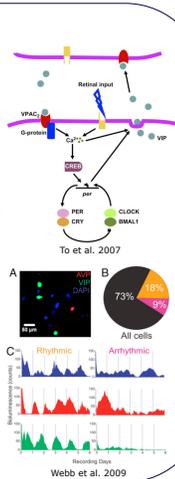


David M Brazel, Stephanie R Taylor

Department of Computer Science, Colby College, Waterville, ME, USA

Biology

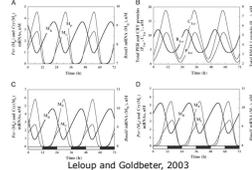
Circadian clocks are molecular clocks found in the cells of animals, plants, fungi, and bacteria which allow organisms to coordinate their behavior on a 24-hour cycle. The mammalian circadian clock is located in the suprachiasmatic nucleus and consists of many individual molecular clocks linked together in a synchronized neural network. Studies have shown that isolated circadian neurons tend to be poor clocks and are frequently either arrhythmic or damped.



Webb et al. demonstrated that in populations of circadian neurons plated at low density, 73% of the cells were damped, while only 18% were rhythmic. Our goal in this study was to develop a computer model of Webb's system, using a single cell mammalian circadian clock model.

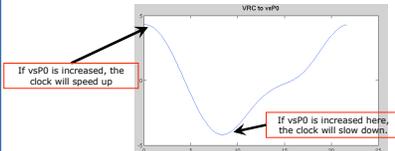
Math

In this study, we used the 16 state, single cell, mammalian circadian clock model developed by Leloup and Goldbeter. This model is composed of 16 ordinary differential equations and their associated parameters (e.g. rate constants). Each equation describes the change in concentration over time of a model component (mRNA or protein), using mathematical models of fundamental cellular processes such as transcription, translation, and phosphorylation. When appropriate parameters are used, the state trajectories of the Leloup and Goldbeter model oscillate on a stable limit cycle, as shown below:



Leloup and Goldbeter, 2003

In order to measure model sensitivity, we used a phase sensitivity analysis, the velocity response curve (VRC). Phase sensitivity is the sensitivity of the clock's internal time or phase to perturbations in parameter value. An example VRC is shown below:



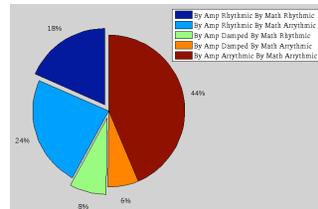
The VRC provides an indication of the effect of each parameter on clock speed at any given time. Theory suggests that more damped clocks should be more sensitive and therefore have greater VRC magnitudes.

We hypothesize that cells that are more damped, as measured by the number of successful cycles, will have greater VRC magnitudes.

Rhythmicity

In order to simulate Webb's experiment, we produced a population of 1,296 simulated cells by varying four parameters, vSP0 (the maximum rate of *Per* transcription), kSP (the rate of PER synthesis), vMP (the maximum rate of *Per* repression), and v3PC (the maximum rate of phosphorylation of the nuclear PER-CRY complex), over a range of 6 values. The cells' rhythmicity was then characterized by two methods, one based on amplitude and one based on the limit cycle definition.

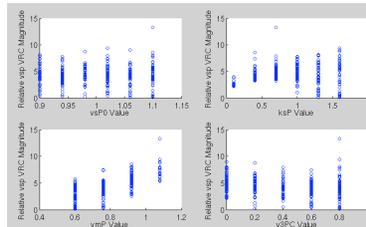
- ◆ **Amplitude** – The amplitude method counts the number of successful cycles, where a successful cycle is defined as having an amplitude no less than 20% of the initial cycle.
 - ◆ >4 cycles – Arrhythmic
 - ◆ 4-12 cycles – Damped
 - ◆ <12 cycles – Rhythmic
- ◆ **Mathematical** – If the cell converges to a limit cycle it is rhythmic. All other cells are arrhythmic.



Rhythmicity classifications – Classifications of all 1,296 cells in the simulated population. The two offset slices represent the portions of the population on which further analysis was conducted.

- ◆ The largest group in the population consists of cells that are considered arrhythmic by both methods.
- ◆ As only cells that converge to a limit cycle can be analyzed with VRCs, the remaining analyses in this study were conducted on 26% of the initial population.

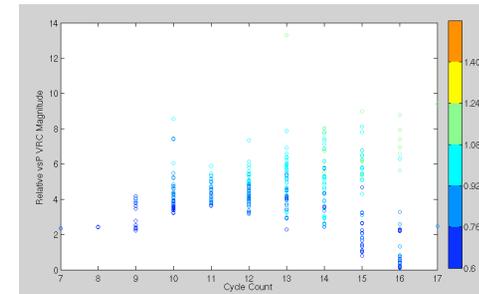
Parameter Value and Sensitivity



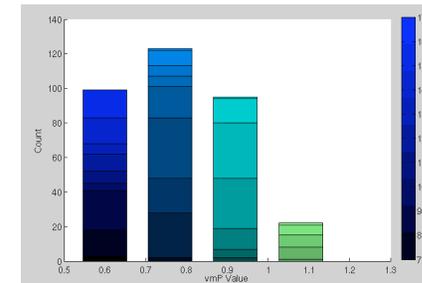
The effect of parameter value on sensitivity – Plots of the varied parameter values and the corresponding relative vsp VRC magnitude which is defined as the maximum value minus the minimum value of the VRC to the parameter vsp, normalized to parameter value. Each point represents one cell.

- ◆ The values of both kSP and vMP have a positive correlation with phase sensitivity.

Rhythmicity and Sensitivity



Rhythmicity and VRC Magnitude – A plot of successful cycle count, as a proxy for "dampedness" and relative vsp VRC magnitude. The color indicates the vmp value of each cell, as shown in the sidebar.



vmp Value and Rhythmicity – A stacked bar graph, showing the number of cells with each vmp value. The color corresponds to vmp value and is identical to the scheme used in the previous figure. The shading of each stacked bar indicates the successful cycle count of the corresponding cells, as shown in the sidebar.

- ◆ The relationship between rhythmicity and sensitivity is more complex than predicted. Cells with a low vmp value show the expected behavior and are more sensitive when damped than when rhythmic. Cells with a high vmp value are more sensitive when rhythmic and less sensitive when damped. In addition, cells at the low end of the damped range are less sensitive than cells at the high end of the damped range.
- ◆ Cells with low vmp values are found in significant numbers at all cycle counts. However, cells with high vmp values are found almost exclusively at higher cycle counts.
- ◆ The unexpected complexity of the model is a challenge for further analysis. It may be possible to identify the specific aspects of the model structure that cause the observed relationship.

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