Entrainment of a cellular circadian oscillator by light

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Abstract

Considering a molecular circadian oscillator described by a sequence of biological reactions at its deterministic kinetics governed by a system of ordinary differential equations, I build this model to simulate jet lag and analyze what kind of light level function would be suitable to use when changing the time zone. The scaling of the noise level is also discussed.

1 Introduction

In this project, I build a four-variable schematic cellular model using the system of ordinary differential equations at its deterministic kinetics in the limit of large numbers of molecules. The model is easily transformed into a fully stochastic version so that reactions occur at random times with specified probabilities per unit time. The focus of the project is to simulate jet lag so I choose the deterministic case where the results are most clear-cut.

The model considered here consists of one gene and four molecular species: the messenger RNA (mRNA) and the protein encoded by the gene, which are separately tracked in the nucleus and in the cytoplasm of the cell. The protein product inhibits the transcription of the gene that encodes it thus providing negative feedback that may lead to oscillations if the parameters of the system are properly chosen. The feedback mechanism in this project is that the protein is itself an inhibitory transcription factor. The effect of light is to modulate the maximal rate of transcription [1].

The outline of the project is as follows. First, I use the deterministic version of the model to find parameters that yield spontaneous limit-cycle oscillation in consistent with the original paper. Next, I study entrainment by different depths of modulation of the ambient light while holding the mean light level constant throughout our studies. Then, I do some experiments on reversing the periodic light signal to simulate jet lags. Finally, I try out different light level functions to test their influence on the oscillation.

2 Basic set-up

2.1 Model

The cellular circadian oscillator is a four-variable schematic cellular model and the oscillation is caused by the negative feedback of the protein.

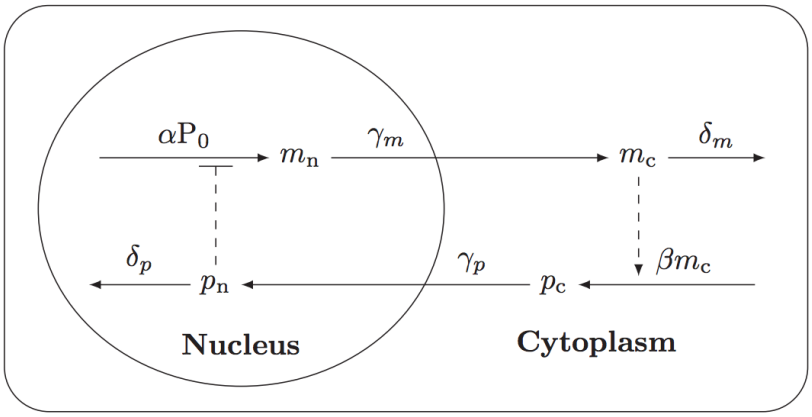

Figure 1: A schematic cellular model

Reaction number	Reaction name	Rate (probability per unit time)	Result
1	Transcription of the *Per* gene	αP_0	$m_n \to m_n + 1$
2	Export of mRNA from the nucleus	$\gamma_m m_n$	$m_n \to m_n - 1,\ m_c \to m_c + 1$
3	Degradation of mRNA (in the cytoplasm)	$\delta_m m_c$	$m_c \to m_c - 1$
4	Translation of *Per* mRNA	βm_c	$p_c \to p_c + 1$
5	Import of protein to the nucleus	$\gamma_p p_c$	$p_c \to p_c - 1,\ p_n \to p_n + 1$
6	Degradation of protein (in the nucleus)	$\delta_p p_n$	$p_n \to p_n - 1$

Figure 2: Reactions in the model

The Per gene is transcribed into Per mRNAs in the nucleus. The Per mRNAs are exported from the nucleus to the cytoplasm to be translated into PER protein and to degrade. The PER protein then enter the nucleus where they inhibit the transcription of the Per gene and degrade in proteasomes in the nucleus.

The model is shown in Fig. 1, and the reactions are defined in Fig. 2, where m represents the number of Per mRNA molecules and p represents the number of PER protein molecules, the subscripts n and c are short for nucleus and cytoplasm, respectively, and P_0 is the probability that transcription happens. The influence of light will be to modulate the maximal transcription rate α as discussed later.

A key assumption of the model is that the PER protein inhibits the transcription of the Per gene. In this model, the inhibition is direct. Assume that there are r protein-binding sites at which this inhibition occurs and that occupation of any one of them is sufficient to block transciption.

Let p_n be the total number of PER protein molecules in the nucleus at some particular time, counting both towards that are free and those that are bound to one of the r protein-binding sites. I consider the model in the deterministic limit when $p_n \gg r$. In this case, the dynamics of the model are approximately governed by the kinetic equations in Fig. 3. The capitalized variables are concentrations of molecules, V_n is the volume of the nucleus, and V_c is the cytoplasmic volume.

$$\frac{dM_{\mathrm{n}}}{dt} = \frac{\alpha}{V_{\mathrm{n}}}\left(\frac{K}{K+P_{\mathrm{n}}}\right)^{r} - \gamma_m M_{\mathrm{n}},$$
$$\frac{dM_{\mathrm{c}}}{dt} = \gamma_m\left(\frac{V_{\mathrm{n}}}{V_{\mathrm{c}}}\right)M_{\mathrm{n}} - \delta_m M_{\mathrm{c}},$$
$$\frac{dP_{\mathrm{c}}}{dt} = \beta M_{\mathrm{c}} - \gamma_p P_{\mathrm{c}},$$
$$\frac{dP_{\mathrm{n}}}{dt} = \gamma_p\left(\frac{V_{\mathrm{c}}}{V_{\mathrm{n}}}\right)P_{\mathrm{c}} - \delta_p P_{\mathrm{n}},$$

Figure 3: Kinetic equations in the deterministic limit

2.2 Stability analysis

To determine the range of parameter values in which the kinetic equations can produce sustained periodic oscillations, need to perform a stability analysis of the system.

I consider the case when $\gamma_m = \gamma_p = \delta_m = \delta_p = v$, which can be shown is the most unstable case, i.e. the case in which it is easiest to obtain oscillations.

To get instability, the model requires:

$$r \geq 5 \tag{1}$$

$$P_n^0 > 4K \tag{2}$$

$$\frac{\alpha}{V_n}\beta > v^2 K 4(1+4)^5 \tag{3}$$

When the last inequality (3) is reversed, the system evolves towards a steady state via damped oscillations. In the original paper [1], the parameters are chosen as $\frac{\alpha}{V_n} = 1800000/(pLh)$, $\beta = 10/h$, $v = \frac{2\pi}{22}/h$, and K = 200/pL, yielding periodic oscillation with a period close to 24 h ($T_0 = 23.2h$) in continuous dark. Also in this case the overall phase is fixed by specifying the following initial conditions: $M_n(0) = 10.9/pL$, $M_c(0) = 1.88/pL$, $P_c(0) = 129.21/pL$, and $P_n(0) = 3531.75/pL$

2.3 Analysis and simulation of the oscillator

In order to vary the free-running period of the oscillator before exposing it to light, I scale all of the rate constants (α, β, and v) by a factor of $\theta = \frac{T_0}{T}$, where T_0 is the autonomous period of the oscillator. This has the effect of setting the period of the oscillator to T.

I put the effect of light into the model by making the the maximal transcription rate α of the Per gene be a function of the light level. The function used in the original paper[1] is:

$$\alpha(t) = \alpha_0 + \bar{\alpha}[1 + \epsilon(t)] \tag{4}$$

In this equation, α_0 denotes the maximum rate of transcription in the dark, and the second term on the right-hand side represents the effect of light on the maximum rate of transcription. In the original paper, α_0 and $\bar{\alpha}$ are constants, and $\epsilon(t)$ is a periodic square wave with a period of 24h such that $\epsilon(t) = +\epsilon_0$

for 12h and $\epsilon(t) = -\epsilon_0$ for 12h, where $\epsilon_0 \in [0, 1]$. The function $\epsilon(t)$ therefore has mean zero, and ϵ_0 is a dimensionless measure of the depth of modulation of the light signal. The parameter ϵ_0 can also be called the relative amplitude of the light signal. The mean light level remains constant as ϵ_0 varies. In this project, I am going to try out various different functions as the function of the light level and simulate jet lag based on the function.

3 Methods

3.1 Numerical Methods

3.1.1 Euler Methods

The model is determined by a system of ODEs describing the change of mRNA and protein in the cell with respect to time. In class we discussed about how to apply Forward Euler and Backward Euler methods to solve a system of ODEs with respect to time. The Euler method is a first-order method, which means that the local error (error per step) is proportional to the square of the step size, and the global error (error at a given time) is proportional to the step size. In the first project we used this method to simulate the effect of earthquakes in a building. In this model, I am trying to use a more accurate method.

3.1.2 ODE45

ode45 is based on an explicit Runge-Kutta formula, the Dormand-Prince pair. It is a single-step solver, i.e. in computing $y(t_n)$, it needs only the solution at the immediately preceding time point, $y(t_{n-1})$. Euler method is one of the routines of Runge-Kutta methods. However, ode45 is a 4th or 5th order method, which is more accurate than forward Euler methods.

[t,y] = ode45(odefun,tspan,y0), where tspan = [t0 tf], integrates the system of differential equations $y' = f(t, y)$ from t0 to tf with initial conditions y0. Each row in the solution array y corresponds to a value returned in column vector t.

3.2 Experiments

3.2.1 Entrainment of the model by light

The first round of the experiments is to observe how the cell would oscillate under different light levels. We mainly consider three cases here:

1. constant light
2. continuous dark
3. 12:12 light-dark (LD) cycles (i.e., 12 hours of light followed by 12 hours of darkness each day

Under each circumstances, the amplitude of the oscillation as well as the phase of the cell (i.e. when the model reaches the largest oscillation point) are different.

3.2.2 Different light level functions

The more direct way to simulate the light level function is to main the light level as a constant for a period of time (12 hours in real life) and then switch the light level as another constant for a period of time. We held the average light level unchanged. In this case, the only states the cell can have are the day and the night.

Another possibility is to get the light keep changing as time goes on. Trigonometric functions would be a reasonable choice to simulate the light level as in real life the light level keeps changing as well. However, in this situation we still hold the average light level constant.

3.3 Jet lag simulation

This is a fun part. In the model we can have different light level functions to represent different time zones. Starting from some given time, the cell is having a flight to a different time zone. After a few hours, the cell reaches the new destination and has to synchronize with the new time zone. Changes in the amplitude and in the phase are quite strong under such simulation.

I want to find several features from the experiments such that I can conclude what can someone do to reduce the effect of jet lag. After a number of trials, there are three features worth noticing:

1. Even flight between two places can be different. For instance, if someone fly from New York to London, the amplitude of the oscillation gets enhanced, which might mean the person would become excited and cannot get to sleep after landed. Conversely, if he is flying back to New York, the amplitude of the oscillation gets reduced, which means he would feel sleepy after the flight landed.

2. Given a flight, the flight time matters. If a person takes the flight at 6 am in the morning, it would be easier for him to recover than that if he takes the flight at 6 pm in the night.

3. The light level during the flight would be important. The amplitude of the oscillation changes to different extent under different light level during the flight. If the flight has strong lights, people would face strong jet lag situation.

There are a lot of other interesting findings and experiments. Here I choose some of them. I do experiments according to this list and compare them to get some results.

4 Results

4.1 Oscillator in continuous dark

Following the stability analysis and choices of parameters, the results resemble those in the original paper much, as the figures show. One tricky thing is to get the autonomous period T_0. Following the parameters used in the paper[1], the period is close to 24 hours. Other choices of parameters can give similar

results but not remarkable enough to beat the choice in the paper. Therefore, I use the corresponding parameters in the paper, which I discussed in section 2.

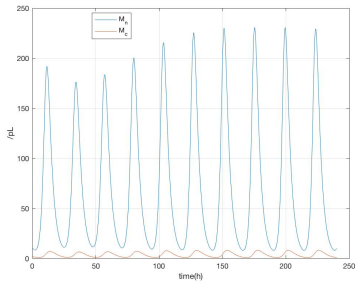

Figure 4: mRNA in continuous dark

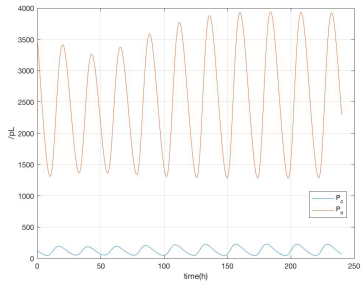

Figure 5: protein in continuous dark

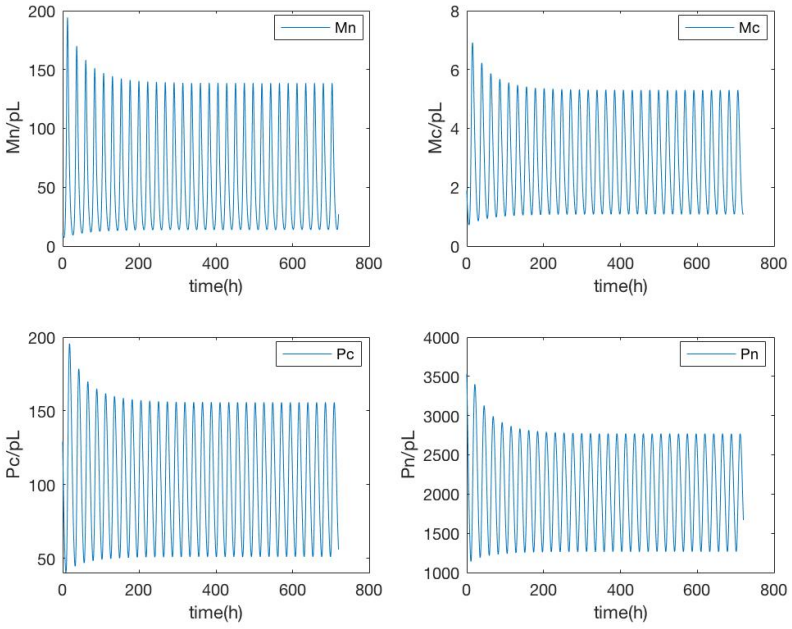

Figure 6: Asymptotic performance

After about 5 or 6 days, the oscillator becomes steady. The concentration of both mRNA and protein in the nucleus are much higher than these in the cytoplasm.

4.2 Oscillator entrained by light

I solve the kinetic equations under continuous dark, constant light conditions and under 12:12 light-dark(LD) cycles (i.e., 12h of light followed by 12h of darkness each day). For the 12:12 LD cycles, the light level is held constant for 12 hours, after that switch to another constant for another 12 hours, and then we start a new period. The mean of light level is also held constant.

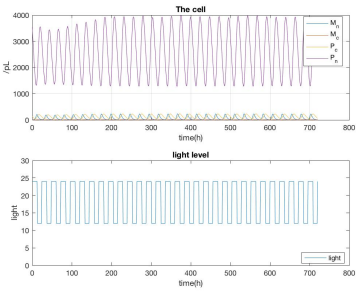

Figure 7: Entrainment for 1 month

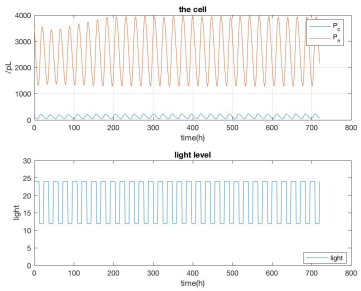

Figure 8: Protein

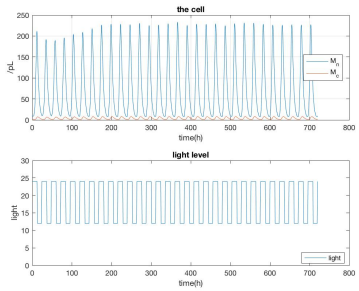

Figure 9: mRNA

Then we do some comparison among entrainment of different light levels. The surprising result is that the amplitude of the oscillation is largest under 12:12 LD cycles, which is even larger than entrained by constant light.

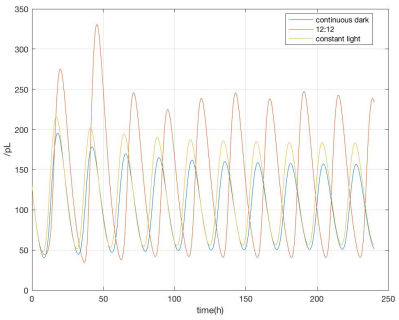

Figure 10: comparison for 10 days

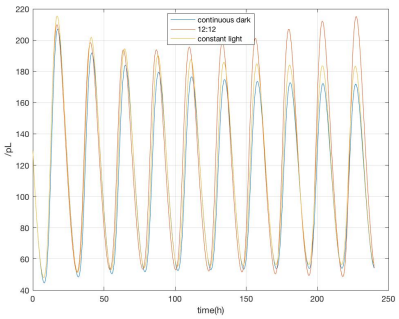

Figure 11: comparison for 10 days

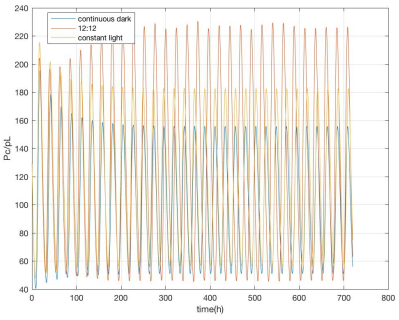

Figure 12: comparison for 30 days

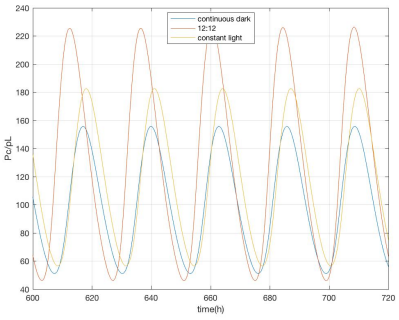

Figure 13: comparison for 30 days

4.3 Different light level functions

Using different light level functions does make a difference. From the results we can see that using a changing light level function is better than just switch the light back and forth.

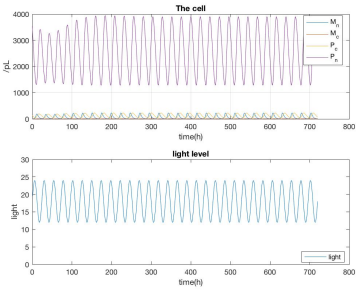

Figure 14: Changing light

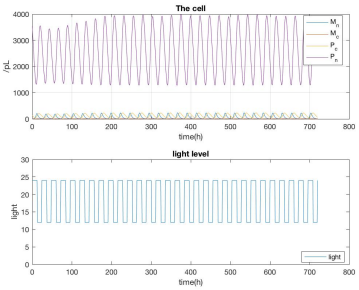

Figure 15: Constant light

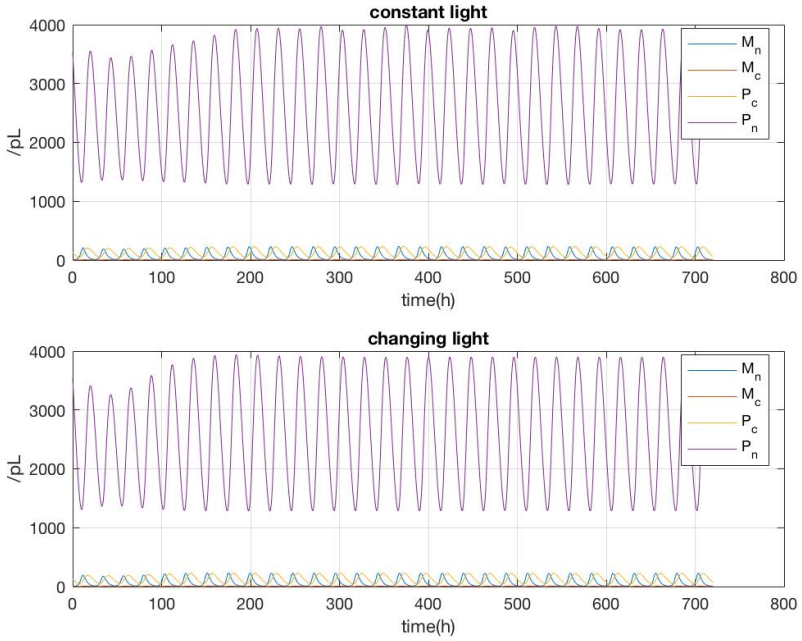

Figure 16: Comparison

As we can see, the oscillation becomes steady faster when the light level function is changing than that when the light level function is held constant.

4.4 Jet lag simulation

One interesting phenomenon to simulate in this model is the jet lag, i.e. the influence of changing the light level functions during the entrainment on the model. From the first group of graphs we can get a feeling of how changing time zones would affect the cell. The origin is the light level function where the cell starts the flight. The destination is the light level function where the cell lands in. We can see that the amplitude of the oscillation becomes much smaller after the flight and it costs the cell about a week to synchronize with the new time zone.

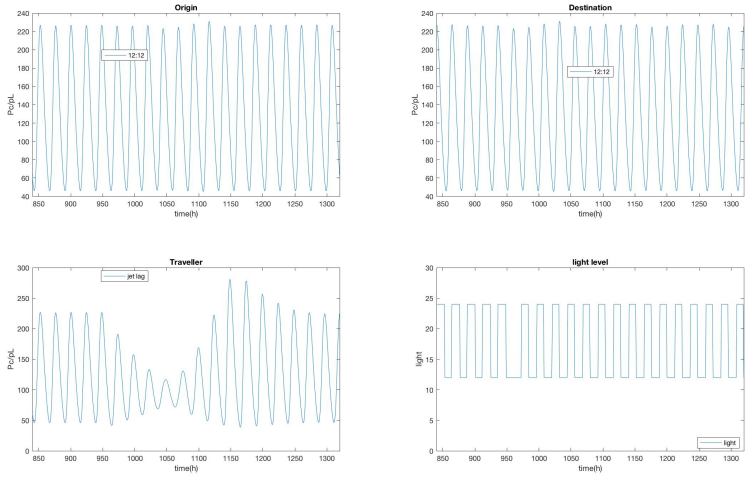

Figure 17: Traveller and all lights

Different light level function seem to have minor influence on how the cell will act under the jet lag circumstances. If using the light that changes as time goes on, the changes in the amplitude of the oscillation is smaller, which means the cell has smaller reactions to jet lag. However, it also takes more time for the cell to go back to steady state when the light keeps changing.

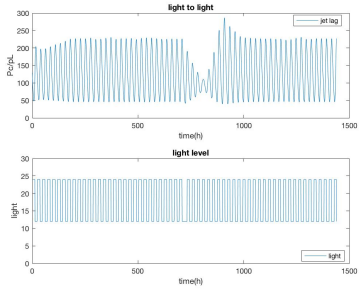

Figure 18: 12 hours difference in time zones

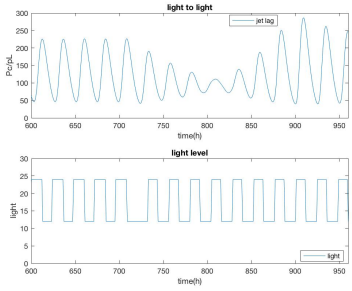

Figure 19: More clear view

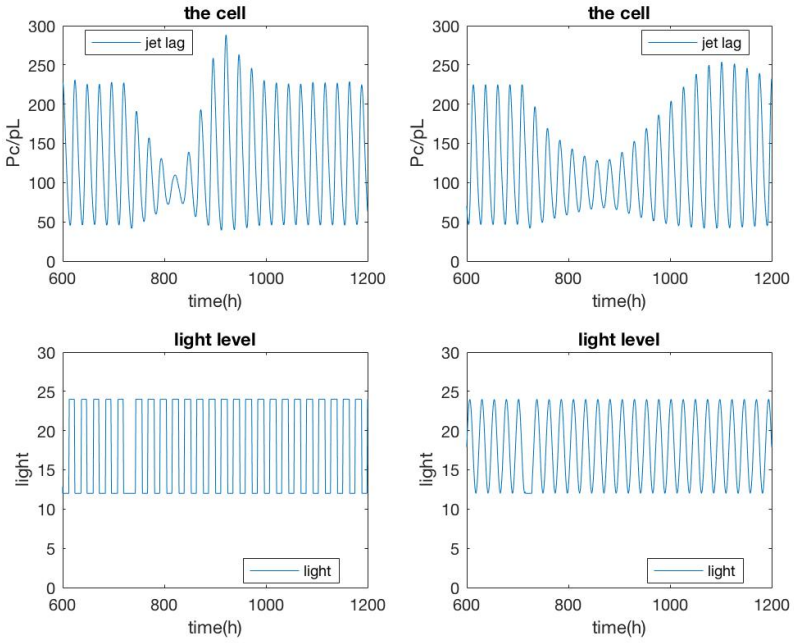

Figure 20: Different light functions comparison

4.4.1 Origin and destination

Travelling between two places can be very different because of different start points and end points. Again the example of travelling between New York and London. If a cell is travelling from New York to London, its amplitude of oscillation becomes larger, which means the cell is more excited than usual. This means a person would get too excited to go to sleep when he landed in London from New York. In contrast, if the person is travelling back to New York, he might be very sleepy since the amplitude of the oscillation in the cell becomes smaller.

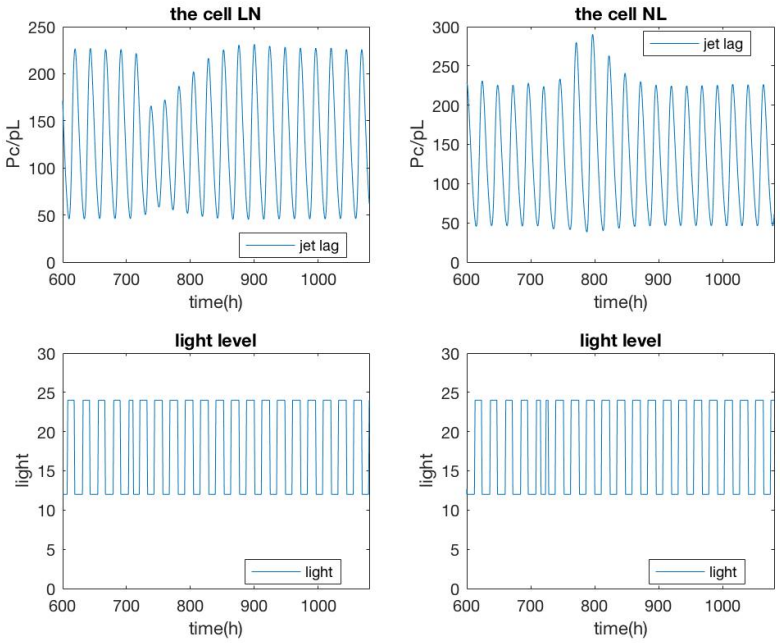

Figure 21: Travel between New York and London

4.4.2 When is the flight start

The amplitude of the oscillation and the phase transition of the oscillation also depend on when the cell starts the flight.

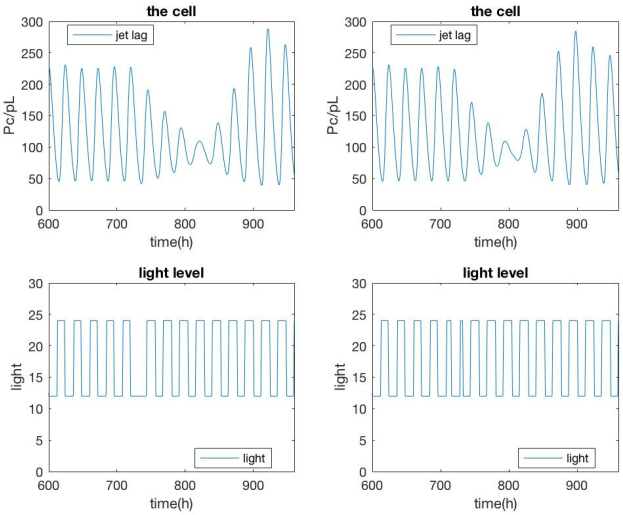

Figure 22: Start time constant light

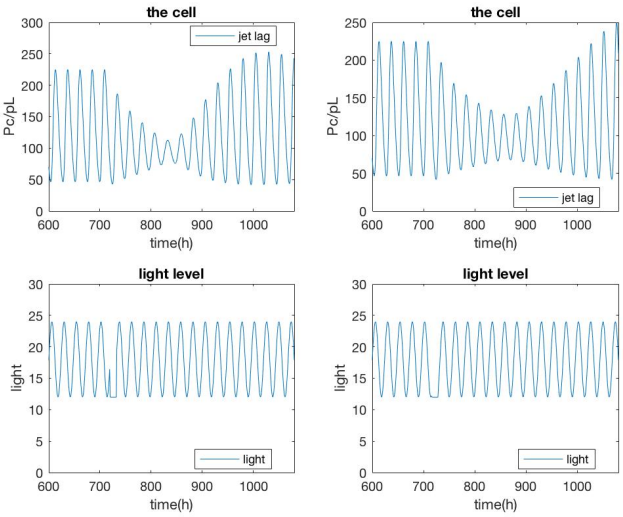

Figure 23: Start time changing light

The experiments show that typically it is better to take a flight to a different time zone during the day instead of the night.

4.4.3 Light during the flight

In order to reduce the effect of jet lag on people. The airlines should choose some kind of appropriate light during the flight. I make a number of experiments on testing different light to use during the flight. For example, we can try to make the light in the flight change so that the light the cell gets keeps continuous, etc. After all, the experiments show that the best light to use during the flight is continuous dark, which means it is better to set less light during the flight so that people can get used to the changes in time zones faster.

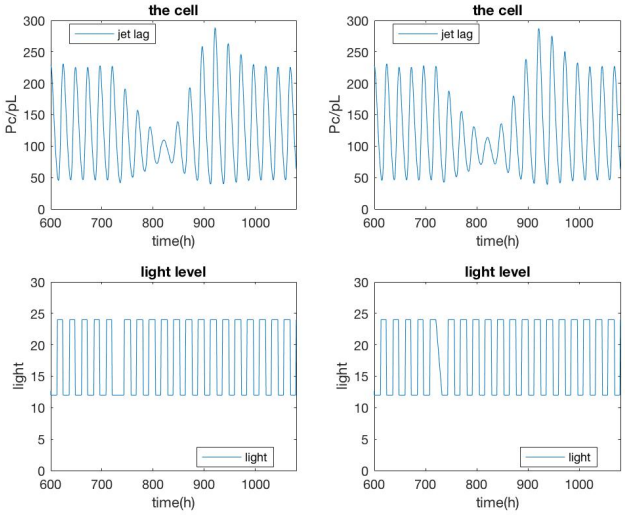

Figure 24: constant light level function

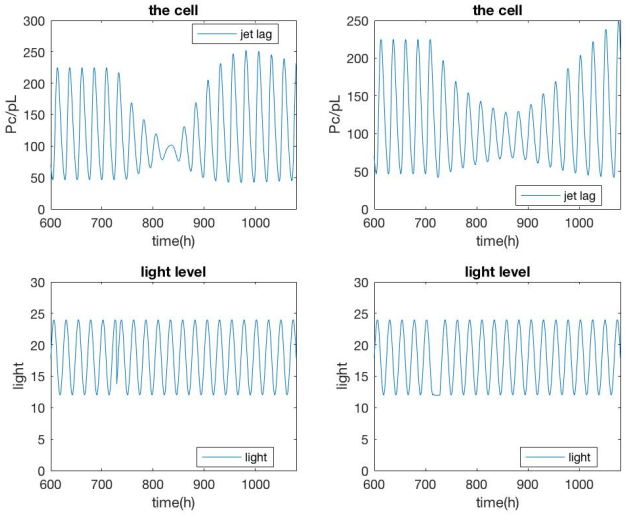

Figure 25: changing light level function

4.4.4 Time shift

After a long flight, the traveller arrives at a different time zone and has to get adjusted to the new time zone. After several days, the traveller would get into a new steady state and have a different phase from he used to have. This is an experiment to check the light signal actually are affecting the cell, which has an important role in making sure the time change is happening. I still use the example of flying between New York and London. From the graphs we can see that the phase does get changed.

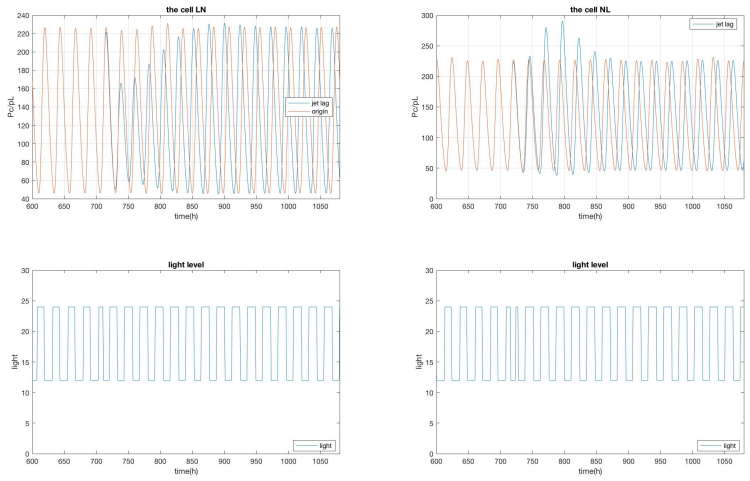

Figure 26: Phase change

4.4.5 Traveller's feeling

Another interesting to observe here is that there is a difference between the actual local time and the time the traveller is feeling like. Using the example of travelling between New York and London again, we can see how the traveller feels based on the reactions. When the traveller travels from London to New York, the time gets 5 hours earlier, which forces the traveller to adjust his bioclock so that the the protein would reach its peak sooner. However, while reaching its peak sooner, the amplitude of the oscillation becomes smaller compared with that in the steady state. This is quite reasonable. When a person arrives New York from London, that day becomes 5 hours longer, which would make him feel sleepy during the day. In order to catch the new peak, the cell sacrifices its amplitude of oscillation a little bit so that it can adjust the phase to the new time zone. Conversely, from New York to London, the cell has to adjust its phase five hours later. The traveller feels the day becomes shorter and the night becomes longer, which would make him more energetic. In the cell, this means the amplitude of the oscillation becomes larger.

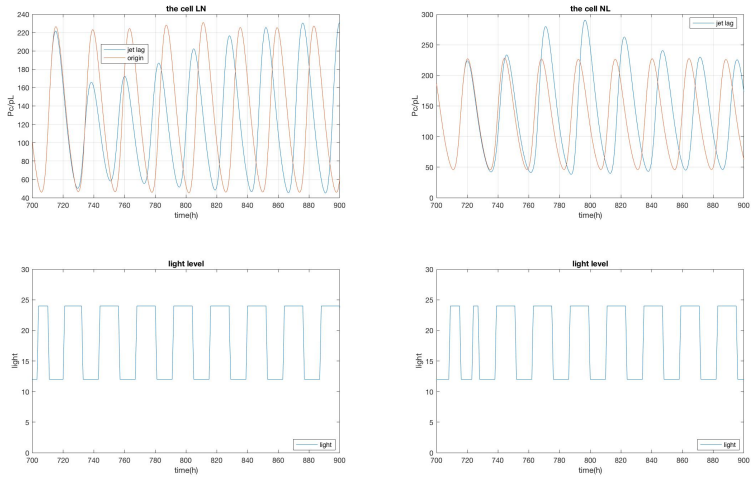

Figure 27: Time "feeling"

5 Discussion

The focus in this project is a schematic model of a cellular circadian clock in which a single gene encodes a protein that inhibits the transcription of that gene. The oscillator is entrained by light, which is represented as a periodic signal that affects the maximal rate at which the gene is transcribed.

Various simulations show the effect of different light level functions on the entrainment of the cell. A large part of the project is devoted to simulating the phenomena of jet lag. In order to reduce the effect of jet lag, experiments show that people can choose a better time to start their trips and the airlines should keep the flight in a dark environment for people to take rest. Moreover, there are different reactions of jet lag when the flight is from East to West or from West to East.

I conducted major experiments in the deterministic case of the cell where the dynamics of the cell are determined by a system of ODEs. Similar experiments can also be performed in the stochastic case but results are expected to more chaotic. Furthermore, we could add more cells instead of just one to make the experiments more sophisticated and realistic.

6 Acknowledgement

I would like to show my gratitude to Professor Charles S. Peskin for his great guidance and encouragement. I would also like to present special thanks to Dr. Charles Puelz. He provides insightful advice to the model and essential help in code implementation.

All my code, notes and graphs are available at `https://github.com/LevineZhou/Cellular-Circadian-Oscillator`. I am more than happy to get suggestions.

References

[1] Wang, Guanyu, and Charles S. Peskin. "Entrainment of a cellular circadian oscillator by light in the presence of molecular noise." Physical Review E 97.6 (2018): 062416.