{dP_p}{dt} = a(P - P_p) \quad (5.5)$$

where $a = 4.46E - 8$ and $n_c = 9$, scaling parameter and parameter of cooperativity respectively. The system produces limit cycle oscillation with a period of 23.6 h, which is the typical free-running period of mammalian SCN neuron [12].

5.3 Results

5.3.1 Coherence resonance in HH model of SCN without periodic forcing by GRN

Codimension-1 bifurcation diagram for the membrane potential V as a function of $g_{Na-leak}$ in the absence of noise ($D = 0$) is shown in Fig 5.2. As $g_{Na-leak}$ increases the stable steady state becomes unstable through subcritical Hopf bifurcation, where the stable steady state is surrounded by the unstable limit cycle, which in turn is surrounded by a stable limit cycle. Further increase in $g_{Na-leak}$, periodic orbits dies through supercritical Hopf bifurcation that has unstable steady state surrounded by stable limit cycle. The parameter region near the onset of subcritical Hopf bifurcation become the focus of attention. In our model simulation, we use the subthreshold value of $g_{Na-leak} = 0.052$ that exhibits only stable steady state. The addition of noise force the system to fluctuate around the fixed point and shift into the limit cycle region, producing the train of action potential. The maxima between two spikes is the interspike interval (ISI) and the histogram of the ISI's for all the spikes gives rise to Interspike interval histogram (ISIH). This is done for different values of noise intensities D , numerical integration has been done in Xppaut [119] using stochastic Euler's method [195] with the integration time step $\Delta t = 0.02ms$ and the resulting ISI histograms are shown in Fig 5.3A. The time taken by the membrane potential to cross the activation threshold of firing is large for a low value of D and hence the number of spike around the mean interspike interval is small. For higher values of D , membrane potential quickly crosses the activation threshold and as a result, the height of the ISI peak increases.

In order to measure the system response to noise, we compute the power spectral density (PSD) of the membrane potential V . Power spectra computed from 25 averages of the firing patterns of membrane potential and smooth PSD curve is obtained by applying Savitzky-Golay filtering method with 100 number of points using Matlab ('sgolay'). For lower values of noise intensity the height of the peak in the power spectra is very small (Fig 5.3 B, $D = 1$, black curve). As noise intensity increases height of the power spectral peak increases and starts to saturate. In order to characterize the system response, we compute the measure of SNR defined in [193] as

$$\beta_s = H Q_s, \quad Q_s = \frac{\omega_p}{\Delta \omega} \quad (5.6)$$

where H is the height of the peak, ω_p is the frequency at which peak occurs and $\Delta \omega$ is the width of the peak at half maximum height. SNR for various noise intensity is shown

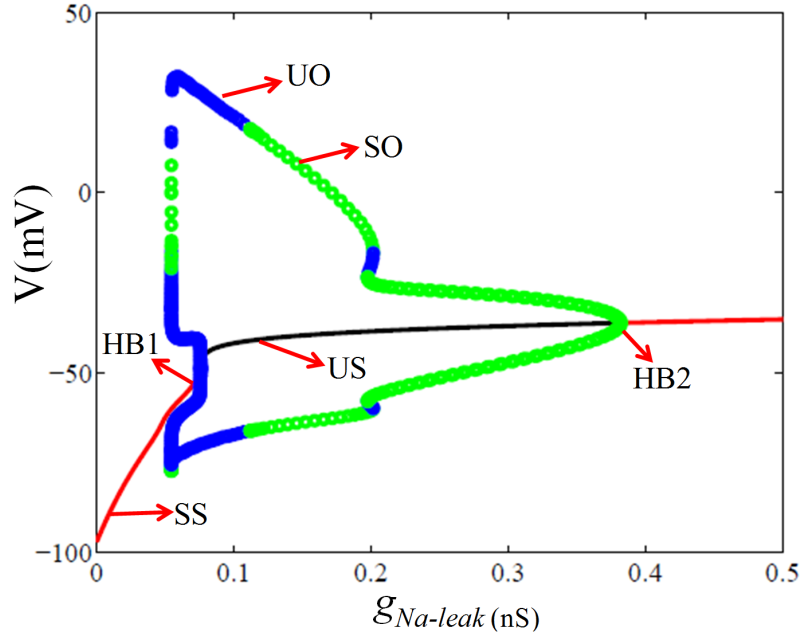


Fig. 5.2 Codimension-1 bifurcation diagram of HH model with $g_{Na-leak}$ as the bifurcation parameter. Lower values of $g_{Na-leak}$ brings the system in the stable steady state which is shown in red line (SS). As $g_{Na-leak}$ increases oscillations arise through subcritical Hopf bifurcation (HB1). Black lines are the unstable steady state (US), green and blue circles are stable (SO) and unstable (UO) oscillation amplitudes respectively. Further increase in $g_{Na-leak}$ leads to a loss of periodic orbits via supercritical Hopf bifurcation (HB2) and the system enters the stable steady state. Xppaut [119] was used for simulating the bifurcation diagram.

Fig 5.3C, coherence resonance take place at optimum noise intensity ($D = 2$), where SNR is maximum and further increase in noise, it starts to decrease. Even though there is a shift in the peaking frequency in power spectra, frequency variation in firing pattern is very less. Hence the coherence resonance is insufficient to convince experimentally observed circadian change in firing rate at SCN.

5.3.2 Circadian dependents of firing frequency variation: Multiscale Coupled oscillator model

Now we are focusing on the role of slow frequency circadian GRN in regulating firing frequency variations by incorporate the slow forcing signal of the gene regulatory network, namely the clock variable M_P that regulates the HH model through conductance of sodium leak $g_{Na-leak}$ as follows:

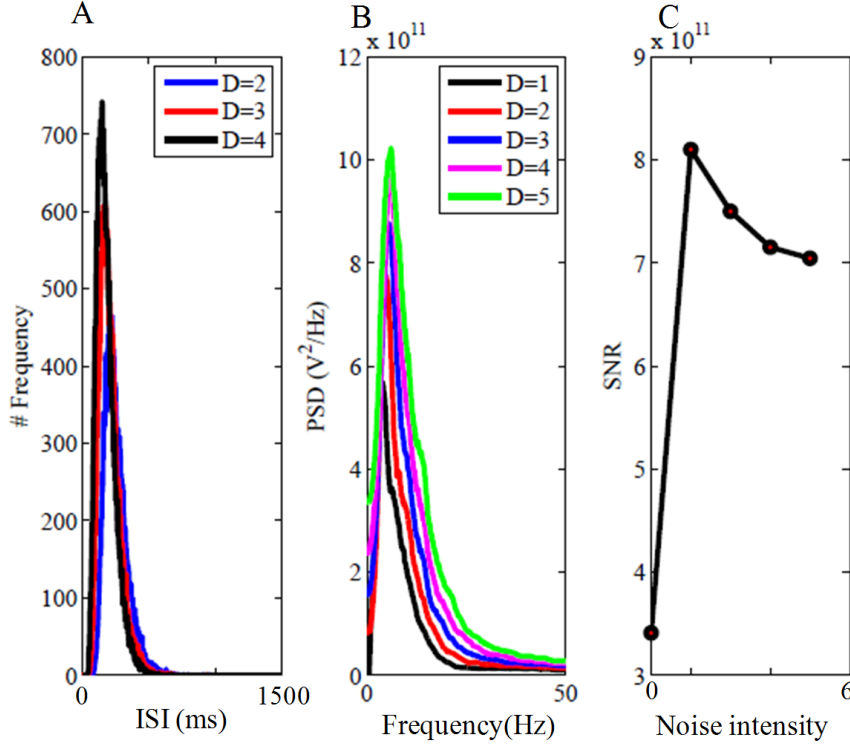


Fig. 5.3 ISIH, PSD, and SNR for HH model without forcing. (A) ISIs are systematically distributed, the majority of firing periods are near the intrinsic periods of the model ($\approx 200ms$) but as the noisy intensity increases the number of spike increases. (B) Averaged power spectral density of membrane potential for different noise intensity. Height of the PSD increases with increase in D and saturated for higher values. Smooth PSD curve is obtained by applying Savitzky-Golay filtering method with 100 number of points. (C) The SNR is calculated from power spectra using the equation 5. As noise intensity increases SNR reaches a maximum then decreases, characteristics of stochastic resonance.

$$gNa_{leak} = gNa_{leakpp} M_{P_{Normalized}} + gNa_{leakbase}$$

where $gNa_{leakpp} = 0.032nS$, $gNa_{leakbase} = 0.02nS$, and $M_{P_{Normalized}}$ is the normalized value of M_P ($M_{P_{Normalized}} = \frac{M_P - M_{P_{min}}}{M_{P_{max}} - M_{P_{min}}}$). The value of gNa_{leak} is varied in near the subcritical Hopf bifurcation. This circadian fluctuation in channel conductance evokes a subthreshold circadian rhythmicity on membrane potential in the absence of noise ($D = 0$) as shown in Fig 5.4A.

We study the consequence of noise at various circadian phases and the corresponding firing patterns for the noise intensity $D = 3$. In order to perform stochastic simulation, we simulated the model only for desired circadian phases (CT0, 6, 12, and 18) instead of continuous 24 h. We first set the integration time step to $0.02ms$ at a desired circadian

phase and run the simulation for one second without adding the noise. We then perform stochastic simulation for the desired time duration by adding the noise. Brief description of the methodology for solving multiscale coupled oscillatory model is given in algorithm 1. The firing patterns recordings for 5s in duration at desired circadian phases(CT_{des}) CT0, CT6, CT12, and CT18 are shown in Fig 5.4B-E. The peaking time of GRN component M_P is taken as CT6 which is the mid of subjective day and CT18 is the mid of subjective night. The spikes frequency at CT18 is less compared to CT6, and this indicates that the firing rate is higher during subjective day compared to the subjective night as observed in the experiments [189–191]. This significant result designate clearly that the slow varying circadian rhythm modulate the SCN firing patterns.

Algorithm 1: Solve the multiscale coupled oscillatory model at desired circadian time to get the firing patterns

Input: multiscale coupled oscillatory model

Input: Gaussian noise w

Input: noise intensity D

Input: step size (Δt), Δt_1 , Δt_2 ($\Delta t_1 \gg \Delta t_2$)

Input: window size ($window$) which compute from experiments

Input: desired circadian time, CT_{des}

If $CT \leq CT_{des}$

$\Delta t = \Delta t_1$ }
 $D = 0$ }

else if $CT > CT_{des}$ & $CT < (CT_{des} + window)$

$\Delta t = \Delta t_2$ }
 $D > 0$ }

end if

$v' = f(v) + D * w$ ——— (I)

print firing pattern according to (I)

ISI histogram for other two D values ($D = 2, 4$) at various circadian phases are shown in Fig 5.5, and it clearly disclose the variation in ISI distribution over the circadian time. Firing periods of membrane voltage show a spread of distribution at CT0, CT12, and CT18, in contrast a narrow distribution is exhibited at CT6, while the firing periods at CT6 are

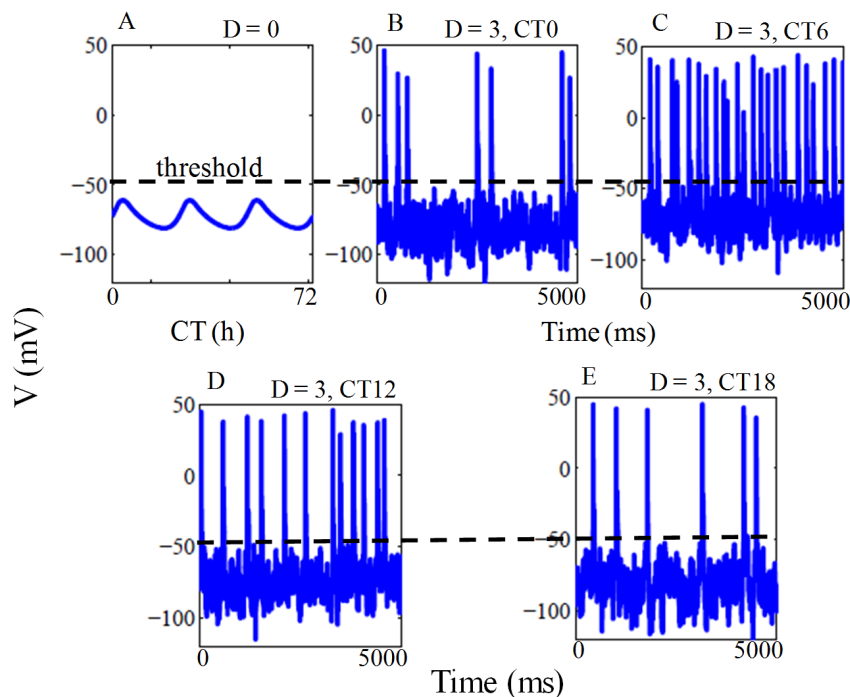


Fig. 5.4 Firing pattern of coupled GRN-HH-noise model. (A) In the absence of noise, subthreshold oscillations are only observed. (B-E) Noise-induced spikes at different circadian time. System shows different firing rates at various circadian phases for the same noise intensity. Black broken lines specify the activation threshold of the HH model.

distributed close to the intrinsic period of the model. At CT6 the HH system crosses the threshold comfortably and results in a narrow and high ISI distribution since the value of conductance g_{N-leak} is very near to Hopf bifurcation point. However, at CT0, CT12, and CT18 the g_{N-leak} value is far away from Hopf bifurcation point and hence the activation time is substantial resulting a wide and short ISI distribution.

5.3.3 Validation with experimental data

In order to validate the simulated firing data with experimentally observed circadian phase dependent firing rate in SCN, we quantify the firing frequency at various circadian time for diverse noise intensities. The results are shown Fig 5.6, which unveil a clear circadian variation in the firing rates. Under DD conditions firing frequency during the subjective day are between 3-10 Hz while during the subjective night it is in the range 0-3 Hz. This is in exceptional agreement with the experimental findings of Jones et al [191].

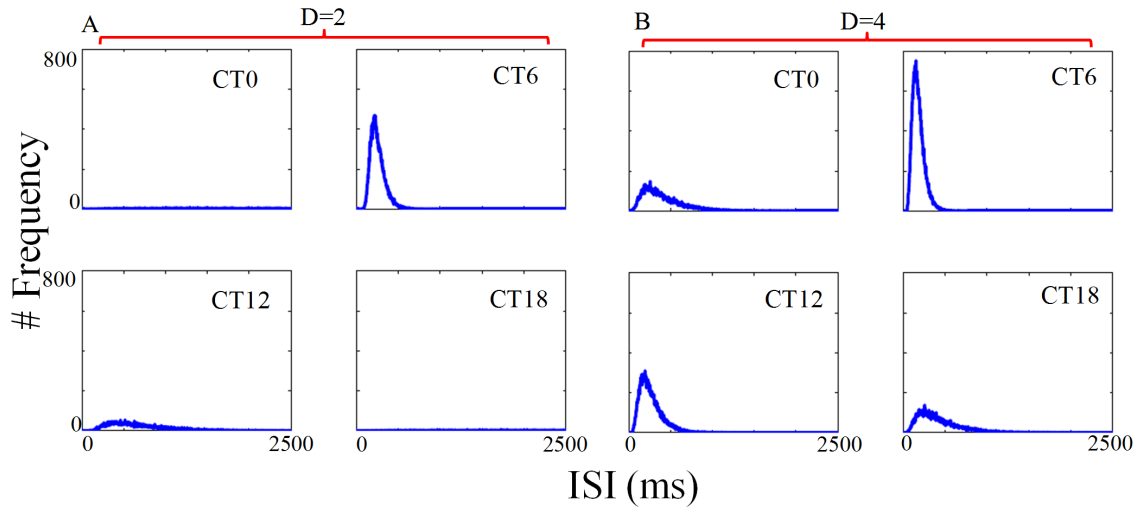


Fig. 5.5 ISIH for GRN-HH-noise model. ISIs are randomly distributed at CT0, CT12, and CT18 for $D = 2$ (A) and $D = 4$ (B). At CT6 majority of the firing periods are well ordered and centered near the intrinsic period.

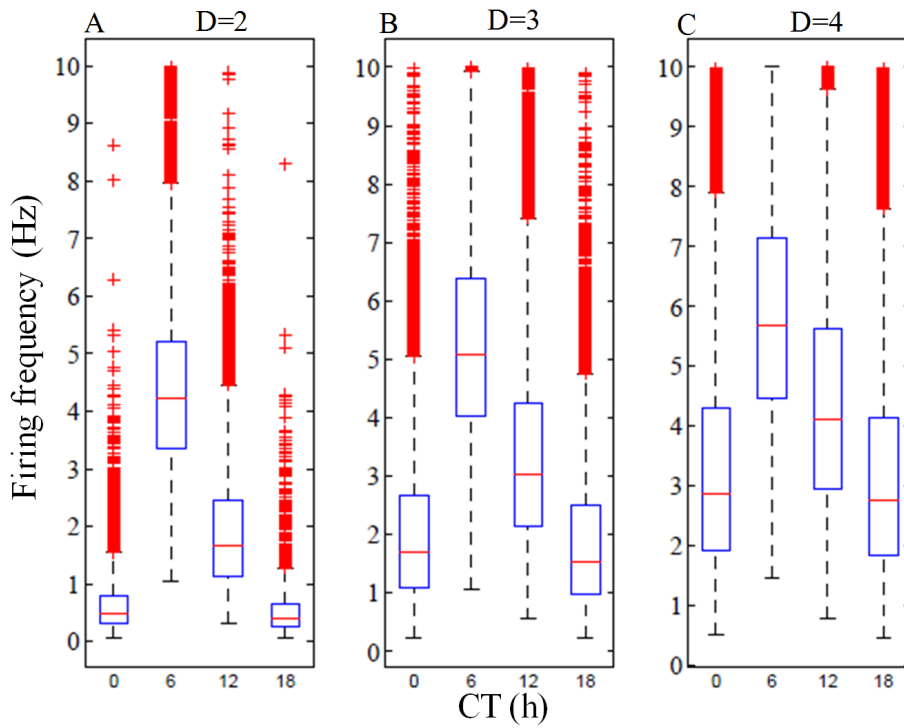


Fig. 5.6 Circadian variation of firing frequency. Firing frequency show circadian variation that is in excellent agreement with the experimental results [191]. On each box, the central mark indicates the median, and the top and bottom edges of the box indicates the 75th and 25th percentiles, respectively, and the outliers are plotted separately using the red '+' symbol.

We calculate the power spectral density of the membrane potential for different noise intensity at various circadian time, and the results are shown in Fig 5.7. Power spectral density of the membrane potential V was determined by averaging out 25 runs of the simulated firing patterns with a time span of 5 min using fast Fourier transform. At CT6 the height of the peak increases with increase in noise intensity and for higher values of D the height starts to saturate and width of the peak increases (Fig 5.7B). Importantly, this trend is not detect at CT0, CT12, and CT18 (Fig 5.7A, C, D).

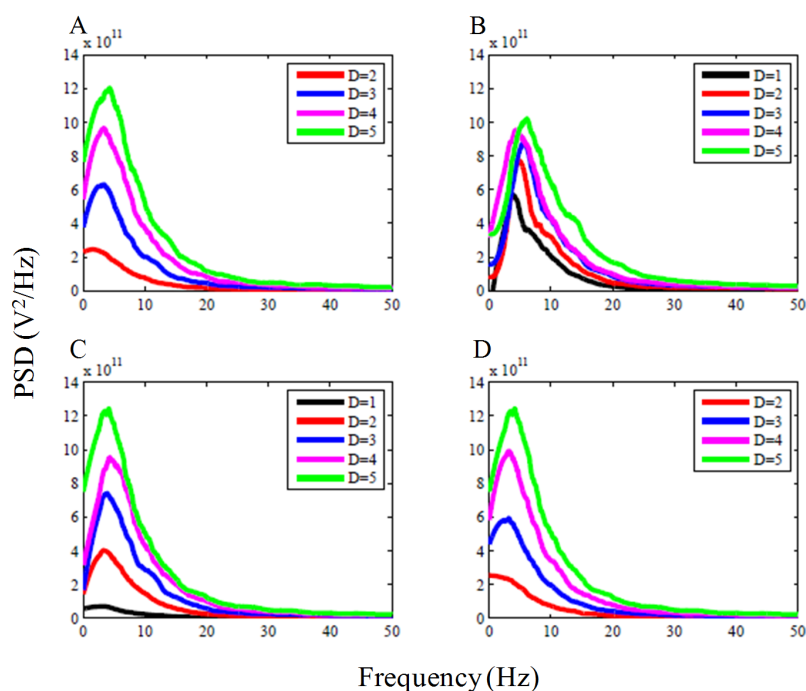


Fig. 5.7 PSD for GRN-HH-noise model. the Height of the peak increases with the increase of noise (A,C,D). However, at CT6, the growth of the height saturates and width of the peak increases for a higher value of noise (B).

We also compute the system's response to noise at various circadian phases for which we quantify the SNR from power spectral density (Fig 5.8). At CT6, the model displays stochastic resonance for the optimum value of noise intensity $D = 2$ and at CT12 stochastic resonance appear for $D = 4$. At CT0 and CT18 we did not see the stochastic resonance for any value of D and currently we do not know why this the case. Altogether, the effect of noise in SCN are circadian phase dependent and we postulate that stochastic resonance is circadian phase dependent because, at CT6, the value of the g_{N-leak} is near to the bifurcation point, in contrast at other phases it is distant from the bifurcation point.

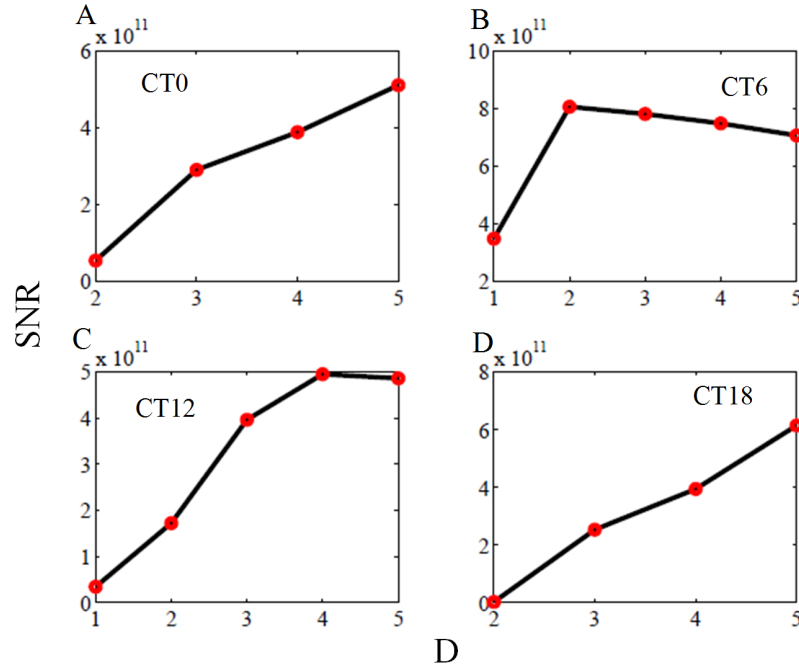


Fig. 5.8 SNR for GRN-HH-noise model. Stochastic resonance shows circadian phase dependency. (A, D) SNR increases with increase in the noise intensity at CT0 and CT18, where the value of $g_{Na-leak}$ is far from the bifurcation point. (B, C) At CT6 and CT12 system exhibit stochastic resonance, where SNR is maximal for some value of noise intensity.

5.4 Discussion

Previous studies have unveiled that the firing rate at SCN shows circadian variation with a period close to 24 h [191, 3]. Though the emergence of firing pattern variations during subjective day and night are not known. In SCN, the mechanisms to regulation of the fast varying ionic channels by slow varying gene regulatory networks are still under study. Presently, there is no strong experimental evidence exists to understand the emergence of firing pattern variations. However, channel noise plays an important role in producing action potentials. This is insufficient to explain firing pattern variations that happen in a circadian phase dependent manner. We postulated and showed the role of noise and the slow varying circadian gene regulatory network in regulating the firing rates at different circadian phases through multiscale simulations of coupled nonlinear ODE model that incorporates both GRN and electrophysiological part of SCN. Here we find that the noise induced firing rate is circadian phase dependent, and the firing rate is higher during the subjective day and lower during the subjective night. This is also in good agreement with the experimental

findings. From the dynamical system perspective, we hypothesize that the firing is fast during subjective day because the peaking of *per* gene take place during the subjective day which in turn takes the conductance of ionic channel near to the bifurcation point and as a result that facilitates noise induces coherent firing pattern. On the other hand, during subjective night *per* concentration is at nadir/trough, the ionic channel conductance is far away from the bifurcation point and hence, firing rate is low and noisy. The window-size taken in the simulation is based on experimental data, and in this window-size, only during certain circadian phase where stochastic resonance is seen. However, we have not looked into the role of window-size in stochastic resonance and firing pattern variations. Currently, we do not have any explanation for the occurrence of stochastic resonance only at certain circadian phases. We expect to look into this characteristics closely in the future work. We are especially interested in modeling the firing pattern variations from the morning and evening oscillator point of view for which the mathematical model of morning and evening oscillators are already discussed in the previous section. In future, we would also like to continue this work further to study rigorously the role of stochastic resonance in circadian rhythms.

Chapter 6

Multiscale modeling of the circadian modulation of learning and memory

Preamble

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6.1 Introduction

The role of circadian rhythms in modulating memory has been studied for a long time, yet molecular mechanisms and networks responsible for the modulation is not well understood. Clock genes that contribute to synaptic plasticity, and fear conditioning processes like acquisition, renewal, and extinction are partially known. Though it is known that circadian rhythm is pervasive all through the system with SCN as a master clock, the regulation of peripheral slave circadian oscillators is far from understood. Besides SCN, circadian rhythmicity have been identified in other brain regions that include amygdala and hippocampus [196, 197, 29, 198–202, 31]. Hippocampus and amygdala are the centers for learning, memory formation, and storage. Presently, memory is understood to be an electrically encoded representation of an event described by LTP and LTD. LTP/LTD refers to the strengthening/weakening of synaptic connection between the neurons and this dynamics is commonly referred to as synaptic plasticity. The measure of LTP (LTD) generally tracks

either an increase (decrease) in the extra-cellular population excitatory postsynaptic potential (EPSP) or population spike (PS) [203]. EPSP makes postsynaptic neuron more likely to fire a spike (action potential), whereas, PS is the measure of the number of cells discharging spikes in response to stimulus [203]. Nabavi et al. [204] showed that associative memory can be activated by LTP and inactivated by LTD, which supported the hypothesis that memories are encoded by synaptic plasticity through LTP and LTD [205]. On the other hand, it is debated whether LTP/LTD is the actual phenomena responsible for memory [206]. The role of circadian rhythms in influencing LTP and LTD has been studied for a long time. Chaudhury et al. [48] found that LTP in the CA1 region of mice showed diurnal variation, where LTP during night is higher than day. Under constant dark (DD) condition, LTP shows higher magnitude during subjective night indicating the endogenous circadian rhythm modulation of the synaptic plasticity [48]. Endogenous circadian rhythm can also modulate different type of hippocampus specific memories and memory processes [207], and, SCN has intricate neural circuit connecting hippocampus that successfully mediates hippocampal activation [29, 31]. These experimental results showed that the circadian rhythm has significant influence on modulating LTP/LTD, but the exact molecular mechanism is not fully understood [196].

LTP and LTD are considered as the principal mechanism underlying learning and memory. Learning is the process of obtaining new information or remodel these existing characters. In associative learning process, a new response becomes associated with a specific event or stimulus. Classical fear conditioning is a form of associative learning in which the conditioned stimulus (CS) such as tone or context acquire the ability to evoke a fear response after the paired exposure of CS with the natural unconditioned stimulus (US) such as foot shock, and this associative learning task is an important technique for exploring the neurobiological basis of learning and memory. The opposite of learning, which is called extinction, is the repeated application of CS alone that results in a declined fear response [208]. Amygdala is responsible for controlling emotional memories and is also the region considered to study the fear conditioning paradigm and extinction [209] extensively. Savalli et al. [202] have shown that the aberrant circadian gene expression and loss of synchrony led to depression in the mouse model, while Chaudhury et al. [31] performed experiments to study the effect of circadian modulation of fear conditioned learning and memory. In particular, Chaudhury et al. [31] showed that during fear conditioning, acquisition is higher in the mice trained during the day than in the night. Recall ability for tone and context also varied as a function of time-of-the-day, and was found to be independent of the time of training. Moreover, mice trained in the night showed a greater degree of extinction than during the day. These results suggest that circadian rhythm modulates acquisition, recall, and extinction in the fear-conditioned mice for which the exact molecular mechanisms are not known.

Based on the above experimental evidences, two explanation about the influence of circadian rhythms on learning and memory are proposed: (i) circadian rhythms only modulate learning and memory, and (ii) clock is embedded in the system and it continuously perceives the time-of-the-day effects. Cain et al. [210] showed that learning and memory depends on the time-of-the-day that training had occurred. In their experiments, learning processes are preserved even when animals have lesioned SCN, indicating strongly that time-of-the-day information is implicitly encoded. They also speculated that the circadian rhythm may modulate the memory process independent of SCN. Also, since the time-scale of circadian rhythm dynamics is around 24 *h* and the learning, memory, and LTP/LTD processes take place at a much smaller time scales, ranging from millisecond to hours, they indicated that the circadian rhythms cannot modulate the learning and memory processes. Therefore, presently, in the absence of any molecular mechanism, it is not clear how a slow circadian process modulates the fast learning and memory processes. These findings suggest that no conclusive evidence can be arrived at to confirm or ignore the hypothesis that circadian rhythm modulates learning, memory, and LTP/LTD processes. However, based on the current newly obtained experimental evidence, we believe that circadian rhythm modulates cognitive functions in a phase-dependent manner [201, 31, 48, 211, 212].

To understand the influence of circadian dynamics on learning and memory, mathematical models can provide considerable insight into intricate working details of the mechanisms. While the mathematical models of circadian rhythms based on nonlinear ordinary differential equation (ODE) models of GRN's has been popular and have been widely used to understand various dynamics of the system, conductance model of circadian rhythms are used to understand the electrophysiological properties [213, 85, 61, 60, 214, 79, 80, 89, 99, 88, 215, 92, 216, 91, 217]. Sim et al. [89] developed electrophysiological model of SCN, where they fit experimentally observed ionic current to Hodgkin-Huxley-type (HH) model. Later, by incorporating GRN of SCN, multi-scale models of SCN were developed and these models reproduced experimentally observed circadian variation of firing patterns [99, 88, 215, 92, 216, 91, 217]. Vasalou et al. [99] incorporated integrate and fire model (IF) and a 16 variable GRN [61] with calcium as a coupling agent. Diekmann et al. [88] and Belle et al. [92] coupled Goodwin type GRN to HH model to explain the daily rhythm in firing patterns. Similarly, Dewoskin et al. [216] proposed an HH model coupled to a detailed mammalian circadian GRN model [60] to explain the firing pattern variations. In the previous chapter, we performed multi-scale stochastic simulation, where we explained the role of noise and GRN in bringing about the circadian variation of SFR. On the other hand, many different types of models are proposed to capture the mechanism of synaptic plasticity, especially the models for LTP/LTD and spike time dependent plasticity (STDP) [218–222].

A detailed review of the models for LTP/LTD/STDP and their characteristics properties were discussed in [51]. Computational models for fear conditioning at amygdala were also previously developed [52–56] to explain the mechanism and dynamics behind learning, recall and the extinction.

Though these models are built and studied to understand specific properties, the interlink between circadian rhythms and synaptic plasticity, learning and memory are not considered. To the best of our knowledge, a multi-scale model that includes circadian GRN and electrophysiology coupled by neurotransmitters is not currently available to explain the mechanism of circadian variation of LTP/LTD, learning and memory. We have therefore in this work built a coupled oscillator model to study three aspects of circadian modulation: (i) SFR in SCN, (ii) LTP/LTD in hippocampus and (iii) fear conditioning, recall and extinction at amygdala. In the previous chapter we built a stochastic model to explain circadian modulation of SFR at SCN. However, in this chapter we use a deterministic models to explain the daily variation of firing frequency at SCN. We also demonstrate through simulations the importance of calcium dynamics, N-Methyl-D-aspartic acid receptor (NMDAR), and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) evoked ionic currents in the circadian modulation of learning and memory. We use experimental data taken from previously published results on C-57/6 or C-3H mice [191, 48, 31] to validate our model.

6.2 Materials and method

We provide in this section the details of models we built to understand the circadian modulation of SFR, LTP/LTD, and fear conditioning. To model the circadian modulation of SFR in SCN, we choose Goodwin type GRN model, and to capture the voltage and current changes we consider the modified version of Morris-Lecar model (ML) [223]. To model the circadian modulation of LTP/LTD in hippocampus, we consider two Goodwin type GRN models— one for SCN and the other one for hippocampus coupled via a yet unknown neurotransmitter. We also couple the GRN to the modified version of ML model to capture the electrophysiological dynamics in hippocampus. Also, we include NMDAR receptor dynamics to explain the occurrence of LTP/LTD and spike time dependent plasticity (STDP). To model the circadian modulation of fear conditioning, recall, and extinction in amygdala, we again consider two Goodwin type GRN model, one for the SCN and the other for amygdala, coupled via a neurotransmitter. We also consider a modified version of ML to capture NMDAR and AMPAR electrophysiological receptor dynamics. In all the three cases we explicitly include calcium

dynamics that plays an important role in the circadian modulation of SFR in SCN, LTP/LTD in hippocampus, learning, and memory in amygdala. Details of the model are given in the subsequent part of this section.

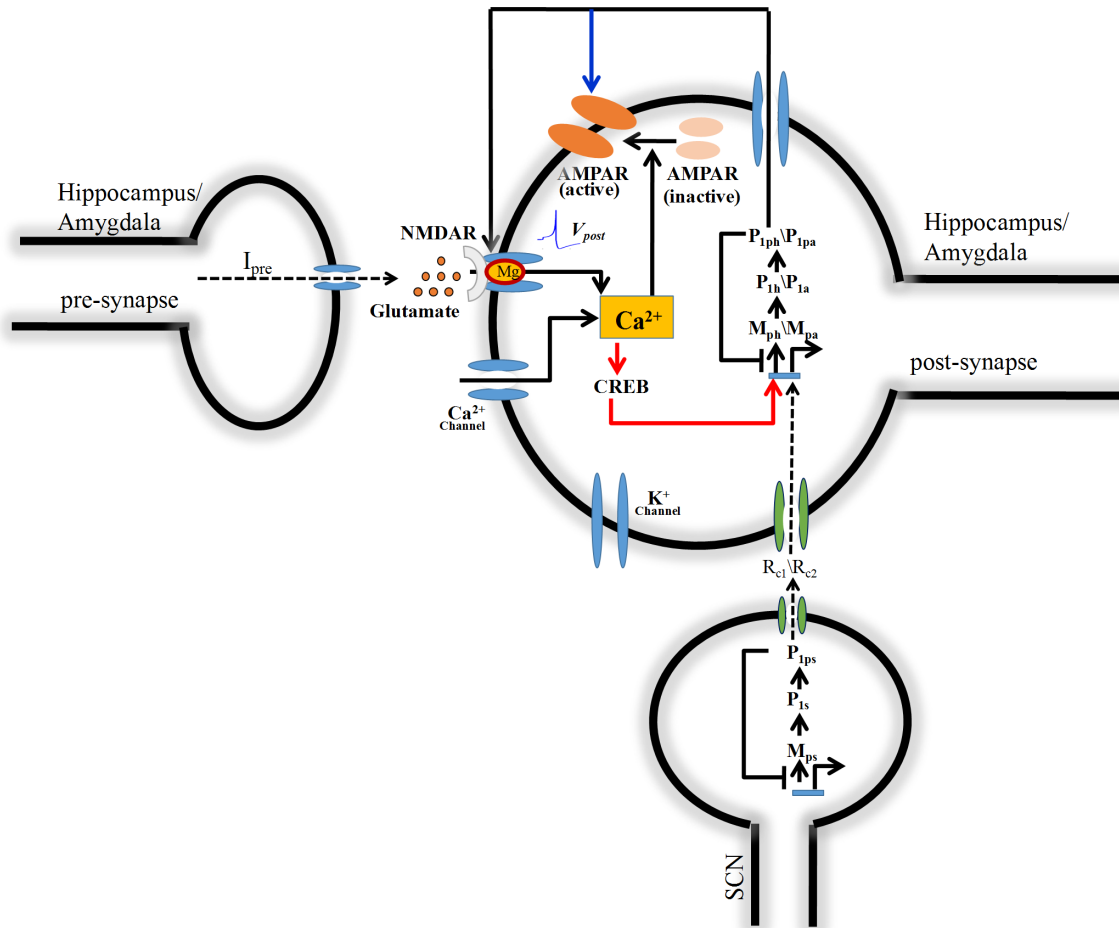


Fig. 6.1 Multi-scale model for LTP/LTD, learning and memory. It is a unidirectionally coupled model with only SCN interacts hippocampus/amygdala and not vice-versa. Its a master-slave oscillator, with SCN acts as a master oscillator and hippocampus/amygdala as a slave oscillator. Goodwin type model is used to capture the SCN and hippocampus/amygdala circadian dynamics [38] which consists of 3 variables. These two Goodwin oscillators are coupled via an intermediate variable R_{C1}/R_{C2} . Hippocampus/amygdala model consists of pre and postsynaptic neurons. Due to presynaptic stimuli, glutamate is released and binds with NMDAR. These are permeable to calcium which is responsible for the insertion and removal of AMPAR. Conductance model of neurons are modified version of ML model. Rate equations and parameters are discuss in Materials and method section. Here red arrows indicate that the regulation is only present for the hippocampus model, while the blue arrows are only taken for amygdala.

6.2.1 Model for circadian modulation of firing pattern variation in SCN

To capture the firing pattern variations in SCN, we choose two models that have disparate time scales: (1) gene regulatory model of Goodwin type circadian oscillator to capture the dynamics of mRNA and proteins in hours and (2) the modified version of ML model to capture the firing patterns in SCN in milliseconds. Goodwin type oscillator [38] consists of dynamical variables *per* mRNA (M_{Ps}), PER protein (P_{1s}), and phosphorylated PER protein (P_{1Ps}). It essentially describes the production of mRNA and protein, their degradation, and delayed-negative feedback of phosphorylated protein using Hills equation. These equations are highly nonlinear, which are given as:

$$\frac{d}{dt}M_{Ps} = A_s \left(v_{s1} \frac{K_{As}^{n_c}}{K_{As}^{n_c} + P_{1s}^{n_c}} - M_{Ps} \right) + L \quad (6.1)$$

$$\frac{d}{dt}P_{1s} = A_s (M_{Ps} - P_{1s}) \quad (6.2)$$

$$\frac{d}{dt}P_{1Ps} = A_s (P_{1s} - P_{1Ps}) \quad (6.3)$$

Where A_s is a scaling parameter and its value is $4.35E - 8 \text{ ms}^{-1}$, L is the parameter responsible for light and its value under DD condition is 0. For LD cycle simulation, L is varied in a square wave manner with period 24 h , and we use $L = 0.5e - 8$ in the light phase, and $L = 0$ at the dark phase. Remaining parameter values are $v_{s1} = 20 \text{ nM}$, $K_{As} = 0.8 \text{ nM}$, $n_c = 9$. The model exhibits limit cycle oscillations with a period of 23.6 h , which is the typical free-running period of a mammalian circadian system [12].

To generate spontaneous firings, we use two-variable ML model, with v as the membrane voltage and w as the recovery variable that represents the fraction of K^+ channel open at a given instant of time. The current-balance equation is given as:

$$\begin{aligned}
C \frac{d}{dt} v &= I_{app} - I_L - I_K - I_{Ca} \\
&= I_{app} - g_L(v - v_L) - g_K w(v - v_k) - g_{Ca} m_\infty(v - v_{Ca})
\end{aligned} \tag{6.4}$$

$$\frac{dw}{dt} = \lambda(w_\infty - w) \tag{6.5}$$

$$m_\infty = 0.5(1 + \tanh(\frac{v - v_1}{v_2})) \tag{6.6}$$

$$w_\infty = 0.5(1 + \tanh(\frac{v - v_3}{v_4})) \tag{6.7}$$

$$\lambda = \phi \cosh(\frac{v - v_3}{2v_4}) \tag{6.8}$$

Where I_L , I_K , and I_{Ca} are the leakage current, potassium current, and calcium current respectively. w_∞ and m_∞ are the open state probability functions of K^+ and Ca^{2+} ions channel respectively. λ represents the voltage dependent time constant for K^+ channel recovery variable. Parameter values of ML model are modified from the original model [223] to get experimentally observed firing patterns at different circadian phases and are given as $C = 20 \text{ pF}$, $I_{app} = 0 \text{ mA}$, $g_L = 2 \text{ nS}$, $g_K = 30 \text{ nS}$, $v_k = -84 \text{ mV}$, $v_{Ca} = 90 \text{ mV}$, $v_L = -60 \text{ mV}$, $v_1 = -1.2 \text{ mV}$, $v_2 = 18 \text{ mV}$, $v_3 = 12 \text{ mV}$, $v_4 = 14.75 \text{ mV}$, $\phi = 0.04$.

To include the circadian modulation of SFR, we modify the conductance of calcium channel (g_{Ca}) as a function of circadian variable M_{ps} :

$$g_{Ca} = g_{cabase} \frac{M_{ps}}{k_{ps} + M_{ps}} \tag{6.9}$$

Where $g_{cabase} = 6.37 \text{ nS}$, $k_{ps} = 0.1 \text{ nM}$. We made this assumption based on the experimental observation that Ca^{2+} current in the SCN shows diurnal variation, and this current strongly contribute to the generation of spontaneous firing in membrane voltage [224, 225]. Previously, several computational models [99, 88, 92, 216, 91] have similarly incorporated circadian variation of calcium conductance based on experimental observation [224, 225].

6.2.2 Model for circadian modulation of LTP/LTD at hippocampus

To capture the circadian modulation of LTP/LTD at hippocampus, we consider the GRN Goodwin model for both SCN and hippocampus, the dynamics of NMDAR, AMPAR, Calcium, CREB, and the postsynaptic action potential. The GRN model of SCN and its

parameter values are the same as described in the previous subsection. The GRN model of hippocampus consists of three dynamical variables, namely *per* mRNA (M_{Ph}), PER protein (P_{1h}), and phosphorylated PER protein (P_{1Ph}). Previously, it was shown that when SCN is lesioned, there is a loss of *per2* oscillation [226] in hippocampus. We, therefore, assume that SCN drives the circadian oscillation in hippocampus that might function as a master-slave oscillator. As a result, we explain the circadian variation of LTP/LTD through coupled GRN model of SCN and hippocampus as shown in Fig 6.1. However, there is no information about how SCN oscillator drives the hippocampal circadian oscillator and maintains the antiphase relationship between them. Therefore, to capture this effect, we include an intermediate protein R_{C1} that couples SCN to hippocampus. It was reported that cAMP response element binding protein (CREB) dependent gene expression play a vital role in circadian system and synaptic plasticity [227, 228]. Therefore, we include the effect of calcium on hippocampal GRN by incorporating the CREB dependent transcription of *per* gene. The full GRN model equations are given as:

$$\frac{d}{dt}M_{Ph} = A_s \left(v_{s2} \frac{K_{Ah}^{n_c}}{K_{Ah}^{n_c} + P_{1h}^{n_c}} - M_{Ph} \right) + v_{ss} \frac{R_{C1}^{n_c}}{K_c^{n_c} + R_{C1}^{n_c}} + K_{scr} S_{CREB} CREB \quad (6.10)$$

$$\frac{d}{dt}P_{1h} = A_s (M_{Ph} - P_{1h}) \quad (6.11)$$

$$\frac{d}{dt}P_{1Ph} = A_s (P_{1h} - P_{1Ph}) \quad (6.12)$$

$$\frac{d}{dt}R_{C1} = (P_{1ps} - R_{C1}) \quad (6.13)$$

$$\frac{d}{dt}S_{CREB} = a_r I_{pre} (1 - S_{CREB}) - a_d S_{CREB} \quad (6.14)$$

We estimate the parameters of hippocampus GRN model so that the oscillators in SCN and hippocampus are antiphase to each other as seen in the experiments [199, 158]. Parameter values are $A_s = 4.35e - 5 \text{ ms}^{-1}$, $v_{s2} = 20 \text{ nM}$, $K_{Ah} = 0.8 \text{ nM}$, $v_{ss} = 1e - 3 \text{ nMms}^{-1}$, $k_{scr} = 1e - 4 \text{ ms}^{-1}$, $K_c = 5 \text{ nM}$, $n_c = 9$, $a_r = 0.01 \text{ pA}^{-1} \text{ ms}^{-1}$, $a_d = 0.07 \text{ ms}^{-1}$.

We do also consider pre and post-synaptic neurons in hippocampus where stimulus current from pre-synaptic neurons triggers the post-synaptic neurons. Postsynaptic neuron contains both AMPAR and NMDAR, and they are activated by binding of neurotransmitter glutamate (Glu) [229]. NMDAR has a high permeability for calcium ion, but it is blocked by magnesium (Mg^{2+}) ion that prevents the calcium ion to enter through the receptor [230, 231]. Application of presynaptic stimulus current triggers the release of glutamate in postsynaptic neuron that

binds to NMDAR and results in a very low permeability of ion that passes through the channel due to the Mg^{2+} blockade [232, 233]. However, when the postsynaptic neurons are sufficiently depolarized, Mg^{2+} blockade is removed from the NMDAR and results in a free flow of calcium into the postsynaptic neuron. Therefore NMDAR acts as a coincident detector since both presynaptic and postsynaptic events are required for the opening of ion channels [234]. The current associated with the ligand (Glutamate) and voltage-gated NMDAR is given as:

$$I_{NMDA} = g_{NMDA} S_G B_v (v_{post} - v_{NMDA}) \quad (6.15)$$

Where v_{NMDA} is the reverse potential of NMDAR current and based on the literature [232, 219, 235] value is taken as 0. The factor B_v is the Mg^{2+} blockade of NMDAR as described by [236], and here we modify the expression as:

$$B_v = \frac{1}{1 + 0.005[Mg^+]e^{-0.2v_{postH}}} \quad (6.16)$$

The dimensionless parameter $[Mg^+]$ has a value of 1. g_{NMDA} is the circadian time dependent conductance associated with the NMDAR, and it is given as:

$$g_{NMDA} = g_N \frac{k_{norm}^{nm}}{P_{1ph}^{nm}} \quad (6.17)$$

We made this assumption based on experimental observation that NMDAR functions exhibit circadian variations [237–239, 198]. Here g_N is the maximal conductance of NMDAR and its value is $12nS$, k_{norm} has a unit of nM and its value is 1, and $n_m = 2$. The dimensionless gating variable S_G representing the fraction of glutamate binding site of NMDAR occupied at any given time. $S_G = 0$ in the absence of glutamate released from presynaptic neuron, and it has a non-zero value when I_{pre} is applied at presynaptic neuron, and its expression is given as:

$$\frac{dS_G}{dt} = a_r I_{pre} (1 - S_G) - a_d S_G \quad (6.18)$$

Where $a_r = 0.01$ and $a_d = 0.07$. For postsynaptic membrane potential (v_{postH}), we modified the ML model to include NMDAR evoked calcium current. Also, we modified the parameters in such a way that neuron produces an action potential when there is a postsynaptic current (I_{post}) applied for a duration of $2ms$.

$$\begin{aligned} C \frac{dv_{postH}}{dt} &= I_{post} - I_L - I_k - I_{Ca} - I_{NMDA} \\ &= I_{post} - g_L(v_{postH} - v_L) - g_K w(v_{postH} - v_k) - \\ &\quad g_{Ca} m_\infty(v_{postH} - v_{Ca}) - I_{NMDA} \end{aligned} \quad (6.19)$$

$$\frac{dw}{dt} = \lambda(w_\infty - w) \quad (6.20)$$

$$m_\infty = 0.5(1 + \tanh(\frac{v_{postH} - v_1}{v_2})) \quad (6.21)$$

$$w_\infty = 0.5(1 + \tanh(\frac{v_{postH} - v_3}{v_4})) \quad (6.22)$$

$$\lambda = \phi \cosh(\frac{v_{postH} - v_3}{2v_4}) \quad (6.23)$$

For the numerical simulation we have $C = 20\mu F/cm^2$, $g_L = 2\mu S/cm^2$, $g_K = 8\mu S/cm^2$, $v_k = -84mV$, $v_{Ca} = 120mV$, $v_L = -60mV$, $v_1 = -12mV$, $v_2 = 18mV$, $v_3 = 2mV$, $v_4 = 30mV$, $\phi = 0.08$. As discussed in the previous section, here also we incorporate the circadian rhythmicity in calcium dynamics by making the conductance of calcium channel dependent on hippocampus GRN variable P_{1ph} :

$$g_{Ca} = g_{Ca-base} \frac{P_{1ph}}{k_{P1ph} + P_{1ph}} \quad (6.24)$$

Where $g_{cabase} = 4.35 nS/cm^2$, $k_{P1ph} = 0.1 nM$. It has been shown that NMDAR are the significant source of Calcium and it is vital for LTP/LTD due to pre-post synaptic activity [240, 241]. Therefore, in our model calcium enters the post synaptic neuron through NMDAR and voltage gated calcium channel. The dynamics of calcium concentration is determined by the following equation:

$$\frac{dCa}{dt} = k_{NMDA} I_{NMDA} - k_{Ca} I_{Ca} + \frac{Ca_0 - Ca}{\tau_{Ca}} \quad (6.25)$$

Where, $Ca_0 = 500nM$, $k_{INMDA} = 1$, $k_{Ca} = 1$, $\tau_{Ca} = 10ms$.

The main procedure to evoke LTP/LTD involves an increase/decrease in the excitatory postsynaptic potential (EPSP) or the number of AMPAR within the postsynaptic membrane. EPSP defined as the potential due to excitatory postsynaptic current (EPSC), the current observed in response to the triggering of glutamate neurotransmitter [99]. In our model, calcium ion (Ca^{2+}) is the major input for synaptic plasticity. Therefore, current due to NMDAR (I_{NMDAR}) is treated as EPSC, and the voltage contributed by EPSC in postsynaptic potential (v_{postH}) is treated as EPSP. Thus dynamics of EPSP is therefore given as:

$$\frac{d}{dt}EPSP = I_{NMDA}/C \quad (6.26)$$

Previously, in a modeling study, Pi and Lisman defined EPSP as proportional to the number of AMPAR on the synaptic membrane [218], where they showed that at the moment of synaptic activation EPSP changed from one stable steady state to another. Similarly, in our model, EPSP defined in equation (6.26) is used to measure LTP/LTD, and this is qualitatively represent the LTP/LTD given in the literature [218, 242, 243].

Ca^{2+} is responsible for the insertion and removal of the AMPAR receptors in the neuronal membrane [241, 244], therefore we define the dynamics of AMPAR as a function of Ca^{2+} , which is given as:

$$\begin{aligned} \frac{d}{dt}AMPAR = & (a_{r1}(Ca - Ca_1)(A_m - AMPAR) - \\ & a_{d1}AMPAR(Ca - Ca_1))S_{AMPA} \end{aligned} \quad (6.27)$$

$$\frac{d}{dt}S_{AMPA} = a_{rs}I_{post}(1 - S_{AMPA} - a_{ds}S_{AMPA}) \quad (6.28)$$

Where, $a_{r1} = 1e-7$, $A_m = 1e4$, $a_{d1} = 1e-5$, $a_{rs} = 0.01$, $a_{ds} = 0.007$, $Ca_1 = 509.5$.

6.2.3 Model for circadian modulation of learning and memory at amygdala

It has been shown that *per* genes in SCN and amygdala peaks during day time [202] and are in phase with each other. We, therefore explain the circadian modulation of learning and memory through a coupled model of SCN and amygdala. We took the same GRN model of SCN as described in the previous subsection and the GRN model of amygdala again consists of three dynamical variables, namely *per* mRNA (M_{pa}), PER protein (P_{1a}), phosphorylated PER protein (P_{1pa}) and the intermediate protein R_{C2} which is responsible for coupling SCN to amygdala. The model equations are:

$$\begin{aligned} \frac{d}{dt}M_{pa} &= A_s(v_{s2} \frac{K_{Ah}^{n_c}}{K_{Ah}^{n_c} + P_{1a}^{n_c}} - M_{pa}) + v_{ss} \frac{K_I^{n_c}}{K_c^{n_c} + R_{C2}^{n_c}} \\ \frac{d}{dt}P_{1a} &= A_s(M_{pa} - P_{1a}) \end{aligned} \quad (6.29)$$

$$\frac{d}{dt}P_{1pa} = A_s(P_{1a} - P_{1pa}) \quad (6.30)$$

$$\frac{d}{dt}R_{C2} = (P_{1ps} - R_{C2}) \quad (6.31)$$

$$(6.32)$$

Parameter values are $A_s = 4.35e - 5 \text{ ms}^{-1}$, $v_{s2} = 20 \text{ nM}$, $K_{Ah} = 0.8 \text{ nM}$, $v_{ss} = 1e - 3 \text{ nMms}^{-1}$, $K_c = 5 \text{ nM}$, $n_c = 9$.

For postsynaptic membrane potential at amygdala (v_{postA}), we modify the ML model to include glutamate controlled NMDAR and AMPAR evoked ionic currents (I_{NMDA} , I_{AMPA}). Also, we modify the parameters in such a way that neurons produce an action potential when there is postsynaptic current (I_{post}) applied for a duration of 2 ms.

$$\begin{aligned} C \frac{dv_{postA}}{dt} &= I_{post} - I_L - I_k - I_{Ca} - I_{NMDA} - I_{AMPA} \\ &= I_{post} - g_L(v_{postA} - v_L) - g_K w(v_{postA} - v_K) - \\ &\quad g_{Ca} m_\infty(v_{postA} - v_{Ca}) - I_{NMDA} - I_{AMPA} \end{aligned} \quad (6.33)$$

$$I_{AMPA} = g_{AMPA} S_G(v_{postA} - v_{AMPA}) \quad (6.34)$$

For the numerical simulation we have $C = 20 \mu F/cm^2$, $g_L = 2 \mu S/cm^2$, $g_K = 8 \mu S/cm^2$, $v_K = -84 \text{ mV}$, $v_{Ca} = 120 \text{ mV}$, $v_L = -60 \text{ mV}$. Here v_{AMPA} is the reverse potential of AMPAR current and its value is taken as zero [219, 235].

It was reported that AMPAR is transported into and out of synapse during learned responses and it strengthens or weakens the synaptic function [245]. Rumpel et al. reported that at amygdala, fear conditioning pushes AMPAR into the synapse of post-synaptic neuron and hence mediate the encoding of memories [246]. Clem and Hugarir found that extinction induced erasure of memory at amygdala was due to the synaptic removal of AMPAR [247]. Therefore, in our model, we assume that AMPAR will be recruit to synaptic membrane during acquisition and removed during extinction. We include the dynamics of addition and

Table 6.1 Model equations and parameters for amygdala

Equations	Parameters
$I_{NMDA} = g_{NMDA} S_G B_v (v_{postA} - v_{NMDA})$ $g_{NMDA} = g_N \frac{k_{norm}^{n_m}}{P_{1pa}^{n_m}}$ $B_v = \frac{1}{1 + 0.005 [Mg^{+}] e^{-0.2 v_{postA}}}$ $\frac{d}{dt} S_G = a_r I_{pre} (1 - S_G) - a_d S_G$ $\frac{dw}{dt} = \lambda (w_{\infty} - w)$ $m_{\infty} = 0.5 (1 + \tanh(\frac{v_{postA} - v_1}{v_2}))$ $w_{\infty} = 0.5 (1 + \tanh(\frac{v_{postA} - v_3}{v_4}))$ $\lambda = \phi \cosh(\frac{v_{postA} - v_3}{2v_4})$ $g_{Ca} = g_{cabase} \frac{M_{pa}}{k_{pa} + M_{pa}}$ $\frac{d}{dt} Ca = k_{NMDA} I_{NMDA} - k_{Ca} I_{Ca} + \frac{Ca_0 - Ca}{\tau_{Ca}}$ $\frac{d}{dt} EPSP = I_{NMDA} / C$ $\frac{d}{dt} S_{AMPA} = a_{rs} I_{post} (1 - S_{AMPA}) - a_{ds} S_{AMPA}$	$v_{NMDA} = 0 \text{ mV}, g_N = 12 \text{ nS}, k_{norm} = 1 \text{ nM}$ $n_m = 2, M g^{+} = 1$ $a_r = 0.01 \text{ pA}^{-1} \text{ ms}^{-1}, a_d = 0.07 \text{ ms}^{-1}$ $v_1 = -1.2 \text{ mV}, v_2 = 18 \text{ mV}, v_3 = 2 \text{ mV}$ $v_4 = 30 \text{ mV}, \phi = 0.08$ $g_{cabase} = 5.3 \text{ nS}, k_{pa} = 0.01 \text{ nM}$ $Ca_0 = 500 \text{ nM}, k_{NMDA} = 10, k_{Ca} = 1$ $\tau_{Ca} = 10 \text{ ms}$ $a_{rs} = 0.01 \text{ pA}^{-1} \text{ ms}^{-1}, a_{ds} = 7e - 4 \text{ ms}^{-1}$

removal of AMPAR in the synapse as a function of both calcium and circadian variables:

$$\begin{aligned} \frac{d}{dt} AMPAR = & A_{MS} ((a_{r1} (Ca - Ca_1) (A_m - AMPAR) - \\ & a_{d1} AMPAR (Ca - Ca_1)) S_{AMPA}) + \\ & P_{1pa}^{s_s} a_{remov} (\frac{I_{pre}}{1 + I_{pre}}) (1 - AMPAR) \end{aligned} \quad (6.35)$$

$$A_m = A_{MS} A_{mx} \quad (6.36)$$

$$A_{MS} = \frac{k_{am1}^{q_q}}{P_{1pa}^{q_q}} \quad (6.37)$$

Where $a_{r1} = 1e - 7 \text{ nM}^{-1} \text{ ms}^{-1}$, $A_m = 1e4$, $a_{d1} = 1e - 5 \text{ nM}^{-1} \text{ ms}^{-1}$, $Ca_1 = 509.5 \text{ nM}$, $A_{mx} = 2500$, $k_{am1} = 1.3 \text{ nM}$, $q_q = 2$, $s_s = 2$.

It was reported that AMPAR levels are high during wake and low during sleep [248], which indicates that AMPAR functioning is also circadian dependent. Therefore we modeled the cumulative AMPAR conductance of postsynaptic neuron (g_{AMPAR}) as a function of circadian variable P_{1pa} , and it is given as:

$$g_{AMPAR} = g_{AM} \frac{k_{am1}^{r_r}}{P_{1pa}^{r_r}} AMPAR \quad (6.38)$$

Where $g_{AM} = 0.2 \text{ nS}$, $k_{am1} = 1.3 \text{ nM}$, $r_r = 3$. We consider AMPAR conductance, g_{AMPAR} , to compare our model output with the experimental data and for model validation. Remaining model equations are the same as that discussed in the previous sections; equations and parameter values are given in the Table6.1.

Algorithm-1: Solve the multi-scale coupled model
at desired circadian time to get SFR, LTP, LTD,
acquisition, recall, and extinction

Input: multi-scale coupled model

Input: step size (δt), δt_1 , δt_2 ($\delta t_1 \gg \delta t_2$)

Input: desired circadian time, t_{des}

Input: time duration for calculation (t_{dur})

Input: presynaptic current, I_{pre}

Input: postsynaptic current, I_{post}

Input: time at which I_{pre} applied, t_{pre}

Input: time at which I_{post} applied, t_{post}

If $t \leq t_{des}$

$\delta t = \delta t_1$

$$\left. \begin{array}{l} I_{post} = 0 \\ I_{pre} = 0 \end{array} \right\} \begin{array}{l} \text{for LTP, LTD,} \\ \text{acquisition, recall, extinction} \end{array}$$

else if $t > t_{des} \ \& \ t < (t_{des} + t_{dur})$

$\delta t = \delta t_2$

$$\left. \begin{array}{l} I_{post} > 0 \quad \text{for } t_{post} < t < (t_{post} + 2ms), \\ I_{pre} > 0 \quad \text{for } t_{pre} < t < (t_{pre} + 2ms) \end{array} \right\} \begin{array}{l} \text{for LTP, LTD,} \\ \text{acquisition} \\ \text{for recall,} \\ \text{extinction} \end{array}$$

end if

$$\left. \begin{array}{l} v' = f(I), \quad \text{for SFR} \\ g_{AMPAR} = f(I_{pre}, I_{post}) \end{array} \right\} \begin{array}{l} \text{for LTP, LTD,} \\ \text{acquisition, recall, extinction} \end{array}$$

6.2.4 Numerical simulation

There are two time scales in the model; circadian gene regulatory model with a slow time scale of 24h, and the conductance model with a time scale of milli-second (ms). We first convert all the circadian dynamics from hours to ms time scale by scaling the GRN model (by parameter A_s in all the GRN equations given in the supporting information). This involves integrating the equations for a long time to get the desired dynamics and leads to a generation of huge amount of data and long time to complete the simulation. Therefore, instead of

performing numerical simulations of SFR, LTP/LTD, acquisition, recall and extinction simulation continuously for 1 circadian cycle, we evolve the equations by integrating the model with two different time steps; one long time step to simulate slow varying circadian dynamics and another with small time step to simulate the conductance model only for the desired circadian phases to get the corresponding voltage dynamics. Initially, we run the simulation with a long time step ($\delta t_1 = 10000ms$, $(0.0028h)$) till we reach the desired circadian phase t_{des} at which we calculate the voltage dynamics. Once we reach $t = t_{des}$, we reduce the time step to $\delta t_2 = 0.01ms$ and run the simulation till the desired time duration (t_{dur}). Reducing δt_1 (long time steps) from $10000ms$ to $1000ms$ and δt_2 (short) from $0.01ms$ to $0.001ms$ yields the same result in our simulation. The summary of the procedure for solving multi-scale coupled oscillatory model is given as Algorithm-1.

We use Xppaut [119] for numerical simulation, and the programs along with the link to the figures are printed in the Appendix-D.4.

6.3 Results

In this section we provide the results of the numerical simulation of the models for the circadian modulation of SFR at SCN, LTP/LTD including STDP at hippocampus, and the fear conditioning paradigm at amygdala. We also compare all the in-silico results with the experiments that are available.

6.3.1 Circadian modulation of SFR at SCN

Codimension-1 bifurcation diagram of the GRN model is shown in Fig 6.2A, with light L as the bifurcation parameter. For lower values of L , the model exhibit sustained oscillations, and when increased further, the system enters into a stable steady state (red line) via Hopf bifurcation, where the unstable steady state (black line) is surrounded by the stable limit cycle. We also similarly construct the codimension-1 bifurcation diagram for modified ML model with g_{Ca} as the bifurcation parameter (Fig6.2B). For small values of g_{Ca} , the system is in stable steady state (Fig6.2B red line) and as g_{Ca} increases stable steady state is approached by an unstable stable steady state (black line), they merge each other, and oscillations are arise (green broken line) via saddle-node of infinite period/on invariant cycle (SNIC) bifurcation [107, 249]. Period of oscillation near SNIC is very large (frequency is small, Fig6.2 C). As g_{Ca} increases, period of oscillation decreases greatly (frequency increases, Fig6.2 C). Further increase in g_{Ca} leads to loss of periodic orbits through subcritical Hopf

bifurcation (HB) and the system enters the stable steady state. We use Xppaut [119] to simulate all the bifurcation diagrams.

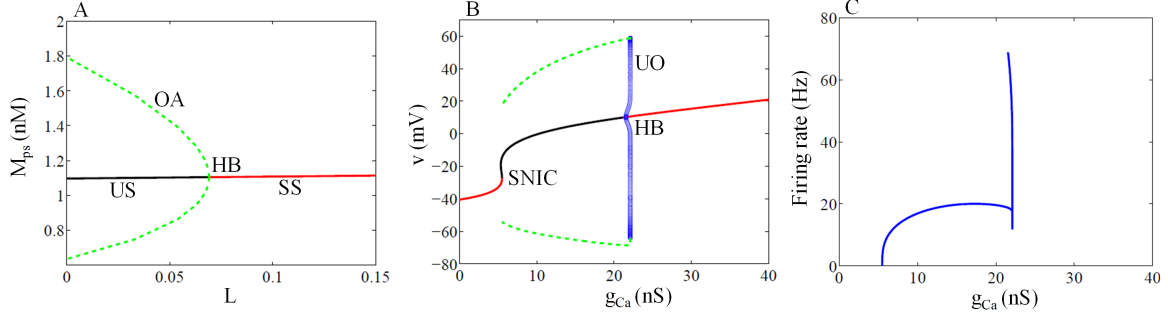


Fig. 6.2 Bifurcation diagram for Goodwin and ML model. (A) Codimension-1 bifurcation diagram of GRN with L as the bifurcation parameter. At lower values of L , the system shows sustained oscillation. Broken green lines are the amplitude of the oscillation (OA), and black lines are the unstable steady state (US). As light intensity increases, sustained oscillation disappears via supercritical Hopf bifurcation (HB), and the system enters the stable steady state (red lines, SS). (B) Codimension-1 bifurcation diagram of modified ML model with g_{Ca} as bifurcation parameter. For lower values of g_{Ca} , the system is in stable steady states (red line). As g_{Ca} increases stable steady state and unstable steady state merge each other and oscillations arise via SNIC bifurcation. Stable oscillations disappeared via subcritical Hopf bifurcation (HB) at $g_{Ca} = 21.5 \text{ nS}$. Blue circles are unstable oscillation amplitude (UO). (C) Firing rate as a function of g_{Ca} . When $g_{Ca} = 5.49 \text{ nS}$, ML model starts firing with a frequency less than 0.5 Hz, when $g_{Ca} = 6.35 \text{ nS}$, its firing rate increased to 10 Hz, and when $g_{Ca} > 6.35 \text{ nS}$, firing rate become greater than 10Hz. Here we take $g_{Ca} = g_{Ca_{base}}$, the rest of the parameter values are the same as described in materials and method section. Xppaut [119] was used for simulating the bifurcation diagram.

We choose the parameter $g_{cabase} = 6.37 \text{ nS}$ for the conductance model, and when coupled with GRN, it produces firing pattern in the frequency range from 0.5 Hz to 8.5 Hz as observed experimentally in SCN [191]. To simulate the firing patterns at different circadian phases, we couple the mRNA (M_{Ps}) of GRN model to the conductance (g_{Ca}) of ML model that modulates calcium conductance at different phases. The coupling term is added on the similar lines as in [99]. In the simulation, peaking of M_{Ps} is taken as CT8, which is the subjective day. We capture the firing rate that varies over 1 circadian cycle. We show in Fig 6.3A-I, the modulation of firing rate by GRN for 1s at different circadian phases in one cycle. For example, the number of spikes at CT18 (night) is less compared to CT6 (day), which agrees with the experimental findings of [191] that SFR is higher during the day than the night. This important result clearly indicates that the circadian rhythm regulates the firing patterns. SFR as a function of circadian time is shown in Fig 6.3J, where firing rates show circadian variation that peaks during the day and reach minimum during the night. This is in good

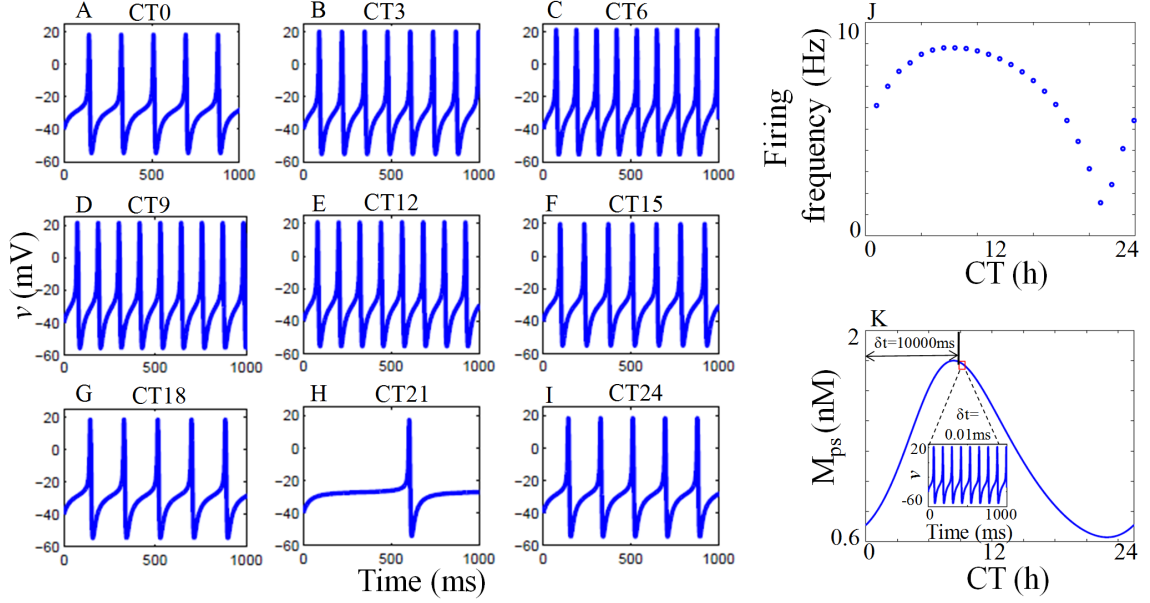


Fig. 6.3 Circadian variation of firing frequency. (A-I) Time series of membrane voltage at different circadian phases. The number of spikes generated during the middle of subjective day (CT6) is higher in comparison to the middle of subjective night (CT18). A minimum number of spike is also observed during subjective night (CT21). (J) SFR as a function of circadian time. Firing rates show circadian variation that peaks during the day and reaches minimum value during the night, and this is in good agreement with the experimental results [191]. (K) Numerical simulation for SFR as described in the Algorithm-1. Here desired circadian time is CT9. From CT0 to CT9, the step size is $1000ms$, and at CT9, the step size is $0.01ms$ for $1000ms$, and the corresponding firing patterns obtained is used to calculate the firing frequency at CT9.

agreement with the experimental results [191]. Fig 6.3K shows the numerical simulation for SFR as described in Algorithm-1. Here, the desired circadian time is CT9. From CT0 to CT9 a step size of $1000ms$ is taken, and at CT9, the step size is reduced to $0.01ms$ for $1000ms$ to get the firing patterns from which the firing frequency at CT9 is calculated.

6.3.2 Circadian modulation of LTP/LTD at hippocampus

Spike time dependent plasticity (STDP) without GRN

In this section, we explain the pre and post-synaptic activity-dependent plasticity, STDP, through our model. STDP links the time difference between pre and postsynaptic spikes ($\Delta t = t_{post} - t_{pre}$) to synaptic changes (*EPSP*). If the presynaptic stimuli (I_{pre}) applied at t_{pre} and postsynaptic spike generated by the application of current (I_{post}) at t_{post} , it evokes

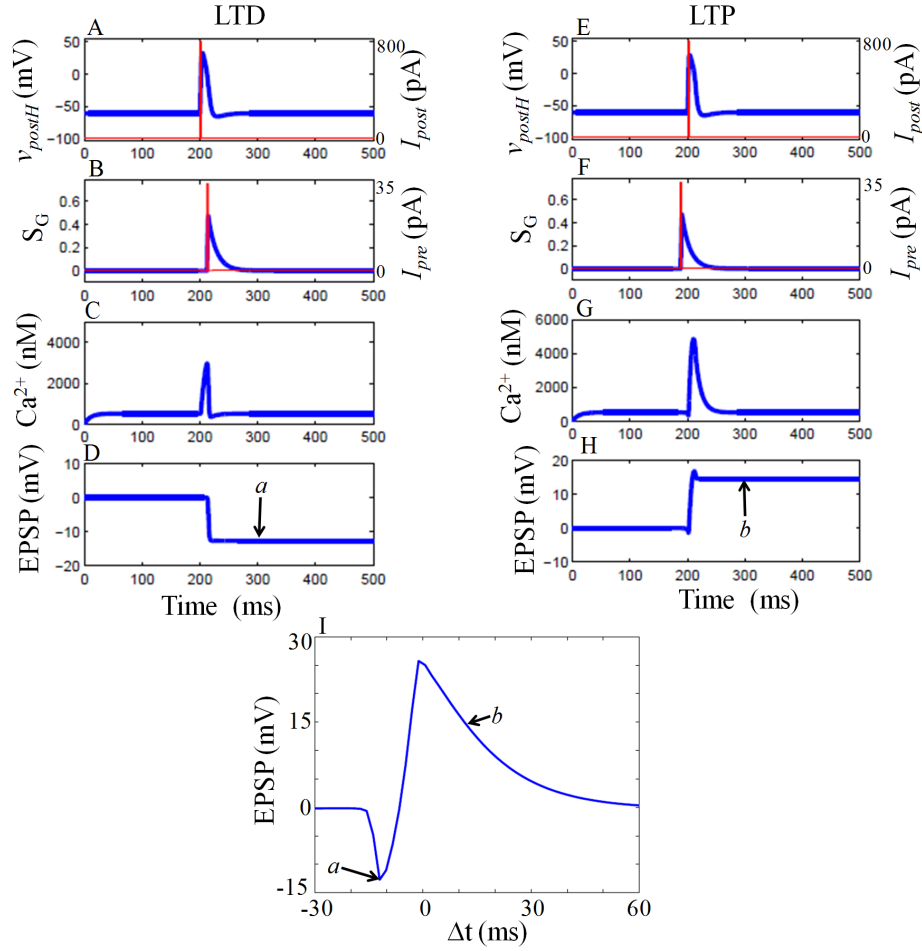


Fig. 6.4 Time series of variables for STDP without GRN. In simulation, we apply I_{post} at $t = 200ms$ (A, E, red line), which induce a spike at post-synaptic neuron (A, E, blue line). (B) When $\Delta t = -12ms$, I_{pre} is applied at $t = 212ms$ (red line). Glutamate is released, and only a fraction of glutamate binds to NMDAR (blue line). (C) Dynamics of the Ca^{2+} . (D) Moderate increase of Ca^{2+} level induce a negative change in the EPSP, which is the measure of LTD. (F) When $\Delta t = +12ms$, I_{pre} applied at $t = 188ms$ (red line), which trigger glutamate release and fraction of glutamate that binds on NMDAR (blue line). (G) Dynamics of Ca^{2+} . In comparison to previous case in (C), increment of Ca^{2+} level is higher that results in a positive change in the EPSP (H), a measure of LTP. (I) EPSP as a function of Δt . LTP and LTD occur when Δt is positive or negative respectively. If the temporal difference between I_{pre} and I_{post} is large, then no LTP/LTD happens. EPSP value obtained at $t = 300ms$ (a , b) is used to construct STDP curve. The magnitude of the I_{pre} and I_{post} are 35 pA and 800 pA respectively, and the duration of the current is 2ms. Here we take $g_{NMDA} = g_N$ and $g_{Ca} = g_{cabase}$.

NMDAR dependent calcium dynamics and induces synaptic plasticity. This is shown in Fig 6.4. When Δt is negative, I_{pre} is applied after I_{post} (Fig 6.4A, B). I_{pre} induces glutamate release at pre-synaptic neuron and I_{post} induces action potential at post-synaptic neuron (Fig

6.4A). Glutamate binds to NMDAR, and the percentage of glutamate that binds to NMDAR shown in Fig 6.4B. This allows NMDAR current to flow to elevate Ca^{2+} to a moderate level (Fig 6.4C) and as a result, induces a negative change in the *EPSP* (Fig 6.4D). Similarly, when Δt is positive, presynaptic spikes arrive before postsynaptic spikes (Fig 6.4E, F). It is clear that the percentage of glutamate starts to bind to the NMDAR (Fig 6.4F) after the postsynaptic spike arrives and induces a higher level of calcium (Fig 6.4G) that in turn cause a positive change in the *EPSP* (Fig 6.4H). The overall change in the *EPSP* as a function of Δt is shown in Fig 6.4I. Pre-synaptic spiking happens before or after the postsynaptic spiking results in a maximal EPSP change, while no EPSP change take place if the time difference between them is larger.

Circadian variation of LTP/LTD under DD condition

Experimental observations suggest that hippocampal LTP/LTD, learning and memory are regulated by circadian rhythm [31, 48]. Rawashdesh et al. [201] suggest that *per1* controls hippocampal rhythm and memory. Wang et al. [199] reported the rhythmic expression of *per2* mRNA and protein at hippocampus. Therefore we speculate that there is a direct link between SCN and hippocampus that regulate LTP/LTD, learning and memory. In our model, we introduce Goodwin type model for SCN that diffusively couples to hippocampus GRN via an intermediate neurotransmitter that is yet unknown. The free running oscillations of SCN and hippocampus GRN is shown in Fig 6.5. We fit the model to the experimentally observed *per2* mRNA and protein data [158, 199]. Under DD condition ($L = 0$), both the oscillators have a free running period of 23.6h, but the hippocampal oscillation is antiphase with SCN. This is in good agreement with the experimental observation [199]. In our model, we assume that NMDAR conductance is controlled by the hippocampal GRN variable P_{1Ph} . The resulting oscillations of other variables in the absence of pre-postsynaptic activities are shown in Fig 6.6.

Now we simulate the circadian dependents of LTP and LTD by introducing pre and postsynaptic spikes. We calculate the EPSP change at two different circadian phases, one at subjective day (CT6) and the other at subjective night (CT18). LTP at subjective night is higher than that of during the subjective day (Fig 6.7A) as seen in the experiments [48]. Again we simulate the complete STDP curve that also shows circadian variation (Fig 6.7B). Maximum LTP that occurs at subjective night is larger in comparison to LTP during subjective day.

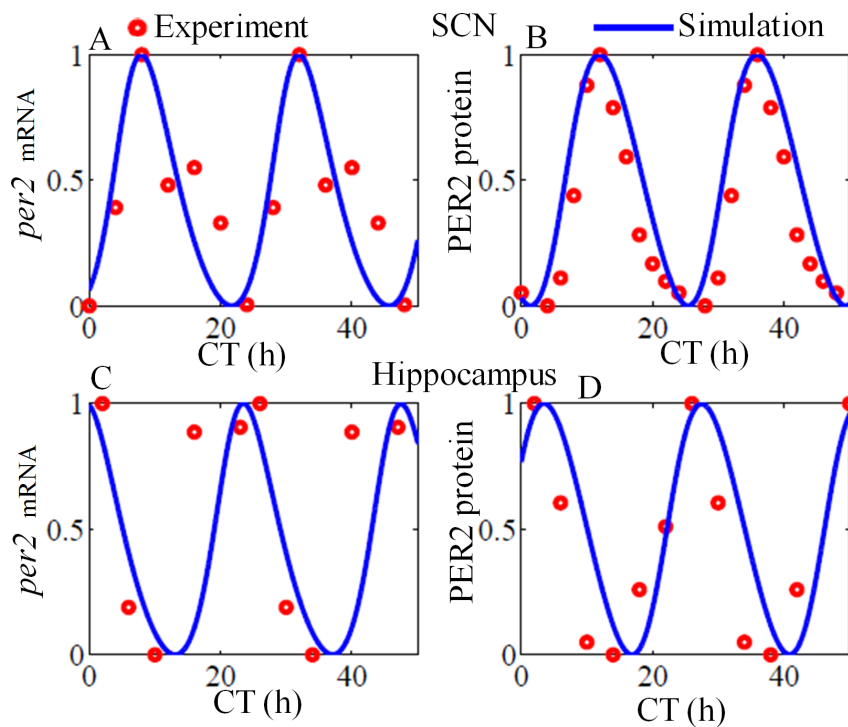


Fig. 6.5 Free running oscillation at SCN and hippocampus under constant darkness condition. Blue curves are from simulation, and red circles are the experimental data points. Simulated *per2* mRNA and protein at SCN (A, C) and hippocampus shows antiphase oscillations, which is good agreement with the experimental observations. Simulation results were obtained by integrating the model equations given in materials and method section with $I_{pre} = 0$. For comparison, the individual time series were normalized to $[0, 1]$. Experimental data points of *per2* mRNA and protein at SCN are extracted from [158] and for hippocampus, the data is extracted from [199].

Circadian variation of LTP/LTD under LD condition

We test the synaptic plasticity under LD conditions with different photoperiods. We compute STDP under different photoperiods ((Fig 6.8), and in all the photoperiods that we consider, the maximum of LTP amplitude occur during night (ZT18), which is larger in comparison to the day (ZT6). This is in good agreement with that experimental results [48], which shows the LTP magnitude is higher at night than during day.

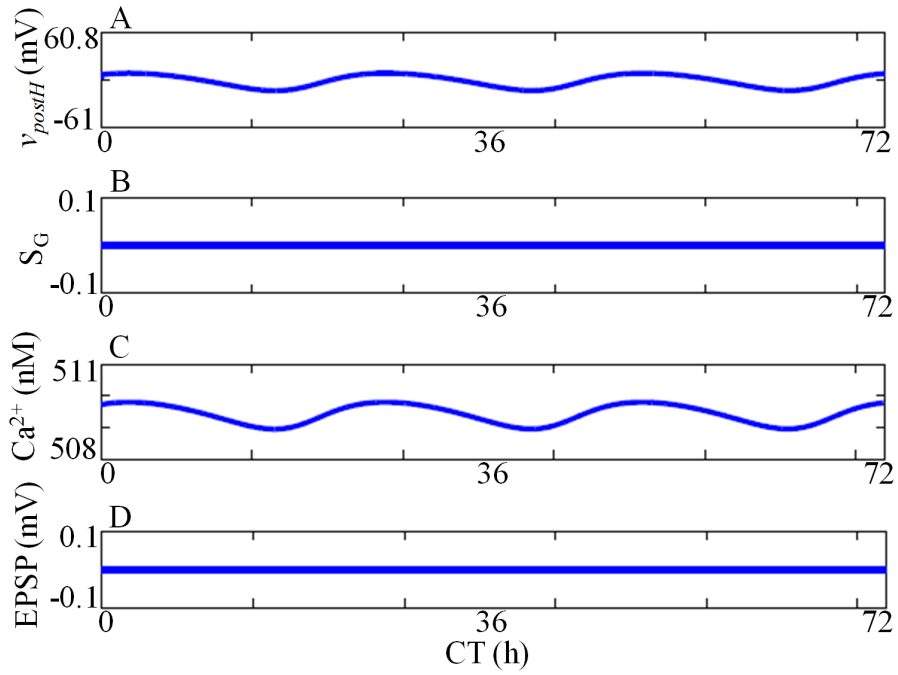


Fig. 6.6 Circadian variation of SCN-hippocampus model variables without any pre-post activity. v_{postH} and calcium concentrations shows circadian variation (A, C), and the corresponding time series of S_G (B) and EPSP (D).

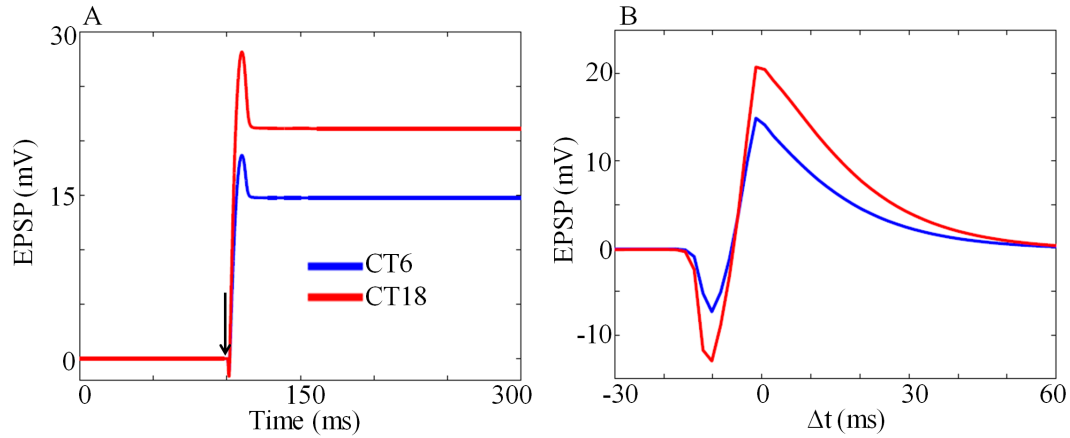


Fig. 6.7 LTP at two different circadian times. (A) $EPSP$ as a function of time at subjective day (CT6) and subjective night (CT18), where $\Delta t = 0$. Larger LTP in simulation seen during subjective night is in good agreement with the experimental results [48]. Arrow head indicate the time at which I_{post} and I_{pre} are applied. (B) $EPSP$ as a function of Δt . The magnitude of the I_{pre} and I_{post} are 35 pA and 800 pA respectively, and the duration of the current is 2ms.

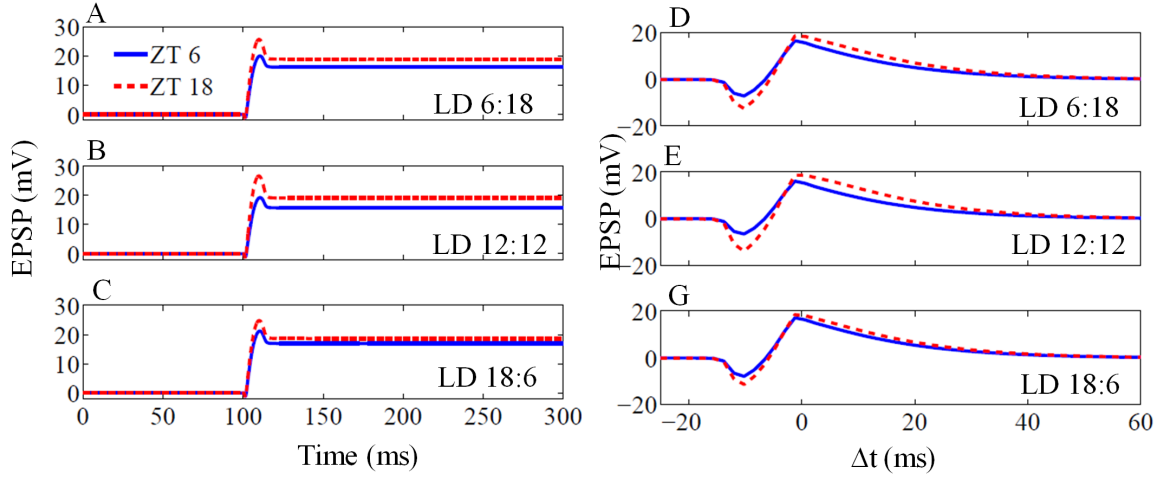


Fig. 6.8 LTP and STDP under different LD cycles. (A-C) LTP for ZT6 (blue line) and ZT18 (red broken line) under different LD cycle with $\Delta t = 0$. For all LD case, LTP during subjective night (ZT18) is higher than subjective day (ZT6). (D-E) STDP curves obtained at ZT6 and ZT18 under different photoperiods. For all the LD case, the magnitude of EPSP amplitude during subjective night is higher than the subjective day.

6.3.3 Circadian modulation of learning and memory at amygdala

Circadian rhythm modulates Acquisition

We test whether our model can learn to simulate the fear conditioning protocol. We first explain the parameters that we use in the model to simulate the CS-US pairings and a unique parameter that we use to capture the percent freezing response in the experiments. Here, we take the spike at the postsynaptic neuron (v_{postA}) responsible for fear response. We consider postsynaptic stimulus (I_{post}) as US, which induces spike in postsynaptic neuron. We take CS as the combination of pre-synaptic stimulus (I_{pre}) and I_{post} to train the postsynaptic neuron to generate a spike in an associative fashion. Whenever I_{pre} is applied, pre-synaptic neuron release glutamate that binds to both NMDAR and AMPAR. I_{post} increase the membrane potential in postsynaptic neuron and produces a spike. If we apply I_{pre} and I_{post} simultaneously, NMDAR conduction induces more amount of Ca^{2+} , and this NMDAR evoked calcium current in turn activate more amount of AMPAR, which eventually increase the AMPAR conductance (g_{AMPA}) to increase the overall synaptic strength. Thus, the paired exposure of CS and US cause LTP, the electrophysiological measure of learning and memory. In the model, we take AMPAR conductance, g_{AMPA} , to compare with the freezing percentage of experimental fear response data.

In experiments conducted with C-3H mice to find the circadian modulation of acquisition, Chaudhury et al. [31] applied 6 pairs of CS-US with an inter-trial interval of 64s. To determine the degree of learning, they calculated the percentage of freezing. In that experiment, US was taken as 1mA footshock and CS was taken as the tone or context. To mimic the experimental procedure in [31] in our simulation, our model training protocol consists of six I_{pre} - I_{post} pairing with 35 pA I_{pre} and 400 pA I_{post} with an inter-trial interval of 500ms. We take the g_{AMPA} level to calculate the degree of learning (acquisition) as shown in Fig6.9. Circadian variation of g_{AMPA} under 12:12 LD cycle is shown Fig6.9A. Application of I_{pre} - I_{post} increases AMPAR, and hence the g_{AMPA} . Enlarged view of g_{AMPA} during training is shown in Fig6.9B. To determine the degree of learning, we calculate the normalized g_{AMPA} value for each training sequence (Fig6.9C) by subtracting the baseline value from the original value we obtain after the training. Normalized g_{AMPA} value a' , b' , c' , d' , e' , f' in Fig6.9C are the corresponding normalized values of a , b , c , d , e , f in Fig6.9B.

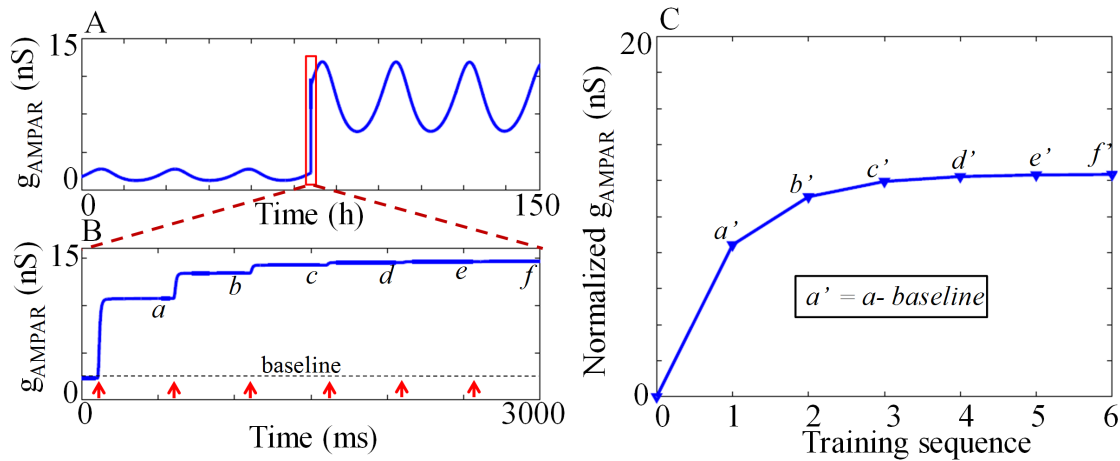


Fig. 6.9 Dynamics of g_{AMPA} . (A) g_{AMPA} as a function of time, which varies in a circadian manner. Model is trained by applying 6 pairs of I_{pre} (CS) and I_{post} (US) at CT3 (CT 75 *mod* 24), which increases the g_{AMPA} level. (B) Enlarged view of the application of 6 pairs of I_{pre} and I_{post} with an inter-trial interval of 500ms. Red arrows indicate the time at which each I_{pre} - I_{post} pair we apply. To determine the degree of learning, we use g_{AMPA} level at the end of the each inter-trial interval (a , b , c , d , e , f). (C) Normalized g_{AMPA} value for each training sequence. a' , b' , c' , d' , e' , f' are the corresponding normalized values of a , b , c , d , e , f in (B). Normalized g_{AMPA} is obtained by subtracting the baseline value from the original value.

To test the circadian rhythm during acquisition, we conduct 3 experiments as shown in Fig 6.10. In the first experiment, we subject the model to 12:12 LD cycle and then we apply CS-US pair at ZT3 (day) and another at ZT15 (night). ZT0 is the taken as light onset. The degree of acquisition captured by g_{AMPA} shows high increment in conductance when we train during subjective day (Fig 6.10A). In the second experiment, we force the model to

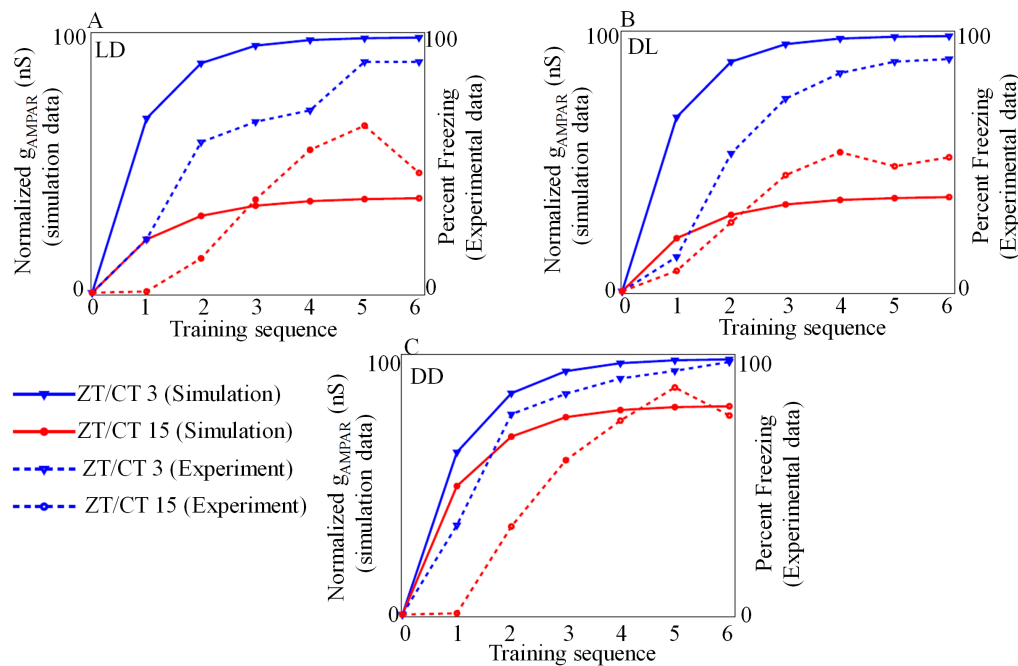


Fig. 6.10 Acquisition. Model is trained by applying I_{pre} (CS) and I_{post} (US) either in the day (ZT/CT 3) or in the night (ZT/CT 15). The model trained in the daytime acquire better synaptic strengthening (g_{AMPA} increment) than trained at night. (A) Model forced by 12:12 LD cycle (B) model forced by 12:12 DL cycle (C) model is under DD condition. In all cases I_{pre} , I_{post} are 35mA, 400mA, respectively with a duration of 2ms. Normalized g_{AMPA} is obtained by subtracting the baseline value from the original value and minimum value of g_{AMPA} is normalized to 0 and maximum value is normalized to 100. This enabled better comparison of experimental and simulation data points, and it is clear that degree of acquisition is more during day. Baseline value for ZT/CT3 is 2.3 and for ZT/CT15 is 1.3. Experimental data points are extracted from [31]

12:12 DL cycle (reversed LD), and we find that the degree of acquisition is still higher during the subjective day (Fig 6.10B) as seen in the 12:12 LD cycle. To test whether the acquisition is modulated by circadian rhythm or controlled by only LD cycle, we again train the model under DD condition, and the results are shown in Fig 6.10C. In DD condition, from simulation, we find the acquisition is higher during the subjective day (CT3) than during the subjective night (CT15). Even though our model fails to capture the exact trend in the degree of acquisition as seen in the experiments [31], qualitatively the trend is captured it is clear that acquisition is modulated by circadian rhythm.

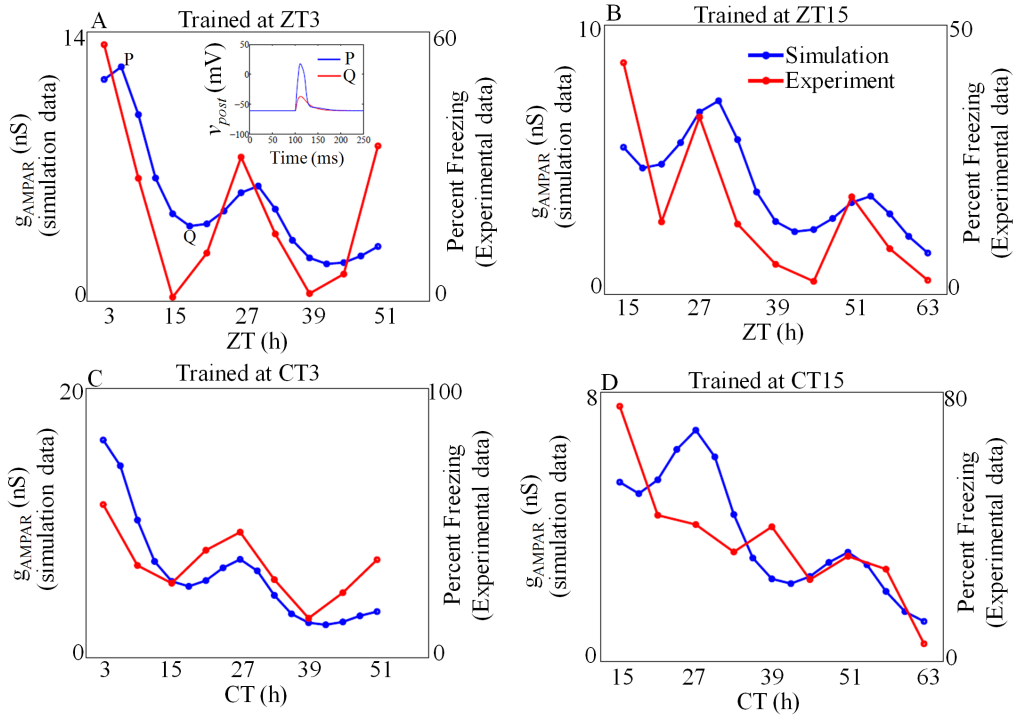


Fig. 6.11 Recall. The model trained either in the day (ZT/CT 3) or night (ZT/CT 15) is tested for recall. The model shows better recall during the day, irrespective of the time of training. (A, B) Model forced by 12:12 LD cycle (C, D) model is under DD condition. Sample spikes generated during the recall test is shown in the inset of A. For training six pairs of I_{pre} and I_{post} were applied and for recall, a single I_{pre} pulse was applied. Magnitude of I_{pre} and I_{post} are 35mA, 400mA respectively with a duration of 2ms. Experimental data points are extracted from [31]

6.3.4 Circadian rhythm modulates Recall

After conditioning, we test whether the model can simulate recall at different circadian phases. Before conditioning, postsynaptic neuron initially responds to the I_{post} (US) by producing an action potential. After conditioning the neuron, it eventually respond to I_{pre} (CS). However, the efficiency of response (recall) is varied as a function of the time-of-the-day in which the recall test is performed. In the recall test, we apply only I_{pre} to generate a spike in the post-synapse, and the corresponding g_{AMPA} values are shown in Fig 6.11. In the first test, when we train the model at ZT3 under 12:12 LD cycle, we test for recall every 3h starting from 24h after the training (Fig 6.11). The g_{AMPA} value peaks during the daytime in each cycle (ZT6, ZT30) and reaches minimum value during the night time (ZT18, ZT42). Two sample spikes generated during recall test is shown in Fig 6.11A inset. A proper spike is generated at ZT6, but only a subthreshold spike is generated at ZT18, and this indicates that

our model obtain better recall during the daytime. We also simulate the recall test trained during the night under 12:12 LD cycle and the results are shown in Fig 6.11B. Again, the maximum values of g_{AMPA} are seen during the daytime, and we only obtain minimum values during the night time. In order to test whether the recall is modulated by circadian rhythm or controlled by only LD cycle, we train and test the model only under DD conditions, and the results are shown in Fig 6.11C, D. When we conduct the recall test at different circadian time, one at subjective day (CT3) and the other at subjective night (CT15), recall process still persists with greater degree during the daytime (Fig 6.11C, D).

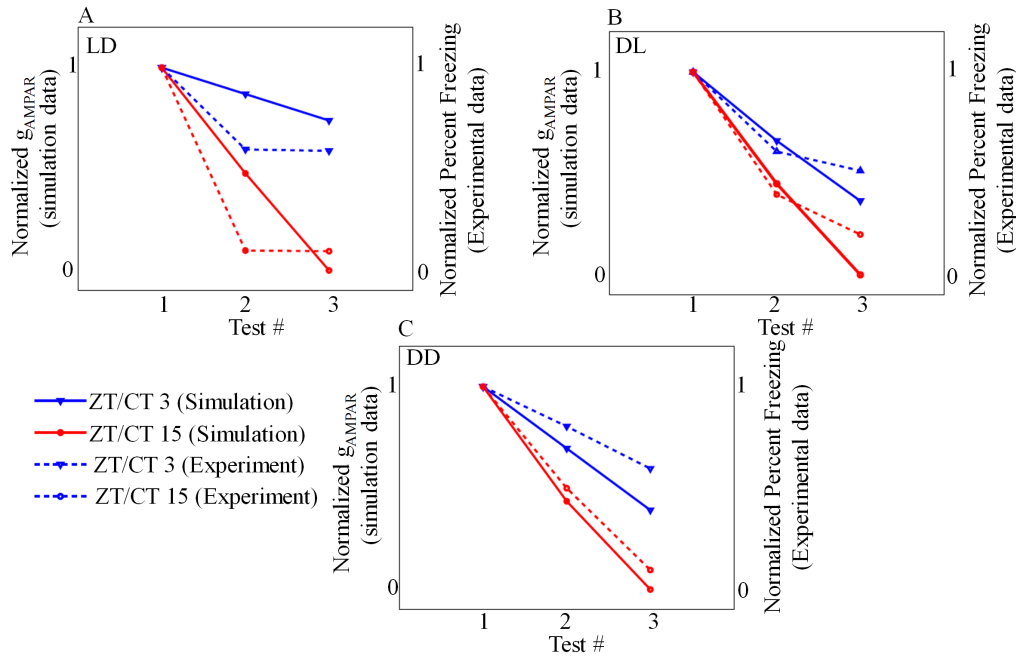


Fig. 6.12 Extinction. Model is trained and tested for extinction either in day or night. Degree of extinction is higher in model trained and tested at night (A-C, red triangle). (A) Model forced by 12:12 LD cycle (B) model forced by 12:12 DL cycle (C) model is under DD condition. For comparison, g_{AMPA} values obtained after the first extinction test at day and night are normalized to 1 and minimum value is normalized to 0. It is clear that degree of extinction is more during night. Experimental data points are extracted from [31].

6.3.5 Circadian rhythm modulates Extinction

Repeated application of CS will decrease the synaptic strength, and as a result, it decreases the conditioned response. We test in our simulation the extinction property by applying repeated I_{pre} at different time of the day, and the results are shown in Fig 6.12. In the first test, we train and test the model either during the day (ZT3) or night (ZT15) under LD

conditions. We found that the model trained and tested during night shows a greater degree of extinction (Fig 6.12A). We obtain similar results even in the reversed LD cycle (DL) (Fig 6.12 B). Finally, when we train and test the model during subjective night, the model exhibit greater degree of extinction under DD condition (Fig 6.12 C). These results once again reinforce that extinction modulated by circadian rhythm is higher during the night, and this is in confirmation with the experiments [31]. Even though our model fails to reproduce the exact trend in the degree of extinction as seen in the experiments [31], the trends in the simulation is similar to the one seen in the experiments. These results suggest that extinction is modulated by circadian rhythm.

6.4 Discussion and conclusion

In this work, through multi-scale modeling, we showed that the circadian rhythm modulates learning and memory. Previous studies have shown that SFR at SCN exhibited circadian oscillation with a period close to 24h. In this study, we incorporated both GRN and conductance model of SCN to show the circadian modulation of SFR. Importantly, we showed the circadian modulation of voltage-gated calcium current (VGCC) played an important role in the modulation of SFR that peaks during day and reach nadir during night.

Though the impact of circadian rhythm on LTP/LTD and memory has long been studied from the molecular mechanism point of view, it is not well understood how circadian rhythms modulated LTP/LTD, and memory formation. However, previous studies [199–201] provided information about how *per* genes (*per1*, *per2*) influenced the synaptic modification and acted as a molecular link between circadian rhythms and synaptic plasticity. In this work we related *per2* in the hippocampus with synaptic plasticity and obtained experimentally observed circadian phase-dependent variation of LTP/LTD under both DD and LD conditions. In our model, calcium that entered the postsynaptic neuron through NMDAR is the key signal for the simulation of synaptic plasticity. Another important regulation we made in our model is that the conductance of the NMDAR channel is controlled by the hippocampal *per2*. Thus the Ca^{2+} dynamics due to the pre and postsynaptic activity is made circadian phase-dependent with the result that it played a strong role in synaptic plasticity.

Based on earlier works, we considered SCN and hippocampus as a master-slave oscillator. However, hippocampal circadian genes showed anti-phase oscillation with respect to SCN GRN's. Presently, how this anti-phase oscillation influences the circadian modulation of LTP/LTD is not known. Simulation results showed variation in LTP and STDP at different circadian phases (Fig 6.7) under DD condition. Maximum EPSP change happened at

subjective night, is in good agreement with the experimental observations [48]. We also tested the model under LD condition, where it showed maximal EPSP change occurred during night time and this simulated results agreed well with the experimental findings [12]. Eckel-Mahan et al. [250] suggested that the memory training may work as zeitgeber for hippocampal neurons. They reported that memory training-induced signaling at hippocampus and light-induced molecular events at SCN are comparable. The phase response curve (PRC) of the mammalian system explained the circadian phase-dependent transient changes in the system in response to perturbation (generally light). The phase responses during the subjective day are different from the subjective night. Similarly, LTP induced by pre-postsynaptic activity also changed in a circadian phase-dependent manner, and our mathematical model faithfully captured this aspect.

Finally, we presented a multi-scale model of the SCN-amygdala to investigate the mechanism behind the circadian modulation of learning and memory at amygdala. We simulated fear conditioning, recall, and extinction at a different time of day and our model qualitatively reproduced the experimental results [31]. The acquisition is maximum when the model is trained during the day, the recall ability is maximum during the day irrespective of the time of training, and the degree of extinction is higher when the model is trained and tested during the night. All these are in good agreement with the experimental results [31]. Experimental studies suggest that activation of NMDAR in the amygdala is necessary for fear conditioning [251], and AMPAR trafficking occurs to synapse during learning [245], and our model used in the simulation also consists of coupled GRN network of SCN and amygdala along with the electrophysiology of spiking neuron and the dynamics of AMPAR, NMDAR, and calcium. In this work, the addition and removal of AMPAR, the conductance of NMDAR, AMPAR, and calcium channel are modeled in such a way that their dynamics oscillate with a circadian period. This modeling approach allowed us to explore the circadian modulation of fear conditioning, recall, and extinction at amygdala. Our model study strongly supports the NMDAR evoked calcium current and AMPAR plays an important role in fear conditioning, and the circadian variation of their dynamics eventually modulated learning and memory.

In summary, our model captures the essence of circadian modulation of learning and memory. However, there are many aspects of this modelling task that we undertook require refinement and modifications. Firstly, since we didn't consider the population of neurons for modelling, the dynamics of population of neurons in SCN, hippocampus, and amygdala may suggest otherwise. Secondly, electrophysiological models that we used in this study are simple, and hence most of the simulation results can only be qualitatively matched with the experimentally observed trends. Thirdly, we focused only on the mice models and have not considered the learning and memory aspects that are studied extensively in other models like *Drosophila*.

Fourth, we have validated our model with only one set of experimental results and training protocols. In order to test the efficiency of our model, cross-validation with different set of experimental results and protocols are required. Finally, our GRN model, though considered *per2*/PER2 dynamics, is very minimalistic. Since our aim is to understand the relationship between circadian dynamics and learning, as a first go, we confined ourselves to a very simple phenomenological model. Also, there is a lack of molecular mechanisms driving these processes and most of the experimental data relating circadian influence on fear conditioning paradigm cannot be correlated to molecular mechanisms. In this work, we assumed most of the molecular mechanisms and therefore, resorted to simple molecular models. We could have considered more realistic models like morning and evening oscillators that we discussed in the Chapter-4, but again to know how the GRN model communicates with the conductance model through neurotransmitters or other coupling agents is a big challenge. This can be at best speculated to model the process. All these limitations can be addressed in the future work only after getting sufficient clarity from the experiments which can provide sufficient insight into the molecular mechanisms operating between the GRN's, channel dynamics and neurotransmitters.

Chapter 7

Conclusion

We provide in this concluding chapter the summary and future work on morning and evening oscillator of circadian rhythm and role of circadian rhythm in generating spontaneous spike with varying frequency during different time of the day and modulating learning and memory event in hippocampus and amygdala. Molecular mechanisms responsible for 24 h circadian oscillations, entrainment to external cues, encoding of day length and the time-of-day effects have been well studied experimentally. However, it is still debated from the molecular network point of view whether each cell in suprachiasmatic nuclei harbors two molecular oscillators, where one tracks dawn and the other tracks dusk activities.

In chapter-4, we presented a realistic dual oscillator model of circadian rhythms based on the series of hypotheses proposed by Daan et al. [43], in which they conjectured that the circadian genes *per1/cry1* track the dawn while *per2/cry2* track the dusk and they together constitute the morning and evening oscillators (dual oscillator). Their hypothesis also provided explanations about the encoding of day length in terms of molecular mechanisms of *per/cry* expression. We framed a minimal mathematical model with the assumption that *per1* acts as a morning oscillator and *per2* acts as an evening oscillator and to support and interpret this assumption we fitted the model to the experimental data of *per1/per2* circadian temporal dynamics, phase response curves (PRC's), and entrainment phenomena under various light-dark conditions. We also captured different patterns of splitting phenomena by coupling two single cell dual oscillators with neuropeptides vasoactive intestinal polypeptide (VIP) and arginine vasopressin (AVP) as the coupling agents and provide interpretation for the occurrence of splitting in terms of ME oscillators, though they are not required to explain the morning and evening oscillators. The proposed dual oscillator model based on Daan's hypothesis supports *per1* and *per2* playing the role of morning and evening oscillators

respectively and this may be the first step towards the understanding of the core molecular mechanism responsible for encoding the day length. To our knowledge, we are the first one to construct the mathematical model of circadian rhythm based on GRN, study the specific role of morning and evening oscillator in SCN, and collaborate with experimental data. Though we are successful, limitation of our modeling study is that it is specified only to the mammalian system, and it has to be extended to other systems like *Drosophila* where abundant experimental data is available. Besides, the complexity of neural circuit is less in *Drosophila* than in SCN.

We studied the cause of spontaneous firing response of the SCN neurons through building electrophysiological models like HH and ML. We use these models to study the daily variation of firing patterns, learning, and memory, which are modulated by circadian rhythm. In chapter-5, we performed multiscale model simulations to study the role of Gaussian noise and slow varying mammalian circadian oscillations (in *hrs*) in modulating the rapidly varying firing patterns (in *ms*) exhibited by the ionic channels of suprachiasmatic nuclei under DD conditions. Hodgkin-Huxley model near subcritical Hopf bifurcation exhibited noise-induced firing patterns and these patterns are highly circadian phase dependent. The simulated firing patterns are also found to be very close to the experimentally observed patterns with a firing rate of 3-10 HZ during subjective day and 0-3 HZ during subjective night. Further, for certain noise intensity, the model's response is maximal, a characteristic feature of stochastic resonance, but surprisingly, we observed it only at certain circadian phases. This is the first instance where it is shown that the slow-varying gene regulatory circadian oscillation along with noise modulates the firing patterns of fast varying voltage-gated channel as observed in experiments and exhibits stochastic resonance only in certain circadian phases.

In chapter-6, we proposed a multiscale model to explain the time-of-day effects on learning and memory. We specifically modeled the circadian variation of hippocampus-dependent long-term potentiation, depression, and fear conditioning paradigm in amygdala. The model we built has both circadian gene regulatory network of Goodwin type and the conductance model of Morris-Lecar model type to explain the spontaneous firing patterns in suprachiasmatic nucleus. We also included NMDAR dynamics in the conductance model to study the circadian dependent changes in LTP/LTD in HC and include both NMDAR and AMPAR dynamics in the conductance model to explain circadian modulation of fear conditioning paradigm in memory acquisition, recall, and extinction seen in amygdala. Our multiscale model captured the essential dynamics seen in the experiments and strongly supports the circadian time-of-day effects on learning and memory.

Apart from mathematical modeling of circadian system, we proposed Hilbert transform based numerical method to analyze the time series of the circadian rhythms in chapter-3. We demonstrated the application of HT by taking both deterministic and stochastic time series that we got from the simulation of the fruit fly model *Drosophila Melanogaster*, and showed how to extract the period, construct phase response curves, determine period sensitivity of the parameters to perturbations and build Arnold tongues to identify the regions of entrainment. We also derived a phase model that we numerically simulate to capture whether the circadian time series entrains to the forcing period completely (phase locking) or only partially (phase slips) or neither. We validated the phase model and numerics with the experimental time series forced under different temperature cycles. Application of HT to the circadian time series appears to be a promising tool to extract the characteristic information about circadian rhythms.

There are few open questions needs to be addressed in the future work. Firstly, ME oscillator and its role in learning and memory have to be taken up. In this thesis, though we addressed these two aspects we have not related the morning and evening oscillators to learning and memory. Particularly it will be interesting to know the contribution of ME oscillators to learning and memory in different chronotypes to study morningness and eveningness. Few other interesting problems that need to be addressed from health perspectives in the near future is to relate ME oscillator to sleep and wake cycle, metabolic syndromes, cancer chronotherapy, etc. Finally, there is hardly any study that explains how SCN integrates the signaling GRN pathways and electrophysiological output. Though the integration of these signals is a complex process, with the advent of high-performance computing, this may no longer be a daunting task. efforts will be made to tackle these problems through data driven modeling approaches.

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Appendix A

Parameters used for single cell dual oscillator

Table A.1 Parameters used for single cell dual oscillator

parameters	Description	Unit	Parameters used for DD and LD	Parameters used for LL condition		
			WT	WT	per1 mutant	per2 mutant
v_{s1}	Maximum rate of per1 mRNA transcription	nMh^{-1}	0.45	0.45	0	4
v_{s2}	Maximum rate of per2 mRNA transcription	nMh^{-1}	1.2	2.08	2	0
v_{s3}	Maximum rate of Bmal1 mRNA transcription (Inhibitor Rev-erb)	nMh^{-1}	4.5	4	4	4.5
v_{s4}	Maximum rate of Bmal1 transcription(activator PER2)	nMh^{-1}	1.23	0.78	0.78	1.23
v_{s5}	Maximum rate of Rev_erb mRNA transcription	nMh^{-1}	0.1	0.1	0.1	0.5
v_1	maximum rate of per1 mRNA degradation	nMh^{-1}	1.47	0.44	0.44	0.44
v_2	maximum rate of PER1 protein (c) degradation	nMh^{-1}	0.49	0.49	0.49	1.38
v_3	maximum rate of PER1 protein (n) degradation	nMh^{-1}	0.42	0.42	0.42	1.67
v_4	maximum rate of per2 mRNA degradation	nMh^{-1}	0.4	0.44	0.44	0.44
v_5	maximum rate of PER2 protein (c) degradation	nMh^{-1}	1.38	1.38	1.38	1.38
v_6	maximum rate of PER2 protein (n) degradation	nMh^{-1}	1.67	1.67	1.67	1.67
v_7	maximum rate of Bmal1 mRNA degradation	nMh^{-1}	4.9	4.9	4.9	4.9
v_8	maximum rate of BMAL1 protein (c) degradation	nMh^{-1}	0.37	0.37	0.37	0.37
v_9	maximum rate of BMAL1 protein (n) degradation	nMh^{-1}	0.6	0.6	0.6	0.6
v_{10}	maximum rate of Rev-erb mRNA degradation	nMh^{-1}	1.51	1.51	1.51	1.51
v_{11}	maximum rate of REV-ERB protein degradation	nMh^{-1}	0.13	0.13	0.13	0.13
k_{d1}	Exponential degradation constant for per1 mRNA	h^{-1}	0.11	0.11	0.11	0.1
k_{d2}	Exponential degradation constant for PER1 protein (c)	h^{-1}	0.09	0.09	0.09	0.09
k_{d3}	Exponential degradation constant for PER1 protein (n)	h^{-1}	1.07	1.07	1.07	0.08
k_{d4}	Exponential degradation constant for per2 mRNA	h^{-1}	0.03	0.01	0.01	0.03
k_{d5}	Exponential degradation constant for PER2 protein (c)	h^{-1}	0.2	0.1	0.1	0.2
k_{d6}	Exponential degradation constant for PER2 protein (n)	h^{-1}	0.08	0.08	0.08	0.08
k_{d7}	Exponential degradation constant for Bmal1 mRNA	h^{-1}	1.34	1.34	1.34	1.34
k_{d8}	Exponential degradation constant for BMAL1 protein (c)	h^{-1}	0.49	0.49	0.49	0.49
k_{d9}	Exponential degradation constant for BMAL1 protein (n)	h^{-1}	0.7	0.7	0.7	0.7
k_{d10}	Exponential degradation constant for Rev-erb mRNA	h^{-1}	0.19	0.19	0.19	0.19
k_{d11}	Exponential degradation constant for Rev-erb protein	h^{-1}	0.92	0.92	0.92	0.92
k_{d12}	Exponential degradation constant for PER1-BMAL complex	h^{-1}	0.29	0.29	0.29	0.29
k_{d13}	Exponential degradation constant for PER2-BMAL complex	h^{-1}	0.07	0.07	0.07	0.07
k_1	Rate constant for synthesis of PER1 protein (c)	h^{-1}	0.33	0.33	0.33	2.5
k_2	Rate constant for synthesis of PER1 protein (n)	h^{-1}	1.7	1.7	1.7	1.7
k_3	Rate constant for synthesis of PER2 protein (c)	h^{-1}	1.44	0.5	0.5	1.44
k_4	Rate constant for synthesis of PER2 protein (n)	h^{-1}	1.65	1	1	1.65
k_5	Rate constant for synthesis of BMAL1 protein (c)	h^{-1}	0.87	0.87	0.87	0.87
k_6	Rate constant for synthesis of BMAL1 protein (n)	h^{-1}	1.6	1.6	1.6	1.6
k_7	Rate constant for synthesis of REV-ERB protein (n)	h^{-1}	3.57	3.57	3.57	3.57
k_{p1}	Rate constant for dissociation of PER1-BMAL1 complex	$\text{nM}^{-1}\text{h}^{-1}$	1.33	0.1	0.1	0.1
k_{p2}	Rate constant for formation of PER1-BMAL complex	$\text{nM}^{-1}\text{h}^{-1}$	0.5	0.17	0.17	1.9
k_{p3}	Rate constant for dissociation of PER2-BMAL1 complex	$\text{nM}^{-1}\text{h}^{-1}$	0.11	0.11	0.11	0.11
k_{p4}	Rate constant for formation of PER2-BMAL complex	$\text{nM}^{-1}\text{h}^{-1}$	0.2	0.6	0.7	0.18
k_{a1}	Activation constant enhancement of per1 mRNA	nM	0.44	0.44	0.44	0.44
k_{a2}	Activation constant enhancement of per2 mRNA	nM	1.66	1.66	1.66	1.66
k_{a3}	Activation constant enhancement of Bmal1 mRNA	nM	1.48	1.48	1.48	1.48
k_{a4}	Activation constant enhancement of Rev-erb mRNA	nM	1.5	1.5	1.5	1.5
k_{I1}	Inhibition constant for repression of Bmal1 (REV-ERB as repressor)	nM	6.63	6.63	6.63	6.63
k_{e1}	Michaelis constant for degradation of per1 mRNA	nM	0.82	0.82	0.82	0.82
k_{e2}	Michaelis constant for degradation of PER1 protein (c)	nM	0.98	0.98	0.98	0.98
k_{e3}	Michaelis constant for degradation of PER1 protein (n)	nM	0.6	0.6	0.6	0.6
k_{e4}	Michaelis constant for degradation of per2 mRNA	nM	1.65	1.65	1.65	1.65
k_{e5}	Michaelis constant for degradation of PER2 protein (c)	nM	1.13	1.13	1.13	1.13
k_{e6}	Michaelis constant for degradation of PER2 protein (n)	nM	1	1	1	1
k_{e7}	Michaelis constant for degradation of Bmal1 mRNA	nM	0.3	0.3	0.3	0.3
k_{e8}	Michaelis constant for degradation of BMAL1 protein (c)	nM	1.05	1.05	1.05	1.05
k_{e9}	Michaelis constant for degradation of BMAL1 protein (n)	nM	1.7	1.7	1.7	1.7
k_{e10}	Michaelis constant for degradation of Bmal1 mRNA	nM	3.58	3.58	3.58	3.58
k_{e11}	Michaelis constant for degradation of BMAL1 protein	nM	0.026	0.026	0.026	0.026
k_x	Scaling constant for <i>rev - erb</i> as a repressor	nM^{-1}	0.29	0.29	0.29	0.29
m	Hills coefficient for per1 and per2 transcription		2.65	2.65	2.65	2.65
s	Hills coefficient for rev-erb transcription due to BMAL1 inhibition		4.07	4.07	4.07	4.07
w	Hills coefficient for Bmal1 transcription due to PER2 activation		5.2	5.2	5.2	5.2

Appendix B

coupled oscillator model

M oscillator

$$\begin{aligned}
\frac{dM_{p1m}}{dt} &= v_{s1m} \frac{B_{Nm}^{m_m}}{k_{a1m}^{m_m} + B_{Nm}^{m_m}} - v_{1m} \frac{M_{p1m}}{k_{e1m} - k_{d1m}M_{p1m}} + L + v_{cm1} \frac{AVP}{k_{cm} + AVP} \\
\frac{P_{1cm}}{dt} &= k_{1m}M_{p1m} - v_{2m} \frac{P_{1cm}}{k_{e2m} + P_{1cm}} - k_{d2m}P_{1cm} \\
\frac{P_{1nm}}{dt} &= k_{2m}P_{1cm} - v_{3m} \frac{P_{1nm}}{k_{e3m} + P_{1nm}} + k_{p1m}PB_{1m} - k_{p2m}P_{1nm}B_{Nm} - k_{d2m}P_{1nm} \\
\frac{dM_{p2m}}{dt} &= v_{s2m} \frac{B_{Nm}^{m_m}}{k_{a2m}^{m_m} + B_{Nm}^{m_m}} - v_{4m} \frac{M_{p2m}}{k_{e4m} - k_{d4m}M_{p2m}} + L + v_{cm2} \frac{AVP}{k_{cm} + AVP} \\
\frac{P_{2cm}}{dt} &= k_{3m}M_{p2m} - v_{5m} \frac{P_{2cm}}{k_{e5m} + P_{2cm}} - k_{d5m}P_{2cm} \\
\frac{P_{2nm}}{dt} &= k_{4m}P_{1cm} - v_{6m} \frac{P_{2nm}}{k_{e6m} + P_{2nm}} + k_{p3m}PB_{2m} - k_{p4m}P_{2nm}B_{Nm} - k_{d6m}P_{2nm} \\
\frac{M_{Bm}}{dt} &= v_{s3m} \frac{k_{I1m}^2}{k_{I1m}^2 + R_m^2 + (\frac{R_m}{k_{xm}})} - v_{7m} \frac{M_{Bm}}{k_{e6m} + M_{Bm}} - k_{d7m}M_{Bm} \\
\frac{B_{cm}}{dt} &= k_{5m}M_{Bm} - v_{8m} \frac{B_{cm}}{k_{e8m} + B_{cm}} - k_{e8m}B_{cm} \\
\frac{B_{Nm}}{dt} &= k_{6m}B_{cm} - v_{9m} \frac{B_{Nm}}{k_{e9m} + B_{Nm}} + k_{p1m}PB_{1m} - k_{p2m}P_{1nm}B_{Nm} + \\
&\quad k_{p3m}PB_{2m} - k_{p4m}P_{2nm}B_{Nm} - k_{d9m}B_{Nm} \\
\frac{M_{Rm}}{dt} &= v_{s5m} \frac{B_{Nm}^{s_m}}{k_{a4m}^{s_m} + B_{Nm}^{s_m}} - v_{10m} \frac{M_{Rm}}{k_{e10m} + M_{Rm}} - k_{d10m}M_{Rm} \\
\frac{R_m}{dt} &= k_{7m}M_{Rm} - v_{11m} \frac{R_m}{k_{e11m} + R_m} - k_{d11m}R_m \\
\frac{PB_{1m}}{dt} &= -k_{p1m}PB_{1m} + k_{p2m}P_{1nm}B_{Nm} - k_{d12m}PB_{1m} \\
\frac{PB_{2m}}{dt} &= -k_{p3m}PB_{2m} + k_{p4m}P_{2nm}B_{Nm} - k_{d13m}PB_{2m}
\end{aligned}$$

E oscillator

$$\begin{aligned}
\frac{dM_{p1e}}{dt} &= v_{s1e} \frac{B_{Nm}^{m_e}}{k_{a1e}^{m_e} + B_{Nm}^{m_e}} - v_{1e} \frac{M_{p1e}}{k_{e1e} - k_{d1e}M_{p1e}} + \frac{\beta + VIP(v_{ce1} + (L/\beta_L))}{k_{ce} + VIP} \\
\frac{P_{1ce}}{dt} &= k_{1e}M_{p1e} - v_{2e} \frac{P_{1ce}}{k_{e2e} + P_{1ce}} - k_{d2e}P_{1ce} \\
\frac{P_{1ne}}{dt} &= k_{2e}P_{1ce} - v_{3e} \frac{P_{1ne}}{k_{e3e} + P_{1ne}} + k_{p1e}PB_{1e} - k_{p2e}P_{1ne}B_{Ne} - k_{d2e}P_{1ne} \\
\frac{dM_{p2e}}{dt} &= v_{s2e} \frac{B_{Ne}^{m_e}}{k_{a2e}^{m_e} + B_{Ne}^{m_e}} - v_{4e} \frac{M_{p2e}}{k_{e4e} - k_{d4e}M_{p2e}} + \frac{\beta + VIP(v_{ce2} + (L/\beta_L))}{k_{ce} + VIP} \\
\frac{P_{2ce}}{dt} &= k_{3e}M_{p2e} - v_{5e} \frac{P_{2ce}}{k_{e5e} + P_{2ce}} - k_{d5e}P_{2ce} \\
\frac{P_{2ne}}{dt} &= k_{4e}P_{1ce} - v_{6e} \frac{P_{2ne}}{k_{e6e} + P_{2ne}} + k_{p3e}PB_{2e} - k_{p4e}P_{2ne}B_{Ne} - k_{d6e}P_{2ne} \\
\frac{M_{Be}}{dt} &= v_{s3e} \frac{k_{I1e}^2}{k_{I1e}^2 + R_e^2 + (\frac{R_e}{k_{xe}})} - v_{7e} \frac{M_{Be}}{k_{e6e} + M_{Be}} - k_{d7e}M_{Be} \\
\frac{B_{ce}}{dt} &= k_{5e}M_{Be} - v_{8e} \frac{B_{ce}}{k_{e8e} + B_{ce}} - k_{e8e}B_{ce} \\
\frac{B_{Ne}}{dt} &= k_{6e}B_{ce} - v_{9e} \frac{B_{Ne}}{k_{e9e} + B_{Ne}} + k_{p1e}PB_{1e} - k_{p2e}P_{1ne}B_{Ne} + \\
&\quad k_{p3e}PB_{2e} - k_{p4e}P_{2ne}B_{Ne} - k_{d9e}B_{Ne} \\
\frac{M_{Re}}{dt} &= v_{s5e} \frac{B_{Ne}^{s_e}}{k_{a4e}^{s_e} + B_{Ne}^{s_e}} - v_{10e} \frac{M_{Re}}{k_{e10e} + M_{Re}} - k_{d10e}M_{Re} \\
\frac{R_e}{dt} &= k_{7e}M_{Re} - v_{11e} \frac{R_e}{k_{e11e} + R_e} - k_{d11e}R_e \\
\frac{PB_{1e}}{dt} &= -k_{p1e}PB_{1e} + k_{p2e}P_{1ne}B_{Ne} - k_{d12e}PB_{1e} \\
\frac{PB_{2e}}{dt} &= -k_{p3e}PB_{2e} + k_{p4e}P_{2ne}B_{Ne} - k_{d13e}PB_{2e}
\end{aligned}$$

coupling

$$\begin{aligned}
\frac{dVIP}{dt} &= \begin{cases} 0, & \text{under DD.} \\ k_{vs1}(P_{1nm} + P_{2nm}) - v_{v1} \frac{VIP}{k_{ev1} + VIP} - k_{dv1}VIP, & \text{under LL and LD.} \end{cases} \\
\frac{dAVP}{dt} &= k_{vs2}(P_{1ne} + P_{2ne}) - v_{v2} \frac{AVP}{k_{ev2} + AVP} - k_{dv2}AVP
\end{aligned}$$

Table B.1 Parameters used for ME oscillator

M oscillator		E oscillator			
parameter	values	parameter	values	Unit	Description
v_{s1m}	4	v_{s1e}	0.2	nMh^{-1}	Maximum rate of per1 mRNA transcription
v_{s2m}	2	v_{s2e}	2	nMh^{-1}	Maximum rate of per2 mRNA transcription
v_{s3m}	3.8	v_{s3e}	4	nMh^{-1}	Maximum rate of Bmal1 mRNA transcription (Inhibitor Rev-erb)
v_{s4m}	1.23	v_{s4e}	0.78	nMh^{-1}	Maximum rate of Bmal1 transcription(activator PER2)
v_{s5m}	0.5	v_{s5e}	0.1	nMh^{-1}	Maximum rate of Rev_erb mRNA transcription
v_{1m}	0.44	v_{1e}	1.47	nMh^{-1}	Maximum rate of per1 mRNA degradation
v_{2m}	1.38	v_{2e}	0.49	nMh^{-1}	Maximum rate of PER1 protein (c) degradation
v_{3m}	1.67	v_{3e}	0.42	nMh^{-1}	Maximum rate of PER1 protein (n) degradation
v_{4m}	0.44	v_{4e}	0.44	nMh^{-1}	Maximum rate of per2 mRNA degradation
v_{5m}	1.38	v_{5e}	1.38	nMh^{-1}	Maximum rate of PER2 protein (c) degradation
v_{6m}	1.67	v_{6e}	1.67	nMh^{-1}	Maximum rate of PER2 protein (n) degradation
v_{7m}	4.9	v_{7e}	4.9	nMh^{-1}	Maximum rate of Bmal1 mRNA degradation
v_{8m}	0.37	v_{8e}	0.37	nMh^{-1}	Maximum rate of BMAL1 protein (c) degradation
v_{9m}	0.6	v_{9e}	0.6	nMh^{-1}	Maximum rate of BMAL1 protein (n) degradation
v_{10m}	1.51	v_{10e}	1.51	nMh^{-1}	Maximum rate of Rev-erb mRNA degradation
v_{11m}	0.13	v_{11e}	0.13	nMh^{-1}	Maximum rate of REV-ERB protein degradation
k_{d1m}	0.05	k_{d1e}	0.11	h^{-1}	Exponential degradation constant for per1 mRNA
k_{d2m}	0.09	k_{d2e}	0.09	h^{-1}	Exponential degradation constant for PER1 protein (c)
k_{d3m}	0.08	k_{d3e}	1.07	h^{-1}	Exponential degradation constant for PER1 protein (n)
k_{d4m}	0.03	k_{d4e}	0.01	h^{-1}	Exponential degradation constant for per2 mRNA
k_{d5m}	0.2	k_{d5e}	0.1	h^{-1}	Exponential degradation constant for PER2 protein (c)
k_{d6m}	0.08	k_{d6e}	0.08	h^{-1}	Exponential degradation constant for PER2 protein (n)
k_{d7m}	1.34	k_{d7e}	1.34	h^{-1}	Exponential degradation constant for Bmal1 mRNA
k_{d8m}	0.49	k_{d8e}	0.49	h^{-1}	Exponential degradation constant for BMAL1 protein (c)
k_{d9m}	0.7	k_{d9e}	0.7	h^{-1}	Exponential degradation constant for BMAL1 protein (n)
k_{d10m}	0.19	k_{d10e}	0.19	h^{-1}	Exponential degradation constant for Rev-erb mRNA
k_{d11m}	0.92	k_{d11e}	0.92	h^{-1}	Exponential degradation constant for Rev-erb protein
k_{d12m}	0.29	k_{d12e}	0.29	h^{-1}	Exponential degradation constant for PER1-BMAL complex
k_{d13m}	0.07	k_{d13e}	0.07	h^{-1}	Exponential degradation constant for PER2-BMAL complex
k_{1m}	2.5	k_{1e}	0.33	h^{-1}	Rate constant for synthesis of PER1 protein (c)
k_{2m}	1.7	k_{2e}	1.7	h^{-1}	Rate constant for synthesis of PER1 protein (n)
k_{3m}	1.44	k_{3e}	0.5	h^{-1}	Rate constant for synthesis of PER2 protein (c)
k_{4m}	1.65	k_{4e}	1	h^{-1}	Rate constant for synthesis of PER2 protein (n)
k_{5m}	0.87	k_{5e}	0.87	h^{-1}	Rate constant for synthesis of BMAL1 protein (c)
k_{6m}	1.6	k_{6e}	1.6	h^{-1}	Rate constant for synthesis of BMAL1 protein (n)
k_{7m}	3.57	k_{7e}	3.57	h^{-1}	Rate constant for synthesis of REV-ERB protein (n)
k_{p1m}	0.1	k_{p1e}	0.1	$\text{nM}^{-1}\text{h}^{-1}$	Rate constant for dissociation of PER1-BMAL1 complex
k_{p2m}	1.9	k_{p2e}	0.17	$\text{nM}^{-1}\text{h}^{-1}$	Rate constant for formation of PER1-BMAL complex
k_{p3m}	0.11	k_{p3e}	0.11	$\text{nM}^{-1}\text{h}^{-1}$	Rate constant for dissociation of PER2-BMAL1 complex
k_{p4m}	0.7	k_{p4e}	0.7	$\text{nM}^{-1}\text{h}^{-1}$	Rate constant for formation of PER2-BMAL complex
k_{a1m}	0.44	k_{a1e}	0.44	nM	Activation constant enhancement of per1 mRNA
k_{a2m}	1.66	k_{a2e}	1.66	nM	Activation constant enhancement of per2 mRNA
k_{a3m}	1.48	k_{a3e}	1.48	nM	Activation constant enhancement of Bmal1 mRNA
k_{a4m}	1.5	k_{a4e}	1.5	nM	Activation constant enhancement of Rev-erb mRNA
k_{I1m}	6.63	k_{I1e}	6.63	nM	Inhibition constant for repression of Bmal1 (REV-ERB as repressor)

Parameters used for ME oscillator

M oscillator		E oscillator			
parameter	values	parameter	values	Unit	Description
k_{e1m}	0.82	k_{e1e}	0.82	nM	Michaelis constant for degradation of per1 mRNA
k_{e2m}	0.98	k_{e2e}	0.98	nM	Michaelis constant for degradation of PER1 protein (c)
k_{e3m}	0.6	k_{e3e}	0.6	nM	Michaelis constant for degradation of PER1 protein (n)
k_{e4m}	1.65	k_{e4e}	1.65	nM	Michaelis constant for degradation of per2 mRNA
k_{e5m}	1.13	k_{e5e}	1.13	nM	Michaelis constant for degradation of PER2 protein (c)
k_{e6m}	1	k_{e6e}	1	nM	Michaelis constant for degradation of PER2 protein (n)
k_{e7m}	0.3	k_{e7e}	0.3	nM	Michaelis constant for degradation of Bmal1 mRNA
k_{e8m}	1.05	k_{e8e}	1.05	nM	Michaelis constant for degradation of BMAL1 protein (c)
k_{e9m}	1.7	k_{e9e}	1.7	nM	Michaelis constant for degradation of BMAL1 protein (n)
k_{e10m}	3.58	k_{e10e}	3.58	nM	Michaelis constant for degradation of Bmal1 mRNA
k_{e11m}	0.026	k_{e11e}	0.026	nM	Michaelis constant for degradation of BMAL1 protein
k_{xm}	0.29	k_{xe}	0.29	nM^{-1}	Scaling constant for <i>rev</i> – <i>erb</i> as repressor
m_m	2.65	m_e	4.07		Hills coefficient for per1 and per2 transcription
s_m	4.07	s_e	2.65		Hills coefficient for rev-erb transcription due to BMAL1 inhibition
w_m	5.2	w_e	5.2		Hills coefficient for Bmal1 transcription due to PER2 activation
v_{cm1}	0.24			nMh^{-1}	Coupling constant
v_{cm2}	0.24			nMh^{-1}	Coupling constant
k_{cm}	2	k_{ce}	2	nM	Activation constant
k_{vs1}	0.01	k_{vs2}	0.05	h^{-1}	Rate constant for synthesis of neuropeptides
k_{ev1}	1	k_{ev2}	1	nM	Michaelis constant for degradation of neuropeptides
v_{v1}	0.01	v_{v2}	0.01	nMh^{-1}	Maximum rate of neuropeptides degradation
k_{dv1}	0.05	k_{dv2}	0.05	h^{-1}	Exponential degradation constant for neuropeptides
		v_{ce1}	0.01		VIP modulation constant
		v_{ce2}	0.01		VIP modulation constant
		β	0.04	nM	Light modulation parameter
		β_L	1	nM^{-1}	Scaling parameter for Light modulation

Appendix C

Sensitivity analysis of Single cell dual ME oscillator

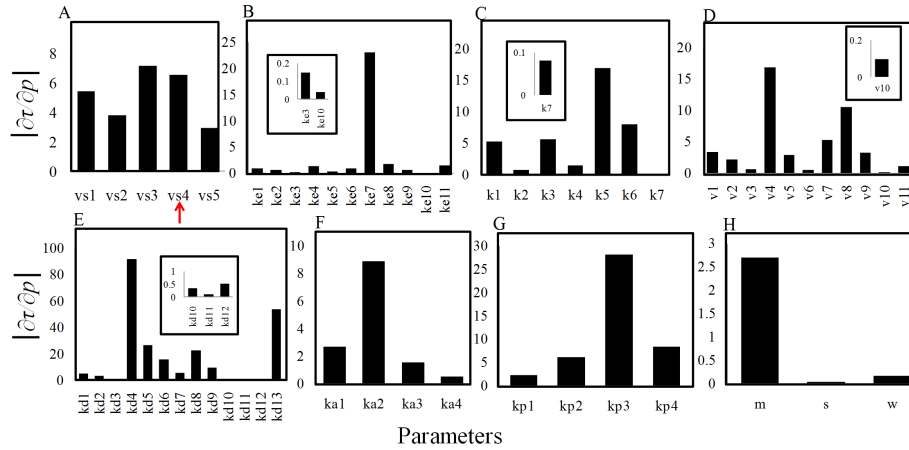


Fig. C.1 Period sensitivity of the WT-LL model parameters. Period sensitivity of transcription rate (A), Michaelis constant (B), protein synthesis (C), enzymatic degradation (D), exponential degradation (E), activation constant (F), complex formation and dissociation (G), and Hill's coefficient (H). While considering transcription rate, *Bmal1* loop shows higher sensitivity (v_{s3} , v_{s4}), and *per2* loop slightly lesser (v_{s2}). Compared with DD parameter, sensitivity of *per1* transcription rate (v_{s1}) is higher than that of *per2* (v_{s2}). For all the other constants, the parameters of the *per2* loop showed higher sensitivity (v_4 , k_5 , k_{d4} , k_{p3} , k_{a2} , m). Red arrows indicate the parameters related to the positive feedback loop between *Bmal1* and PER2 (v_{s4}) which the period is shown to be highly sensitive to changes in the parameter.

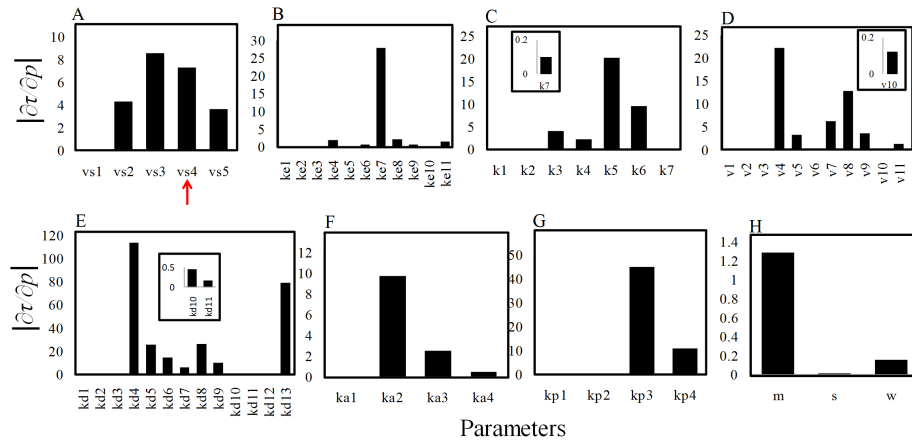


Fig. C.2 Period sensitivity of the *per1* mutant-LL model parameters. Period sensitivity of transcription rate (A), Michaelis constant (B), protein synthesis (C), enzymatic degradation (D), exponential degradation (E), activation constant (F), complex formation and dissociation (G), and Hill's coefficient (H). Here all parameters related to *per1* is completely insensitive. While considering transcription rate, *Bmal1* loop shows higher sensitivity (v_{s3} , v_{s4}), and *per2* loop slightly lesser (v_{s2}). For all the other constants, the parameters of the *per2* loop showed higher sensitivity (v_4 , k_5 , k_{d4} , k_{p3} , k_{a2} , m). Red arrows indicate the parameters related to the positive feedback loop between *Bmal1* and PER2 (v_{s4}) which the period is shown to be highly sensitive to changes in the parameter.

Appendix D

Programs

D.1 Chapter-3 programs

Program-1

This is a function file that contain ODE of Tyson et.al (1999) model

```
%*****
Program name : Tyson_ode.m
This function is called by the programs named 'Arnold_tonguel.m',
'Devil_staircase.m', 'period_det_Tyson1999.m'.
This function requires 5 input arguments to execute and they are
t: Time
B:Initial points of the variable
a: amplitude of the forcing signal
tau: period of forcing signal
PP: photo period of the forcing signal
If it is free run (no external signal), then a=0;
%*****

function dadt=Tyson_ode(t,B,a,tau,PP)
dadt=zeros(size(B));
M=B(1);Pt=B(2);
vm=1;km=0.1;vp=0.5;kp1=10;kp2=0.03;kp3=0.1;Pcrit=0.1;Jp=0.05;
```

```

keq=(-100*a*square(2*pi*(1/tau)*t,PP)+100*a)+(200-200*a);

q=2/(1+sqrt(1+8*keq*Pt));
P1=q*Pt;
P2=keq*P1^2;

dadt(1)=vm/(1+(Pt*(1-q)/(2*Pcrit))^2)-km*M;
dadt(2)=vp*M-(kp1*Pt*q+kp2*Pt)/(Jp+Pt)-kp3*Pt;

```

Program-2

This is the program to determine the period of the tyson et al. model using Hilbert transform (Fig. 3.4)

```

%*****
Program name: period_dt_Tyson1999.m
This program calls the function file Tyson_ode.m
%*****

dt=0.01;
fs=1/dt;
t=0:dt:7000;

B=0.1*ones(1,2);% initial condition
a=0;%amplitude of forcing signal
tau=24;%period of forcing signal
PP=50;%photoperiod;

[t,A]=ode23s(@Tyson_ode,t,B,[],a,tau,PP);

% period calculation using HT

x=A(:,1);
x1=detrend(x);

```

```
z = hilbert(x1);
phi=angle(z);
xp=phi;

zerIdx1=[];
for i=1:length(xp)-1
    if (abs(xp(i)-xp(i+1))>(1.5*pi))
        zerIdx1(end+1)=i; % save index of zero-crossing
    end
end

t1=t(zerIdx1);
Period_switching=[];
for j=1:length(t1)-1
    period3(j)=t1(j+1)-t1(j);

    if period3(j)>5
        Period_switching(end+1)=period3(j);
    end

end

subplot(311)
plot(t,x)
axis([2000 2072 0 3])
subplot(312)
plot(t,phi);
axis([2000 2072 -4 4])
subplot(313)
hist(Period_switching(5:end-5),1)
axis([22 26 0 300])
```

Program-3

This is the program to determine the period of the stochastic version of Tyson et al. model using Hilbert transform. (Fig. 3.5)

```
% *****
Program Name : Tyson_stochastic_period.m
Here we use a parameter 'Omg' for representing various number
of molecules.
%*****

clc;clear all;
warning off;

% initialization

%%general simulation parameters
t=0; %start time
t_end=2500; %end time
t_sample=1; %sample interval for gathering data
k=1; %counter for waitbar update

%initial numbers for each chemical species
M = 1; %mRNA
Pt = 5; %protien

%model parameters
vm=1;km=0.1;vp=0.5;kp1=10;kp2=0.03;kp3=0.1;Pcrit=0.1;Jp=0.05;keq=200;

%%arrays to store results
j=1; %counter for arrays
t_array(1,t_end/t_sample+1)=0; t_array(1,j)=t; %time array and initial value
M_array(1,t_end/t_sample+1)=0; M_array(1,j)=M; %M array and initial value
Pt_array(1,t_end/t_sample+1)=0; Pt_array(1,j)=Pt; %Pt array and initial value
```

```
Omg=1e2;% Number of molecules
```

```
% SSA
```

```
while t < t_end,
    %calculate rxn propensities
    h=[Omg*vm/(1+(Pt*(1-(2/(1+sqrt(1+8*(keq/Omg)*Pt)))))/...
        (2*Omg*Pcrit))^2) km*M...
        vp*M (Omg*kp1*Pt*(2/(1+sqrt(1+8*(keq/Omg)*Pt)))+...
        Omg*kp2*Pt)/(Jp*Omg+Pt) kp3*Pt];
    %combined rxn hazard
    h0 = sum(h);
    %calculate time to next event
    r1=rand;
    while r1 == 0,
        r1=rand;
    end
    t_next = ((1/h0)*(log(1/r1)));

    %update time
    t = t + t_next;
    %determine next reaction
    i=1; mu=0; amu=0; r2=rand;
    while amu < r2*h0,
        mu = mu + 1;
        amu = amu + h(i);
        i = i + 1;
    end

    %reactions
    if mu == 1 %Reaction 1
        M = M + 1;
    elseif mu == 2 %Reaction 2
        M = M - 1;
    elseif mu == 3 %Reaction 3
```

```

        Pt = Pt + 1;
    elseif mu == 4 %Reaction 4
        Pt = Pt - 1;
    elseif mu == 5 %Reaction 5
        Pt = Pt - 1;
    end

    %store/output time and species
    if t >= j*t_sample
        j=j+1;
        t_array(1,j)=j;
        M_array(1,j)=M;
        Pt_array(1,j)=Pt;

    end
end

x2=M_array;
t=t_array;
x1=detrend(x2);
z = hilbert(x1);
Fs=1;
fs=1;
phi=(angle(z));

%period calculation method1: positive mean crossing of M
xp=M_array;
N=mean(xp);
zerIdx=[];

for i=1:length(xp)-1
    if ((xp(i)<N && xp(i+1)>N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end

```

```

end
t1=t(zerIdx);

Period_zero_crossing=[];
for j=1:length(t1)-1
    period2(j)=t1(j+1)-t1(j);

%    if period2(j)>5
%        Period_zero_crossing(end+1)=period2(j);
%    end
end
% hist(Period_zero_crossing(5:end-5))

% period calculation method2: switching time of phase (HT)

xp=phi;
zerIdx1=[];
for i=1:length(xp)-1
    if (abs(xp(i)-xp(i+1))>(1.5*pi))
        zerIdx1(end+1)=i; % save index of zero-crossing
    end
end

t1=t(zerIdx1);
Period_switching=[];
for j=1:length(t1)-1
    period3(j)=t1(j+1)-t1(j);
    Period_switching(end+1)=period3(j);
end

subplot(411)
plot(t_array,x2)
subplot(412)
plot(t_array,phi)
subplot(413)
hist(Period_zero_crossing(5:end-5))

```

```
subplot(414)
hist(Period_switching(5:end-5))
```

Program-4

This is the program to find the period sensitivity using Hilbert transform (Fig. 3.6)

```
%*****
Program Name: Period_sensitivity_HT.m
% *****

function Period_sensitivity_HT
dt=0.01;
fs=1/dt;
t=0:dt:2000;
B=0.1*ones(1,2);
dP=0.01;% change in the parameter (10% of the parameter of interest)
[t,A]=ode45(@ode1,t,B,[],dP);
x=A(:,1);
x1=detrend(x);
z = hilbert(x1);
phi=angle(z);
instfreq = fs/(2*pi)*diff(unwrap(angle(z)));
period1=1/mean(instfreq(100000:190000));

period_sensitivity=(period1-24.2)/dP

period1=1/mean(instfreq(100000:190000));

function dadt=ode1(t,B,dP)

dadt=zeros(size(B));

M=B(1);Pt=B(2);
km=0.1+dP;% Here parameter of interest is km. For other
%parameter sensitivity, please add dP to that parameter value.
```

```
vm=1;vp=0.5;kp1=10;kp2=0.03;kp3=0.1;Pcrit=0.1;Jp=0.05;keq=200;
```

```
q=2/(1+sqrt(1+8*keq*Pt));
```

```
P1=q*Pt;
```

```
P2=keq*P1^2;
```

```
dadt(1)=vm/(1+(Pt*(1-q)/(2*Pcrit))^2)-km*M;
```

```
dadt(2)=vp*M-(kp1*Pt*q+kp2*Pt)/(Jp+Pt)-kp3*Pt;
```

Program-5

This is the program to construct PRC of the Tyson et al. model using HT (Fig. 3.9).

```
%*****
Program Name : PRC_Tyson_HT
%*****

function PRC_Tyson_HT

clc;clear all;

time=0:0.01:2500;
number_of_points_required=12;%How many points required for PRC

N_rep=1;%Number of PRC cycle required

Number_cycle=5;%Number of cycle after which phase calculating

T_ref=3.5 ;%specify the circadian time of peak value of the first variable

P_light=198; %Intensity of perturbation(For type 1 change P_light=185)

N_light=0; %Unperturbed intensity

P_time=2;%perturbation duration (For type 1 change P_time=0.7)
```

```
B=0.1*ones(1,2);

option = odeset('RelTol', 1e-8);

[t1,A1]=ode23s(@ode2,time,B,option,[],N_light,N_light,[]);

% Period calculation

x1=find(time>500);
x=A1(:,1);
A_ref=x;
x_new=x(x1);
x=x_new;
N=mean(x_new);
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)<N && x(i+1)>N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end

t1=time(zerIdx); % display timepoints of zero-crossings

for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
period1;
period=mean(period1)%find out the mean period

kk1=find(time>=1000 & time<=1000+period);

Time_new=time(kk1);
```

```

kk2=find((A_ref(kk1))==max(A_ref(kk1)));

CT0=Time_new(kk2)-T_ref*(period/24);

T_initial=CT0;

time_interval=(period*N_rep/number_of_points_required);
Tp=T_initial:time_interval:T_initial+period*N_rep;
%Tp is the time at which perturbation applied

for i=1:length(Tp)

    perturbation_time=Tp(i);

    [time,A]=ode23s(@ode2,time,B,option,perturbation_time,P_light,N_light,P_time);

    T_phase_calculation=T_initial+Number_cycle*period;%Phase calculation after N
    %cycle

    % phase difference calculation using hilbert transform

    T1=find(time>=T_phase_calculation);%phase shift calculate after 20 cycle
    X=A1(:,2);Y=A(:,2);
    x_pt=X(T1);y_pt=Y(T1);
    T_new=time(T1);

    h1=hilbert(detrend(x_pt));
    h2=hilbert(detrend(y_pt));

    p1 = unwrap(angle(h1)); p2 =unwrap(angle(h2)); % Instantaneous phase
    % (wrapped)
    tau=period/(2*pi);

    p=mean(p2-p1);

    phase_shift(i)=p*tau;

```

```

    if phase_shift(i) > period/2
        phase_shift(i)=phase_shift(i)-period;
    elseif phase_shift(i) < -period/2
        phase_shift(i)=phase_shift(i)+period;
    end

end

Tp_new=0:time_interval:period*N_rep;
plot(Tp_new,phase_shift);

function dadt=ode2(time,B,perturbation_time,P_light,N_light,P_time)

dadt=zeros(size(B));
M=B(1);Pt=B(2);

    if time>=perturbation_time & time<=perturbation_time+P_time
        a=P_light;
    else
        a=N_light;
    end

vm=1;km=0.1;vp=0.5;kp1=10;kp2=0.03;kp3=0.1;Pcrit=0.1;Jp=0.05;
keq=200-a;

q=2/(1+sqrt(1+8*keq*Pt));

P1=q*Pt;
P2=keq*P1^2;

dadt(1)=(vm)/(1+(Pt*(1-q)/(2*(Pcrit)))^2)-km*M;
dadt(2)=vp*M-(kp1*Pt*q+kp2*Pt)/((Jp)+Pt)-kp3*Pt;

```

Program-6

This is the program to construct Arnold's tongue of the Tyson model using HT (Fig. 3.10 B).

```

%*****
Program Name: Arnold_tonguel.m
This program calls the function Tyson_ode.m
%*****

clc;clear all;warning off
fs=100;
t=0:1/fs:25000;

B=0.2*ones(1,2);% initial condition
a=0:0.1:0.6;%amplitude of forcing signal
tau=23:0.5:26;%period of forcing signal
PP=50;%photoperiod;

period1=[];
Entrainment_amplitude1=[];
period2=[];
Entrainment_amplitude2=[];
period3=[];
Entrainment_amplitude3=[];
for i=1:length(tau);
    for j=1:length(a)
        [t,A]=ode45(@Tyson_ode,t,B,[],a(j),tau(i), PP);
        x=A(:,1);

x1=detrend(x);
z = hilbert(x1);
instfreq = fs/(2*pi)*diff(unwrap(angle(z)));
period=1/mean(instfreq(100000:245000))

if abs(2*period-tau(i))<=0.1
    Entrainment_amplitude1(end+1)=a(j);
    period1(end+1)=tau(i);

```

```

elseif abs(period-tau(i))<=0.1
    Entrainment_amplitude2(end+1)=a(j);
    period2(end+1)=tau(i);

elseif abs(0.5*period-tau(i))<=0.1
    Entrainment_amplitude3(end+1)=a(j);
    period3(end+1)=tau(i);
end
end
end
    plot(period1,Entrainment_amplitude1,'b.')
hold on
    plot(period2,Entrainment_amplitude2,'r.')
hold on
    plot(period3,Entrainment_amplitude3,'k.')

```

Program-7

This is the program to construct Devil staircase of the Tyson model using HT (Fig. 3.10 A).

```

%*****
Name of the program : Devil_staircase.m
%*****

clc;clear all;warning off
fs=100;
t=0:1/fs:2500;

B=0.2*ones(1,2);% initial condition
a=0.5;%amplitude of forcing signal
tau=0:0.5:60;%period of forcing signal
PP=50;%photoperiod;

for i=1:length(tau);

    [t,A]=ode23s(@Tyson_ode,t,B,[],a,tau(i),PP);

```

```

        x=A(:,1);
        tau(i)

x1=detrend(x);
z = hilbert(x1);
instfreq = fs/(2*pi)*diff(unwrap(angle(z)));
period1=1/mean(instfreq(100000:245000))

period1=1/mean(instfreq(100000:245000))

period_number(i)=period1/tau(i);

end
period_number';

plot(1./tau,period_number);
xlabel('forcing frequency')
ylabel('Period number')

```

Program-8

This is the program to calculate the phase slip of the Tyson model using HT. (lfig. 3.12).

```

% *****
Program Name: Tyson_Phaseslip
%*****

function Tyson_Phaseslip

clc;clear all;

dt=0.01;
Fs=1/dt;
t_end=6000;
t1=0:dt:t_end;
B=0*ones(1,2);

```

```

T_E=23;% forcing period
a=0.19;% forcing amplitude

[t,A]=ode23s(@ode1,t1,B,[],T_E,a);
x=A(:,1);

x2=x;
x1=detrend(x2);
z = hilbert(x1);
phi=(angle(z));
% period calculation
x=phi;
N=0;
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)>N && x(i+1)<N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end
t1=t(zerIdx);
for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
period1;
M1=mean(period1);%find out the mean period

% period calculation (cycle by cycle)

cycle=round(t_end/T_E);
period_bin=[];
%
for td=1:cycle-20
    ynm=period1(td)*ones(1,(T_E/0.01));
    period_bin=[period_bin ynm];
end

```

```

period_bin=period_bin';

slip(1)=0;

for i=1:length(period_bin);
    slip(i+1)=slip(i)+dt*((period_bin(i)-T_E)./period_bin(i));
end

plot(t(1:length(slip))/24,slip);
xlabel('day')
ylabel('\psi');
slip_per_day=(period1-T_E)*24/period1

function dadt=ode1(t,B,T_E,a);

dadt=zeros(size(B));
M=B(1);Pt=B(2);
vm=1;km=0.1;vp=0.5;kp1=10;kp2=0.03;kp3=0.1;Pcrit=0.1;Jp=0.05;

keq=(-100*a*square(2*pi*(1/T_E)*t,50)+100*a)+(200-200*a);

q=2/(1+sqrt(1+8*keq*Pt));

dadt(1)=vm/(1+(Pt*(1-q)/(2*Pcrit))^2)-km*M;
dadt(2)=vp*M-(kp1*Pt*q+kp2*Pt)/(Jp+Pt)-kp3*Pt;

```

Program-9

This is the program to calculate period and phase slip of the SCN experimental data. (Fig. 3.14)

```

% *****
Program Name : Slip_experimental.m
% *****

clc;clear all;warning off;

```

% Experimental data collected from

% For T24 temperature cycle

```
A=xlsread('SCN_Entrainment_T24.xlsx');
T_E=24;t=A(:,1);xs=A(:,2);LD=A(:,3);
```

% For T26 temperature cycle

```
% A=xlsread('SCN_Entrainment_T26_T22.xlsx');
% T_E=26;t=A(:,1);xs=A(:,2);LD=A(:,3);
```

% For T22 temperature cycle

```
% A=xlsread('SCN_Entrainment_T26_T22.xlsx');
% T_E=22;t=A(:,1);xs=A(:,4);LD=A(:,5);

dt=1/12;
t_end=t(end);

x2=x;
x1=detrend(x2);
z = hilbert(x1);
phi=(angle(z));
% period calculation
x=phi;
N=0;
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)>N && x(i+1)<N))
        zerIdx(end+1)=i; % save index of phase switching
    end
end
t1=t(zerIdx);
for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
```

```
period1;
```

% period calculation (cycle by cycle)

```
period_bin=period1(1)*ones(1,(t1(1)/dt));% initilizing the Instantaneous period  
%  
for td=1:length(period1)  
    ynm=period1(td)*ones(1,round((period1(td)/dt)));  
    period_bin=[period_bin ynm];  
end
```

```
period_bin=period_bin';  
slip(1)=0;
```

```
for i=1:length(period_bin);  
    slip(i+1)=slip(i)+dt*((period_bin(i)-T_E)./period_bin(i));  
end
```

% Plotting commands

```
subplot(411)  
plotyy(t/24,xs,t/24,LD);  
grid on  
ylabel('Bioluminescence')
```

```
subplot(412)  
plot(t/24,phi)  
grid on  
ylabel('\theta');
```

```
subplot(413)  
plot((t(1:length(period_bin)))/24,period_bin)  
grid on  
ylabel('T_{inst}');
```

```
subplot(414)
```

```
plot(t(1:length(slip))/24,slip/24);  
grid on  
ylabel('\psi');
```

D.2 Chapter-4 programs

Program-1

This is the program for simulating single cell dual oscillator model (WT) in DD along with experimental data (Fig. 4.2).

```
%*****
Program Name: New_model_WT_DD.ode
This program requires experimental data file, 'Wild_type_Data.xlsx'
%*****

function New_model_WT_DD

clc;clear all;warning off;

step=0.01;
fs=100;
t_end=2500;
t=0:1/fs:t_end;

B=1*ones(1,13);%Initial condition
option = odeset('RelTol', 1e-8);

[t,A]=ode45(@ode1,t,B,option);

k1=find(t>3000 & t<3500);

x1=find(t>1000);%Inorder to avoide transient

% Normalization between 0 to 1

x_new1=(A(x1,1)-min(A(x1,1)))/(max(A(x1,1))-min(A(x1,1)));
x_new2=(A(x1,2)-min(A(x1,2)))/(max(A(x1,2))-min(A(x1,2)));
```

```

x_new3=(A(x1,3)-min(A(x1,3)))/(max(A(x1,3))-min(A(x1,3)));
x_new4=(A(x1,4)-min(A(x1,4)))/(max(A(x1,4))-min(A(x1,4)));
x_new5=(A(x1,5)-min(A(x1,5)))/(max(A(x1,5))-min(A(x1,5)));
x_new6=(A(x1,6)-min(A(x1,6)))/(max(A(x1,6))-min(A(x1,6)));
x_new7=(A(x1,7)-min(A(x1,7)))/(max(A(x1,7))-min(A(x1,7)));
x_new8=(A(x1,8)-min(A(x1,8)))/(max(A(x1,8))-min(A(x1,8)));
x_new9=(A(x1,9)-min(A(x1,9)))/(max(A(x1,9))-min(A(x1,9)));
x_new10=(A(x1,10)-min(A(x1,10)))/(max(A(x1,10))-min(A(x1,10)));
x_new11=(A(x1,11)-min(A(x1,11)))/(max(A(x1,11))-min(A(x1,11)));
x_new12=(A(x1,12)-min(A(x1,12)))/(max(A(x1,12))-min(A(x1,12)));
x_new13=(A(x1,13)-min(A(x1,13)))/(max(A(x1,13))-min(A(x1,13)));

```

% period calculation

```

x1=find(t>2000);
x=A(:,7);
x_new=x(x1);
x=x_new;
N=mean(x_new);
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)<N && x(i+1)>N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end
t1=t(zerIdx);
for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
period1;
M1=mean(period1);%find out the mean period
Period_of_oscillation=M1

```

% plotting of time series

```

data=xlsread('Wild_type_Data.xlsx'); %Loading Experimental data

```

```

T_per1_WT3=data(:,5);
T_per2_WT3=data(:,11);
T_Bmal1_WT3=data(:,17);
T_Rev_WT2=data(:,21);
T_PER1_WT1=data(:,23);
T_PER2_WT1=data(:,27);

per1_WT3=(data(:,6)-min(data(:,6)))/(max(data(:,6))-min(data(:,6)));

per2_WT3=(data(:,12)-min(data(:,12)))/(max(data(:,12))-min(data(:,12)));

bmal1_WT3=(data(:,18)-min(data(:,18)))/(max(data(:,18))-min(data(:,18)));

rev_WT2=(data(:,22)-min(data(:,22)))/(max(data(:,22))-min(data(:,22)));

PER1_WT1=(data(:,24)-min(data(:,24)))/(max(data(:,24))-min(data(:,24)));

PER2_WT1=(data(:,28)-min(data(:,28)))/(max(data(:,28))-min(data(:,28)));

T_shift=2000-9+0.6;%To avoid transient;

%To normalize maximum vallue between 0 and 1

T1=find(t>=T_shift & t<=T_shift+M1);

X1=A(:,1);X3=A(:,4); X2=A(:,2); X4=A(:,5);X5=A(:,7);X6=A(:,10);

x1_shift=X1(T1);x2_shift=X2(T1);x3_shift=X3(T1);
x4_shift=X4(T1);x5_shift=X5(T1);x6_shift=X6(T1);

PER1_normalized=((A(:,2))-min(x2_shift))/(max(x2_shift)-min(x2_shift));
PER2_normalized=((A(:,5))-min(x4_shift))/(max(x4_shift)-min(x4_shift));

```

```
per1_normalized=(A(:,1)-min(x1_shift))/(max(x1_shift)-min(x1_shift));

per2_normalized=(A(:,4)-min(x3_shift))/(max(x3_shift)-min(x3_shift));

bmal1_normalized=(A(:,7)-min(x5_shift))/(max(x5_shift)-min(x5_shift));

Rev_normalized=(A(:,10)-min(x6_shift))/(max(x6_shift)-min(x6_shift));


subplot(321)
plot(T_per1_WT3,per1_WT3,'ro')
hold on
plot((t-T_shift)*(24/M1),per1_normalized)
title('Per1 mRNA')
axis([0 50 0 1.1])


subplot(322)
plot(T_per2_WT3,per2_WT3,'ro')
hold on
plot((t-T_shift)*(24/M1),per2_normalized)
axis([0 50 0 1.1])
title('Per2 mRNA')


subplot(323)
plot(T_Bmal1_WT3,bmal1_WT3,'ro')
hold on
plot((t-T_shift)*(24/M1),bmal1_normalized)
title('Bmal1 mRNA')
axis([0 50 0 1.1])


subplot(324)
plot(T_Rev_WT2,rev_WT2,'ro')
hold on
plot((t-T_shift)*(24/M1),Rev_normalized)
```

```

title('Rev-erb\alpha mRNA')
axis([0 50 0 1.1])

subplot(325)
plot(T_PER1_WT1,PER1_WT1,'ro')
hold on
plot((t-T_shift)*(24/M1),PER1_normalized)
title('PER1 protein')
axis([0 50 0 1.1])

subplot(326)
plot(T_PER2_WT1,PER2_WT1,'ro')
hold on
plot((t-T_shift)*(24/M1),PER2_normalized)
title('PER2 protein')
axis([0 50 0 1.1])

function dadt=ode1(t,B)

dadt=zeros(size(B));

Mp1=B(1);P1c=B(2);P1n=B(3);
Mp2=B(4);P2c=B(5);P2n=B(6);
Mb=B(7);B1=B(8);B2=B(9);
Mr=B(10);R=B(11);PB=B(12);PB2=B(13);

% parameters

vs1=0.45;vs2=1.2;vs3=4.5;vs4=1.23;vs5=0.1;
v1=1.47;v2=0.49;v3=0.42;v4=0.4;v5=1.38;v6=1.67;v7=4.9;v8=0.37;v9=0.6;
v10=1.51;v11=0.13;kd1 =0.11;kd2=0.09;kd3=1.07;kd4=0.03;kd5=0.2;kd6
=0.08;
kd7=1.34;kd8 =0.49;kd9 =0.7;
kd10 =0.19;kd11 =0.92;kd12=0.29;kd13 =0.07;k1 =0.33;k2 =1.7;k3
=1.44;k4 =1.65;k5=0.87;

```

```

k6=1.6;k7 =3.57; kp1 =1.33;kp2 =0.5;kp3 =0.11;kp4=0.2;ka1=0.44;ka2=1.66;
ka3=1.48;ka4 =1.5;kI1=6.63;ke1=0.82;ke2 =0.98;ke3 =0.6;ke4 =1.65;
ke5 =1.13;ke6 =1;ke7 =0.3;ke8 =1.05;ke9 =1.7;ke10=3.58;ke11=0.026;
kx1 =0.29;m =2.65;s =4.07;w =5.2;L=0;

```

```

L=0;

```

```

% per1 loop

```

```

dad1(1)=(vs1)*B2^m/(ka1^m+B2^m)-v1*Mp1/(ke1+Mp1)-kd1*Mp1+L;

```

```

dad1(2)=k1*Mp1-v2*P1c/(ke2+P1c)-kd2*P1c;

```

```

dad1(3)=k2*P1c-v3*P1n/(ke3+P1n)-kd3*P1n-kp2*P1n*B2+kp1*PB;

```

```

% per2 loop

```

```

dad2(4)=(vs2)*B2^m/(ka2^m+B2^m)-v4*Mp2/(ke4+Mp2)-kd4*Mp2+L;

```

```

dad2(5)=k3*Mp2-v5*P2c/(ke5+P2c)-kd5*P2c;

```

```

dad2(6)=k4*P2c-v6*P2n/(ke6+P2n)-kd6*P2n-kp4*P2n*B2+kp3*PB2;

```

```

%Bmal loop

```

```

dad3(7)=vs3*kI1^2/(kI1^2+R^2+(1/kx1)*R)-v7*Mb/(ke7+Mb)-kd7*Mb+...
(vs4)*P2n^w/(ka3^w+P2n^w);

```

```

dad3(8)=k5*Mb-v8*B1/(ke8+B1)-kd8*B1;

```

```

dad3(9)=k6*B1-v9*B2/(ke9+B2)-kd9*B2-kp2*P1n*B2+kp1*PB-kp4*P2n*B2+...
kp3*PB2;

```

```

%rev-erb

```

```

dad4(10)=vs5*B2^s/(ka4^s+B2^s)-v10*Mr/(ke10+Mr)-kd10*Mr;

```

```
dadt(11)=k7*Mr-v11*R/(ke11+R)-kd11*R;
```

```
%PER-BMAL complex
```

```
dadt(12)=kp2*P1n*B2-kp1*PB-kd12*PB;
```

```
dadt(13)=kp4*P2n*B2-kp3*PB2-kd13*PB2;
```

Program-2

This is the program for PRC of WT (Fig. 4.8).

```
%*****
Program Name: New_model_WT_PRC.m
Number of points in the simulation can be increased by changing
the variable 'number_of_points_required'
The program requires excel file, 'PRC_data.xlsx' for experimental PRC data
%*****
```

```
function New_model_WT_PRC
```

```
time=0:0.01:3500;
```

```
number_of_points_required=12;%How many points required for PRC
```

```
N_rep=1;%Number of PRC cycle required
```

```
Number_cycle=10;%Number of cycle after which phase calculating
```

```
T_ref=6;%specify the circadian time of peak value of the reference variable
```

```
P_light=0.35; %Intensity of perturbation
```

```
N_light=0; %Unperturbed light intensity
```

```

P_time=0.5;%perturbation duration

B=1*ones(1,13);%Initial condition

option = odeset('RelTol', 1e-12);

[t1,A1]=ode45(@ode2,time,B,option,[],N_light,N_light,[],[]);

%*****

% Period calculation

x1=find(time>500);
x=A1(:,1);
A_ref=x;
x_new=x(x1);
x=x_new;
N=mean(x_new);
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)<N && x(i+1)>N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end

t1=time(zerIdx); % display timepoints of zero-crossings

for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
period1;
period=mean(period1);%find out the mean period

% calculating CT0

kk1=find(time>=3000 & time<=3000+period);

```

```

Time_new=time(kk1);

kk2=find((A_ref(kk1))==max(A_ref(kk1)));

CT0=Time_new(kk2)-T_ref*(period/24);

T_initial=CT0;

time_interval=(period*N_rep/number_of_points_required);

Tp=T_initial:time_interval:T_initial+period*N_rep;
%Tp is the time at which perturbation applied

h = waitbar(0,'Please wait...');

for i=1:length(Tp)

    perturbation_time=Tp(i);
    [time,A]=ode45(@ode2,time,B,option,perturbation_time,P_light,N_light,...
    P_time,T_initial);

    %Phase calculation after N cycle
    T_phase_calculation=T_initial+Number_cycle*period;

    %phase shift calculate after 20 cycle
    T1=find(time>=T_phase_calculation & time<=T_phase_calculation+period*N_rep);

    X=A1(:,1);Y=A(:,1);
    x_pt=X(T1);y_pt=Y(T1);
    T_new=time(T1);

    % Phase difference calculation

    T_W0_perturbation=find(x_pt==max(x_pt));
    T_With_perturbation=find(y_pt==max(y_pt));

```

```

%Phase difference
phase_shift(i)=T_new(T_WO_perturbation)-T_new(T_With_perturbation);

if phase_shift(i) > period/2
    phase_shift(i)=phase_shift(i)-period;
elseif phase_shift(i) < -period/2
    phase_shift(i)=phase_shift(i)+period;
end

waitbar(i/length(Tp),h)

end

Tp_new=0:time_interval:period*N_rep;

plot(Tp_new*(24/period),phase_shift,'b');
xlabel('Initial phase (CT)')
ylabel('Phase shifts (hr)')
hold on

data=xlsread('PRC_data.xlsx');%for plotting Experimental data

T_wild=data(:,1);
P_wild=data(:,2);
T_per2mut=data(:,5);
P_per2muta=data(:,6);

% Plot PRC:

plot(T_wild,P_wild,'ro')

legend('Simulation','experiment')

function dadt=ode2(time,B,perturbation_time,P_light,N_light,...
P_time,T_initial)

```

```

dadt=zeros(size(B));

Mp1=B(1);P1c=B(2);P1n=B(3);
Mp2=B(4);P2c=B(5);P2n=B(6);
Mb=B(7);B1=B(8);B2=B(9);
Mr=B(10);R=B(11);PB=B(12);PB2=B(13);

t=time;

if time>=perturbation_time & time<=perturbation_time+(P_time)
    Lp=P_light;
else
    Lp=N_light;
end

% without gating

L1=1;L2=1;

% with gating variable 1

%T=23.76;%free running period CT0=3013; L_max=1;
%t1=3.5*T/24;t2=15*T/24;t3=22*T/24;t4=T;
%y1=(L_max)*((t2)/(T/1.3));
%y2=(L_max)*((t1)/(T/1.3));
%L1=L_max/2; if t<=CT0
    %L2=0;
%elseif t>CT0 & t<=CT0+t1
    % L2=y2;
%elseif t>CT0+t1 & t<=CT0+t2
    %L2=(L_max)*((t-CT0)/(T/1.3));
%elseif t>CT0+t2 & t<=CT0+t3
    %L2=y1;
%elseif
    %t>CT0+t3 & t<=CT0+t4
    %L2=(y2-y1)*(((t-CT0)-t3)/(t4-t3))+y1;

```

```
%else
```

```
%L2=y2;
```

```
%end
```

% Gating variable 2

```
%Bmal_N=(Mb-1.2981)/(1.3014-1.2981);
```

```
%L1=(Bmal_N)^10*1.3014;
```

```
%L2=L1;
```

% parameters

```
vs1=0.45;vs2=1.2;vs3=4.5;vs4=1.23;vs5=0.1;
```

```
v1=1.47;v2=0.49;v3=0.42;v4=0.4;v5=1.38;v6=1.67;v7=4.9;v8=0.37;v9=0.6;
```

```
v10=1.51;v11=0.13;
```

```
kd1 =0.11;kd2 =0.09;kd3 =1.07;kd4 =0.03;kd5 =0.2;kd6 =0.08;kd7=1.34;
```

```
kd8 =0.49;kd9 =0.7;kd10 =0.19;kd11 =0.92;kd12 =0.29;kd13 =0.07;
```

```
k1 =0.33;k2 =1.7;k3 =1.44;k4 =1.65;k5 =0.87;k6 =1.6;k7 =3.57;
```

```
kp1 =1.33;kp2 =0.5;kp3 =0.11;kp4=0.2;
```

```
ka1=0.44;ka2=1.66;ka3=1.48;ka4 =1.5;
```

```
kI1=6.63;ke1=0.82;ke2 =0.98;ke3 =0.6;ke4 =1.65;
```

```
ke5 =1.13;ke6 =1;ke7 =0.3;ke8 =1.05;ke9 =1.7;ke10=3.58;ke11=0.026;
```

```
kx1 =0.29;m =2.65;s =4.07;w =5.2;
```

```
dadt(1)=(vs1)*B2^m/(ka1^m+B2^m)-v1*Mp1/(ke1+Mp1)-kd1*Mp1+Lp*L1;
```

```
dadt(2)=k1*Mp1-v2*P1c/(ke2+P1c)-kd2*P1c;
```

```
dadt(3)=k2*P1c-v3*P1n/(ke3+P1n)-kd3*P1n-kp2*P1n*B2+kp1*PB;
```

```
% per2 loop
```

```
dadt(4)=(vs2)*B2^m/(ka2^m+B2^m)-v4*Mp2/(ke4+Mp2)-kd4*Mp2+Lp*L2;
```

```

dadt(5)=k3*Mp2-v5*P2c/(ke5+P2c)-kd5*P2c;

dadt(6)=k4*P2c-v6*P2n/(ke6+P2n)-kd6*P2n-kp4*P2n*B2+kp3*PB2;

%Bmal loop

dadt(7)=vs3*kI1^2/(kI1^2+R^2+(1/kx1)*R)-v7*Mb/(ke7+Mb)-kd7*Mb+...
(vs4)*P2n^w/(ka3^w+P2n^w);

dadt(8)=k5*Mb-v8*B1/(ke8+B1)-kd8*B1;

dadt(9)=k6*B1-v9*B2/(ke9+B2)-kd9*B2-kp2*P1n*B2+kp1*PB-kp4*P2n*B2+...
kp3*PB2;

%rev-erb

dadt(10)=vs5*B2^s/(ka4^s+B2^s)-v10*Mr/(ke10+Mr)-kd10*Mr;

dadt(11)=k7*Mr-v11*R/(ke11+R)-kd11*R;

%PER-BMAL complex

dadt(12)=kp2*P1n*B2-kp1*PB-kd12*PB;

dadt(13)=kp4*P2n*B2-kp3*PB2-kd13*PB2;

```

Program-3

This is the program to simulate entrainment of the single cell oscillator (Fig. 4.10).

```

%*****
The program requires the following excel files:
'Photo period wild.xlsx' : For wild type experimental data points
(LD 18:6, LD 6:18)

```

```

'Photo period per2M.xlsx' : For mutant experimental data points
(LD 18:6, LD 6:18)

'per1_per2_LD1212.xlsx' : For wild type experimental data points
(LD 12:12)
%*****

function New_model_Entrainment

clear all;
warning off;
data=xlsread('Photo period wild.xlsx');%Wild type data
T_SD_per1=data(:,1);
T_LD_per1=data(:,3);
T_SD_per2=data(:,5);
T_LD_per2=data(:,7);

SD_per1=(data(:,2)-min(data(:,2)))/(max(data(:,2))-min(data(:,2)));
LD_per1=(data(:,4)-min(data(:,4)))/(max(data(:,4))-min(data(:,4)));
SD_per2=(data(:,6)-min(data(:,6)))/(max(data(:,6))-min(data(:,6)));
LD_per2=(data(:,8)-min(data(:,8)))/(max(data(:,8))-min(data(:,8)));

data1=xlsread('Photo period per2M.xlsx');%Mutant data
T_SD_per1_per2M=data1(:,1);
T_LD_per1_per2M=data1(:,3);
T_SD_per2_per1M=data1(:,5);
T_LD_per2_per1M=data1(:,7);

SD_per1_per2M=(data1(:,2)-min(data1(:,2)))/(max(data1(:,2))-min(data1(:,2)));
LD_per1_per2M=(data1(:,4)-min(data1(:,4)))/(max(data1(:,4))-min(data1(:,4)));
SD_per2_per1M=(data1(:,6)-min(data1(:,6)))/(max(data1(:,6))-min(data1(:,6)));
LD_per2_per1M=(data1(:,8)-min(data1(:,8)))/(max(data1(:,8))-min(data1(:,8)));

data2=xlsread('per1_per2_LD1212.xlsx');
T_1212_per1=data2(:,1);

```

```

T_1212_per2=data2(:,3);

per1_1212=(data2(:,2)-min(data2(:,2)))/(max(data2(:,2))-min(data2(:,2)));
per2_1212=(data2(:,4)-min(data2(:,4)))/(max(data2(:,4))-min(data2(:,4)));

step=0.01;
fs=100;
t_end=7000;
t=0:1/fs:t_end;

B=1*ones(1,13);%Initial condition

option = odeset('RelTol', 1e-8);

k1=find(t>= 1197+4800 & t<= 1221+4800);
k2=find(t>= 1215+4800 & t<= 1239+4800);
k3=find(t>= 1194+4800 & t<= 1218+4800);

H=0.25;

L1=H*square(2*pi*(1/24)*t,75)+H;
L2=H*square(2*pi*(1/24)*t,25)+H;
L3=H*square(2*pi*(1/24)*t,50)+H;

t_new=t(k1);
t_plot=t_new-t_new(1);

% for short day WT

LD=1;
[t,A]=ode45(@ode1,t,B,option,LD);

```

```

per1_SD=(A(k2,1)-min(A(k2,1)))/(max(A(k2,1))-min(A(k2,1)));

per2_SD=(A(k2,4)-min(A(k2,4)))/(max(A(k2,4))-min(A(k2,4)));
subplot(331)
plot(t_plot,per1_SD)
hold on
plot(t_plot,per2_SD,'r')
legend ('per1','per2')
hold on
plot(t_plot,L2(k2),'k');
hold on
plot(T_SD_per1,SD_per1,'bo')
hold on
plot(T_SD_per2,SD_per2,'ro')
xlabel('Time(h)')
axis([0 24 -0.1 1.2])

```

% for Long day WT

```

LD=0;

[t,A]=ode45(@ode1,t,B,option,LD);

per1_LD=(A(k1,1)-min(A(k1,1)))/(max(A(k1,1))-min(A(k1,1)));

per2_LD=(A(k1,4)-min(A(k1,4)))/(max(A(k1,4))-min(A(k1,4)));

subplot(333)
plot(t_plot,per1_LD)
hold on
plot(t_plot,per2_LD,'r')
legend ('per1','per2')
hold on
plot(t_plot,L1(k1),'k');
hold on
plot(T_LD_per1,LD_per1,'bo')

```

```

hold on
plot(T_LD_per2,LD_per2,'ro')
xlabel('Time(h)')
axis([0 24 -0.1 1.2])

% %% for 12:12 WT
LD=2;

[t,A]=ode45(@ode1,t,B,option,LD);

per1_1212s=(A(k3,1)-min(A(k3,1)))/(max(A(k3,1))-min(A(k3,1)));

per2_1212s=(A(k3,4)-min(A(k3,4)))/(max(A(k3,4))-min(A(k3,4)));

subplot(332)
plot(t_plot,per1_1212s)
hold on
plot(t_plot,per2_1212s,'r')
legend('per1','per2')
hold on
plot(t_plot,L3(k3),'k');
hold on
plot(T_1212_per1,per1_1212,'bo')
hold on
plot(T_1212_per2,per2_1212,'ro')
xlabel('Time(h)')
axis([0 24 -0.1 1.2])

% for short day per1 mutant

LD=1;
[t,A]=ode45(@ode2,t,B,option,LD);

per1_SD=(A(k2,1)-min(A(k2,1)))/(max(A(k2,1))-min(A(k2,1)));

per2_SD=(A(k2,4)-min(A(k2,4)))/(max(A(k2,4))-min(A(k2,4)));

```

```

subplot(334)
plot(t_plot,per2_SD,'r')
hold on
plot(T_SD_per2_per1M,SD_per2_per1M,'ro')
hold on
plot(t_plot,L2(k2),'k');
xlabel('Time(h)')
axis([0 24 -0.1 1.2])
%
% %% for long day per1 mutant
LD=0;

[t,A]=ode45(@ode2,t,B,option,LD);

per2_LD=(A(k1,4)-min(A(k1,4)))/(max(A(k1,4))-min(A(k1,4)));

subplot(336)
plot(t_plot,per2_LD,'r')
hold on
plot(T_LD_per2_per1M,LD_per2_per1M,'ro')
axis([0 24 -0.1 1.2])
hold on
plot(t_plot,L1(k1),'k');
xlabel('Time(h)')
axis([0 24 -0.1 1.2])

% for per1 mutant

LD=2;

[t,A]=ode45(@ode2,t,B,option,LD);

per2_1212s=(A(k3,4)-min(A(k3,4)))/(max(A(k3,4))-min(A(k3,4)));

```

```

subplot(335)
plot(t_plot,per2_1212s,'r')
hold on
plot(t_plot,L3(k3),'k');
axis([0 24 -0.1 1.2])

```

% for short day per2 mutant

```

LD=1;
[t,A]=ode45(@ode3,t,B,option,LD);

per1_SD=(A(k2,1)-min(A(k2,1)))/(max(A(k2,1))-min(A(k2,1)));

per2_SD=(A(k2,4)-min(A(k2,4)))/(max(A(k2,4))-min(A(k2,4)));

```

```

subplot(337)
plot(t_plot,per1_SD,'b')
hold on
plot(T_SD_per1_per2M,SD_per1_per2M,'bo')
hold on
plot(t_plot,L2(k2),'k');
xlabel('Time(h)')
axis([0 24 -0.1 1.2])
%
% %% for long day per2 mutant

```

```

LD=0;

[t,A]=ode45(@ode3,t,B,option,LD);

per1_LD=(A(k1,1)-min(A(k1,1)))/(max(A(k1,1))-min(A(k1,1)));

per2_LD=(A(k1,4)-min(A(k1,4)))/(max(A(k1,4))-min(A(k1,4)));

subplot(339)
plot(t_plot,per1_LD,'b')

```

```

hold on
plot(T_LD_per1_per2M,LD_per1_per2M,'bo')
hold on
plot(t_plot,L1(k1),'k');
xlabel('Time(h)')
axis([0 24 -0.1 1.2])
%
% %% 1212 per1 mutant

LD=2;

[t,A]=ode45(@ode3,t,B,option,LD);

per1_1212s=(A(k3,1)-min(A(k3,1)))/(max(A(k3,1))-min(A(k3,1)));

subplot(338)

plot(t_plot,per1_1212s)
hold on
plot(t_plot,L3(k3),'k');
xlabel('Time(h)')
axis([0 24 -0.1 1.2])

function dadt=ode1(t,B,LD)

dadt=zeros(size(B));

Mp1=B(1);P1c=B(2);P1n=B(3);
Mp2=B(4);P2c=B(5);P2n=B(6);
Mb=B(7);B1=B(8);B2=B(9);
Mr=B(10);R=B(11);PB=B(12);PB2=B(13);

% parameters

vs1=0.45;vs2=1.2;vs3=4.5;vs4=1.23;vs5=0.1;

```

```

v1=1.47;v2=0.49;v3=0.42;v4=0.4;
v5=1.38;v6=1.67;v7=4.9;v8=0.37;v9=0.6;v10=1.51;v11=0.13;

kd1 =0.11;kd2 =0.09;kd3 =1.07;kd4 =0.03;kd5 =0.2;kd6 =0.08;kd7 =1.34;
kd8 =0.49;kd9 =0.7;kd10 =0.19;kd11 =0.92;kd12 =0.29;kd13 =0.07;

k1 =0.33;k2 =1.7;k3 =1.44;k4 =1.65;k5 =0.87;k6 =1.6;k7 =3.57;

kp1 =1.33;kp2 =0.5;kp3 =0.11;kp4=0.2;

ka1=0.44;ka2=1.66;ka3=1.48;ka4 =1.5;

kI1=6.63;

ke1=0.82;ke2 =0.98;ke3 =0.6;ke4 =1.65;
ke5 =1.13;ke6 =1;ke7 =0.3;ke8 =1.05;ke9 =1.7;ke10=3.58;ke11=0.026;

kx1 =0.29;
m =2.65;s =4.07;w =5.2;

% without gating

L1=1;L2=1;

% with gating

% T=24;L_max=1;
%
% L1=L_max/2;
%
% t1=3.5*T/24;t2=15*T/24;t3=22*T/24;t4=T;
%
% y1=(L_max)*((t2)/(T/1.3));
% y2=(L_max)*((t1)/(T/1.3));
%
```

```

%
% if mod(t,T)<=t1
%     L2=y2;
% elseif mod(t,T)>t1 & mod(t,T)<=t2
%     L2=(L_max)*mod(t,T)/(T/1.3);
% elseif mod(t,T)>t2 & mod(t,T)<=t3
%     L2=y1;
% elseif mod(t,T)>t3 & mod(t,T)<=t4
%     L2=(y2-y1)*((mod(t,T)-t3)/(t4-t3))+y1;
% else
%     L2=y2;
% end

a=0.1;

if LD==1
L=(a/2)*square(2*pi*(1/24)*t,25)+(a/2);
elseif LD==0
    L=(a/2)*square(2*pi*(1/24)*t,75)+(a/2);
else
    L=(a/2)*square(2*pi*(1/24)*t,50)+(a/2);
end

% per1 loop

dad1(1)=(vs1)*B2^m/(ka1^m+B2^m)-v1*Mp1/(ke1+Mp1)-kd1*Mp1+L*L1;

dad1(2)=k1*Mp1-v2*P1c/(ke2+P1c)-kd2*P1c;

dad1(3)=k2*P1c-v3*P1n/(ke3+P1n)-kd3*P1n-kp2*P1n*B2+kp1*PB;
% per2 loop

dad2(4)=(vs2)*B2^m/(ka2^m+B2^m)-v4*Mp2/(ke4+Mp2)-kd4*Mp2+L*L2;

dad2(5)=k3*Mp2-v5*P2c/(ke5+P2c)-kd5*P2c;

dad2(6)=k4*P2c-v6*P2n/(ke6+P2n)-kd6*P2n-kp4*P2n*B2+kp3*PB2;

```

```

%Bmal loop

dadt(7)=vs3*kI1^2/(kI1^2+R^2+(1/kx1)*R)-v7*Mb/(ke7+Mb)-kd7*Mb+
(vs4)*P2n^w/(ka3^w+P2n^w);

dadt(8)=k5*Mb-v8*B1/(ke8+B1)-kd8*B1;

dadt(9)=k6*B1-v9*B2/(ke9+B2)-kd9*B2-kp2*P1n*B2+kp1*PB-kp4*P2n*B2+
kp3*PB2;

%rev-erb

dadt(10)=vs5*B2^s/(ka4^s+B2^s)-v10*Mr/(ke10+Mr)-kd10*Mr;

dadt(11)=k7*Mr-v11*R/(ke11+R)-kd11*R;

%PER-BMAL complex

dadt(12)=kp2*P1n*B2-kp1*PB-kd12*PB;

dadt(13)=kp4*P2n*B2-kp3*PB2-kd13*PB2;

function dadt=ode2(t,B,LD)

dadt=zeros(size(B));

Mp1=B(1);P1c=B(2);P1n=B(3);
Mp2=B(4);P2c=B(5);P2n=B(6);
Mb=B(7);B1=B(8);B2=B(9);
Mr=B(10);R=B(11);PB=B(12);PB2=B(13);

vs2=1.2;vs3=4.5;vs5=0.1;vs4=1.23;

v1=1.47;v2=0.49;v3=0.42;v4=0.44;
v5=1.38;v6=1.67;v7=4.9;v8=0.37;v9=0.6;v10=1.51;v11=0.13;

```

```
kd1 =0.11;kd2 =0.09;kd3 =1.07;kd4 =0.03;kd5 =0.2;kd6 =0.08;kd7 =1.34;
kd8 =0.49;kd9 =0.7;kd10 =0.19;kd11 =0.92;kd12 =0.29;kd13 =0.07;
```

```
k1 =0.33;k2 =1.7;k3 =1.44;k4 =1.65;k5 =0.87;k6 =1.6;k7 =3.57;
```

```
kk=1.05;
```

```
kp1 =1.33;kp2 =0.5;kp3 =0.11;kp4 =0.2;
```

```
ka1=0.44;ka2=1.66;ka3=1.48;ka4 =1.5;
```

```
kI1=6.63;
```

```
ke1=0.82;ke2 =0.98;ke3 =0.6;ke4 =1.65;
```

```
ke5 =1.13;ke6 =1;ke7 =0.3;ke8 =1.05;ke9 =1.7;ke10=3.58;ke11=0.026;
```

```
kx1 =0.29;
```

```
m =2.65;s =4.07;w =5.2;
```

% per1 mutant

```
kp2=0.5;
```

```
v4=0.43;
```

```
kp4=0.19;
```

```
vs1=0;
```

% without gating

```
L1=1;L2=1;
```

% with gating

```
% T=23.76;%free running period CT0=3013; L_max=1;
```

```
% t1=3.5*T/24;t2=15*T/24;t3=22*T/24;t4=T; y1=(L_max)*((t2)/(T/1.3));
```

```
% y2=(L_max)*((t1)/(T/1.3));
```

```
% L1=L_max/2;
```

```

% if t<=CT0
%     L2=0;
% elseif t>CT0 & t<=CT0+t1
%     L2=y2;
% elseif t>CT0+t1 & t<=CT0+t2
% L2=(L_max)*((t-CT0)/(T/1.3));
% elseif t>CT0+t2 & t<=CT0+t3
% L2=y1;
% elseif
% t>CT0+t3 & t<=CT0+t4
% L2=(y2-y1)*(((t-CT0)-t3)/(t4-t3))+y1;
% else
% L2=y2;
% end

a=0.1;
if LD==1
L=(a/2)*square(2*pi*(1/24)*t,25)+(a/2);
elseif LD==0
    L=(a/2)*square(2*pi*(1/24)*t,75)+(a/2);
else
    L=(a/2)*square(2*pi*(1/24)*t,50)+(a/2);
end

% per1 loop

dadt(1)=(vs1)*B2^m/(ka1^m+B2^m)-v1*Mp1/(ke1+Mp1)-kd1*Mp1;

dadt(2)=k1*Mp1-v2*P1c/(ke2+P1c)-kd2*P1c;

dadt(3)=k2*P1c-v3*P1n/(ke3+P1n)-kd3*P1n-kp2*P1n*B2+kp1*PB;
% per2 loop

dadt(4)=(vs2)*B2^m/(ka2^m+B2^m)-v4*Mp2/(ke4+Mp2)-kd4*Mp2+L*L2;

dadt(5)=k3*Mp2-v5*P2c/(ke5+P2c)-kd5*P2c;

```

```

dadtt(6)=k4*P2c-v6*P2n/(ke6+P2n)-kd6*P2n-kp4*P2n*B2+kp3*PB2;

%Bmal loop

dadtt(7)=vs3*kI1^2/(kI1^2+R^2+(1/kx1)*R)-v7*Mb/(ke7+Mb)-kd7*Mb+
(vs4)*P2n^w/(ka3^w+P2n^w);

dadtt(8)=k5*Mb-v8*B1/(ke8+B1)-kd8*B1;

dadtt(9)=k6*B1-v9*B2/(ke9+B2)-kd9*B2-kp2*P1n*B2+kp1*PB-kp4*P2n*B2+kp3*PB2;

%rev-erb

dadtt(10)=vs5*B2^s/(ka4^s+B2^s)-v10*Mr/(ke10+Mr)-kd10*Mr;

dadtt(11)=k7*Mr-v11*R/(ke11+R)-kd11*R;

%PER-BMAL complex

dadtt(12)=kp2*P1n*B2-kp1*PB-kd12*PB;

dadtt(13)=kp4*P2n*B2-kp3*PB2-kd13*PB2;

function dadtt=ode3(t,B,LD)

dadtt=zeros(size(B));

Mp1=B(1);P1c=B(2);P1n=B(3);
Mp2=B(4);P2c=B(5);P2n=B(6);
Mb=B(7);B1=B(8);B2=B(9);
Mr=B(10);R=B(11);PB=B(12);PB2=B(13);

vs4=1.23;

v4=0.4;v5=1.38;v6=1.67;v7=4.9;v8=0.37;v9=0.6;v10=1.51;v11=0.13;

```

```

kd1 =0.11;kd2 =0.09;kd4 =0.03;kd5 =0.2;kd6 =0.08;kd7 =1.34;
kd8 =0.49;kd9 =0.7;kd10 =0.19;kd11 =0.92;kd12 =0.29;kd13 =0.07;

k2 =1.7;k3 =1.44;k4 =1.65;k5 =0.87;k6 =1.6;k7 =3.57;

kp3 =0.11;kp4=0.2;ka1=0.44;ka2=1.66;ka3=1.48;ka4=1.5;kI1=6.63;

ke1=0.82;ke2 =0.98;ke3 =0.6;ke4 =1.65;
ke5 =1.13;ke6 =1;ke7 =0.3;ke8 =1.05;ke9 =1.7;ke10=3.58;ke11=0.026;
kx1 =0.29;
m =2.65;s =4.07;w =5.2;

```

% per2 mutant (Rhythmic)

```

vs2=0;
vs3=4.5;vs5=0.5;vs1=0.7;
v1=0.4400;v2 = 1.3800; v3 = 1.6700;
k1= 1.4400;kd3 = 0.0800;
kp1 = 0.1100; kp2=0.18;

```

% without gating

```

L1=1;L2=1;

```

% with gating

```

% T=24;%free running period CT0=3013; L_max=1;
% t1=3.5*T/24;t2=15*T/24;t3=22*T/24;t4=T; y1=(L_max)*((t2)/(T/1.3));
% y2=(L_max)*((t1)/(T/1.3));
% L1=L_max/2;
% if t<=CT0
%     L2=0;
% elseif t>CT0 & t<=CT0+t1
%     L2=y2;
% elseif t>CT0+t1 & t<=CT0+t2

```

```

% L2=(L_max)*((t-CT0)/(T/1.3));
% elseif t>CT0+t2 & t<=CT0+t3
% L2=y1;
% elseif
% t>CT0+t3 & t<=CT0+t4
% L2=(y2-y1)*(((t-CT0)-t3)/(t4-t3))+y1;
% else
% L2=y2;
% end

a=0.1;
if LD==1
L=(a/2)*square(2*pi*(1/24)*t,25)+(a/2);
elseif LD==0
    L=(a/2)*square(2*pi*(1/24)*t,75)+(a/2);
else
    L=(a/2)*square(2*pi*(1/24)*t,50)+(a/2);
end

% per1 loop

dad1(1)=(vs1)*B2^m/(ka1^m+B2^m)-v1*Mp1/(ke1+Mp1)-kd1*Mp1+L*L1;

dad1(2)=k1*Mp1-v2*P1c/(ke2+P1c)-kd2*P1c;

dad1(3)=k2*P1c-v3*P1n/(ke3+P1n)-kd3*P1n-kp2*P1n*B2+kp1*PB;
% per2 loop

dad1(4)=(vs2)*B2^m/(ka2^m+B2^m)-v4*Mp2/(ke4+Mp2)-kd4*Mp2;

dad1(5)=k3*Mp2-v5*P2c/(ke5+P2c)-kd5*P2c;

dad1(6)=k4*P2c-v6*P2n/(ke6+P2n)-kd6*P2n-kp4*P2n*B2+kp3*PB2;

%Bmal loop

dad1(7)=vs3*kI1^2/(kI1^2+R^2+(1/kx1)*R)-v7*Mb/(ke7+Mb)-kd7*Mb+

```

```

(vs4)*P2n^w/(ka3^w+P2n^w);

dadt(8)=k5*Mb-v8*B1/(ke8+B1)-kd8*B1;

dadt(9)=k6*B1-v9*B2/(ke9+B2)-kd9*B2-kp2*P1n*B2+kp1*PB-kp4*P2n*B2+
kp3*PB2;

%rev-erb

dadt(10)=vs5*B2^s/(ka4^s+B2^s)-v10*Mr/(ke10+Mr)-kd10*Mr;

dadt(11)=k7*Mr-v11*R/(ke11+R)-kd11*R;

%PER-BMAL complex

dadt(12)=kp2*P1n*B2-kp1*PB-kd12*PB;

dadt(13)=kp4*P2n*B2-kp3*PB2-kd13*PB2;

```

Program-4

This is a function file to plot the double actogram of coupled ME oscillator. This function is called by the program named 'coupledModel.m'

```

%*****
Program Name: actogram_R.m
This function requires 11 input arguments to execute and they are
t: Time
x1:Time series-1 for M activity
th1: Threshold for defining M activity
per1 = period (typically 24h)
days1: Number of days for M activity
rep1: Number of repetition for M activity (1 for single actogram,
      2 for double actogram)
x2:Time series-2 for E activity

```

```

th2: Threshold for defining E activity
per2 = period (typically 24h)
days2: Number of days for E activity
rep2: Number of repetition for E activity (1 for single actogram,
2 for double actogram)
%*****

function actogram_R(t,x1,th1,per1,days1,rep1,x2,th2,per2,days2,rep2)

clf;

% Plot data

for td=0:days1-1

    t1=td*per1;
    t2=t1+(rep1*per1);

    k=find(t>t1 & t<t2);

    y=x1(k);

    k=find(y>th1);

    ta=t(k);

    h=days1-td;

    plot(ta,h*ones(1,length(k)),'b.')
    hold on;

end
%*****

for td=0:days2-1

```

```

    t1=td*per2;
    t2=t1+(rep2*per2);

    k=find(t>t1 & t<t2);

    y=x2(k);

    k=find(y>th2);

    ta=t(k);

    h=days2-td;

    plot(ta,h*ones(1,length(k)),'r. ')
    hold on;

end

%*****

% Axis limits and labels

xlim([0 per1*rep1])
ylim([0.01 days1+0.99])

set(gca,'YTickLabel',{' '})

xlabel('Time','fontsize',18);
ylabel('Days','fontsize',18);

ylabh = get(gca,'yLabel');
set(ylabh,'Position',get(ylabh,'Position') - [2.5 0 0]); % shift y-label to left

```

Program-5

This program is for the splitting pattern (Fig. 4.17).

```

%*****
Program Name: coupled__Model.m
This program requires 'actogram_R.m' file for plotting
actogram of activity pattern
%*****

function coupled__Model

clc;clear all;close all;

t_end=7000;
dt=0.01;
t=0:dt:t_end;
B=0*ones(1,28);
[t,A]=ode15s(@ode1,t,B);

% Normalization (cycle by cycle)

cycle=round(t_end/24);

%variables used for actogram

x2_M=A(:,3);%PER1
x2_E=A(:,16);

PER1norm_M=[];
PER1norm_E=[];

for td=0:cycle-1

    t1=td*24;
    t2=t1+(24);

    k=find(t>t1 & t<=t2);

```

```

    ym2=x2_M(k);
    ye2=x2_E(k);

    ynm2=(ym2-min(ym2))/(max(ym2)-min(ym2));
    yne2=(ye2-min(ye2))/(max(ye2)-min(ye2));

    PER1norm_M=[PER1norm_M ynm2'];
    PER1norm_E=[PER1norm_E yne2'];

end

%period calculation
x1=find(t>2000);

x=A(:,1);
x_new=x(x1);
x=x_new;
N=mean(x_new);
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)<N && x(i+1)>N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end

t1=t(zerIdx); % display timepoints of zero-crossings
for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
period1;
M1=mean(period1);%find out the mean period
Period_of_oscillation=M1;

% period of E oscillator

x=A(:,14);

```

```

x_new=x(x1);
x=x_new;
N=mean(x_new);
zerIdx=[];
for i=1:length(x)-1
    if ((x(i)<N && x(i+1)>N))
        zerIdx(end+1)=i; % save index of zero-crossing
    end
end

t1=t(zerIdx); % display timepoints of zero-crossings
for j=1:length(t1)-1
    period1(j)=t1(j+1)-t1(j);
end
period1;
M1=mean(period1);%find out the mean period

Period_of_oscillation=M1;

% actogram generation

k1=find(t>2500);

t_act=t(k1);
xref_M=PER1norm_M(k1(1:end-1));
xref_E=PER1norm_E(k1(1:end-1));

th=1.8*mean(xref_M);
rep=2; %Number repetation
days=100;
per=24;%period
th1=1.8*mean(xref_E);

rep1=rep;days1=days;per1=per;

```

```

actogram_R(t,xref_M,th,per,days,rep,xref_E,th1,per1,days1,rep1);

function dadt=ode1(t,B)

dadt=zeros(size(B));

Mp1m=B(1);P1cm=B(2);P1nm=B(3);
Mp2m=B(4);P2cm=B(5);P2nm=B(6);
Mbm=B(7);B1m=B(8);B2m=B(9);
Mrm=B(10);Rm=B(11);PBm=B(12);PB2m=B(13);

Mp1e=B(14);P1ce=B(15);P1ne=B(16);
Mp2e=B(17);P2ce=B(18);P2ne=B(19);
Mbe=B(20);B1e=B(21);B2e=B(22);
Mre=B(23);Re=B(24);PBe=B(25);PB2e=B(26);
% CB=B(27);
VIP=B(27);AVP=B(28);

% M oscillator

vs1m=4;vs2m=2;vs3m=3.8;vs5m=0.5;vs4m= 1.2300;

ka1m= 0.4400;ka2m  = 1.6600;ka3m= 1.4800;ka4m  = 1.5000;

kI1m= 6.6300;

ke1m = 0.8200;ke2m = 0.9800;ke3m = 0.6;ke4m  = 1.6500;
ke5m  = 1.1300;ke6m  = 1;
ke7m  = 0.3000;ke8m = 1.0500;ke9m  = 1.7000;ke10m = 3.5800;
ke11m  = 0.0260;

kx1m= 0.2900;sm  = 4.0700;mm  = 2.6500;wm= 5.2000;

k1m=2.5;k2m= 1.7000;k3m  = 1.4400;k4m  = 1.6500;k5m  = 0.8700;
k6m  = 1.6000;k7m  = 3.5700;

```

```
kd1m=0.05;kd2m = 0.0900;kd3m = 0.0800;kd4m = 0.0300;kd5m = 0.2000;
kd6m = 0.0800;kd7m= 1.3400;
kd8m = 0.4900;kd9m = 0.7000;kd10m = 0.1900;
kd11m = 0.9200;kd12m=0.29;kd13m=0.07;
```

```
v1m= 0.4400;v2m = 1.3800;v3m = 1.6700;v4m= 0.4400;v5m = 1.3800;
v6m = 1.6700;
v7m= 4.9000;v8m = 0.3700;v9m = 0.6000;v10m = 1.5100;
v11m = 0.1300;
```

```
kp1m=0.1;kp2m=1.9;kp3m=0.11;kp4m=0.7;
```

% E oscillator

```
kI1e= 6.6300;
```

```
kx1e= 0.2900;se = 4.0700;me = 2.6500;we= 5.2000;
```

```
ka1e= 0.4400;ka2e = 1.6600;ka3e= 1.4800;ka4e = 1.5000;
```

```
ke1e = 0.8200;ke2e = 0.9800;ke3e = 0.6000;ke4e = 1.6500;
ke5e = 1.1300;
ke6e = 1;ke7e = 0.3000;
ke8e = 1.0500;ke9e = 1.7000;ke10e = 3.5800;ke11e = 0.0260;
```

```
v1e= 1.4700;v2e = 0.4900;v3e = 0.4200;v4e= 0.4400;v5e = 1.3800;
v6e = 1.6700;v7e= 4.9000;v8e = 0.3700;v9e = 0.6000;v10e = 1.5100;
v11e = 0.1300;
```

```
kd1e= 0.1100;kd2e = 0.0900;kd3e= 1.0700;kd4e=0.01;kd5e = 0.1000;
kd6e = 0.0800;kd7e= 1.3400;kd8e = 0.4900;
kd9e = 0.7000;kd10e = 0.1900;kd11e = 0.9200;kd12e=0.29;kd13e=0.07;
```

```
k1e= 0.3300;k2e= 1.7000;k3e=0.5;k4e=1;k5e = 0.8700;k6e = 1.6000;
```

```
k7e = 3.5700;

vs1e=0.2;vs2e=2;vs3e= 4;vs4e= 0.7800;vs5e=0.1;

kp1e=0.1;kp2e=0.17 ;kp3e=0.11;kp4e=0.7;

vv1=0.01;kev1=1;kdv1=0.05;
vv2=0.01;kev2=1;kdv2=0.05;

kcm=2;kce=2;

beta=0.01;

vcm1=0.24;vcm2=0.24;

vce1=0.01;vce2=0.01;

kvs1=0.01;kvs2=0.05;

vcm1=0.23;vcm2=0.23;

vce1=0.005;vce2=0.005;

kvs1=0.01;kvs2=0.05;

% for DD

L=0;

%LD condion
%
% a=0.01;
% L=(a/2)*square(2*pi*(1/24)*t,50)+(a/2);
% %
```

```
vcm1=0.24;vcm2=0.24;
vce1=0.01;vce2=0.01;
kvs1=0.01;kvs2=0.05;
```

% DD condition

```
L=0;
```

```
%LD condion
% a=0.02;
%  $L=(a/2)*\text{square}(2*\pi*(1/24)*t,50)+(a/2);$ 
```

% stable split

```
L=0.02;
vce1=0.01;vce2=0.01;
beta=0.04;

if t<3215
    vcm1=0.35;vcm2=0.25;
    kvs1=0.001;kvs2=0.015;
else
    vcm1=0.28;vcm2=0.25;
    kvs1=0.001;kvs2=0.009;
end
```

% stable split and re-fuse

```
% L=0.02; vce1=0.01;vce2=0.01; beta=0.04;
%
% if t<=2700
% vcm1=0.35;vcm2=0.25;
% kvs1=0.001;kvs2=0.015;
% elseif t>2700 & t<3500
% vcm1=0.28;vcm2=0.25;
% kvs1=0.001;kvs2=0.009;
```

```

% else
% vcm1=0.35;vcm2=0.25;
% kvs1=0.004;kvs2=0.025;
% end

% %% split and re-fuse

% L=0.02;
% vce1=0.01;vce2=0.01;
% beta=0.04;
%
% if t<=3400
%     vcm1=0.35;vcm2=0.25;
%     kvs1=0.001;kvs2=0.015;
% elseif t>=3400 & t<4300
%     vcm1=0.01;vcm2=0.25;
%     kvs1=0.001;kvs2=0.01;
% else
%     vcm1=0.35;vcm2=0.25;
%     kvs1=0.001;kvs2=0.015;
% end

%M oscillator

dadt(1)=(vs1m)*B2m^mm/(ka1m^mm+B2m^mm)-v1m*Mp1m/(ke1m+Mp1m)-...
kd1m*Mp1m+vcm1*AVP/(kcm+AVP)+L;

dadt(2)=k1m*Mp1m-v2m*P1cm/(ke2m+P1cm)-kd2m*P1cm;

dadt(3)=k2m*P1cm-v3m*P1nm/(ke3m+P1nm)-kd3m*P1nm-kp2m*P1nm*B2m+...
kp1m*PBm;

%per2 loop
dadt(4)=(vs2m)*B2m^mm/(ka2m^mm+B2m^mm)-v4m*Mp2m/(ke4m+Mp2m)-...
kd4m*Mp2m+vcm2*AVP/(kcm+AVP)+L;

```

```

dadtt(5)=k3m*Mp2m-v5m*P2cm/(ke5m+P2cm)-kd5m*P2cm;

dadtt(6)=k4m*P2cm-v6m*P2nm/(ke6m+P2nm)-kd6m*P2nm-kp4m*P2nm*B2m+...
kp3m*PB2m;

%Bmal loop
dadtt(7)=vs3m*kI1m^2/(kI1m^2+Rm^2+(1/kx1m)*Rm)-v7m*Mbm/(ke7m+Mbm)-...
kd7m*Mbm+(vs4m)*P2nm^wm/(ka3m^wm+P2nm^wm);

dadtt(8)=k5m*Mbm-v8m*B1m/(ke8m+B1m)-kd8m*B1m;

dadtt(9)=k6m*B1m-v9m*B2m/(ke9m+B2m)-kd9m*B2m-kp2m*P1nm*B2m+...
kp1m*PBm-kp4m*P2nm*B2m+kp3m*PB2m;

%rev-erb
dadtt(10)=vs4m*B2m^sm/(ka4m^sm+B2m^sm)-v10m*Mrm/(ke10m+Mrm)-...
kd10m*Mrm;

dadtt(11)=k7m*Mrm-v11m*Rm/(ke11m+Rm)-kd11m*Rm;

%PER-BMAL complex

dadtt(12)=kp2m*P1nm*B2m-kp1m*PBm-kd12m*PBm;

dadtt(13)=kp4m*P2nm*B2m-kp3m*PB2m-kd13m*PB2m;

% E oscillator

dadtt(14)=(vs1e)*B2e^me/(ka1e^me+B2e^me)-v1e*Mp1e/(ke1e+Mp1e)-kd1e*Mp1e+...
(beta+VIP*(vce1+L))/(kce+VIP);

dadtt(15)=k1e*Mp1e-v2e*P1ce/(ke2e+P1ce)-kd2e*P1ce;

```

```

dadt(16)=k2e*P1ce-v3e*P1ne/(ke3e+P1ne)-kd3e*P1ne-kp2e*P1ne*B2e+kp1e*PBe;

%per2 loop

dadt(17)=(vs2e)*B2e^me/(ka2e^me+B2e^me)-v4e*Mp2e/(ke4e+Mp2e)-kd4e*Mp2e+...
(beta+VIP*(vce2+L))/(kce+VIP);

dadt(18)=k3e*Mp2e-v5e*P2ce/(ke5e+P2ce)-kd5e*P2ce;

dadt(19)=k4e*P2ce-v6e*P2ne/(ke6e+P2ne)-kd6e*P2ne-kp4e*P2ne*B2e+...
kp3e*PB2e;

%Bmal loop
dadt(20)=vs3e*kI1e^2/(kI1e^2+Re^2+(1/kx1e)*Re)-v7e*Mbe/(ke7e+Mbe)-...
kd7e*Mbe+(vs4e)*P2ne^we/(ka3e^we+P2ne^we);

dadt(21)=k5e*Mbe-v8e*B1e/(ke8e+B1e)-kd8e*B1e;

dadt(22)=k6e*B1e-v9e*B2e/(ke9e+B2e)-kd9e*B2e-kp2e*P1ne*B2e+...
kp1e*PBe-kp4e*P2ne*B2e+kp3e*PB2e;

%rev-erb
dadt(23)=vs5e*B2e^se/(ka4e^se+B2e^se)-v10e*Mre/(ke10e+Mre)-kd10e*Mre;

dadt(24)=k7e*Mre-v11e*Re/(ke11e+Re)-kd11e*Re;

%PER-BMAL complex

dadt(25)=kp2e*P1ne*B2e-kp1e*PBe-kd12e*PBe;

dadt(26)=kp4e*P2ne*B2e-kp3e*PB2e-kd13e*PB2e;

dadt(27)=((kvs1)*(P2nm+P1nm)-vv1*VIP/(kev1+VIP)-kdv1*VIP);

% dadt(27)=0;

```

```
dadt(28)=((kvs2)*(P1ne+P2ne)-vv2*AVP/(kev2+AVP)-kdv2*AVP);
```

D.3 Chapter-5 programs

Program-1

This is a xppaut file that is used for for the bifurcation diagram and stochastic simulation of Dickman et.al (2013) HH model. (Fig. 5.2).

```
% *****
For bifurcation diagram set D=0. For stochastic simulation,
Just run the program in xppaut. for different intensity,
change the parameter 'D'=1,2,3,...
%*****

init v=0,m=0,h=0,n=0,rl=0,rnonl=0,fnonl=0,s=0,ca_shell=0

par g_naleak=0.052

par g_kca=180,

par g_kleak=0.15,

par D=0

wiener w

par g_na=229,g_cal=6,

par C=5.7,Ena=45,Ek=-97,Eca=54,

par g_canonl=20,g_k=3,

par K1=3.93e-5,K2=6.55e-4,

par k_shell=1.65e-4,tau_shell=0.05,b_shell=5.42510e-4,
```

```
# m
m_inf=1/(1+exp(-(v+35.2)/8.1))

tau_m=exp(-(v+286)/160)

# h
h_inf=1/(1+exp((v+62)/2))
tau_h=0.51+exp(-(v+26.6)/7.1)

# n
n_inf=1/(((1+exp((v-14)/(-17))))^0.25)
tau_n=exp(-(v-67)/68)

# rl
rl_inf=1/(1+exp(-(v+36)/5.1))

tau_rl=3.1

# rnonl
rnonl_inf=1/(1+exp(-(v+21.6)/6.68))
tau_rnonl=3.1

# fl
fl=K1/(K2+ca_shell)

# fnonl
fnonl_inf=1/(1+exp((v+260)/65))
tau_fnonl=exp(-(v-444)/220)

# s
s_inf=((10^7)*ca_shell^2)/((10^7)*ca_shell^2+5.6)
tau_s=500/((10^7)*ca_shell^2+5.6)

## Currents
```

```

Iapp=0
Ina=g_na*(m^3)*h*(v-Ena)
Ik=g_k*(n^4)*(v-Ek)
Ical=g_cal*rl*fl*(v-Eca)
Icanonl=g_canonl*rnonl*fnonl*(v-Eca)
Ikca=g_kca*(s^2)*(v-Ek)
Ikleak=g_kleak*(v-Ek)
Inaleak=g_naleak*(v-Ena)

Sr=1

## Differential Equations

dv/dt=Sr*((Iapp-Ina-Ik-Ical-Icanonl-Ikca-Ikleak-Inaleak)/C)+D*w

dm/dt=Sr*((m_inf-m)/tau_m)

dh/dt=Sr*((h_inf-h)/tau_h)

dn/dt=Sr*((n_inf-n)/tau_n)

drl/dt=Sr*((rl_inf-rl)/tau_rl)

drnonl/dt=Sr*((rnonl_inf-rnonl)/tau_rnonl)

dfnonl/dt=Sr*((fnonl_inf-fnonl)/tau_fnonl)

ds/dt=Sr*((s_inf-s)/tau_s)

```

```
dCa_shell/dt=Sr*(-k_shell*(Ical+Icanonl)-ca_shell/tau_shell+b_shell)
```

```
@ total=2e3,dt=0.02,xp=t,yp=v,ylo=-100,yhi=50,xlo=0,xhi=2e3,bounds=100000
@ MAXSTOR=10e200000, method=Eulers
```

Program-2

This xppaut program is for the stochastic simulation of Diekman et.al (2013) HH model with periodic forcing by GRN (5.4).

```
%*****
Simulation procedure:
1. First choose the desired time at which stochastic simulation
has to perform.
2. If the circadian time is less than desired time, choose a higher
time step (10ms),and perform simulation without adding noise
3. If the circadian time is greter than desired time choose a smaller
time step (0.02ms),for a 5s time perform simulation with noise
%*****

init v=0,m=0,h=0,n=0,rl=0,rnonl=0,fnonl=0,s=0,ca_shell=0,
init Mp=0.0010, P=0.0019, P1=0.0024

mpn=(mp-0.000867)/(0.00415-0.000867)

aux gnl=Ag1*mpn+Ag2

par Ag1=0.032,Ag2=0.02

g_naleak=Ag1*mpn+Ag2
```

```
par g_kca=180,g_kleak=0.15,
par D=0

wiener w

par g_na=229,g_cal=6,C=5.7,Ena=45,Ek=-97,Eca=54,

par g_canonl=20,g_k=3, K1=3.93e-5,K2=6.55e-4,

par k_shell=1.65e-4,tau_shell=0.05,b_shell=5.42510e-4,

# m
m_inf=1/(1+exp(-(v+35.2)/8.1))

tau_m=exp(-(v+286)/160)

# h
h_inf=1/(1+exp((v+62)/2))
tau_h=0.51+exp(-(v+26.6)/7.1)

# n
n_inf=1/((1+exp((v-14)/(-17)))^0.25)
tau_n=exp(-(v-67)/68)

# rl
rl_inf=1/(1+exp(-(v+36)/5.1))

tau_rl=3.1

# rnonl
rnonl_inf=1/(1+exp(-(v+21.6)/6.68))
tau_rnonl=3.1

# fl
fl=K1/(K2+ca_shell)
```

```

# fnonl
fnonl_inf=1/(1+exp((v+260)/65))
tau_fnonl=exp(-(v-444)/220)

# s
s_inf=((10^7)*ca_shell^2)/((10^7)*ca_shell^2+5.6)
tau_s=500/((10^7)*ca_shell^2+5.6)

## Currents
Iapp=0
Ina=g_na*(m^3)*h*(v-Ena)
Ik=g_k*(n^4)*(v-Ek)
Ical=g_cal*rl*fl*(v-Eca)
Icanonl=g_canonl*rnonl*fnonl*(v-Eca)
Ikca=g_kca*(s^2)*(v-Ek)
Ikleak=g_kleak*(v-Ek)
Inaleak=g_naleak*(v-Ena)

Sr=1

## Differential Equations
# dv/dt
dv/dt=Sr*((Iapp-Ina-Ik-Ical-Icanonl-Ikca-Ikleak-Inaleak)/C)+D*w
# dm/dt
dm/dt=Sr*((m_inf-m)/tau_m)
# dh/dt
dh/dt=Sr*((h_inf-h)/tau_h)
# dn/dt
dn/dt=Sr*((n_inf-n)/tau_n)
# drl/dt
drl/dt=Sr*((rl_inf-rl)/tau_rl)
# drnonl/dt
drnonl/dt=Sr*((rnonl_inf-rnonl)/tau_rnonl)
# dfnonl/dt
dfnonl/dt=Sr*((fnonl_inf-fnonl)/tau_fnonl)

```

```
# ds/dt
ds/dt=Sr*((s_inf-s)/tau_s)
# dca_shell/dt
dCa_shell/dt=Sr*(-k_shell*(Ical+Icanonl)-ca_shell/tau_shell+b_shell)

par a=4.46e-8,nn=9

dMp/dt=(a*((0.001^nn/(0.001^nn+P1^nn))-Mp))
dP/dt=a*(Mp-P)
dP1/dt=a*(P-P1)

@ total=1e9,dt=0.02,xp=t,yp=v,ylo=-100,yhi=50,xlo=0,xhi=1e9,bounds=100000,
@ MAXSTOR=10e200000, method=eulers
```

D.4 Chapter-6 programs

Program-1

This is a xppaut file that is used for simulating circadian modulation of SFR (Fig. 6.3).

```
%*****
Initially we run the simulation with a high time step
(dt=10000 ms, 0.0028 h) till we reach the desired circadian phase
at which we calculate the voltage dynamics. When we start the simulation
of voltage dynamics at desired time, we reduce the time step to
dt=0.01ms and run the simulation for the desired time duration.
%*****

SCN_ML_SFR.ode

par g_cabase=1,

# for spiking (After set the desired CT change 1 to 6.37)

##g_cabase=6.37

#####
# GRN For SCN

dMps/dt=As*((vs1*kas^nc/(kas^nc+P1ps^nc))-Mps)+L

dP1s/dt=As*(Mps-P1s)

dP1ps/dt=As*(P1s-P1ps)

init Mps=0.709, p1s=0.8639, P1ps=1.088
par nc=9,As=4.35e-8,kas=0.8,vs1=20, L=0

#####
# Modified ML model
```

```

dv/dt=(I_app-gL*(v-vL)-gk*w*(v-vk)-gca*minf(v)*(v-vca))/C

dw/dt=lamw(v)*(winf(v)-w)

minf(v)=.5*(1+tanh((v-v1)/v2))

winf(v)=.5*(1+tanh((v-v3)/v4))

lamw(v)=phi*cosh((v-v3)/(2*v4))

init v=-60.899, w=0.014873

par I_app=0, vk=-84,vL=-40,vca=90, gk=30,gL=2,C=20, phi=.04,

par v1=-1.2,v2=18,v3=12,v4=14.75,kph=0.1,

gca=g_cabase*Mps/(kph+Mps)

aux gc=gca

@ total=8.64e8,dt=10000, bounds=1e25, method=stiff, yp=mps, xhi=1e9

```

Program-2

This is a xppaut file that is used for simulating circadian modulation of LTP/LTD at HC (Fig. 6.5).

```

%*****
Initially we set I_pre_max=0, I_post_max=0, and we run the simulation with a
high time step (dt=10000 ms) till we
reach the desired circadian phase at which we calculate the EPSP.
When we start the simulation of LTP/LTD at desired time, we reduce the time
step to dt=0.01ms,
and set I_pre_max=35, I_post_max=800, and run the simulation for the desired
time duration.

```

```

%*****

## pre-post activity##*****

LTP_LTD_HC.ode

par del_t=0

t_on1=t_on-del_t

t_off1=t_on1+2

par I_pre_max=0, I_post_max=0,

#par I_pre_max=35, I_post_max=800

I_pre(t)=I_pre_max*heav(t_off1-t)*heav(t-t_on1)

par t_on=100

t_off=t_on+2

I_post(t)=I_post_max*heav(t_off-t)*heav(t-t_on)

##*****GRN for SCN *****

#init mps=0.64, p1s=1.006,p1ps=1.2, Rc1=1.2, mph=1.53, p1h=1.03, p1ph=0.99

init mps=0.704, p1s=0.865,p1ps=1.09, Rc1=1.1807, mph=1.738, p1h=1.281, p1ph=1.059

dMps/dt=As*((vs1*kas^nc/(kas^nc+P1ps^nc))-Mps)+L

dP1s/dt=As*(Mps-P1s)

dP1ps/dt=As*(P1s-P1ps)

```

```

par nc=9,As=4.35e-8,kas=0.8,vs1=20, L=0

##*****GRN for HC *****

dMph/dt=As*((vs2*kAh^nc/(kAh^nc+P1ph^nc))-Mph)+vss*Rc1^nc/(kc^nc+Rc1^nc)+
(Kscr*s_CREB)*CREB

dP1h/dt=As*(Mph-P1h)

dP1ph/dt=As*(P1h-P1ph)

# for higher coupling
#par vss=1e-2

# for lower coupling

par vss=1e-3

par kAh=0.8,vs2=20, Kscr=1e-4, kc=5

## *****Coupling *****

dRc1/dt=AS*(P1ps-Rc1)

ds_CREB/dt=ar*I_pre(t)*(1-s_CREB)-ad*s_CREB

CREB=ca*kcre1-kcre2

par ar=0.01,ad=0.07,

## *****NMDA Current*****

I_NMDA=g_nmda*sG*Bv*(v-v_NMDA)

g_nmda=g_N*knorm^nm/(P1ph^nm)

```

```

Bv=1/(1+0.005*Mg*exp(-0.2*v))

dsG/dt=ar*I_pre(t)*(1-sG)-ad*sG

par nm=2, knorm=1, g_N=12, v_NMDA=0, Mg=1,

##*****Modified ML model*****

dv/dt=(I_post(t)-gl*(v-vl)-gk*w*(v-vk)-gca*minf(v)*(v-vca)-I_NMDA)/c

dw/dt=lamw(v)*(winf(v)-w)

minf(v)=.5*(1+tanh((v-v1)/v2))

winf(v)=.5*(1+tanh((v-v3)/v4))

lamw(v)=phi*cosh((v-v3)/(2*v4))

par vk=-84,vl=-60,vca=120, gk=8,gl=2,c=20, phi=.08,

par v1=-1.2,v2=18,v3=2,v4=30,

init v=-60.899, w=0.014873

## *****Calcium dyndamics*****

gca=g_cabase*Mph/(kph+Mph)

dca/dt=k_nmda*I_NMDA-k_ca*I_ca+(Ca0-ca)/tau_c

I_ca=gca*minf(V)*(V-Vca)

par k_nmda=10, k_ca=1, tau_c=10, kph=0.1, g_cabase=4.35, ca0=500

## *****EPSP and AMPAR dynamics*****

```

```

dEPSP/dt=I_NMDA/c

dAMPA/dt=(ar1*(ca-Ca1)*(A_m-AMPAR)-ad1*AMPAR*(ca-509.5))*s_AMPA

ds_AMPA/dt=ars*I_post(t)*(1-s_AMPA)-ads*s_AMPA

init AMPAR=6

par kcre1=0.03,kcre2=1e-5, Ca1=509.5, ar1=1e-7,ad1=1e-5,A_m=1e4,
par ars=0.01,ads=0.0007

## *****

@ total=2e8,dt=10000, bounds=1e25, method=stiff, yp=mps, xhi=9e9

```

Program-3

This is a xppaut file that is used for simulating circadian modulation of Acquisition, Recall, extinction at amygdala (Figs 6.10, 6.11, 6.12).

```
% *****
```

This is a xppaut file that is used for simulating circadian modulation of Acquisition, Recall, extinction at amygdala.

Initially we set $I_{pre_max}=0$, $I_{post_max}=0$, and we run the simulation with a high time step ($dt=10000$ ms) till we reach the desired circadian phase at which we calculate the g_AMPAR .

(a) When we start the simulation of acquisition at desired time, we reduce the time step to $dt=0.01$ ms, and set $I_{pre_max}=35$, $I_{post_max}=400$, and run the simulation for the desired

time duration.

(b) When we start the simulation of recall and extinction at desired time, we reduce the time step to $dt=0.01ms$, initially perform acquisition. Then set $I_{pre_max}=35$, $I_{post_max}=0$, and run the simulation for the desired time duration.

```
%*****
```

```
Aq_Re_Ex_Amygdala ode
```

```
# LD cycle
```

```
par del=0, PP=12
```

```
L(t)=if(mod(t-(del*60*60*1000),24*60*60*1000)<(PP*60*60*1000))then(0.5e-8)else(0)
```

```
## DL cycle
```

```
#L(t)=if(mod(t-(del*60*60*1000),24*60*60*1000)<(PP*60*60*1000))then(0)else(0.5e-8)
```

```
## for DD
```

```
# L(t)=0
```

```
## ***** Initial conditions*****
```

```
#init mps=1.79, P1s=1.2, P1ps=1.01, Rc2=1.06, Mpa=1.79, P1a=1.21,
```

```
# init P1pa=1.02
```

```
# for DD
```

```
#init mps=1.3085, P1s=0.9234, P1ps=0.9869, Rc2=1.1079, Mpa=0.8659,
```

```
# init P1a=0.8968, P1pa=1.0668
```

```
# for LD
```

```

init mps=0.5981, P1s=0.8412, P1ps=1.1159, Rc2=1.2199, Mpa=0.6418,
  init P1a=0.9212, P1pa=1.1512

#for DL

#init mps=1.5428, P1s=1.5153, P1ps=1.2213, Rc2=1.0959, Mpa=1.6256,
# init P1a=1.4213, P1pa=1.1475

## pre-post activity##*****

par del_t=0, t_duration=2

t_on1=t_on-del_t

t_off1=t_on1+t_duration

par I_pre_max=0, I_post_max=0,

#par I_pre_max=35, I_post_max=400

I_pre(t)=I_pre_max*heav(t_off1-t)*heav(t-t_on1)

par t_on=100

t_off=t_on+t_duration

I_post(t)=I_post_max*heav(t_off-t)*heav(t-t_on)

##*****GRN for SCN *****

dMps/dt=As*((vs1*kas^nc/(kas^nc+P1ps^nc))-Mps)+L(t)

dP1s/dt=As*(Mps-P1s)

```

```

dP1ps/dt=As*(P1s-P1ps)

par nc=9,As=4.35e-8,kas=0.8,vs1=20,

##*****GRN of Amygdala *****

dMpa/dt=As*((vs2*kah^nc/(kah^nc+P1pa^nc))-Mpa)+vss*KI^nc/(kc^nc+Rc2^nc)

dP1a/dt=As*(Mpa-P1a)

dP1pa/dt=As*(P1a-P1pa)

par kah=0.8,vs2=20,vss=1e-7,kc=1,KI=0.8

## ***** coupling term *****

dRc2/dt=As*(P1pa-Rc2)

## ***** NMDA, AMPAR current*****

Inmda=g_nmda*sG*Bv*(v-v_NMDA)

g_nmda=g_N*knorm^nm/(P1pa^nm)

Bv=1/(1+0.005*Mg*exp(-0.2*v))

dsG/dt=ar*I_pre(t)*(1-sG)-ad*sG

Iampa=g_AMPAR*sG*(v-v_ampa)

g_AMPAR=gAM*(K_am1^rr/P1pa^rr)*AMPAR

aux g_am=g_AMPAR

par ar=0.01,ad=0.07, nm=2,knorm=1, g_N=12,
par v_NMDA=0, Mg=1, k_am1=1.3,rr=3,gAM=0.2,v_ampa=0

```

```

## *****Modified ML model *****

dv/dt=(1/c)*(I_post(t)-IL-Ik-ICa-Inmda-Iampa)

dw/dt=lamw(v)*(winf(v)-w)

minf(v)=.5*(1+tanh((v-v1)/v2))

winf(v)=.5*(1+tanh((v-v3)/v4))

lamw(v)=phi*cosh((v-v3)/(2*v4))

IL=g1*(v-v1)

Ik=gk*w*(v-vk)

ICa=gca*minf(v)*(v-vca)

par vk=-84,v1=-60,vca=120, gk=8,g1=2,c=20, phi=.08, v1=-1.2,
par v2=18,v3=2,v4=30,

v(0)=-60.899

w(0)=0.014873

##***** Calcium ydnamics*****

gca=g_cabase*Mpa/(kph+Mpa)

dca/dt=k_nmda*INMDA-k_ca*Ica+(Ca0-ca)/tau_ca

par g_cabase=5.3,kph=0.01

par ca0=500, k_nmda=1, k_ca=1, tau_ca=10,

```

```

#####AMPA dynamics #####

dEPSP/dt=(1/C)*(INMDA)

dAMPA/dt=AMS*(ar1*(ca-ca1)*(Am-AMPAR)-ad1*AMPAR*(ca-ca1))*s_AMPA+
(P1pa^ss)*(a_remov*(I_pre(t)/(1+I_post(t)))*(1-AMPAR))

Am=AMS*Amx

AMS=(K_am1^qq/P1pa^qq)

ds_AMPA/dt=ars*I_post(t)*(1-s_AMPA)-ads*s_AMPA

par ar1=1e-7,ad1=1e-5, ars=0.01, ads=0.0007,Ca1=509.5,Amx=2500, qq=2,
par ss=3, K_am2=1, a_remov=0.001,

init AMPAR=6

@ total=1.728e8,dt=10000, bounds=1e25, method=stiff, yp=mps, xhi=1e9

```