

# A Parameter-Based Investigation of Oscillatory Behavior in a Model of the Mammalian Circadian Clock

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## Abstract

Circadian clocks regulate the day-night biological cycle in living organisms, including plants, animals, fungi, and some bacteria. Mammalia have similar endogenous circadian gene regulatory networks located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus, so the study of their clocks is generally conducted as a class. Henry Mirsky (2009) developed a mathematical model of *Mus musculus* single cell rhythms by describing 21 biological species with 132

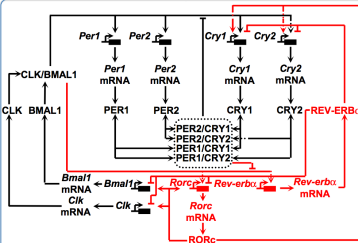


Figure 1. A diagram of the gene regulatory network, developed by Mirsky et al (2009). The primary positive feedback loop is black and the ancillary is red.

parameters in a system of ordinary differential equations (ODEs), using standard Michaelis-Menten, Hill, and mass action enzyme kinetics. The model consists of two regulatory loops, which we name the primary positive feedback loop and the ancillary negative feedback loop. We perturbed the model in MATLAB (MathWorks, Inc) in separate sensitivity and bifurcation analyses. We found that the ancillary loops relatively insensitive to parameter perturbation while the primary loop is highly sensitive to perturbations. We also confirmed that parameters related to cellular function, such as

gene transcription rates, were more sensitive than those exclusive to the model, such as protein-specific rates of dimerization. These data suggest that the positive regulatory loop is primarily responsible for maintaining oscillations in vivo and that the negative regulatory loop confers non-robustness-related characteristics to the model. Further research will perform similar analysis on multi-cellular tissue models whose parameter vectors are currently being discovered.

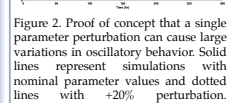


Figure 2. Proof of concept that a single parameter perturbation can cause large variations in oscillatory behavior. Solid lines represent simulations with nominal parameter values and dotted lines with +20% perturbation.

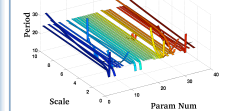


Figure 3. Bifurcation plot of ancillary loop parameters. The majority of parameter perturbations can be seen to have little effect on the periodicity of the system.

## Perturbation Analyses

### Sensitivity Analysis

- Uses single-parameter, percent-based perturbations to compare state values of the system when running nominal versus perturbed parameter vectors

### Bifurcation Analysis

- Uses larger parameter perturbations to discover the stability of the "bifurcation point" (i.e. state change) in system behavior

## Systems Biology

### What we do

- Model biological systems using data collected from cultured cells and tissue samples
- Work in collaboration with scientists from other disciplines to gain a comprehensive understanding of biology

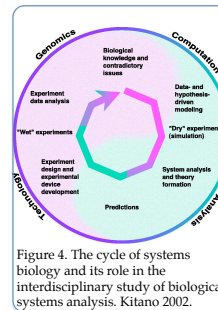


Figure 4. The cycle of systems biology and its role in the interdisciplinary study of biological systems analysis. Kitano 2002.

- Analyze the system against other observed data to ensure that it is a good description (e.g. knockout experiments)

- Refine the system by what was discovered, then repeat these steps again

### How we do it

- Propose and test enzymatic rate velocities defined using standard biochemical equations: Michaelis-Menten, Hill, and mass action kinetics
- Optimize parameters of enzymatic rate velocities to fit observed data using iterative algorithms based on nature's innovation of evolution

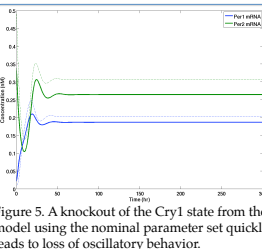


Figure 5. A knockout of the Cry1 state from the model using the nominal parameter set quickly leads to loss of oscillatory behavior.

## Why This Model?

Single-cell vs. multi-cell oscillators: In SCN tissue, many oscillating cells are sending and receiving signals. These signals allow cells to oscillate in synchrony and with a high amplitude. When cells are isolated from the network, their oscillations drift out of phase and lose amplitude (some become damped, and some even become arrhythmic). Knockout experiments (e.g. cry1-/-) show that intercellular signaling should confer robustness to the system (without communication there are no oscillations, but with communication there are synchronized oscillations).

## Results

Our single cell model nearly reproduces these data. However, our related multi-cell model fails. We want to know why. We investigate this through parameter-based sensitivity and bifurcation analyses.

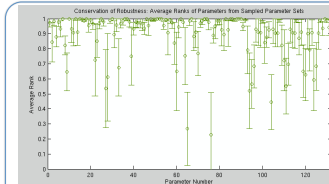


Figure 6. Mean sensitivity rank of each parameter, based on Stelling (2004). A rank of 1 shows low sensitivity and a rank of 0 indicates the opposite.

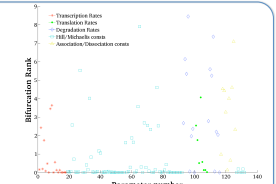


Figure 7. Clustering parameters according to five parameter types. A rank of 0 indicates low sensitivity.

## Conclusions

Mean parameter sensitivity data do not show correlations or clear clustering. Groupings designed to cluster by parameter type also do not show strong correlation of sensitivity. From this, we conclude one of two possibilities:

- The system is too robust to parameter perturbations using the current parameter set, or
- We can not analyze the model using the parameter grouping ideas of Stelling et al (2004).

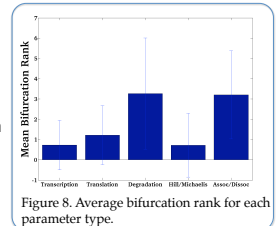


Figure 8. Average bifurcation rank for each parameter type.

### Future Goals

Our next goal is to refit parameters so that wild type model is less robust (i.e. it is easy to get a population of damped/arrhythmic/sustained oscillators) and so that the Cry1 knock-out is damped, rather than arrhythmic. This should show proper multi-cell entrainment behavior.

## A little bit of math

### A portion of one ODE

$$\frac{dPer1}{dt} = \underbrace{v_{0,Per1}}_{\text{basal}} + \underbrace{v_{1,Per1}}_{\text{maximal}} \cdot \underbrace{\frac{CLK/BMAL1^{n_{H,Per1}}}{K_{A1,Per1} + CLK/BMAL1^{n_{H,Per1}}}}_{\text{Hill term}}$$

Hill coefficient for Per1 transcription due to CLK/BMAL1 activation:  $n_{H,Per1} = 3.28$   
Hill coefficient for Per1 transcription due to PER1/CRY1 inhibition:  $n_{I1,Per1} = 3.93$   
Hill coefficient for Per1 transcription due to PER1/CRY2 inhibition:  $n_{I2,Per1} = 2.61$   
Hill coefficient for Per1 transcription due to PER2/CRY1 inhibition:  $n_{I3,Per1} = 1.66$   
Hill coefficient for Per1 transcription due to PER2/CRY2 inhibition:  $n_{I4,Per1} = 4.85$   
Hill coefficient for Per2 transcription due to CLK/BMAL1 activation:  $n_{H,Per2} = 4.50$   
Hill coefficient for Per2 transcription due to PER1/CRY1 inhibition:  $n_{I1,Per2} = 4.45$   
Hill coefficient for Per2 transcription due to PER1/CRY2 inhibition:  $n_{I2,Per2} = 3.70$

### References

- Kitano, H. et al (2002) Systems Biology: A Brief Overview. *Science*, 295, 1662-1664.  
Mirsky, H. P., A. C. Liu, D. K. Welsh, S. A. Kay & F. J. Doyle (2009) A model of the cell-autonomous mammalian circadian clock. *PNAS*, 106, 11107-11112.  
Stelling, J., E.D. Gilles, F.J. Doyle III (2004) Robustness priorities of circadian clock architectures. *PNAS*, 101, 13210-13215.

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