

**AC 2008-1367: NICOTINIC ACETYLCHOLINE RECEPTOR KINETICS OF THE
NEUROMUSCULAR JUNCTION SIMULATED USING SPICE - AN
ILLUSTRATION OF PHYSIOLOGICAL PROCESS SIMULATION WITH
CONVENTIONAL CIRCUIT SIMULATION SOFTWARE**

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Nicotinic Acetylcholine Receptor Kinetics of the Neuromuscular Junction Simulated Using SPICE – An Illustration of Physiological Process Simulation with Conventional Circuit Simulation Software

Abstract

With the advent of modern day computational power, there is a great deal of interest in the simulation and modeling of complex biological systems. A significant effort is being made to develop generalized software packages for the simulation of cellular processes, metabolic pathways and complex biochemical reaction systems. The advantages to being able to implement and simulate complex biological systems in a virtual environment are several. Simulations of this type, if sufficiently detailed, provide experimental physiologists with the ability to visualize the dynamics of a given biological system of interest. The validity of hypotheses related to the system under study can be tested in a virtual environment prior to carrying out experimental studies. We discuss a systematic approach by which certain reaction balance equations can be transformed into equivalent circuit models that may then be implemented and simulated using SPICE (Simulation Program with Integrated Circuit Emphasis). To introduce the methodology, we develop a simulation for a single ligand-receptor interaction and then we utilize this framework to implement a simulation of nicotinic acetylcholine receptor kinetics at the postsynaptic membrane of the neuromuscular junction. Although the example studies that we present are specific to biochemical reaction systems associated with cellular processes, the procedure is equally applicable to any biochemical or chemical process for which analogous systems of mass balance equations exist that have an equivalent circuit analog. The overall approach described above is useful from the biomedical engineering educational perspective because SPICE simulators are readily accessible to students in freeware versions that they can use to simulate and visualize relatively complex physiological processes such as neurotransmitter/receptor dynamics.

Introduction

During the past several years, there has been an increasing interest in the development of generalized software packages for simulating biological systems of varying complexity. Increasingly, experimental physiologists are relying on these software packages to assist with validation of empirical results. The public domain software packages NEURON and GENESIS are two good examples of simulation programs that are utilized with increasing frequency by experimental electrophysiologists.^{1,2} Other packages have been developed that focus on simulation of biochemical system dynamics³ and still other tools, such as Bio-Spice, have been written for genetic circuit analysis but are being adapted to function as generic modeling and simulation environments.⁴ An excellent review of available software is provided by Takahashi *et al.* along with discussion of related software development issues.⁵

The use of virtual environments to simulate complex biological systems has advantages from the perspective of the ability to theoretically validate experimental results.⁶ Equally importantly, virtual studies provide physiologists with the opportunity to test hypotheses prior to undertaking experimental investigations which can be both expensive and time consuming.

It has long been recognized that chemical networks are mathematically equivalent to a specific class of multiport network allowing for the application of abstract circuit theory to the study of chemical systems.⁷ Many physical systems, such as mechanical or electro-optic systems, can be modeled as equivalent circuits and their dynamics may be determined through the transient solution of the applicable equivalent circuit model.^{8,9} The utility of SPICE in simulation and modeling is not limited to physical systems but has been expanded to the realm of complex biological systems. Hodgkin-Huxley active membrane models have been implemented in SPICE at both the netlist level as well as hard coded into specific variants of the SPICE program.^{10,11} These models have been upgraded and utilized in simulation studies with artificial electronic circuitry to demonstrate neural-electronic inhibition.¹² The utility of SPICE software in simulating the interaction of biological and synthetic electronic components over and above conventional solution of the related transient problem with ordinary differential equation solvers is realized in the ease with which multi-node problems may be implemented.¹³

We describe a systematic approach for converting a system of biochemical balance equations into an equivalent circuit model. Once converted to an equivalent circuit, the associated dynamics of the biochemical system can easily be simulated using the computational infrastructure provided by the SPICE simulator. Quantitative results can also be readily visualized with graphical post processors that are available with most public domain or commercial versions of SPICE. We discuss this systematic approach in the context of two examples of biochemical reaction systems associated with cellular processes. Although the examples presented are related to cellular processes, the methodology outlined is equally applicable to reaction systems in organic and inorganic chemistry.

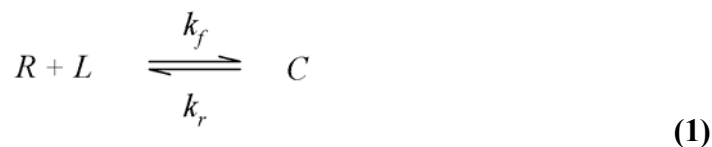
Method

The two examples that will be discussed consist of a simple ligand-receptor interaction neglecting endocytosis adapted from Fogler *et al.*¹⁴ Based on the methodology illustrated, the framework associated with the first example will be used to develop a model of the reaction kinetics of nicotinic acetylcholine receptors at the postsynaptic membrane of the neuromuscular junction.

A. Ligand-Receptor Interaction

Figure 1 is a diagram of a simple ligand-receptor interaction where the rate constants k_f ($M^{-1}s^{-1}$) and k_r (s^{-1}) model the reaction associated with the ligand binding to the receptor and the reverse dissociation reaction respectively.

The associated equilibrium reaction and the concomitant reaction rate equation are shown in (1) where R is the number of unoccupied receptors per cell, L is the free ligand concentration (M/dm^3) and C is the number of bound receptor ligand complexes per cell.



$$\frac{dC}{dt} = k_f RL - k_r C$$

The total number of receptors per cell R_T is equal to the number of free receptors and the number of receptors associated with bound receptor ligand complexes C such that $R_T = R + C$. The values of the rate constants and species concentrations are shown in Table 1.

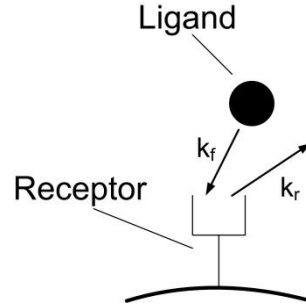


Figure 1. Diagram of simple ligand-receptor interaction. The rates shown k_f ($M^{-1}s^{-1}$) and k_r (s^{-1}) are associated rates for the reaction where the ligand binds with the receptor and dissociates.

Table 1 Receptor Ligand Interaction Variables and Parameters

R_T	Total Number of Receptors Per Cell	1×10^4 #/cell
R	Number of Unoccupied Receptors Per Cell	#/cell
C	Number of Bound Receptor Ligand Complexes Per Cell	#/cell
k_f	Receptor Ligand Binding Reaction Rate Constant	$1.2 \times 10^{-6} M^{-1}s^{-1}$
k_r	Receptor Ligand Dissociation Reaction Rate Constant	$5.67 \times 10^{-3} s^{-1}$
L	Ligand Concentration	$1 \times 10^{-8} M/dm^3$
C_d	Dummy Capacitance	$1 \mu F$

Development of a SPICE equivalent circuit model proceeds directly from the form of (1). If we multiply the ordinary differential equation in (1) by a dummy capacitance value C_d then the resultant equation (2) can be interpreted as a statement of Kirchhoff's Current Law.

$$C_d \frac{dC}{dt} - C_d k_f (R_T - C)L + C_d k_r C = 0 \quad (2)$$

Equation (2) can be modeled using an equivalent circuit where each term corresponds to a branch current in the network of Figure 2 as shown.

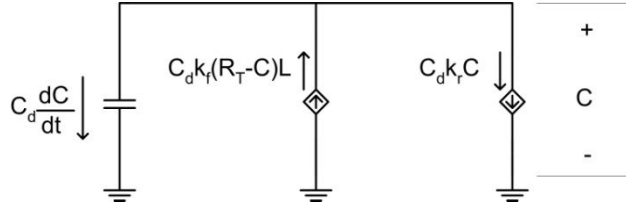


Figure 2. Circuit model of the differential equation describing the ligand-receptor interaction. The branch currents shown form the terms in the equation where the variables and parameters are as per Table 1. The potential across the three elements models the number of bound receptor ligand complexes per cell. The voltage controlled current source in the center of the circuit generates a product, using the SPICE POLY command, between the potential across the resistor of the circuit in Figure 3 (a) and the potential across the resistor in Figure 3 (e). The voltage controlled current source on the right hand side generates a product, using the SPICE POLY command, between the potential across the resistor in Figure 3 (b) and the potential across the circuit elements in Figure 2. The initial potential on the capacitor is zero which constitutes the initial condition $C(0) = 0$ #/cell.

Formulation of an equivalent circuit model that encompasses the dynamic behavior of the system is not unique. Figure 3, parts (a) through (e), illustrate the generation of the various circuit parameters associated with the network shown in Figure 2. Branch current and node potential relationships to specific parameters or variables are indicated on the circuit diagrams as required for clarity.

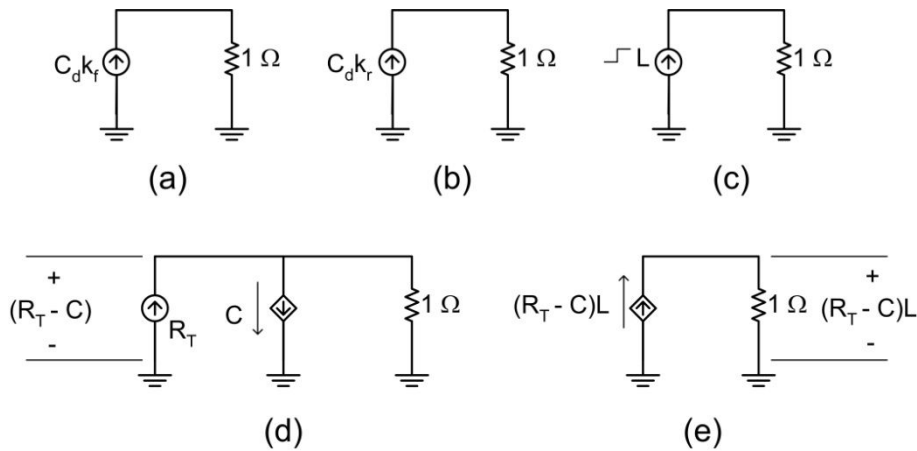


Figure 3. Equivalent circuits for generation of parameters and branch currents for the network in Figure 2. a) Generation of $C_d k_f$ as the voltage across the 1Ω resistor. b) Generation of $C_d k_r$ as the voltage across the 1Ω resistor. c) Generation of L as a voltage step across the 1Ω resistor from 0 to 1×10^{-8} M/dm³. d) Generation of the $(R_T - C)$ term as the voltage across the 1Ω resistor. The voltage controlled current source takes as its control voltage the potential across the circuit in Figure 2. e) Generation of the product term $(R_T - C)L$ using a voltage controlled current source and the control voltages across the resistor in c) and the three elements in d). The SPICE POLY statement is used to generate the product between the control voltages.

A systematic procedure can be applied to formulation of the equivalent circuit models for equations of the form shown in (2). The terms associated with branch currents in (2) are formed

by the product of several parameters and reactants having a general form shown in (3) where k_1 through k_n are parameters and Ψ_1 through Ψ_n are reactants.

$$k_1 k_2 \dots k_n \cdot \Psi_1 \Psi_2 \dots \Psi_n \quad (3)$$

Individual reactant concentrations may be generated using a general circuit of the form shown in Figure 3 (c). The voltage across the resistor in Figure 3 (c) may then be used as a control variable in conjunction with an alternate reactant voltage from another circuit and the product of these two control voltages can be implemented using the SPICE POLY command. Assuming a two reactant system with a series of parameter products, the POLY statement would take on the following form where k_p consists of one or the product of several parameters.

POLY (2) (V_{Ψ_1} , 0) (V_{Ψ_2} , 0) 0 0 0 0 k_p

Alternatively, the multiplicative constant k_p shown in the POLY statement can be incorporated in a circuit such as Figure 3 (a) or (b). The placeholder for the gain constant in the POLY statement above, in this case, would be set to unity.

A flowchart for the proposed procedure is shown in Figure 4.

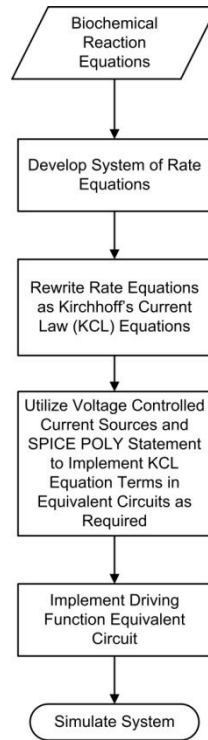
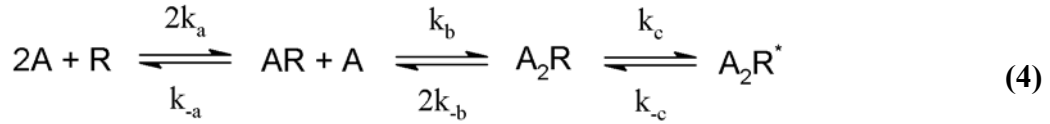


Figure 4. Flow chart of process for implementing an equivalent circuit simulation in SPICE of a biochemical reaction system.

B. Nicotinic Acetylcholine Receptor Kinetics of the Neuromuscular Junction

Equation (4) describes the mass action kinetics of the interaction between acetylcholine and the nicotinic acetylcholine receptors at the postsynaptic membrane of the neuromuscular junction discussed by Rosenberry¹⁵ and used by others.¹⁶



In this reaction, two acetylcholine molecules $2A$ can react with a nicotinic receptor R forming a single bound receptor AR and a free acetylcholine molecule. The free acetylcholine molecule can then react with the single bound receptor forming a double bound closed receptor complex A_2R . A double bound closed receptor complex can then undergo a further conformational change to a double bound open state A_2R^* . The variables and parameters in (4) are defined in Table 2. The coupled system of differential equations describing the reactions outlined by the combined mass balance equation in (4) is shown in (5) through (8).

$$\frac{dR}{dt} = -2k_a 2A \cdot R + k_{-a} A \cdot AR \quad (5)$$

$$\frac{dAR}{dt} = 2k_a 2A \cdot R - k_{-a} A \cdot AR - k_b A \cdot AR + 2k_{-b} A_2R \quad (6)$$

$$\frac{dA_2R}{dt} = k_b A \cdot AR - 2k_{-b} A_2R - k_c A_2R + k_{-c} A_2R^* \quad (7)$$

$$\frac{dA_2R^*}{dt} = k_c A_2R - k_{-c} A_2R^* \quad (8)$$

Equations (5) through (8) can be modeled using the equivalent circuits shown in Figure 5 where the differential equations have been multiplied by the dummy capacitance C_d and all terms have been moved to one side of the equation.

Table 2 Acetylcholine and Nicotinic Acetylcholine Receptor Interaction Simulation

A	Acetylcholine Concentration	33.2 mM
R	Unbound Nicotinic Acetylcholine Receptors	M
AR	Single Bound Nicotinic Acetylcholine Receptors	M
A_2R	Double Bound Closed Nicotinic Acetylcholine Receptors	M
A_2R^*	Double Bound Open Nicotinic Acetylcholine Receptors	M
R_T	Total Receptor Concentration	664 μ M
k_a	Kinetic Reaction Constant	30×10^6 ($M^{-1}s^{-1}$)
k_{-a}	Kinetic Reaction Constant	10×10^6 ($M^{-1}s^{-1}$)
k_b	Kinetic Reaction Constant	20×10^6 ($M^{-1}s^{-1}$)
k_{-b}	Kinetic Reaction Constant	10×10^3 (s^{-1})
k_c	Kinetic Reaction Constant	20×10^3 (s^{-1})
k_{-c}	Kinetic Reaction Constant	5×10^3 (s^{-1})
C_d	Dummy Capacitance	1 μ F

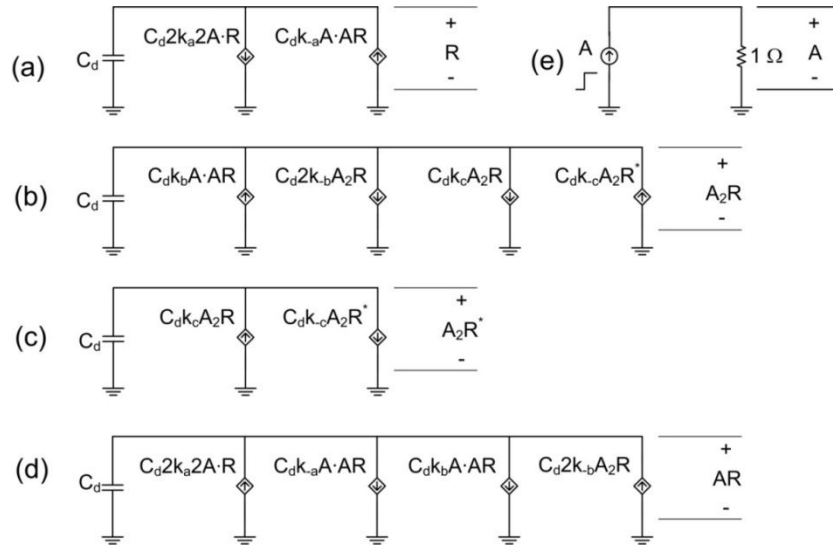


Figure 5. Equivalent circuits used to model the dynamics of the nicotinic acetylcholine receptors at the postsynaptic membrane of the neuromuscular junctions. Circuits a) through d) implement equations (5) through (8). The circuit shown in e) represents the acetylcholine forcing function which, in the case of the simulation results presented below, is a step function. The relevant parameter values for the simulation are shown in Table 2.

Results

The two models discussed above were implemented in the student version of ORCAD PSPICE 9.1. For the ligand-receptor model, a transient simulation was carried out using the SPICE STEP command to vary the extracellular ligand concentration L . Figure 6 is a plot of the variation in the number of free receptors R and bound ligand-receptor complexes C with time taking the extracellular ligand concentration L as a parameter that is varied between 0.02 and 0.1 μ M/dm³.

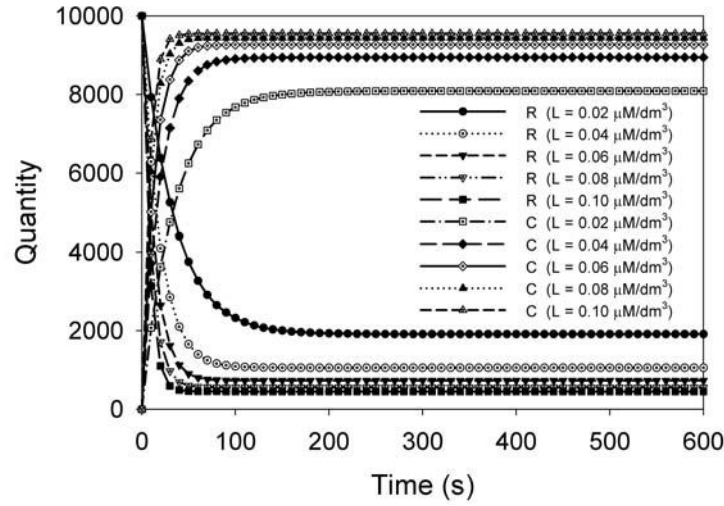


Figure 6. Variation of free receptor R and bound ligand-receptor complex C quantities per cell with time where the extracellular ligand concentration L is taken as a parameter. The SPICE transient simulations were done using the equivalent circuit model described above and the SPICE STEP command.

A SPICE netlist file of the circuits shown in Figure 5 was implemented. Utilizing a step excitation of acetylcholine concentration from a value of zero to 33.2 mM, the SPICE netlist file was simulated using the Student Version of OrCAD PSPICE 9.1. Plots of the receptor complexes R , AR , A_2R and A_2R^* were generated from the simulation and are shown in Figure 7.

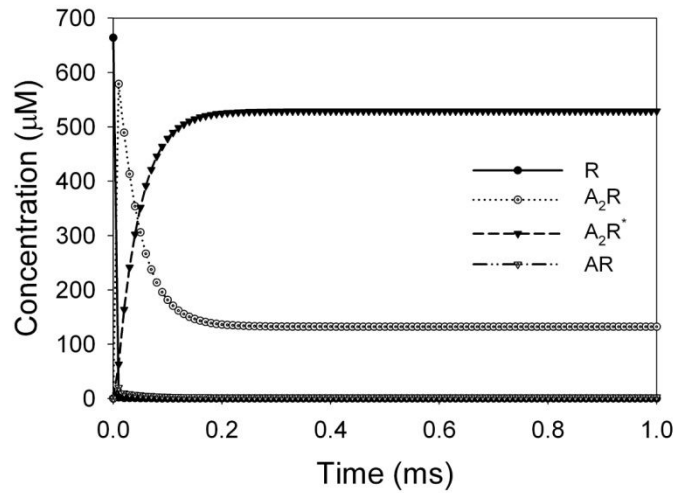


Figure 7. Plot of the variation with time in receptor complexes R , AR , A_2R and A_2R^* given a step excitation in the acetylcholine concentration A . The other relevant parameters for this simulation are given in Table 2.

The total number of receptors $R_T = R + AR + A_2R + A_2R^*$ for all time t . Consistent with the equilibrium described in equation (4), the values of the various receptor complexes approach steady state with increasing time t .

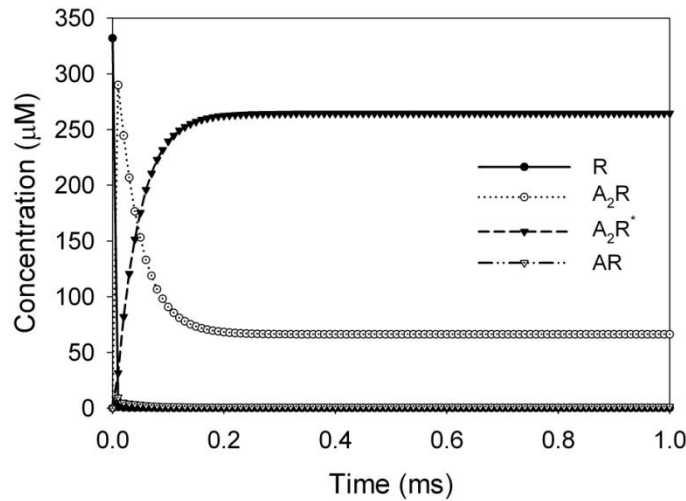


Figure 8. Plot of the variation with time in receptor complexes R, AR, A₂R and A₂R* given a step excitation in the acetylcholine concentration A. To simulate the effect of the myasthenia gravis disease process, the total number of free receptors was reduced to $R_T(0) = 332 \mu\text{M}$. The other relevant parameters for this simulation are given in Table 2.

A further simulation study was carried to simulate the situation where the neuromuscular junction has been compromised by myasthenia gravis.¹⁷ A concomitant reduction, by one half, in the number of free nicotinic acetylcholine receptors yielding a total free initial receptor concentration of $R_T(0) = 332 \mu\text{M}$ was used in a subsequent simulation. Data from this simulation study is shown in Figure 8.

Discussion

The SPICE simulator provides a versatile tool for simulation and modeling of cellular process dynamics where these processes can be modeled using systems of chemical balance equations. Provided that the equations describing the system can be represented using equivalent circuits, the efficiency of the SPICE simulation platform at solving nonlinear ordinary differential equations can be utilized to provide the computational infrastructure for rapid calculation of system dynamics. Most modern implementations of SPICE provide powerful graphics post processor applications that may be further utilized for rapid and efficient graphical visualization of simulation results.

Due to its optimization as a circuit simulation tool, SPICE can provide a unifying simulation framework for complex biological systems such as those routinely studied in electrophysiology. Models of Hodgkin-Huxley dynamics have been presented in the literature by several authors.^{10,11,12} These models may now be enhanced, using the techniques described in this paper related to simulation of biochemical systems and cellular processes, to include effects of neurotransmitters-receptor interaction. Recent efforts in neural-electronics, which specifically relates to the interaction of neurons with synthetic electronics, would benefit from an efficient and unified simulation and modeling tool.^{13,18,19,20,21,22} The SPICE simulation platform provides an environment where complex systems involving synthetic electronic components, cells with

active biological membranes and neurochemical processes can be modeled and simulated within a single unifying framework.

The principal inhibiting factor to the utilization of the equivalent circuit modeling approach is the concomitant formulation of a circuit that encompasses the same dynamic behavior as the biochemical system that is being modeled. As the approach outlined herein has demonstrated, the technique for realization of the equivalent circuit model is, in many cases, systematic and amenable to implementation in an algorithmic form. The overall utility of the approach could be greatly enhanced through the development of a front end schematics capture type application that generates the relevant SPICE netlist code in a manner that is transparent to the user.

The technique proposed herein can be utilized as the framework for useful virtual laboratory exercises in biomedical engineering courses that discuss neurotransmitter/receptor interactions as well as other biochemical reaction processes or paths. One of the attractive features of this approach is the utilization of the simulator that can allow students to change input parameters and very rapidly visualize the impact that these changes have on the dynamics of the system under study.

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