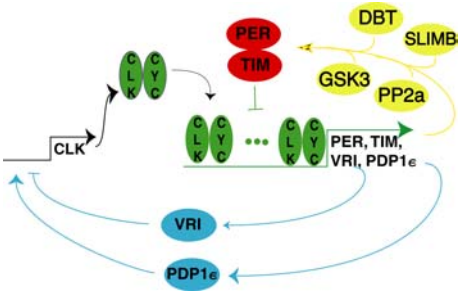
Research Summary Daniel Forger

Unicellular organisms time biological events (e.g. luminescence or oxygen consumption) with a circadian (24-hour) clock consisting of a network of genes and proteins. Higher organisms use a network of neurons, some of which contain intracellular molecular clocks similar to those in unicellular organisms, to time biological events (e.g. sleep-wake cycles, the release of hormones). Due to their relatively simple function (24-hour timekeeping) and a wealth of biological data, circadian clocks are an ideal model system for understanding genetic and neural networks. Research on circadian clocks also has many clinical applications (e.g. countermeasures for jet-lag).

My research uses mathematical models and analysis to understand timekeeping by circadian clocks. In particular, I have 1) Studied how individual parts of the intracellular circadian clock in *Drosophila* contribute to circadian timekeeping 2) Developed a biologically accurate, detailed mathematical model of the circadian system in mammals, and 3) Developed analytic tools which help us understand complex models and data.

Aim 1: Design Principles of the *Drosophila* Circadian Clock

Intracellular circadian clocks show autonomous oscillations and function accurately at a wide range of temperatures (temperature compensation). Their structure is complex (see figure) and it is not fully understood what aspects of their structure contribute to their ability to show autonomous oscillations or temperature compensation. Justin Blau and I have developed conceptual and mathematical models to understand autonomous oscillations and temperature compensation in the *Drosophila* circadian clock. These bring parsimony to available data, and make experimental predictions that we are testing.



The period of an intracellular clock depends on the rates of individual biochemical reactions within the clock, each of which probably change by a factor of 2 to 4 for every 10± C temperature increase. While increasing many of these reactions speeds up the clock (shorter period), increases in some reaction rates (temperature compensation elements) may slow the clock. Our simulations of available clock models challenged a widely held view that any biochemical oscillator has temperature compensation elements. We propose that an "opposing" reaction can act as a temperature compensation element, and that opposing reaction may be needed for temperature compensation. We identified the VRI feedback loop (see above schematic of the *Drosophila* molecular clock) as an opposing reaction and showed that the *Drosophila* molecular clock looses temperature compensation without this loop. Future work will test the role opposing kinases or phosphatases in the *Drosophila* molecular clock (shown in yellow above).

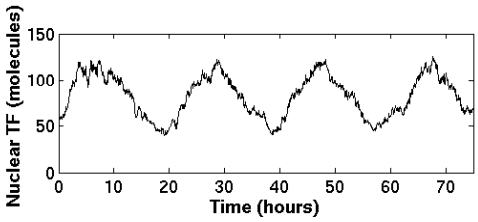
Genetic networks are mathematically dissipative, and most are therefore incapable of showing sustained oscillations. We have developed two models for how oscillations in cellular circadian clocks are sustained: 1) a fast positive feedback loop involving the PDP1ε protein adds gain to the PER-TIM negative feedback loop, 2) Transcription events on E-box promoters act in an all or none manner. Experiments are ongoing to test these two hypotheses.

Aim 2: Modeling the Mammalian Circadian System

A Cellular Model:

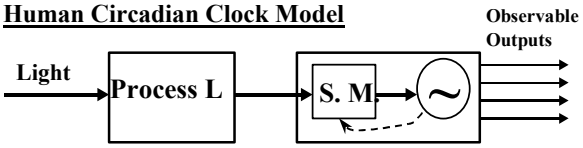
Charles Peskin and I have developed a mathematical model of the mammalian circadian clock that functions within individual neurons of the mammalian circadian pacemaker. Unlike the models in Aim 1 in which I sought simple explanations for complex data, here we tried to incorporate all of the available experimental data as directly as possible. This resulted in the most detailed and accurate model of a circadian clock yet derived. Since the specific biochemical rates of reaction in our model have not yet been experimentally determined, we estimated the parameters of the model as an inverse problem on the experimentally determined time courses of mRNAs and proteins within the clock. We found that this model is accurate in its predictions about the behavior of the clock with clock gene mutations and we used it understand key questions about clock structure and phase resetting.

With an experimental estimate of the number of molecules of key proteins within the clock, we used the model to study the effect of stochastic molecular interactions in the mammalian intracellular circadian clock. Amazingly, interactions between transcription factors and promoters must occur rapidly (several minutes or faster) for accurate 24-hour timekeeping. When the stochastic model was simulated with the PER2 mutation (which stops sustained oscillations in the deterministic model) accurate 24-hour oscillations are seen due to molecular noise (see right). Although published just a year ago, other modelers and experimentalists are already using the model.



A Human Performance Model:

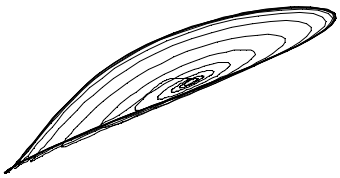
A large collection of phase response and dose response data is available on the effect of light exposure on the human circadian clock. Using these data and a specially designed inverse method, Richard Kronauer, Megan Jewett and I developed a model of the human circadian system (see schematic below). Using nonlinear analysis, I developed a mathematically simpler version of this model which required less model assumptions about the underlying physiology. The original and simpler models both accurately predict the phase response and dose response data and have similar signal-to-noise ratios when fit to core body temperature data with the Kalman filter and an ARMA model of temperature regulation.



Two key predictions of this model are: 1) the effect of bright light on the human circadian clock saturates within 20 minutes and 2) the human circadian pacemaker is particularly sensitive to late night light exposure. These models has been incorporated into a software package CPSS, a web site (www.lightmodel.com), and are being used to design schedules for NASA and Air Force missions.

Aim 3: General Mathematical Methods for Understanding and Building Models

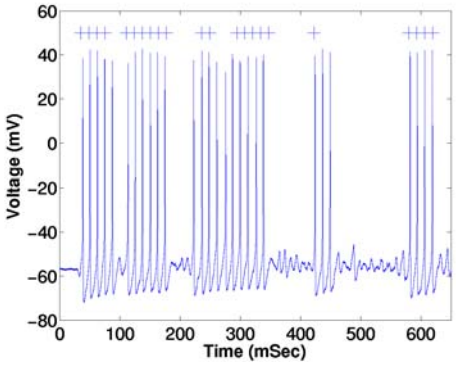
A Simple Description of Oscillations in Complex Networks:



Biochemical networks can be very complex, often involving tens or hundreds of reactions and chemical species. Despite their complexity, these networks show behaviors that can also be seen in relatively simple systems (e.g. oscillations). We have developed a method, by which quasi-linear oscillations in high dimensional systems may be approximated by a two variable (amplitude and phase) model. This method has been used to study the Forger-Peskin model of the intracellular mammalian circadian clock, and several models of the intracellular circadian clock in *Drosophila*. In each model, one finds a two dimensional manifold in phase space on which oscillations exist (see above for the > 70 variable Forger-Peskin model), and to which the rest of the phase space relaxes. Averaging methods on an approximation of this manifold can then be used to describe the oscillations dynamics. Using this method, we found that Goldbeter's original *Drosophila* circadian clock model is almost identical in behavior, on a manifold, to the simpler model of the human circadian system I have developed.

Signal Processing by Biological Systems:

There are many examples of biological systems which interpret external signals. For instance, a neuron can use signals from neighboring neurons to time its action potentials. David Paydarfar and I have developed an experimental protocol to understand how biological systems respond to environmental signals. This protocol first finds random test stimuli that are matched to the biological oscillator's relevant time scales and the range of stimuli that are physiologically reasonable. After testing these stimuli, patterns are found among the most effective test stimuli. These patterns can then be translated into a mathematical model, or used to find an optimal stimulus. If a good mathematical model already exists, geometric arguments about the phase space, or the calculus of variations can also be used to determine which stimuli may be optimal.



Using this protocol in an experimental preparation (with John Clay), we found separate mechanisms in a neuron (the squid giant axon with raised pH) for processing signals comprised of short or long post-synaptic potentials. We were also able to develop a model for the signal processing of the squid giant axon with raised pH (see Figure which shows the firing pattern of this neuron and predicted (+) action potential peaks to an applied stimulus).

Teaching Statement — Daniel Forger

As an undergraduate, I was the teaching assistant for Richard Kronauer's course on nonlinear dynamics and chaos at Harvard. This course enrolled both graduate and undergraduate students, and I was responsible for grading assignments and teaching sections that reviewed the material presented in lecture. When Prof. Kronauer was away, I lectured. The course material was advanced and much of it was not found in the course texts. Much preparation was required to make the subject material clear to the students who struggled with the course material and answer advanced questions from the students with strong backgrounds. To help motivate the students, I developed a computer lab, and presented many examples. Teaching in this course was a rewarding experience and helped convince me to pursue an academic career.

While my graduate and post-doctoral work was not funded by teaching fellowships, I have taken advantage of every opportunity to guest teach. I have given guest lectures in undergraduate and graduate course in the mathematics and biology departments at NYU. Each year, I also give guest lectures on mathematical modeling at Stuyvesant High School. My work is very interdisciplinary, and I spend much time thinking about how to explain mathematics to biologists, and biology to mathematicians. The better I am at this, the quicker my collaborative research proceeds.

One of my favorite activities has been mentoring students in the Intel Science Talent Search. For three of the past four years, I have met with a high school about once a week to model a problem in biology. In addition to learning about a new topic (e.g. cancer or Gaucher Disease) and practicing my modeling skills, I feel invigorated when working with students one-on-one. Two of these students were chosen as finalists, and the third just submitted his project. For this work, I was awarded the Stuyvesant High School mentor award.

There are many courses I would enjoy teaching. Of particular interest are courses in mathematical modeling, nonlinear dynamics, techniques in applied mathematics, genetic regulatory networks and circadian rhythms.

Proposed Research Daniel Forger

During the next five to seven years, I plan to develop detailed and conceptual models of circadian timekeeping, and develop mathematical tools that can help us understand complex mathematical models and complex genetic networks. In particular, I aim to:

1) Use circadian clocks to study how genetic networks function accurately in the presence of molecular noise
2) Develop a model of the neural communication between pacemaker neurons
3) Develop a detailed multi-scale model of the human circadian system
4) Develop tools which help design schedules that minimize jet lag
5) Develop mathematical tools which help understand complex genetic networks.

The following summaries of these projects describe the work to be done, preliminary results, and potential collaborations.

Project 1: The Behavior of Circadian Clocks in the Presence of Molecular Noise

The timing of individual molecular interactions of molecules, within a cell, is stochastic. Genetic networks, and in particular molecular circadian clocks, must function accurately in the presence of this "molecular noise". One proposed design principle, which can be used to overcome molecular noise in genetic networks, is to have multiple redundant molecular species perform the same function. This increases the number of individual molecular interactions, and reduces stochasticity by the principle of averaging.

Charles Peskin and I have several model-based predictions on the accuracy of intracellular circadian timekeeping. Although the mammalian molecular circadian clock has many proteins that are often considered redundant (e.g. CRY1 and CRY2), simulations of our model showed that small differences between these proteins (e.g. differences in their rates of degradation) do not reduce molecular noise. In fact, we found that removing either CRY1 or CRY2 from our model allows for more accurate timekeeping. Increasing the number of individual molecular interactions by proportionally increasing the numbers of molecules of all species and the reaction rates with the promoter causes the observed variability in our model to diminish in a characteristic way (the standard deviation of the model's period scales as $1/n^{0.5}$, where n is the number of molecules of any species).

New experimental protocols can be developed to directly measure the variability of intracellular circadian timekeeping. Mammalian pacemaker neurons can be pharmacologically isolated; *Drosophila* neurons can be grown in isolation; cell cultures may be able to be developed where individual cells do not synchronize. Circadian rhythms can be measured from the firing rates of action potentials or by luminescence. Measurements can be made from both wild-type and mutant pacemaker cells. Several experimental groups are interested in helping me develop these protocols.

These protocols will allow us to directly test design principles that may reduce the stochasticity of intracellular circadian timekeeping. The frequency spectra of measured rhythms can also be analyzed and compared with model simulations to provide information on which parts of the molecular circadian clock contribute to its observed variability. These measurements will also help us validate and refine our model of the mammalian molecular clock.

Project 2: Communication and Signal Processing in Pacemaker Neurons

(A collaboration with the Allen Lab, Oregon Health Sciences University)

Mammalian pacemaker neurons can synchronize rhythms through electrical signals (post-synaptic potentials) and these signals can lead to more accurate timekeeping. Disrupting electrical signals by 1) Stopping action potential firing in mammalian cultured neurons, 2) expressing open-rectifying ion channels in *Drosophila* pacemaker neurons 3) blocking the production of the neuropeptide PDF in *Drosophila* or 4) blocking the neuropeptide receptor $VPAC_2$ in mammals, all stop the internal molecular clock within pacemaker neurons. Thus, neural communication is central to circadian timekeeping itself. Electrical signals also likely communicate the time of the external day to pacemaker neurons, and form the main output of pacemaker neurons.

I have developed an experimental protocol and mathematical methods that can be used to uncover the signal processing within pacemaker neurons (See Reseach Summary). First, we plan to pharmacologically isolate pacemaker neurons in slices of the suprachiasmatic nucleus (SCN, the core circadian pacemaker in mammals) from the signals of other neurons. Test stimuli (simulated combinations of post-synaptic potentials) will then be input into these neurons and their responses recorded. From these responses, we will find patterns of signal processing, and models will be developed and validated. This procedure will be repeated for several cell types in the SCN and at several times of the day.

Project 3: A Multi-Scale Model of the Mammalian Circadian Clock

Models of circadian timekeeping are greatly limited by their scale. Detailed intracellular models do not incorporate the synchronizing or desynchronizing effects of other pacemaker cells and the processing of visual stimuli by the retina and optic nerve. Intracellular models therefore cannot predict whole organism behavior. Phenomenological model of human behavior typically assume that all SCN pacemaker neurons have the same clock state. However, this is rarely the case *in vivo* or *in vitro*. SCN heterogeneity likely plays a large role in the processing of visual information, the timing of diverse biological events, and the adaptation of the SCN to new schedules. Phenomenological models of human performance also cannot make predictions about the effects of pharmacological treatments or genetic mutations on human behavior.

To bridge these levels of organization I plan to develop a multi-scale model of the human circadian clock. This model will include the light preprocessor of the Forger-Jewett-Kronauer models, and contain a network of hundreds or thousands of neurons some of which will contain a copy of the Forger-Peskin model of the molecular clock within mammalian pacemaker cells. A new description of the electrical coupling will also be required and will be based on the experimental data of Project 2, data on the morphology and heterogeneity of the SCN and may also take into account recent data on specific ion channels in SCN neurons. Special numerical techniques may be required to solve this model, potentially including dimensional reduction of the Forger-Peskin model, and tau-leaping stochastic methods.

Project 4: Designing Optimal Schedules for Shift Work and Travel Across Time Zones

(with members of the Division of Sleep Medicine, Harvard Medical School)

The Forger-Jewett-Kronauer model of the human circadian system predicts the response of the human circadian system to a schedule of light exposure. Most real life applications ask the inverse problem, "What schedule best adapts a person's circadian clock to a new time zone?" This question can be answered mathematically with the Calculus of Variations. Genetic algorithms might also be used to speed up these calculations. We aim to develop a method, implemented in a software package, which can optimize schedules for shift work and overseas travel. The Division of Sleep Medicine already has some funding for this work.

Project 5: Mathematical Decomposition of Genetic Networks

Although a genetic network may consist of many genes and proteins, the behavior of some parts of the network may not have a large effect on the behavior of other parts of the network. I hope to develop and apply mathematical techniques that can help decompose genetic networks into independent (or approximately independent) subsystems, which can then be studied individually. One possible method is to decompose networks into fast and slow subsystems where, on a fast time scale, the slow subsystem can be considered quiescent, and, on a slow time scale, the fast subsystem's dynamics may be averaged or treated at equilibrium. Kalman's theory of observability can be also used to determine which parts of the network have no effect (i.e. not observable) on the dynamics of another part of the network. These ideas can be used to develop a general theory of the behavior of coupled transcription-translation feedback loops similar to what has been found for other coupled oscillators (e.g. the work of Rand and Holmes on coupled van der Pol oscillators).

One example of the interesting behavior that can result from coupled genetic networks is shown below. Although the A and B feedback loops share a common element (the AB complex), they can each produce relatively independent oscillations.