Local Excitation, Global Inhibition Mechanism for Gradient Sensing: An Interactive Applet

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Abstract

We have proposed a model in which cells detect gradients of chemoattractant by balancing a fast local excitation and a slower global inhibition. To illustrate this general mechanism, we have developed an interactive applet that mimics laboratory experiments in which either spatially homogeneous or heterogeneous stimuli of chemoattractant are applied.

Introduction

Directional sensing refers to the ability of a cell to detect an asymmetric extracellular cue and generate an internal response (*1*, *2*). This is a necessary first step in chemotaxis or chemotropism. However, as demonstrated in cells that have been treated with inhibitors of actin polymerization and are unable to polarize, directional sensing can be observed independently of movement.

The ability of cells to generate localized internal responses at their leading edges (*3*) requires a means to compare receptor occupancy at various points along the cell membrane and a mechanism for amplifying differences that are detected. Cells also possess a powerful mechanism for adaptation. When stimulated uniformly and persistently by a chemoattractant, cells will respond transiently. However, within a few minutes their response will subside and will return to prestimulus levels. Subsequent increases in receptor occupancy will elicit new transient responses. This empowers cells with a mechanism for background subtraction, so that chemoattractant gradients with a large range of midpoint concentrations can be sensed.

To elucidate these mechanisms, we need to appreciate how temporal biochemical responses triggered by stimulus increments relate to the spatial responses of cells in gradients. Many models explaining how cells can sense gradients have been proposed [see (*2*) for a review]. We have proposed a general model that can explain both sensing of spatial gradients and adaptation to uniform temporal stimuli (*1*, *4*, *5*). In the local excitation, global inhibition (LEGI) model, temporal and spatial sensing involve two opposing processes representing local excitation (E) and global inhibition (I). Receptor occupancy controls the steady-state activity of the two processes, and the balance between the two regulates the activity of the response regulator (RR). Upon chemoattractant stimulation, receptor occupancy increases, eliciting a fast rise in excitation. This induces a transient change in activity of RR. The activity of RR peaks as excitation plateaus. A slower but equal increase in I then causes a decrease in the activity of RR. If the relative change in activity of both E and I is the same, then the activity of RR will return exactly to what it was before the stimulus was applied.

One of the challenges of integrating mathematical models into the toolbox of the biological community is overcoming existing discomfort with complicated mathematical descriptions. It is important that models be made easily available to researchers for evaluation, testing, and generating testable predictions. Methods that allow one to explain the precepts of models without becoming mired in the mathematical underpinnings are critical. To meet these needs, we have created an interactive Java applet to illustrate the basic premise of the LEGI model of gradient sensing.

The applet allows the user to test the effects of varying different conditions on the model without having to perform any programming. It also provides an extremely fast means for determining the effects of parameter variations, both in the kinetic parameters as well as in the physical constraints such as the gradient steepness. Although other interactive Java applet–based models of signal transduction pathways are available [for examples, see (*6–8*)], these are primarily for spatially uniform systems and do not incorporate partial differential equation models. In contrast, our applet allows for spatially inhomogeneous stimuli and responses. Moreover, we designed the display to provide the user with a two-dimensional image that closely parallels the response of real cells.

Equipment

Internet access

> Note: No special computer equipment is needed; the Java applet can be accessed using any Java-enabled Web browser from http://stke.sciencemag.org/cgi/content/full/sigtrans;2004/219/pl3/DC1 or http://www.ece.jhu.edu/~pi/LEGI/legi.html.

Java plug-in (http://java.sun.com)

Instructions

Using the labels from a screen shot of the applet (Fig. 1), we describe each of the user options. Choosing from the three possible buttons in this panel (Fig. 1A), the user can select one of three different types of chemotactic cues: a homogeneous source, or a directional source coming from either one or two micropipettes. When using the pipettes, the user can change the relative con-

centration of the chemoattractant coming from each of the pipettes with the slide bars provided (Blue conc or Red conc). The units associated with these concentrations are arbitrary.

When a homogeneous input is chosen, the "virtual" cell is stimulated uniformly by an abrupt step-increase in chemoattractant. The default start is from zero, but the user can generate multiple concentration steps by unselecting the "Reset cell between simulations" box in section A. In this case, the data display will be normalized to the new values.

When one pipette is chosen, the position of the pipette tip (Fig. 1B) relative to the cell is used to generate a gradient along the perimeter of the cell. The profile of this gradient along the cell's perimeter is computed using the formula for diffusion near an impermeable sphere [Eq. 1 in (9)]. For two pipettes, their respective contributions are computed and summed.

Fig. 1. Interactive Java applet. (**A**) Available input sources include homogeneous additions of chemoattractant, or gradients formed by either one or two pipettes. (**B**) The chemoattractant-filled micropipettes can be moved with the mouse. (**C**) Kinetic constants and the diffusion coefficient are adjustable. (**D**) The cell membrane is compartmentalized into 12 sections. (**E** and **F**) Each section can be "clicked" to display the time course of excitation, inhibition, or response. (**G**) Various display options are also provided. In this case, we have plotted time courses for two different positions on the membrane corresponding to the sections approximately at 5 and 9 on the face of a clock. The applet can be accessed at http://stke.sciencemag.org/cgi/content/full/sigtrans;2004/219/pl3/DC1 or www.ece.jhu.edu/~pi/LEGI/legi.html.

The panel in the top left corner provides the model of the virtual cell and provides the palette in which the pipettes can be moved. Cells are modeled as two-dimensional spheres; however, all reactions are assumed to occur on the membrane. The membrane is divided into 12 separate compartments. The chemoattractant concentration around the cell's perimeter can be viewed by pressing the "Signal Lvls" button, which will open a new window called "Signal Levels by Partition" and provide a graphical representation of the concentration of the chemoattractor(s) and the response regulator (labeled as R* in the graph) along the 12 membrane sections.

With the mouse, the user can click and drag the micropipette(s) to move it around the outside of the cell. Because the room on the screen is limited, clicking on the extracellular region opens a "zoomed-out" depiction of the cell and pipette(s) in a separate window in which the user can more conveniently move the pipettes. This allows analysis of shallower gradients as the pipette is moved farther away from the cell. The cell's response is only displayed on the main window.

The mathematical description of the LEGI is based on (5). Three differential equations are solved for each of the $n = 1$, …, 12 compartments (Fig. 2). Thus, the system is characterized by seven parameters. Six describe on-off rates for each of the three processes: E (k_e, k_{-e}), I (k_i, k_{-i}), and activity of RR (k_r, k_{-r}) (in the applet, these are labeled as Ke, K-e, Ki, K-i, Kr, and K-r). The remaining parameter is the diffusion coefficient for the inhibition process (D_i). The respective values can be changed using the scroll bars or by typing in numbers (Fig. 1C). (The allowable values for each of the parameters in Fig. 1C are 0.0 to 9.0.)

$$\frac{dE_n}{dt} = -k_{-e}E_n + k_e S_n$$

$$\frac{dI_n}{dt} = -k_{-i}I_n + k_i S_n + D_i \left(I_{n+1} + I_{n-1} - 2I_n \right)$$

$$\frac{dR_n}{dt} = -k_{-r}I_n R_n + k_r E_n$$

Fig. 2. Differential equations for the LEGI model.

The response can be observed directly on each of the compartments of the cell membrane (Fig. 1D) for E, I, or RR. The default selection is RR, but the user can select to show the virtual cell's changes in E or I by clicking the buttons next to the "Video Shows" (Fig. 1G). Moreover, clicking on any one of the compartments on the membrane of the virtual cell plots the response-time curves for that compartment in graphs shown in the lower left corner (Fig. 1, E and F). Curves for the activity of RR (Fig. 1E) and for E and I (Fig. 1F) are shown. Data from any one or more of the 12 compartments can be plotted simultane-
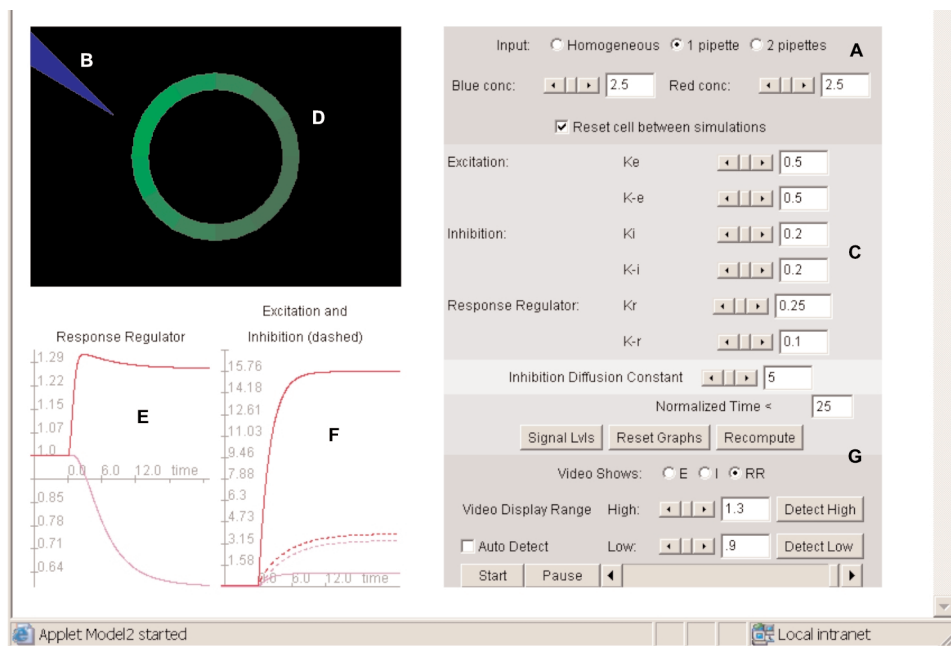
ously and are color coded. In the example shown, from the excitation curves, the local aspect of the response can be seen. The inhibition at the front and the inhibition at the back of the cell approach similar values. This manifests itself in a graded RR in that, at the front, the response increases from the basal level, whereas at the back, it decreases.

Finally, the display is adjustable (Fig. 1G). Three radio buttons allow the user to change the display on the virtual cell to show changes in the activity of E, I, or RR. The display range (the range of units that will be displayed in shades of green on the cell surface segments) can be changed using the parameters provided. The "Detect High" and "Detect Low" buttons automatically adjust the display range so that the high and low ranges match the highest and lowest activities of RR, respectively. The appropriate range can also be automatically selected by clicking the Auto Detect box, which selects the high and low ranges before playing the video. Finally, the user has the ability to replay the video using the Start and Pause/Resume buttons, the scroll bar, or the arrows provided.

To illustrate the use of the applet, we consider three possible "experiments." Although explicit instructions for achieving the virtual experiments are listed, users are encouraged to consider these as just a starting point and to try different values and combinations of the adjustable parameters.

Adaptation to Homogeneous Stimuli

The applet can be used to stimulate a cell responding to a homogeneous source. After the simulation, the video can be stepped through to observe the status of E, I, and RR at three time points as stimulation proceeded. The video output at three different times for each of the three responses is shown in Fig. 3. Before the stimulus (left column), all three responses [excitation (E), top row; inhibition (I), second row; response regulator (RR), bottom row] are "off." Upon stimulation, E is rapidly turned on, causing the cell to respond (middle column). Finally, as the slower I catches up, E and I are both high, but cancel each other, and the response returns to basal levels. The reader is invited to verify using the applet that in order to achieve the rapid response, only $k_{-e} > k_{-i}$ is required. Moreover, when this condition is violated, a transient decrease in response is elicited.
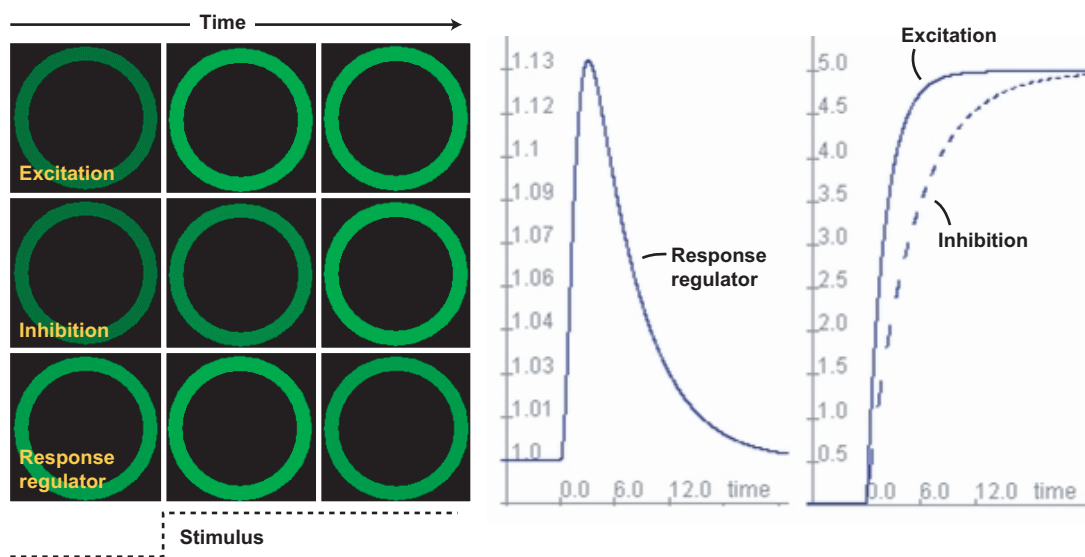

Fig. 3. Homogeneous stimuli. In the model, the balance between excitation and inhibition regulates the response. Images at the left: Before stimulation (left column), the excitation (top row) and inhibition (middle row) processes are at low levels, but their balance determines the low basal level in the response (bottom row). A stimulus-induced change in receptor occupancy (dotted line below images) triggers a fast increase in excitation, eliciting a fast response (middle column). As the slower inhibition catches up in steady state (right column), the balance between excitation and inhibition is restored, and the response element returns to basal values. Graphs on the right show quantitative changes in excitation, inhibition, and response as a function of time.

1. Choose Homogeneous for the input.

2. Set k_{-e} to a value greater than k_{-i}; for example, set k_{-e} to 0.5 and k_{-i} to 0.2.

   Note: If you set the values of k_{-e} smaller than those of k_{-i}, a window will pop up warning you that a transient decrease in RR will result.

3. Click anywhere on the membrane of the virtual cell to select a region to display on the graphs below.

4. Press the "Start" button to view the change in the virtual cell during the response.

5. To view the changes in E, I, or RR, click the appropriate radio buttons in Video Shows.

   Note: To take snapshots at different times, press the "Pause" button or use the slide at the bottom to select a specific point in time. The cell display can then be saved as a screen capture while the simulation is paused. This can be done for each value of E, I, and RR, using the Video Shows buttons.

Response to Varying Chemotactic Gradients

Using the one-pipette option, the user can verify that the steepness of the gradient determines the degree of localization of the response (Fig. 4A). In particular, creating a steeper gradient by moving the pipette closer to the cell leads to a more localized activation of RR. Additionally, the user can easily visualize the gradient-sensing mechanism of the LEGI model by clicking on the head and tail of the cell to display graphs of the processes in those areas. As expected, E is high at the head and low at the tail of the cell. However [whenever the inhibition diffusion constant (D_i) > 0], I is more uniform throughout the cell. The varying difference between the magnitude of E and I around the cell causes RR to be higher at the side of the cell facing the pipette.

Fig. 4. Spatial stimuli. The absolute value of the response—measured in terms of the brightness and width of the observed crescent—depends on the steepness of the chemoattractant gradient. Micropipettes that are farther away (**A**) elicit a smaller difference in receptor occupancy and therefore a weaker response than those that are close by (**B**). (**C**) Response to multiple stimuli. The LEGI model also predicts that the cell will create two localized response regions when stimulated by two micropipettes at opposite ends.

1. Choose "1 pipette" for the Input.

2. Set the value of "Blue conc" to any value between 0.1 and 4.9.

3. Click and drag the pipette far from the cell to observe the response to a shallow gradient.

4. Click on a portion of the cell membrane closest to the pipette and distal from the pipette to graph the changes in RR, E, and I.

5. Move the pipette close to the cell to observe the changes in activity in response to a steeper gradient.

6. Click a portion of the membrane close to the pipette and distal from the pipette to graph the changes in RR, E, and I in those parts of the cell.

7. Compare the responses to those obtained in step 4.

   Note: When it is desirable to compare results of different conditions, it may be useful to open the browser in multiple windows.

8. Change the value of the inhibition diffusion constant (D_i) to a value greater than 1.

   Note: When a parameter is changed that requires the simulation to be recomputed, the "Recompute" button will flash. Click the button to set the system to the new parameters. If you choose values that are inappropriate, a dialog box will appear requesting you to make a change.

9. Press the "Recompute" button.

10. Click a portion of the membrane close to the pipette and distal from the pipette to graph the changes in RR, E, and I in those parts of the cell.

11. Compare to the responses observed for step 4 and step 6 to see how a more uniform I affects the response.

Response to Multiple Sources

As can be readily verified using the two-pipette option, the LEGI model predicts that cells exposed to more than one source will respond to both and form two crescent-shaped responding regions of the membrane, one facing each of the two pipettes (Fig. 4B). The localization of a molecule is related to its spatial dispersion $(D_i/k_{-i})^{-1/2}$. This can be checked by observing how the degree of localized response diminishes as either D_i is increased or k_{-i} decreased. As the user can verify using the applet itself, as long as $D_i > 0$, directional sensing is achieved.

1. Choose "2 pipettes" for the Input.

2. Position the two pipettes on opposite sides of the virtual cell by clicking the mouse and dragging the pipettes.

3. To observe similar intensities in response at opposite sides of the cell, place the pipettes at an equal distance from the cell membrane and set the Blue Conc and Red Conc to equal values.

4. Click a portion of the membrane close to each of the pipettes to graph the changes in RR, E, and I in those parts of the cell.

5. Increase the value of the inhibition diffusion constant (D_i).

6. Press "Recompute" and notice that the RR activity of the cell becomes more broadly distributed than it was when D_i was set to the default value of 0.8.

7. Reset D_i to 0.8.

8. Set the value of k_{-i} to a value less than the default value of 0.2.

9. Press "Recompute" and notice that the RR activity of the cell becomes more broadly distributed than it was when k_{-i} was set to the default value of 0.2.

Troubleshooting

The numerical integration is done with fixed time-steps. This can lead to numerical errors if kinetic parameters are chosen that are larger than the ranges allowed by the slide bars. If these problems do occur, the applet should simply be reloaded. STKE will also host a Forum (http://stke.sciencemag.org/cgi/forum-display/stkeforum;12?FORUM_ID=stkeforum;12) to allow users to discuss their experiences and ask questions. The authors will monitor that online discussion and respond as needed for at least the next 3 months after publication (10 February 2004).

Notes and Remarks

The interactive applet presented here is meant to illustrate the LEGI mechanism and not the specific biochemical schemes that may be used by various chemotactic cells to implement it. It allows the user to visualize the different components of the model both temporally and spatially for varying parameter values, and to do so with minimal effort. After simulating the model for various inputs and combinations of parameters, it should be clear that the model's ability to detect spatial gradients is not based on specific parameter choices but on some broad general principles. Moreover, by changing some of these relations, the model can account for transient decreases in chemotactic response. Using this applet, we have been able to predict some cellular responses that have now been tested experimentally (*10*).

It should also be clear, however, that because of the simplicity of the model presented in this applet, the results cannot be used quantitatively to predict cellular behavior. For example, we have not incorporated receptor-ligand interactions, nor have we attempted to explain the large amplification seen in the spatial response of chemotactic cells. For the latter, we advocate a modular concept in which the LEGI model acts as a gradient-detection module feeding a separate amplification module (*11*). The applet is not meant as a substitute for advanced simulation software (*12–14*). However, for illustrating mathematical concepts, Java applets provide several advantages over other possible avenues (*15*). As an instructional tool, these applets provide the capability to engage the user in control of the simulation parameters—including such aspects as the location of the virtual micropipette. The ease of use of the applet and its ability to provide rapid feedback to the user make it an invaluable tool for understanding gradient detection.

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