Mathematical Modelling of Circadian Rhythms and Drug Interactions

Team Number - 24

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Github Repository
Video Link
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1. Abstract. Circadian rhythms are internal 24-hour oscillations that are typically synchronized with daily light-dark environmental cycles. These rhythms influence numerous biological processes and physiological functions, including body temperature regulation, the cell cycle, sleep-wake patterns, neurobehavioral performance, and various diseases such as metabolic, cardiovascular, and psychiatric disorders. Circadian clocks operate at the cellular level and emerge as complex properties through the interaction of cellular oscillators, extending to tissues and the entire organism. Mathematical models of circadian rhythms have been developed using various proteins and genes to enhance understanding and predict various aspects of this intricate physiological system.

In this project, our team aims to model the mammalian circadian rhythm and explore the interactions between circadian proteins, genes, and lithium-based drugs. The influence of drugs and medication on various aspects of the mammalian circadian rhythm, including but not limited to the concentration and expression of proteins and genes, remains an understudied domain, and we seek to address this gap. Drawing on existing literature and research exploring the interaction of the circadian rhythm on other drugs, we have developed a system of ODEs that incorporates the influence of lithium-based drugs.

2. Project Description.

2.1. Goal of the Project.

This project investigates the impact of drug interactions on the sleep cycle of an organism, exploring how such interactions can alter circadian rhythms and influence sleep patterns. We aim to understand how modifications to the circadian model, influenced by varying drug concentrations, impact the biological clock.

2.2. Description.

This project centers on the circadian rhythm, a biological clock intrinsic to all organisms. The circadian rhythm is like the body's internal timer, regulating essential functions such as sleep, wakefulness, eating, and activity levels. This clock operates on a 24-hour cycle, primarily synchronized with natural light and dark patterns in the environment. Think of it as a rhythm that controls various physiological processes, including energy levels, hunger, and metabolism. It aligns our body's functions with the Earth's day-night cycle, ensuring optimal performance and health. At a molecular level, circadian rhythms involve proteins that accumulate and then inhibit their own production, creating a repeating cycle. This self-regulating loop helps the body anticipate and adapt to regular environmental changes.

Impact on DailyLife: The circadian rhythm influences various physiological processes, including:

- Hormone production
- Sleep-Wake Cycle
- Metabolism
- Body Temperature
- Cognitive Function
- Mood and Behavior

From figure 1, one can understand the complex molecular machinery behind the circadian rhythm, The diagram shows two main feedback loops (RBR and PC loops) that work together like the gears of a clock inside our cells. These loops involve various proteins (shown in different

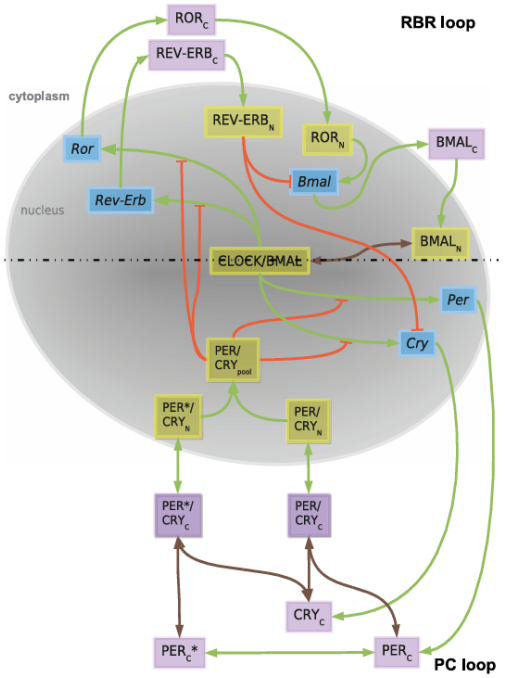

Figure 1. *The biological clock mechanism [16]*

colored boxes) that interact with each other to maintain our daily rhythm.

How it Works:

- Genes in the nucleus (shown in blue boxes) produce proteins
- These proteins move between the nucleus and cytoplasm (the cell's interior)
- The proteins undergo modifications and form complexes (shown by different colored boxes)
- The arrows show how these components influence each other, creating a self-regulating system

2.3. RBR Loop Components. The RBR loop consists of several key proteins:

- ROR_c and ROR_n: Nuclear receptors acting as transcriptional activators
- $REV\text{-}ERB_c$ and $REV\text{-}ERB_n$: Transcriptional repressors
- $BMAL_c$ and $BMAL_n$: Core clock transcription factors

2.4. PC Loop Components. The PC loop includes:

- PER_c and PER_n: Period proteins
- CRY_c: Cryptochrome proteins
- PER*/CRY complexes: Regulatory protein complexes

2.5. Transcriptional Regulation. The CLOCK/BMAL complex serves as the primary transcriptional activator, initiating the expression of target genes. Green arrows indicate activation pathways, while red arrows represent inhibitory relationships.

2.6. Protein Transport. Brown arrows demonstrate protein movement between cytoplasm and nucleus, showing:

- Nuclear import of PER/CRY complexes
- Cytoplasmic-nuclear shuttling of regulatory proteins
- Complex formation and dissociation

3. Feedback Mechanisms. The system maintains rhythmicity through:

- Positive feedback through ROR activation, ROR activation promotes the transcription of Bmal1 and other genes involved in the circadian clock, which enhances the expression of clock genes like Per (period) and Cry (cryptochrome). As these clock genes accumulate, they begin to exert effects on the system, leading to the formation of protein complexes (such as PER/CRY), which later inhibit the activity of ROR and other transcription factors, completing the feedback loop.
- Negative feedback through REV-ERB repression, REV-ERBs bind to specific DNA sequences near the Bmal1 promoter, leading to transcriptional repression of Bmal1 expression. As Bmal1 levels fall, the levels of Per and Cry proteins increase, and they form complexes that inhibit the activity of RORs (which are part of the positive feedback loop), reducing the activation of Bmal1 transcription. This feedback represses the system's activity at the peak of the circadian cycle, allowing the system to "reset" and prepare for the next cycle. This repression is crucial to maintain the 24-hour cycle by ensuring that the system does not over-activate at any point.
- Together, the positive feedback through ROR activation and negative feedback through REV-ERB repression help to regulate the oscillations of the biological clock.
- Hence, a comprehensive idea can be obtained from this description of the project. In real-world applications, aligning drug administration with the body's natural rhythms can improve therapeutic outcomes and reduce side effects.

Key words. CLOCK-BMAL, PER/CRY, ROR, Repressors, Therapeutic

4. Literature Review. Researchers have observed circadian rhythms/clocks in almost all life forms — from simple cyanobacteria to complex multicellular mammals. Additionally, these clocks are present as oscillators at the system, tissue, and cellular levels which affect several functions in a living creature. In humans, the circadian rhythms affect the sleep and awake cycles, drug response, immune system reactions, metabolism, and more. These wide-range implications, it has attracted researchers to study and unravel the mysteries of the circadian clocks. These studies have several learning outcomes for human civilization such as optimal periods to administer drugs and vaccines and changes in the sleep cycles of humans during different stages of evolution. We can attribute some of our understanding of the circadian clock to the intuitions derived from the mathematical modeling of these clocks.

Circadian rhythms arise from protein feedback cycles. [17] Asgari-Targhi et al. provide a brief overview of various mathematical approaches employed to model these systems. [3] The methods vary from deterministic to stochastic, interpretable with physiological linkage to purely mathematical, and with different levels of detail. Additionally, Brown et al. discuss the deterministic models for circadian clocks. [5] The Goodwin oscillator with three states

has been widely used due to its limit cycle properties, whereas the two-state Kronauer model provides an interpretable output in the presence of light. Several other models/oscillators account for higher states and capture multiple processes — for example, the model proposed by Kim et al. uses 181 states to capture multiple feedback loops, kinase enzymes, and more. [11] The deterministic models are useful for understanding the dynamics of the circadian clock. These models accurately model the clock at the macro or organism level but fail to capture any fluctuations in the clock at the micro or cellular level as shown by Herzog et al. [8] The fluctuations are a result of intrinsic noise, and multiple studies have shown that stochastic models perform better at the cellular level. Researchers have also observed the importance of stochastic versions and compared them with the traditional deterministic versions. Gonze et al. [7] back in 2001 compared the deterministic and stochastic versions of a core molecular model for circadian rhythms based on negative autoregulation of gene expression by modeling two stochastic models - a non-developed version keeping the nonlinear terms from the deterministic model and a developed version for the decomposition reactions into the elementary steps. The stochastic simulations were performed using the Gillespie algorithm, which mainly focuses on an exact stochastic time evolution for reacting molecules. According to Gonze et al., [7] robust circadian rhythms will occur even with a low number of molecules, and stochastic models are much preferred if the number of molecules is low. The stochastic models too depicted the bifurcation behavior similar to the deterministic models. And stressed the molecular noise on the biological oscillators and its effect on how it increases the thickness of the limit cycle trajectory. From [7] one can see how the factors of light-dark cycles and protein complex formation play a major role in the biological oscillators.

Elowitz and Leibler showed that only three proteins are enough to make biological clocks by describing and constructing a synthetic genetic clock circuit called the repressilator [6]. They modeled a dynamical system with three repressor proteins and showed that it is periodic with a stable steady state, and then introduced it in real E.coli cells using DNA recombination techniques. The network was then tweaked to periodically induce the synthesis of green fluorescent protein as a readout of its state in individual cells. The resulting oscillations had a time period of a few hours. The clock displayed noisy behavior, probably due to the stochastic nature of the biological components.

Drug pharmacokinetics are described by two differential equations, one for its absorption and one for its excretion. [2] Drug absorption and excretion rate in the blood have been shown to be modulated by circadian changes in organisms (Reinberg, A., Smolensky, 1982). [15] The changes in the pharmacokinetics are modeled by changing the parameters of the absorption and excretion equations. Additionally, the time of day can affect the efficacy, absorption, and toxicity of a drug. This has been substantiated by JC Walton et al. in their paper surveying how the time of day affects the response of the drug by the body. [18] They reviewed the clinical data from a plethora of studies examining the treatment of various immunological, metabolic, and endocrinological disorders. It also showed how it can reduce the impact of side effects faced by patients after taking certain drugs. Hesse et al. is one of the few studies that examine a drug called "irinotecan" for colorectal cancer patients while optimizing their administration. [9] However, a combined system of drug pharmacokinetics and circadian network protein equations have not been modeled for most drugs. This presents an exciting and novel opportunity.

Although we have found promising data for a variety of agents including (but not limited to) Paracetamol (acetaminophen), Erlotinib, Capecitabine, Loratadine and Melatonin [4] we decided to go with Lithium as our drug of choice. Lithium has a profound impact on circadian rhythms, affecting both the period and amplitude of circadian gene expression. This is unlike drugs like Paracetamol (acetaminophen) whose in-vivo concentrations are only impacted by circadian proteins, that do not modulate the biological clock directly. Lithium's effects are mediated through molecular pathways involving key clock components like PER2 and potentially through the inhibition of GSK3. While higher concentrations are needed to observe effects in peripheral tissues, lithium's modulation of the central and peripheral clocks highlights its significance in understanding and potentially treating circadian-related disorders.

5. Conceptual Model.

5.1. Basline Circadian Model. As mentioned in our literature review and previous checkpoint submissions, we have used the model proposed by Hesse et al. [9] as the baseline model to implement the Mammalian circadian rhythm. This model consists of 18 different ODEs and 64 parameters. This model was the modified form of Relogio et al. [16] based on the improvements in the following ways:

- Relogio et al. [16] included two PER/CRY loops to represent phosphorylated and unphosphorylated forms of PER proteins. Due to a lack of quantitative data on PER phosphorylation, they simplified the model by merging these forms into single variables representing the total PER proteins. This simplification also led to the removal of phosphorylation-related parameters, as they became redundant.
- The degradation and dissociation of CLOCK/BMAL and PER/CRY cytoplasmic complexes were simplified due to the lack of data distinguishing these two processes. The degradation parameters were removed to improve model identifiability.
- CLOCK/BMAL dynamics were refined to better represent their influence on downstream processes like gene expression. The variable for the CLOCK/BMAL nuclear complex was highlighted as an important link to external networks. Additionally, the *Clock* gene was reintroduced to reflect its significance in other tissues, such as the liver.
- This model accounted for the differing volumes of the nucleus and cytoplasm (the nucleus occupies around 10% of the cell's volume). To ensure species conservation during cytoplasm-nucleus transport, the cytoplasm-to-nucleus volume ratio adjusted transport terms.

The 18 equations from the above model are shown below:

$$\frac{dz_9}{dt} = k_{fz9} z_8 z_5 + \frac{v_c}{v_n} k_{ex1} x_1 - \frac{v_c}{v_n} k_{iz9} z_9 - k_{dz9} z_9 \tag{5.1}$$

$$\frac{dx_1}{dt} = k_{iz9} z_9 - k_{ex1} x_1 - d_{x1} x_1 \tag{5.2}$$

$$\frac{dz_5}{dt} = k_{p6} y_6 + k_{dz9} z_9 - k_{fz9} z_8 z_5 - d_{z5} z_5 \tag{5.3}$$

$$\frac{dy_3}{dt} = V_{3\max}\frac{1+g\left(\frac{x_1}{k_{t3}}\right)^b}{1+\left(\frac{x_2}{k_{i3}}\right)^c\left(\frac{x_1}{k_{t3}}\right)^b+\left(\frac{x_1}{k_{t3}}\right)^b} - d_{y3}y_3 \tag{5.4}$$

$$\frac{dy_4}{dt} = V_{4\max}\frac{1+h\left(\frac{x_1}{k_{t4}}\right)^b}{1+\left(\frac{x_2}{k_{i4}}\right)^c\left(\frac{x_1}{k_{t4}}\right)^b+\left(\frac{x_1}{k_{t4}}\right)^b} - d_{y4}y_4 \tag{5.5}$$

$$\frac{dz_6}{dt} = k_{p3}y_3 - \frac{v_c}{v_n}k_{iz6}z_6 - d_{z6}z_6 \tag{5.6}$$

$$\frac{dz_7}{dt} = k_{p4}y_4 - \frac{v_c}{v_n}k_{iz7}z_7 - d_{z7}z_7 \tag{5.7}$$

$$\frac{dx_5}{dt} = k_{iz6}z_6 - d_{x5}x_5 \tag{5.8}$$

$$\frac{dx_6}{dt} = k_{iz7}z_7 - d_{x6}x_6 \tag{5.9}$$

$$\frac{dy_6}{dt} = V_{6\max}\frac{1+j\left(\frac{x_6}{k_{t6}}\right)^b}{1+\left(\frac{x_5}{k_{i6}}\right)^c+\left(\frac{x_6}{k_{t6}}\right)^b} - d_{y6}y_6 \tag{5.10}$$

$$\frac{dy_5}{dt} = V_{5\max}\frac{1+i\left(\frac{x_6}{k_{t5}}\right)^b}{1+\left(\frac{x_5}{k_{i5}}\right)^c+\left(\frac{x_6}{k_{t5}}\right)^b} - d_{y5}y_5 \tag{5.11}$$

$$\frac{dz_8}{dt} = k_{p5}y_5 + k_{dz9}z_9 - k_{fz9}z_8z_5 - d_{z8}z_8 \tag{5.12}$$

$$\frac{dy_1}{dt} = V_{1\max}\frac{1+a\left(\frac{x_1}{k_{t1}}\right)^b}{1+\left(\frac{x_2}{k_{i1}}\right)^c\left(\frac{x_1}{k_{t1}}\right)^b+\left(\frac{x_1}{k_{t1}}\right)^b} - d_{y1}y_1 \tag{5.13}$$

```
# odes for nuclear proteins and protein complexes
dx1dt = params['impr_z9'] * z9 - params['expr_x1'] * x1 - params['dr_x1'] * x1
dx2dt = params['impr_z4'] * z4 - params['expr_x2'] * x2 - params['dr_x2'] * x2
dx5dt = params['impr_z6'] * z6 - params['dr_x5'] * x5
dx6dt = params['impr_z7'] * z7 - params['dr_x6'] * x6

# odes for core genes for the circadian clock
dy1dthelper = (x1 / params['ar_y1'] / 1e-9)**params['b']
dy1dt = params['tr_y1'] * 1e-9 * (1 + params['a'] * dy1dthelper) / (1 + dy1dthelper * (1 + (x2 / params['ir_y1'] / 1e-9)**params['c'])) - params['dr_y1']
dy2dthelper = (x1 / params['ar_y2'] / 1e-9)**params['e']
dy2dt = params['tr_y2'] * 1e-9 * (1 + params['d'] * dy2dthelper) / (1 + dy2dthelper * (1 + (x2 / params['ir_y2'] / 1e-9)**params['f'])) * (1 / (1 + (x5 /
dy3dthelper = (x1 / params['ar_y3'] / 1e-9)**params['b']
dy3dt = params['tr_y3'] * 1e-9 * (1 + params['g'] * dy3dthelper) / (1 + dy3dthelper * (1 + (x2 / params['ir_y3'] / 1e-9)**params['c'])) - params['dr_y3']
dy4dthelper = (x1 / params['ar_y4'] / 1e-9)**params['b']
dy4dt = params['tr_y4'] * 1e-9 * (1 + params['h'] * dy4dthelper) / (1 + dy4dthelper * (1 + (x2 / params['ir_y4'] / 1e-9)**params['c'])) - params['dr_y4']
dy5dthelper = (x6 / params['ar_y5'] / 1e-9)**params['b']
dy5dt = params['tr_y5'] * 1e-9 * (1 + params['i'] * dy5dthelper) / (1 + dy5dthelper + (x5 / params['ir_y5'] / 1e-9)**params['c']) - params['dr_y5'] * y5
dy6dthelper = (x6 / params['ar_y6'] / 1e-9)**params['b']
dy6dt = params['tr_y6'] * 1e-9 * (1 + params['j'] * dy6dthelper) / (1 + dy6dthelper + (x5 / params['ir_y6'] / 1e-9)**params['c']) - params['dr_y6'] * y6

# odes for cytoplasmic proteins and protein complexes
dz1dt = params['pr_z1'] * y2 + params['kd_z4'] * z4 - params['kf_z4'] * 1e9 * z1 * z2 - params['dr_z1'] * z1
dz2dt = params['pr_z2'] * y1 + params['kd_z4'] * z4 - params['kf_z4'] * 1e9 * z1 * z2 - params['dr_z2'] * z2
dz4dt = params['kf_z4'] * 1e9 * z1 * z2 + ratio * params['expr_x2'] * x2 - ratio * params['impr_z4'] * z4 - params['kd_z4'] * z4
dz5dt = params['pr_z5'] * y6 + params['kd_z9'] * z9 - params['kf_z9'] * 1e9 * z8 * z5 - params['dr_z5'] * z5
dz6dt = params['pr_z6'] * y3 - ratio * params['impr_z6'] * z6 - params['dr_z6'] * z6
dz7dt = params['pr_z7'] * y4 - ratio * params['impr_z7'] * z7 - params['dr_z7'] * z7
dz8dt = params['pr_z8'] * y5 + params['kd_z9'] * z9 - params['kf_z9'] * 1e9 * z5 * z8 - params['dr_z8'] * z8
dz9dt = params['kf_z9'] * 1e9 * z8 * z5 + ratio * params['expr_x1'] * x1 - ratio * params['impr_z9'] * z9 - params['kd_z9'] * z9
```

Figure 2. *A screen grab of the code implementing the system of ODEs in the baseline model.*

$$\frac{dy_2}{dt} = V_{2\max} \frac{1 + d\left(\frac{x_1}{k_{t2}}\right)^e}{\left(1 + \left(\frac{x_2}{k_{i2}}\right)^f \left(\frac{x_1}{k_{t2}}\right)^e + \left(\frac{x_1}{k_{t2}}\right)^e\right)\left(1 + \left(\frac{x_5}{k_{i21}}\right)^{f1}\right)} - d_{y2} y_2 \tag{5.14}$$

$$\frac{dz_1}{dt} = k_{p2} y_2 + k_{dz4} z_4 - k_{fz4} z_1 z_2 - d_{z1} z_1 \tag{5.15}$$

$$\frac{dz_2}{dt} = k_{p1} y_1 + k_{dz4} z_4 - k_{fz4} z_1 z_2 - d_{z2} z_2 \tag{5.16}$$

$$\frac{dz_4}{dt} = k_{fz4} z_1 z_2 + \frac{v_c}{v_n} k_{ex2} x_2 - \frac{v_c}{v_n} k_{iz4} z_4 - k_{dz4} z_4 \tag{5.17}$$

$$\frac{dx_2}{dt} = k_{iz4} z_4 - k_{ex2} x_2 - d_{x2} x_2 \tag{5.18}$$

To ensure the validity of the model we implemented the model and obtained stable circadian rhythms, one can witness from figure 3.

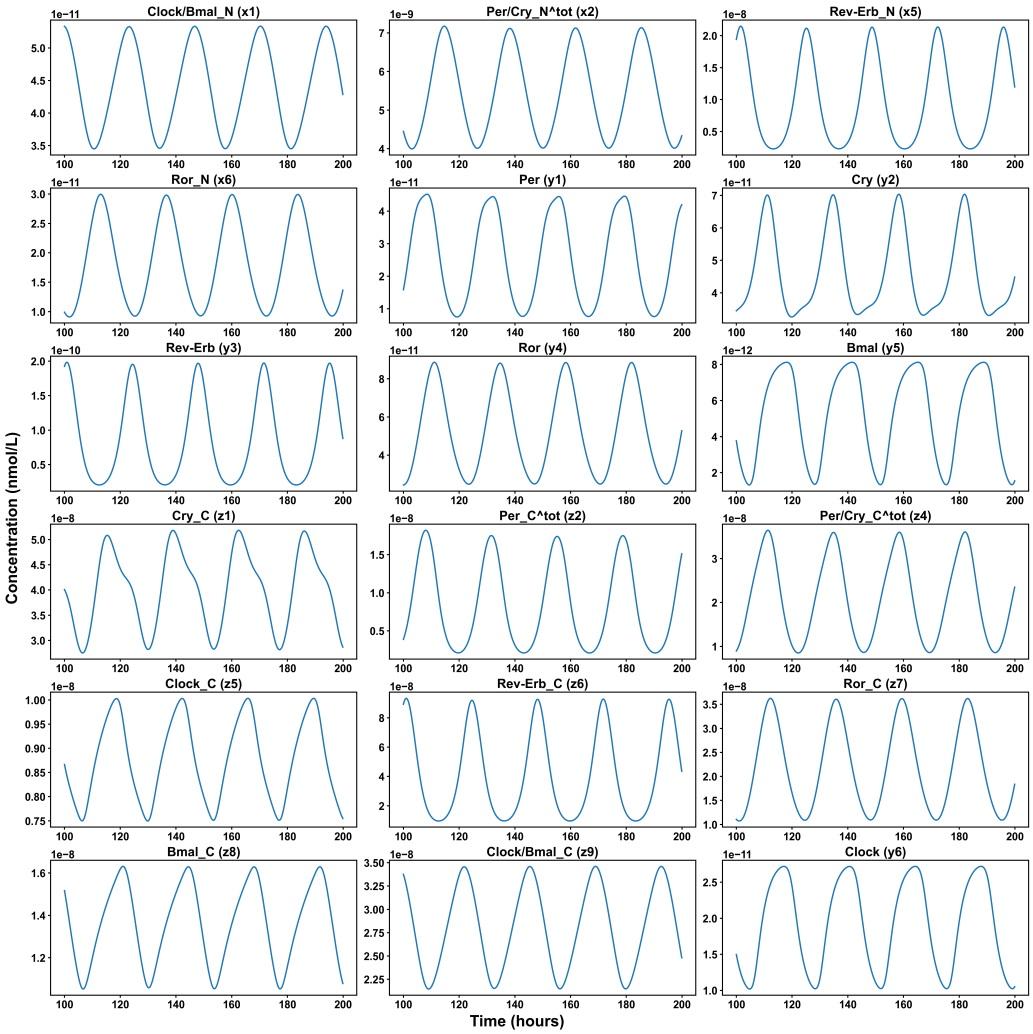

Figure 3. *Periodicity in the circadian clock as an outcome of solving the system of ODEs.*

5.2. Finalized Circadian Model. When we implemented the above model for lithium-based drugs and performed parameter optimization on the resulting equations using randomly initialized parameters, we encountered significant computational resource limitations and instability in the parameter values obtained after the optimization. The final values of parameters obtained after the optimization led to results that did not resemble a circadian rhythm. However, when these parameters were constrained to similar ranges as given by the baseline model we observed stable circadian outputs. We think this might be due to certain biological and genomic constraints we forgot to consider. This prompted us to create a simplified system of equations with fewer equations and parameters. On analyzing the behavior and mechanics of various Li-based drugs we developed a simplified circadian model as shown below.

5.3. 4-ODE model. The circadian model has been refined and will only concentrate on the PC loop and the CLOCK-BMAL complex as the lithium-based drug has major interaction on the PC loop proteins, so the model is modified and investigated on the rhythmic effects of the sleep cycle, by showing a qualitative validation.
The system of ODEs is as follows,

$$\frac{dx_1}{dt} = V_{1max}\frac{(K_{i_{z1}}^m + C^m)K_{i_{z1}}^n}{K_{i_{z1}}^m(K_{i_{z1}}^n + (z1)^n)} - dx_1x_1 \tag{5.19}$$

$$\frac{dx_2}{dt} = V_{2max}\frac{(K_{i_{z1}})^n}{(K_{i_{z1}})^n + (z1)^n} - dx_2x_2 \tag{5.20}$$

$$\frac{dx_3}{dt} = k_{f_{x3}}x_1x_2 - k_{d_{x3}}x_3 + k_{exp_{z1}}z_1 - k_{imp_{x3}}x_3 - dx_3x_3 \tag{5.21}$$

$$\frac{dz_1}{dt} = -k_{exp_{z1}}z_1 + k_{imp_{x3}}x_3 - dz_1z_1 \tag{5.22}$$

Here,
C: Concentration of Li dose
d_{x_i}: degradation rate of protein x_i
n, m: Hill Coefficients
d_{z_i}: degradation rate of protein z_i
V_{imax}: transcription rate of gene/mRNA x_i
$K_{i_{z1}}$: inhibition rate for the transcription due to z_1
$k_{f_{x3}}$: formation rate of PER/CRY complex
$k_{d_{x3}}$: dissociation rate of PER/CRY complex
$k_{exp_{z1}}$: export rate for PER/CRY from nucleus to cytoplasm
$k_{imp_{x3}}$: import rate for PER/CRY from cytoplasm to nucleus

In our literature review, we observed that Li has a strong impact on only one protein, PER2. [12] In the literature, we often find PER1, PER2 and PER3 as three separate proteins. However, for the sake of simplicity of the model and a general practice in the domain, they are combined as PER. Coming to the circadian clock, it is made of two feedback loops which are driven by the CLOCK/BMAL protein. PER is a part of the negative feedback loop which revolves around PER and CRY proteins and genes with CLOCK/BMAL as the activator (think as driving force for transcription of genes) for the Cry and Per genes.

We thus chose to isolate the negative feedback loop and model it instead of the bigger circadian clock. The isolated loop is further simplified to use only proteins as the variables and the effects and reactions of genes consumed in the protein variables. Here, x_1 and x_2 are variables which account for the PER and CRY proteins and genes in a combined form. We can do this simplification provided we account for the transcription of genes and production of proteins in the x_1 and x_2 variables.

We apply the design principles from the 18 variable model to create a simpler model. The principles are listed below.

```
# ode for lithium concentration
dcdt = -K * c + inp

# odes for nucleic proteins and their complexes
dz1dt = params['impr_x3'] * x3 - params['expr_z1'] * z1 - params['dr_z1'] * z1

# odes for cytoplasmic proteins and their complexes
dxidthelper = params['ir_z1']**params['n'] / (z1**params['n'] + params['ir_z1']**params['n'])
if inp == 0:
    dx1dthelper = 1
else:
    dx1dthelper = (c**params['m'] + params['ir_z1']**params['m']) / params['ir_z1']**params['m']
dx1dt = params['tr_x1'] * dxidthelper * dx1dthelper + params['kd_x3'] * x3 - params['kf_x3'] * x1 * x2 - params['dr_x1'] * x1
dx2dt = params['tr_x2'] * dxidthelper + params['kd_x3'] * x3 - params['kf_x3'] * x1 * x2 - params['dr_x2'] * x2
dx3dt = params['kf_x3'] * x1 * x2 - params['kd_x3'] * x3 + ratio * params['expr_z1'] * z1 - ratio * params['impr_x3'] * x3 - params['dr_x3'] * x3
```

Figure 4. *A screen grab of the code implementing the system of ODEs in 4-ODE model.*

- degradation of the genes and proteins
- formation and dissociation of protein complexes (as some protein tend to combine and form complexes)
- transcription of genes based on proteins in the system (think as production)
- activating and inhibiting behaviour of the proteins for the genes
- production of proteins from genes
- export and import of these proteins, in and out of the nucleus (cells are made of cytoplasm and nucleus, and these species can exist inside the nucleus or in the cytoplasm)

Based on these principles, we arrived at the conclusion that the derivative of the concentration for the variables would take the following forms:

$$\frac{dx_1}{dt} = \text{transcription of Per gene} + \text{dissociation of PER/CRY complex} - \text{formation of PER/CRY complex - degradation of PER protein}$$

$$\frac{dx_2}{dt} = \text{transcription of Cry gene} + \text{dissociation of PER/CRY complex} - \text{formation of PER/CRY complex - degradation of CRY protein}$$

$$\frac{dx_3}{dt} = \text{formation of PER/CRY complex - dissociation of PER/CRY complex} + \text{export from nucleus - import to nucleus - degradation of protein}$$

$$\frac{dz_1}{dt} = \text{import from nucleus - export to nucleus - degradation of protein}$$

Now, the activator for gene transcription (CLOCK/BMAL) is not in the loop but the inhibitor (nucleic complex of PER/CRY) is in the loop. So we simplify the expression from

$$\frac{1 + a\left(\frac{x_a}{ar}\right)^b}{1 + \left(\frac{x_a}{ar}\right)^b + \left(\frac{x_a}{ar}\right)^b \left(\frac{x_i}{ir}\right)^c}$$

to

$$\frac{1}{1+\left(\frac{x_i}{ir}\right)^c}$$

to negate the impact of the activator. The above expressions have x_i and x_a to denote inhibitor and activator concentrations respectively. Additionally, they have ir and ar to denote inhibition and activation rate. The exponents are Hill coefficients. The expression can finally be written as

$$\frac{ir^c}{ir^c + x_i^c}$$

which is also the form we are using the code.

Thus, the simplified model has 4 variables and 13 parameters. (Much less than the large model in Hesse et al.[9] which has 18 variables and 64 parameters.)

Lastly, we need to account for the change in concentration of Li in the body/cell. We have observed in Wood et al.[19] that concentration shows an exponential reduction with time and spikes when we administer a dose. We thus have the following derivative for Li concentration:

$$\frac{dC}{dt} = -kC + input$$

Even though we have the system of ODEs for the 4-ODE model, we need to estimate the parameters for the system. We chose to estimate these parameters using data-based approaches. The approaches has the following pseudo code:

- **Step 1:** Initialize a guess for the parameters.
- **Step 2:** Solve the system of ODEs for the guess and obtain a prediction.
- **Step 3:** Compute the objective/loss function using the prediction and the data.
- **Step 4:** Update the guess based on the loss function and go to Step 2.
- **Step 5:** Stop the iterations when the desired tolerance for the error is reached.

We borrowed data from Narumi et al.[13] The protein data for PER and CRY was processed such that,

$$PER = PER1 + PER2 + PER3$$

$$CRY = CRY1 + CRY2 + CRY3$$

These measured values are in attomol/microgram which was then converted to mol/L.

Additionally, the measured values are all forms of the species. PER measurement accounts for PER and PER/CRY complexes inside and outside nucleus. So the parameter optimization has to happen on an updated form of the predictions.

$$PER_{tot} = v_c\left(PER + PER/CRY_C\right) + v_n PER/CRY_N$$

Here, v_c and v_n denote the volume proportion of the cytoplasm and nucleus. We found that the value of v_n is roughly 0.1, so we have stuck with this estimated value.[1]

6. Simulation Model. For the Verification of the circadian rhythm model, a steady state and stability analysis is presented, Steady state and stability analysis are crucial techniques in understanding the behavior of dynamical systems. Steady state analysis involves finding the equilibrium points of the system, where all state variables remain constant over time. For the circadian clock model, this means finding the concentrations of proteins and genes that remain stable.

Purpose

The purpose of steady state analysis is to:

- Identify the long-term behavior of the system
- Determine the baseline concentrations of proteins and genes
- Provide a starting point for stability analysis

Stability analysis examines how the system responds to small perturbations around the steady state. It involves calculating the eigenvalues of the Jacobian matrix at the steady state point.

Jacobian Matrix

The Jacobian matrix J is a matrix of all first-order partial derivatives of the system. For our circadian clock model with 18 variables, the Jacobian is an 18x18 matrix:

$$J = \begin{bmatrix} \frac{\partial f_1}{\partial x_1} & \frac{\partial f_1}{\partial x_2} & \cdots & \frac{\partial f_1}{\partial y_6} \\ \frac{\partial f_2}{\partial x_1} & \frac{\partial f_2}{\partial x_2} & \cdots & \frac{\partial f_2}{\partial y_6} \\ \vdots & \vdots & \ddots & \vdots \\ \frac{\partial f_{18}}{\partial x_1} & \frac{\partial f_{18}}{\partial x_2} & \cdots & \frac{\partial f_{18}}{\partial y_6} \end{bmatrix}$$

where f_i represents the right-hand side of the i-th differential equation in the system.

Purpose

The purpose of stability analysis is to:

- Determine whether the steady state is stable or unstable
- Identify the types of dynamics (e.g., oscillations, exponential growth) near the steady state
- Assess the robustness of the circadian rhythm to small disturbances

The results for the baseline circadian model,

```
# Perform steady state analysis
steady_state = steady_state_analysis(parameters)
print("Steady state values:", steady_state)

# Perform stability analysis
stable, eigenvalues = stability_analysis(parameters, steady_state)
print("Stable:", stable)
print("Eigenvalues:", eigenvalues)
```

```
Steady state values: [ 2.76898093e-03  1.02509379e-01  1.24283348e-06  6.09500561e-10
  6.63647404e-09 -3.04387471e-09 -1.41903689e-09  3.52151541e-09
 -4.45592918e-08  4.52816418e-09 -7.12906352e-02  1.07129064e+00
  3.94856839e-01  1.04624798e-01  5.28898046e-06 -1.49477695e-06
  8.95375208e-01  1.76877137e+00]
Stable: True
Eigenvalues: [-6.57011653e+07+0.j -6.67446377e-01+0.j -5.26718891e-03+0.j
 -3.35085738e-01+0.j -1.73833003e+00+0.j -3.17607610e+07+0.j
 -5.72797079e+00+0.j -1.31747557e+00+0.j -4.86932614e-02+0.j
 -6.74259656e-01+0.j -1.25004007e-01+0.j -3.23526259e-01+0.j
 -3.19761723e-01+0.j -8.05946413e-01+0.j -4.97657310e-01+0.j
 -4.19468851e-01+0.j -4.58837454e-01+0.j -2.35195833e-01+0.j]
```

Figure 5. *The equilibrium points and the jacobian matrix for baseline model*

It turns out to be a stable model. For the modified and refined 4-ODE model,

In [40]:
```
if __name__ == "__main__":
    # Parameters
    V1max, V2max, Ki_z1, n, m, C = 3.931358e+00, 4.560146e+00, 1.861609e-02, 9, 1, 0.001
    dx1, dx2, dx3, kf_x3, kd_x3 = 2.453857e+00, 1.293570e+00, 1.000000e-04, 1.178044e+00, 2.154028e-01
    kexp_z1, kimp_x3, dz1 = 4.328731e-18, 2.260046e-01, 1.630935e-01

    # Initial conditions and time
    initial_conditions = [0.1504302, 0.47560800000000003, 0.0752151, 0.0250717]
    t = np.linspace(0, 100, 1000)

    # Create system and solve
    ode_system = modified_ODE_System(V1max, V2max, Ki_z1, n, m, C, dx1, dx2, dx3, kf_x3, kd_x3, kexp_z1, kimp_x3, dz1)
    solution = ode_system.solve(initial_conditions, t)

    # Find steady state and check stability
    steady_state = ode_system.steady_state(initial_conditions)
    if steady_state is not None:
        print("Steady state:", steady_state)
        eigenvalues = ode_system.stability(steady_state)
        print("Eigenvalues:", eigenvalues)
        print("Stable" if all(np.real(eigenvalues) < 0) else "Unstable")
    else:
        print("Failed to find steady state.")
```

```
Steady state: [0.05904066 0.12328853 0.01942215 0.02691398]
Eigenvalues: [-3.8333544 +0.j         -2.34695139+0.j          1.56092394+3.06861626j
  1.56092394-3.06861626j]
Unstable
```

Figure 6. *The equilibrium points and the jacobian matrix for 4-ODE model*

It appears to be unstable. This result can be qualitatively validated with the figure from the Experimental section, Where the steady state values are expected to be close enough to the values where the first derivatives are zero for all the 4 cases, as **scipy.optimize.root** solves a system of nonlinear equations by using numerical methods, such as Newton-Raphson or other root-finding techniques. So from the experimental section we can see that the derivative becomes zero at around 0.05 for the 1mmol concentration of Li dosage which is what we expected from the steady state analysis, and one can see the perturbations are more around 0.05 which turns out to be unstable, from stability analysis.

7. Experimental Results and Validation.

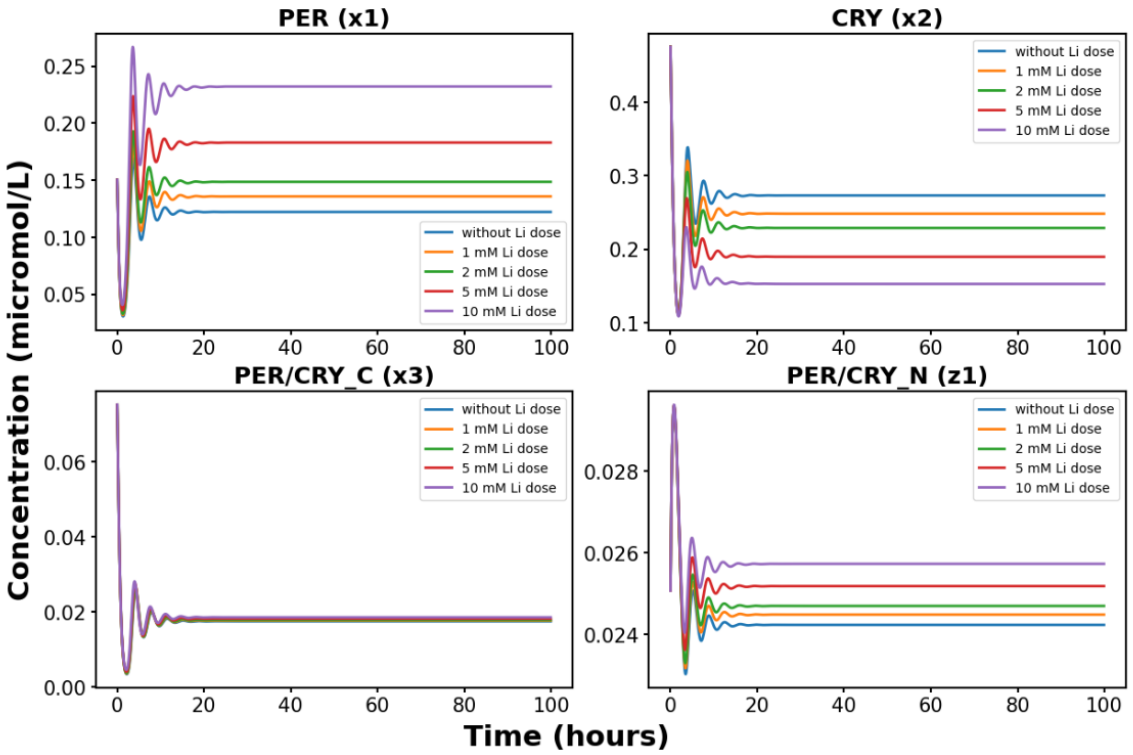

Figure 7. *The rhythms of 4-ODE model*

Mechanism of PER Increase. Lithium enhances the transcription rate of PER protein (x_1) through the term:

$$V_{1\max}\frac{(K_{i_{z1}}^m + C^m)}{K_{i_{z1}}^m} \tag{7.1}$$

This expression models the cooperative effect of lithium on PER transcription, with the Hill coefficient m capturing the degree of cooperativity. As lithium concentration increases, the transcription rate of PER increases non-linearly due to cooperative binding, leading to higher steady-state concentrations of PER protein.

Lithium-Induced Changes in CRY Protein Concentration. The model predicts a dose-dependent decrease in CRY protein concentration

- **1 mM Lithium**: CRY (x_2) concentration decreases from 0.28 μM to 0.26 μM.
- **5 mM Lithium**: CRY (x_2) concentration decreases from 0.28 μM to 0.20 μM.
- **10 mM Lithium**: CRY (x_2) concentration decreases significantly from 0.28 μM to 0.15 μM.

Mechanism of CRY Decrease. Although lithium does not directly affect CRY transcription in the model, the decrease in CRY levels can be explained by:

- **Increased PER Levels**: Elevated PER levels lead to more PER/CRY complex formation, sequestering CRY proteins and reducing their free concentration.
- **Feedback Regulation**: The increased nuclear PER/CRY complex (z_1) enhances negative feedback on CRY transcription indirectly, contributing to decreased CRY protein synthesis.

Stability of Cytoplasmic PER/CRY Complex (x_3). The concentration of the cytoplasmic PER/CRY complex remains unchanged across different lithium doses. Despite changes in PER and CRY concentrations, the formation and dissociation rates adjust to maintain a constant level of the cytoplasmic complex.

Consistency with Experimental Data. Experimental studies have demonstrated that lithium treatment lengthens the circadian period and increases the amplitude of PER2 expression [10][12]. The significant increase in PER concentration at higher lithium doses in the model aligns with these observations, supporting the validity of the model.

Li et al. [12] conducted experiments to evaluate the impact of lithium on the amplitude and period of the molecular circadian clockwork in mice. Their key findings relevant to our model include the lengthening of the circadian period in behavioral rhythms and molecular pacemaking in both the SCN and peripheral mice tissues. They also indicate that lithium increases PER2 expression and oscillation amplitude in ectopic tissue explants and isolated cells. There were with significant effects observed at higher lithium concentrations (10 mM), with negligible effects at lower levels (1mM). Our model is able accurately encapsulate this data.

Ono and Honma [14] investigated the effects of lithium on the circadian period of peripheral clock gene expression in vivo using *Per2Luc* mice. They reported that Lithium treatment (10 mM) extended the circadian period of clock gene expression in the kidney by approximately 0.41 hours (from 23.93 h to 24.34 h). Our model's prediction of increased PER protein concentration at 10 mM lithium is consistent with the observed period lengthening. The elevated PER levels enhance the negative feedback loop, which can slow down the molecular clock and extend the circadian period, as reported in the study. However we do not model the circadian explicitly and cannot verify the exact magnitude of the period increase.

While specific experimental data on CRY protein levels under lithium treatment are limited [10] [12], the model's prediction of decreased CRY concentrations is plausible. It suggests that lithium's impact on PER proteins indirectly affects CRY proteins through complex formation and feedback mechanisms.

Experimental observations suggest that lithium does not significantly alter the cytoplasmic PER/CRY complex levels [10] [12]. The model's prediction aligns with this, indicating that the cytoplasmic complex remains stable despite lithium treatment.

8. Discussions, Conclusion and Summary. Experimental studies have shown that lithium lengthens the circadian period in behavioral rhythms and molecular pacemaking [10]. The increase in nuclear PER/CRY complex supports this observation, as it enhances transcriptional repression, slowing down the molecular clock.

Our simplified 4-ODE model effectively captures the dose-dependent effects of lithium on key circadian clock components. The analysis demonstrates that lithium enhances PER protein levels, decreases CRY protein levels, and increases the nuclear PER/CRY complex at higher doses, consistent with experimental data. This leads to lengthening of the circadian period and increased amplitude of molecular oscillations.

Consistent with the experimental data, our model shows minimal changes in PER concentration at 1 mM lithium, increasing only from 0.12 mM to 0.13 mM. This suggests that therapeutic concentrations of lithium may not significantly impact circadian rhythm parameters in peripheral cells, as observed in fibroblasts by Dokucu et al.

We were able to model the dose-dependent non-linear pharmacokinetics of lithium by including a Hill Coefficient in the interaction term between PER and Lithium. As a result our model was able to provide a strong fit with the experimental data observed by Jian et. al [12].

By focusing on the primary components affected by lithium, the model provides valuable insight into the molecular mechanisms underlying lithium's modulation of circadian rhythms. It also highlights the importance of cooperative interactions and feedback regulation in the circadian clock.

Appendix A. Division of labor.

- **Aditya:** Development of Equations and parametrization, Programming, Visualization, Report Writing, Video Editing
- **Aman:** Biological research, Development of Equations, Literature Review, Report Writing, Video Editing
- **Prabhas:** Biological Research, Programming and visualization for steady state and stability analysis, Literature Review, Report Writing, Video Editing
- **Prajeet:** Model Training, Programming, Development of Equations and parametrization, Literature Review, Report Writing, Video Editing

REFERENCES

[1] https://bionumbers.hms.harvard.edu/bionumber.aspx?id=113848&ver=0&trm=cytoplasm+nucleus+ratio&org=.

[2] J. Aronson, M. Chappell, K. Godfrey, and M. Yew, *Modelling circadian variation in the pharmacokinetics of non-steroidal anti-inflammatory drugs*, European journal of clinical pharmacology, 45 (1993), pp. 357–361.

[3] A. Asgari-Targhi and E. B. Klerman, *Mathematical modeling of circadian rhythms*, Wiley Interdisciplinary Reviews: Systems Biology and Medicine, 11 (2019), p. e1439.

[4] F. A. F. A. Bicker J, Alves G, *Timing in drug absorption and disposition: The past, present, and future of chronopharmacokinetics*, British Journal of Pharmacology, 177 (2020), p. 2215–2239.

[5] L. S. Brown, J. H. Abel, E. B. Klerman, and F. J. Doyle III, *Mathematical modeling of circadian rhythms*, in Circadian Clocks, Springer, 2022, pp. 403–425.

[6] M. B. Elowitz and S. Leibler, *A synthetic oscillatory network of transcriptional regulators*, Nature, 403 (2000), pp. 335–338.

[7] D. Gonze, J. Halloy, and A. Goldbeter, *Deterministic versus stochastic models for circadian rhythms*, Journal of biological physics, 28 (2002), pp. 637–653.

[8] E. D. Herzog, S. J. Aton, R. Numano, Y. Sakaki, and H. Tei, *Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons*, Journal of biological rhythms, 19 (2004),

pp. 35–46.

[9] J. Hesse, J. Martinelli, O. Aboumanify, A. Ballesta, and A. Relógio, *A mathematical model of the circadian clock and drug pharmacology to optimize irinotecan administration timing in colorectal cancer*, Computational and Structural Biotechnology Journal, 19 (2021), pp. 5170–5183.

[10] O. M. . C. F. R. Johnston, J. D., *Lithium lengthens circadian period and increases amplitude of per2::luc rhythms in mice.*, PloS one, 4 (2009).

[11] J. K. Kim and D. B. Forger, *A mechanism for robust circadian timekeeping via stoichiometric balance*, Molecular systems biology, 8 (2012), p. 630.

[12] J. Li, W.-Q. Lu, S. Beesley, A. S. I. Loudon, and Q.-J. Meng, *Lithium impacts on the amplitude and period of the molecular circadian clockwork*, PLOS ONE, 7 (2012), p. e33292, https://doi.org/10.1371/journal.pone.0033292, https://doi.org/10.1371/journal.pone.0033292.

[13] R. Narumi, Y. Shimizu, M. Ukai-Tadenuma, K. L. Ode, G. N. Kanda, Y. Shinohara, A. Sato, K. Matsumoto, and H. R. Ueda, *Mass spectrometry-based absolute quantification reveals rhythmic variation of mouse circadian clock proteins*, Proceedings of the National Academy of Sciences, 113 (2016), pp. E3461–E3467.

[14] D. Ono and K. Honma, *In vivo evaluation of the effect of lithium on peripheral circadian clocks by real-time monitoring of clock gene expression in near-freely moving mice*, Scientific Reports, 3 (2013), p. 2378, https://doi.org/10.1038/srep02378, https://doi.org/10.1038/srep02378.

[15] A. Reinberg and M. Smolensky, *Circadian changes of drug disposition in man*, Clinical pharmacokinetics, 7 (1982), pp. 401–420.

[16] A. Relógio, P. O. Westermark, T. Wallach, K. Schellenberg, A. Kramer, and H. Herzel, *Tuning the mammalian circadian clock: robust synergy of two loops*, PLoS computational biology, 7 (2011), p. e1002309.

[17] M. H. Vitaterna, J. S. Takahashi, and F. W. Turek, *Overview of circadian rhythms*, Alcohol research & health, 25 (2001), p. 85.

[18] J. C. Walton, W. H. Walker, J. R. Bumgarner, O. H. Meléndez-Fernández, J. A. Liu, H. L. Hughes, A. L. Kaper, and R. J. Nelson, *Circadian variation in efficacy of medications*, Clinical Pharmacology & Therapeutics, 109 (2021), pp. 1457–1488.

[19] A. Wood, G. M. Goodwin, R. De Souza, and A. Green, *The pharmacokinetic profile of lithium in rat and mouse; an important factor in psychopharmacological investigation of the drug*, Neuropharmacology, 25 (1986), pp. 1285–1288.