Investigating Cell Differentiation in the Brain with a Computational Model of Delta-Notch Signaling and Dynamical System Analysis

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Abstract
This project investigates the role of Delta-Notch signaling in neural cell differentiation. This signaling mechanism allows for neighboring cells to interact to regulate their protein concentration, resulting in a diverse pattern of functionally different cells in the brain. The mechanism involves a ligand-receptor binding system with Delta as a transmembrane ligand and Notch as a receptor. Upon binding, the receptor is activated, releasing Notch intracellular domain (NICD) into the cell cytoplasm. NICD increases Notch transcription and inhibits Delta expression, leading to a regulation in Delta-Notch binding rate in neighboring cells. This lateral inhibition mechanism induces divergent cell fates, with cells expressing high Delta levels becoming neurons and those with high Notch levels becoming glial cells. A Python-based simulation model using differential equations and agent-based dynamical network is developed to visualize and analyze the cell differentiation process in the brain over time.

1 Introduction

During embryonic development, neural precursors in the brain differentiate into functionally diverse neurons and glial cells. This differentiation is made possible by a mechanism of signaling between neighboring embryonic stem cells in the brain, called Delta-Notch signaling. This signaling mechanism allows cells to receive input from their neighbors and regulate their protein concentration, similar to how leaky and integrating neurons compute their membrane potentials. The change in protein concentration within a cell subsequently affects the protein level of neighboring cells. As a result, a lateral inhibition mechanism acts to differentiate the concentration of Delta ligands and Notch receptors within the system, leading to a diverse pattern of functionally different cells in the brain. In this project, I aim to construct a network of embryonic stem cells in the brain and model the Delta-Notch signaling system to investigate its role in cell differentiation.

Delta-Notch signaling utilizes a ligand-receptor binding mechanism to induce divergent cell fates among neighboring cells. Delta is a transmembrane ligand while Notch is a transmembrane receptor. Upon binding of a neighboring Delta ligand to a cell's Notch receptor, the receptor is activated, which results in the release of the Notch intracellular domain (NICD) into the cell cytoplasm. NICD acts to upregulate the transcription of Notch receptors while downregulating the transcription of Delta ligands within the cell. Consequently, this inhibition of Delta ligand expression leads to the reduction of Delta-Notch binding and NICD release in neighboring cells. In summary, when a cell's Notch copy number increases, the Notch transcription rates of adjacent cells are inhibited, while the Delta transcription is encouraged. Eventually, the cells expressing high Notch level become glial cells, while those with high Delta level become neurons. Figure 1 summarizes this modula-

tory mechanism.

In this project, I developed a Python-based dynamical network of cell differentiation with the Delta-Notch signaling model. To achieve this, I generated an agent-based network model that imitates the grid of cells. The model incorporates a system that uses differential equations and pre-established functions to compute the copy number of Delta and Notch in each cell, considering factors such as protein production rate, ligand-receptor binding rate, and degradation rate. The simulation continually updates the protein level of each cell agent by utilizing information from neighboring cells as well as cell-intrinsic mechanisms. Using this model, I was able to visualize the process of cell differentiation in the brain and analyze its rate over time.

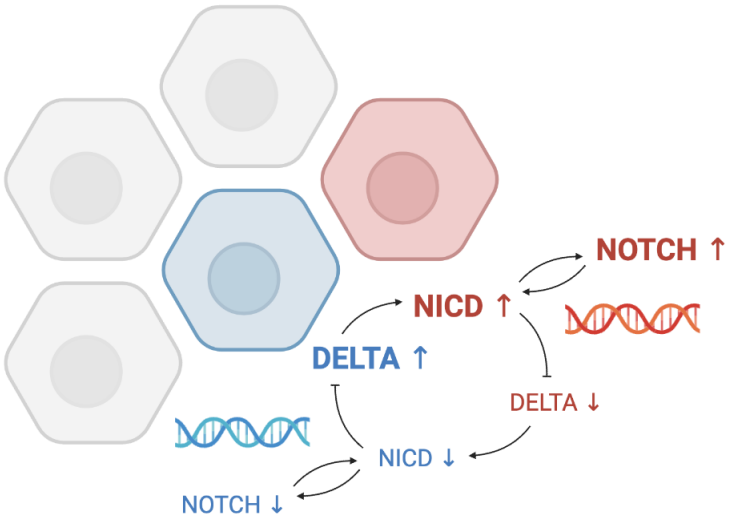

Figure 1: Summary of Delta-Notch Signaling Mechanism

2 Methods

2.1 Mathematical Model

This project utilizes a mathematical model of Delta-Notch signaling, which involves several functions to determine the protein production rate, ligand-receptor binding rate, and protein degradation rate. Each cell agent has its own Delta, Notch, and NICD levels that are computed every time step through differential equations and the aforementioned functions. The Delta, Notch, and NICD levels of adjacent cells significantly impact a cell's rate of protein concentration change through various mechanisms.

2.2 Protein Production Rate

To begin with, the Hill function is employed to compute the production rate of Delta ligands and Notch receptors as a function of NICD copy number (Bocci, 2020).

$$N_p = N_0 \cdot \frac{1 + \lambda_N (\frac{NICD}{I_0})^n}{1 + (\frac{NICD}{I_0})^n}$$

$$D_p = D_0 \cdot \frac{1 + \lambda_D (\frac{NICD}{I_0})^n}{1 + (\frac{NICD}{I_0})^n}$$

In these equations, N_p and D_p represent the production rate of Notch and Delta. The

basal transcription rates are referred to as N_0 and D_0, and these values have been set to 500 and 1000 in the simulation to correspond with previous research (Boareto, 2015). λ_N and λ_D are fold-change, while n is the Hill coefficient ($\lambda_N = 2$, $\lambda_D = 0$, $n = 2$). The fold-change parameters incorporate the fact that NICD production upregulates Notch transcription and downregulates Delta transcription. Additionally, the threshold of the NICD concentration is represented by I_0, which is set to 200 to reflect the range of actual NICD numbers found in the nucleus, which can vary up to a few hundred ng/ml (Boareto, 2015).

Calculating the production rate of NICD does not involve the Hill function. Instead, it is modulated by the concentration of Delta ligands in neighboring cells and Notch receptors within the cell.

$$I_p = k_T N_1 D_2$$

The trans-activation rate, represented by k_T, determines the rate of ligand-receptor binding events between neighboring cells. By multiplying the trans-activation rate constant with the concentration of Notch receptors within the cell (N_1) and the concentration of Delta ligands in neighboring cells (D_2), we can determine the degree to which NICD gets released into the cytoplasm.

2.3 Protein Degradation Rate

Furthermore, the degradation rate resulting from the ligand-receptor binding mechanism was modeled using a chemical reaction term (Bocci, 2020). To determine the number of degraded Notch receptors resulting from binding with Delta ligands, the concentration of Delta ligands in neighboring cells as well as within the same cell were taken into account.

$$N_b = N_1 \cdot (k_C D_1 + k_T D_2)$$

As indicated in this equation, the ultimate degradation level of Notch receptors (N_b) was determined by adding the contributions from the two distinct ligand-receptor binding mechanisms (Boareto, 2015). Firstly, the concentration of Notch receptors lost through binding within the same cell was calculated. This process is called cis-inhibition, with k_C as the rate constant. The cis-inhibition degradation rate of Notch receptors is computed by multiplying the concentration of Notch receptors within the cell (N_1) with the concentration of Delta receptors (D_1), along with the cis-inhibition rate constant (k_C). Then, the concentration of Notch receptors lost through binding with neighboring cells was calculated. This process is called trans-activation, with k_T as the rate constant. The trans-activation degradation rate of Notch receptors is computed by multiplying the concentration of Notch receptors within the cell (N_1) with the concentration of Delta receptors in the neighboring cell (D_2), along with the trans-activation rate constant (k_T). The values of both rate constants were determined during the simulation tuning process to ensure that the protein levels reached equilibrium after multiple time steps. The cis-inhibition rate was set to be 10 times higher than the trans-activation rate based on previous research findings (Bray, 2006). Furthermore, the calculation was done vice versa for the ligand-receptor binding rate of Delta ligands.

$$D_b = D_1 \cdot (k_C N_1 + k_T N_2)$$

When calculating the trans-activation binding rate in the simulation, I linearly superpose the protein concentration levels for all neighboring cells. The model was designed to consider the contact area of neighboring cells so that cells with more contact area have a greater influence on the calculation of the binding rate for its neighbor (Shaya, 2017).

To account for protein degradation beyond the ligand-receptor binding mechanisms, a linear model is utilized with a single parameter of inverse half-life. This model calculates the gradual reduction of protein levels in each cell

due to molecule degradation and dilution.

$$N_d = \gamma N_1$$
$$D_d = \gamma D_1$$
$$I_d = \gamma_I I_1$$

The amount of degradation for Notch, Delta, and NICD is represented by N_d, D_d, and I_d, respectively. The inverse half-life of proteins (γ) is typically set to 0.1, with the exception of NICD (γ_I) which has a five times higher degradation rate due to its crucial role in the signaling mechanism that requires rapid degradation (Manderfield, 2012).

2.4 Equations

In summary, the differential equations used to compute each cell's protein level can be expressed as follows. It should be noted that H^S is the abbreviation of the Hill function.

$$\frac{dN_1}{dt} = N_0 \cdot H^S(I_1) - N_1 \cdot (k_C D_1 + k_T D_2) - \gamma N_1$$

$$\frac{dD_1}{dt} = D_0 \cdot H^S(I_1) - D_1 \cdot (k_C N_1 + k_T N_2) - \gamma D_1$$

$$\frac{dI_1}{dt} = k_T N_1 D_2 - \gamma_I I_1$$

2.5 Cell Grid Generation

This project investigates the dynamics of intracellular signaling using an agent-based cell network model (Reynolds, 2019). In this model, a cell is defined as a set of contiguous grid points sharing the same cell identity number (Bocci, 2020). Employing object-oriented programming in Python, I assigned attributes to cells indicating their protein levels (i.e. Delta, Notch, NICD), neighboring cells, and the grid points that comprise them.

To generate a randomized cell grid, I developed an algorithm inspired by the random walk process. The algorithm starts by randomly selecting a set of initial points within the grid, while ensuring that they are not too close to each other. Each initial point is assigned a unique cell ID. The initial grid points then randomly select a neighboring point to expand to. If the chosen neighbor does not have a cell ID yet, the original grid point 'conquers' it and assigns its cell ID to the neighbor. This process continues with either the initial grid point or the conquered points expanding in the next round. The expansion continues until all grid points are assigned a cell ID. Figure 2 summarizes this cell grid generation process. The color of the grid points in the figure represents unique cell IDs.

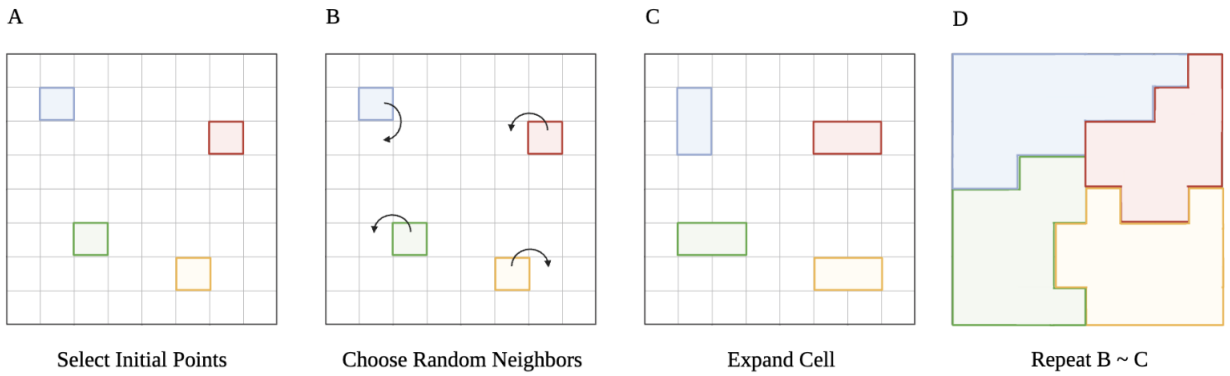

Figure 2: Cell Grid Generation Algorithm

3 Results

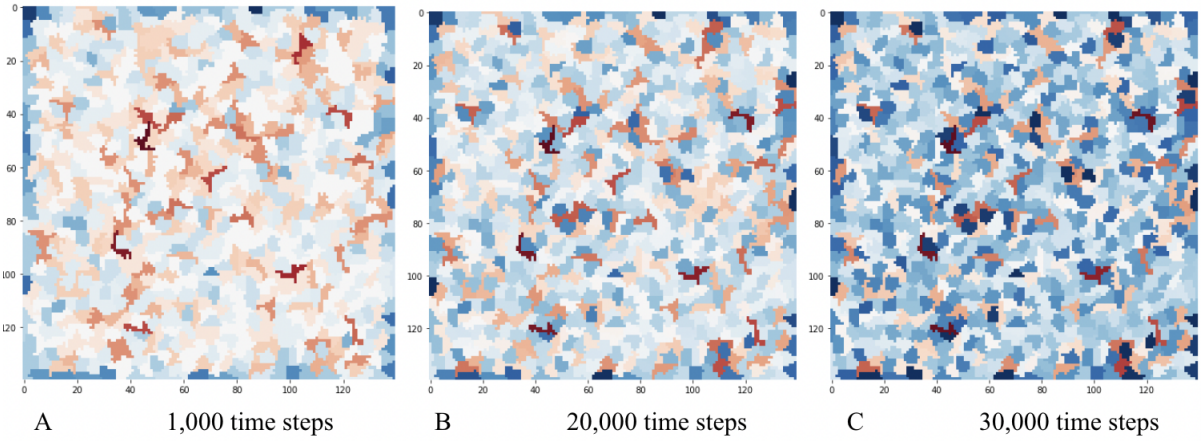

Figure 3: Visualization of the Cell Differentiation Process

3.1 Cell Differentiation Process

To ensure that the simulation was stable and the protein levels were not fluctuating significantly from their initial concentrations, I ran 50 time steps while monitoring Delta, Notch, and NICD levels prior to commencing the simulation. During this tuning phase, the binding rate constants were adjusted, while adhering to a 10 to 1 ratio between cis-inhibition and trans-activation, as suggested by previous research (Bray, 2006). I also fine-tuned the extent of change in each time step. Once equilibrium was achieved, the simulation was then run for 30,000 time steps. At each time step, the aforementioned differential equations were used to calculate the protein concentration levels of each cell agent, while accounting for the signaling interactions with neighboring cells.

Figure 3 above shows the results of the dynamical network simulation of the cell differentiation process. The subplots illustrate the Notch level of every cell after 1,000, 20,000, and 30,000 time steps. The color red denotes high Notch levels, which correspond to low Delta levels, while the color blue denotes low Notch levels, which correspond to high Delta levels. The visualization clearly demonstrates that as the cells interact with each other over time, the differentiation of Delta and Notch concentration levels becomes more pronounced.

A notable observation from the cell grid visualization figure is that the cells situated on the outermost periphery of the network are more prone to expressing high Delta levels. This phenomenon may be linked to the fact that these cells have fewer neighbors, resulting in a reduced probability of Notch receptors binding with Delta ligands. Consequently, there is a decreased release of NICD, which typically downregulates Delta production. Thus, cells on the outer border area, where there are fewer neighbors, tend to exhibit high Delta levels.

Correspondingly, cells with a high number of contacting cells tend to exhibit high concentrations of Notch even in the early stages of the simulation. After only 1,000 time steps, these cells exhibit a deep red color, indicating high Notch levels (Figure 3A). Interestingly, these cells tend to have a slender and elongated shape, giving them a greater contact area relative to their actual size. This increased surface area likely promotes more frequent trans-activation binding events with Delta ligands from neighboring cells, leading

to a higher Notch concentration.

It is evident that after 30,000 time steps, the cells have taken on distinct cell fates (Figure 3C). The cells are displaying either a vivid red or blue color, indicating a clear divergence from neighboring cells and a more bipolar concentration in terms of Notch and Delta levels. It is worth noting that prior mathematical modeling research on Delta-Notch signaling found a ratio of 3 to 1 between high-expressing Notch cells and high-expressing Delta cells. However, the present model displays the opposite result, with a high-expressing Notch cell typically surrounded by high-expressing Delta cells. Possible limitations of the model that could have contributed to this result are discussed later in the "Discussion" section.

3.2 Protein Level Distribution

Figure 4 shows density plots of protein level distribution across different time steps. The three plots represent the distribution of Notch, Delta, and the difference between Notch and Delta (Notch - Delta) levels across all cells at a specific time point. The color in the figure legend represents the corresponding time step. The dispersion of protein levels is seen to increase with time in all three plots. The plot depicting the difference in level between Notch and Delta concentrations took the longest time to disperse, and thus displays the analysis results at time step 11,000, 14,000, and 17,000, unlike the other two (Figure 4C).

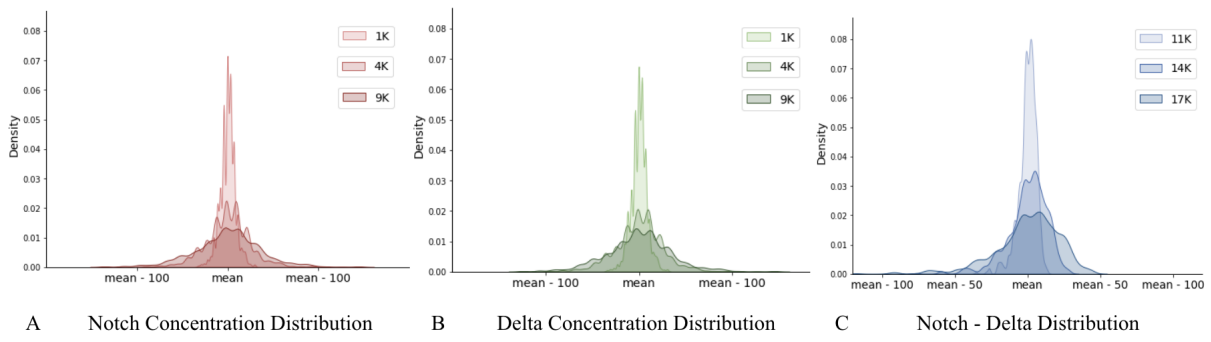

Figure 4: Density Plot of Protein Level Distribution

4 Discussion

Delta-Notch signaling is a crucial mechanism that differentiates cells during neural development. Varying protein levels induced through lateral inhibition results in distinct fates for neurons and glial cells. To explore the role of Delta-Notch signaling in cell differentiation, we developed a simulation model that uses an algorithm to generate a randomized cell grid. The signaling system is then implemented using differential equations of protein concentration change rate. The simulation model was utilized to visualize the cell differentiation process in the brain by displaying Notch concentration levels in all cells within the network. This observation of divergent cell fate is consistent with previous experimental findings.

As the model is a simplification of the actual biological system, there are several limitations to our study. First, the model did not capture the intricate details of actual embryonic cells. Cell-intrinsic properties other than the Delta, Notch, and NICD protein levels were not considered. Furthermore, the model assumed that all cells in the system are identical, which did not account for the effects of cell heterogeneity on the differentiation process. Additionally, other

important actors related to the Delta-Notch pathway were not factored in. For instance, some organisms have a ligand called Jagged that binds to the Notch receptors to affect cell differentiation. The Fringe effect, which is a regulatory mechanism of the Notch signaling pathway, was also not acknowledged in this model.

In addition, the model utilizes a two-dimensional grid of cells, where each cell is represented as a randomized contiguous region in the grid. The actual biological system is in a much more complex form and exists in three-dimensional space. Exploring how the three-dimensional cell-to-cell contact area influences the differentiation pattern could be an exciting area for further research. Future studies could develop more realistic models of the cell grid that account for the complex spatial organization of neural precursor cells. To accomplish this, more sophisticated models of the cell grid, such as off-lattice models, that account for the intricate spatial structure of neural precursor cells could be utilized (Bocci, 2020).

In conclusion, this study has provided valuable insights into the role of Delta-Notch signaling in neural cell differentiation. The development of a dynamic network model has enabled the demonstration of the interactions of the cell agents and tracking of the changes in the network over time. However, there is still much to learn, and future studies can build on this work to achieve a more in-depth comprehension of the complex mechanisms that regulate cell differentiation in the brain.

Acknowledgments

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