Graphical Simulation of Early Development of the Cerebral Cortex

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Abstract

Much experimental data exists concerning the development of the cerebral cortex. There is a need for a common vehicle to integrate this data, and to allow the testing of hypotheses concerning development. Computer simulation and visualization are powerful mechanisms for hypothesis testing. Our long-term goal is to create a robust, extensible, portable tool for simulation and visualization of cortical development to serve both research and educational purposes.

This paper describes a simulation program, SimCortex, which models the early stages of development of the cerebral cortex of the mouse. Version 1.0 of SimCortex models the proliferation of progenitor cells in the pseudostratified ventricular epithelium (PVE), the generation of young neurons, and their migration into the cortical plate, which is the forerunner of cell layers II through VI of the adult cortex. We present an overview of the design and implementation of SimCortex, sample output of the system, and a comparison of our results with experimental data. We conclude with a brief overview of proposed future enhancements of the system.

Keywords: development, cerebral cortex, simulation, visualization, particle systems

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1 Introduction

The cerebral cortex carries out higher level processing in the brain necessary for such functions as sensory perception, motor output, and conscious thought. The cortex is composed of six cell layers, each containing different types of neurons, with morphology, connectivity, and function specific to each type (for review, see [1]). All of these specialized cells with their complex connections arise initially from an epithelial sheet of morphologically homogeneous progenitor cells. How does this highly organized tissue develop?

The development of the cerebral cortex is a very complex process, involving proliferation of cells, determination of numerous neuronal and glial cell types, migration of cells to appropriate locations in the mature cortex, and formation of correct synaptic connections between neurons (for review, see [2]). By necessity, these processes are usually studied in isolation from each other. Generally, genes or factors are manipulated one at a time in a given system, and their effects are examined individually. In order to generate a comprehensive model of cortical development, it would be useful to have a way to combine data from multiple types of studies, and to test hypotheses concerning the effects of multiple factors at once upon the system.

Computer simulation and visualization are powerful tools for studying complex systems. Simulations can be designed to gather information from many studies into a database for ease of comparison and hypothesis generation [3]. Simulation of cell growth and motion has proven useful in a number of systems. Silveira and Massad [4] developed a simulation of morphological evolution based on a number of parameters and rules for updating the model at each cycle. Similarly, Vawer and Rashbass developed a Cell Description Language for defining rules for cell behavior [3]. Rules for cell division, migration, and death are specified by users, and the system generates an MPEG animation based on this specification. Cell-based simulation has also been used to study cell patterns in the growth of different types of tomato roots [5]. Simulation of nervous system development in particular has proven useful in the past to study the many factors involved in the formation of topographic maps [6, 7] and ocular dominance columns [8]. In both of these cases, the simulations were important in the interpretation of existing experimental data, as well as in the generation of new hypotheses that could then be tested both experimentally and by simulation.

The goal of this project is to create a robust, extensible, portable tool for simulation and visualization of cortical development for use in both research and education. We envision a model that will eventually contain data on known gene expression patterns and cell behavior in response to various factors, and will allow the user to manipulate parameters to test hypotheses of interest (e.g., proposed effects of particular genes; the amount of cell death occurring during development). The initial version of the simulation presented here models simplified cell behavior during early development of the cerebral cortex in the mouse.

2 Background

The cerebral cortex arises from a single layer of proliferating cells, the PVE, which surrounds the fluid-filled ventricles of the brain. As development progresses, the progenitor cells of the PVE give rise to post-mitotic neurons that migrate radially away from the ventricles along radial glial cell processes to form the cortical plate (CP), which eventually becomes cell layers II through VI of the mature cerebral cortex. A neuron's final mitosis ('birthday') is highly correlated with its final location in the cortex; neurons whose birthdays occur late in development migrate past those with earlier birthdays, producing the so-called 'inside-out' pattern of development of the tissue [9, 10]. Thus, layer VI, the deepest layer of the mature cortex, contains the oldest neurons derived from the CP; while layer II, the most superficial layer deriving from the CP, contains the youngest neurons. The most superficial layer of the cortex, layer I, is generated earlier, before the CP arises [11]. Eventually, the PVE produces no more proliferative cells, and thus disappears at the end of the time of neuron generation. In the mouse, this neurogenetic period extends from embryonic days 11 to 17 (E11 to E17).

Several different kinds of data have been reported that bear on this process. Using ^3H-Thymidine or Bromodeoxyuridine to label the DNA of populations of dividing cells, Caviness and colleagues have determined birthdays of neurons of each layer of the cortex [10], as well as cell cycle parameters [12], and the percentage of progenitor divisions giving rise to post-mitotic cells (percentage Q, for quiescent) [13], throughout the neurogenetic period. Chenn and McConnell have studied individual cell divisions in cortical slices *in vitro*, and reported changes during the neurogenetic period in the percentages of progenitors that divide along an axis perpendicular to the PVE (vertical divisions) or parallel to the PVE (horizontal divisions) [14]. These investigators hypothesize that horizontal divisions give rise to one proliferative cell and one post- mitotic neuron, while vertical divisions give rise to two proliferative cells or two post-mitotic neurons. Finally, several laboratories have used retroviral vectors to label all the progeny of single progenitor cells in order to study both cell fate determination and cell division and migration patterns (for a review, see [15]).

Our simulation brings these different types of data together. It models the generation of the cortex using the population properties defined by Caviness and colleagues (cell cycle parameters, Q values, neuron birthdays). The model assumes that daughters of an individual mitosis become post-mitotic or remain proliferative independently of each other (which may not be true; see discussion). Given this assumption, and assuming Chenn and McConnell's hypothesis is correct, the simulation calculates the percentage of horizontal and vertical divisions as the neurogenetic interval progresses. Finally, the model keeps track of all the progeny of each original E11 progenitor (founder cell), and allows the highlighting of these clones as the simulation proceeds.

The results of our simulations show that this initial model generates numbers of neurons per founder cell in reasonable agreement with experimental studies [13], and that cell layers are appropriately populated with neurons. In addition, the numbers of vertical and horizontal divisions generated by the simulation are in approximate agreement with experimental data [14]. Finally, clones of cells generated from founder cells are distributed throughout layers II-VI of the cortex, as expected [15].

3 Methods

The basic structure of the simulation combines concepts from artificial life [16] and particle systems [17, 18]. In these systems, a separate data structure is generated for each particle, and this particle exhibits birth, migration, evolution, reproduction, and death based on probabilistic or deterministic rules and processes. In our case, each cell is assigned a particular behavior, which determines its migration and division characteristics. For each phase (G1, S, G2, and M) of a cell's cycle, there is a mean and deviation which depends on the cell's creation time. Table 1 gives the values for each simulation day currently being used in SimCortex. The table includes mean cell cycle times from the literature [12]. G2 and M times are combined for technical reasons relating to the methodology used to calculate cell cycle parameters. In order to include some variability in the cell cycle length, the simulation currently assumes standard deviations for the G1 phase of about 10% of the mean. When a mitotic cell is born, it is assigned a cell cycle length based on a G1 length that is chosen randomly in the interval $G1_{mean}(T) \pm 2G1_{dev}(T)$, where T is the cell's birthdate. Future releases of SimCortex will provide users the capability to easily modify the cycle and phase behavior of cells.

An initial set of user-specified founder progenitor cells are scheduled to divide based on the phase times for the first day of the simulation (E11). At the end of the G2 phase, the cell enters the mitotic (M) phase, and the cell divides. A division can result in either two progenitor cells, one progenitor and one post-mitotic neuron, or two post-mitotic neurons. The probability of a cell being post-mitotic (Q) is currently implemented as a monotonically increasing function which reaches 100 percent at a specified date. We use a set of known Q values at the end of each simulation day (from [13]), and interpolate between these values to compute Q at an arbitrary time. The specific probabilities for days E11-E17 are (0.0, 0.11, 0.19, 0.36, 0.67, 0.79, and 1.0). This function will be user-customizable in future releases. Each progenitor cell then is assigned phase times based on interpolating values from Table 1.

The simulation models what would occur in a section of cortex that is essentially 2-dimensional, with a width of about 25 cell diameters. New progenitor cells migrate laterally within the PVE until unoccupied space is located (in reality, this process should include tissue growth). Post-mitotic neurons, colored according to the cortical layer to which they are assigned, migrate out of the PVE. Layer assignment is currently performed in a rather simplistic manner, comparing the Q value at the current time with a set of four non-overlapping ranges. At present, cells born when Q is between 0 and .35 are assigned to layer VI, between .35 and .46 to layer V, between .46 and .75 to layer IV and from .75 to 1.0 to layer III-II. These thresholds were set by examining figures in [13]. Experimental data shows that the inside-out pattern of neurogenesis is not strictly adhered to during development; there is overlap in the final layer destinations of populations of cells born on different days [10, 19]. Thus, the stepwise method of assigning cells to layers used in the simulation is oversimplified. In future releases of SimCortex we will examine alternate theories regarding layer formation.

Outward-moving cells can collide with existing (older) cells, which causes some lateral movement of the newer cell (in reality, collisions may be infrequent, with lateral motion being caused by other forces). This outward motion ceases when a cell reaches an unobstructed position. The result of this behavior is layers with a fair amount of bumpiness. We are investigating alternate migration strategies, including the modeling of radial glial cells for transporting and depositing neurons.

To start the simulation, the user indicates the initial number of founder cells, their diameter (for drawing and collision detection), and the step size (in minutes) of the simulation. Once the *Go* button is clicked, the cells begin to divide and migrate. During the simulation, a continuous readout is provided, showing the current date/time, cell counts, and Q value (probability of a division resulting in a post-mitotic cell). At any time, the user can click on a cell to get its birthdate. Clicking on a founder cell, either in the PVE or after it has migrated out of the PVE, highlights all of its progeny. The founder cells are drawn with a white ring around them to ease their selection by the user. The simulation halts when the PVE is empty.

In addition to the main visualization showing cell proliferation and movement over time, SimCortex provides the following supplemental graphs, which can be displayed at any time by pausing the simulation (hitting the *Stop* button) and selecting the desired plot from a menu:

- total cells over time
- number and percentage of proliferative cells over time
- post-mitotic cells over time
- layer populations
- percent horizontal/vertical divisions over time
- number of horizontal/vertical divisions per simulation day

Some examples of these plots are shown in the next section.

SimCortex was designed to be highly portable and easily extended. The source code, documentation, and a working version is freely available via the Web at http://davis.wpi.edu/˜matt/projects/SimCortex/. The program is implemented in Java 1.1.2 to facilitate portability, and should compile readily with newer releases of Java. It runs on any computer (PC, workstation, mainframe) and operating system (Windows, UNIX, MacOS) which has Java installed. It executes under most popular web browsers, including Netscape and Internet Explorer. The program consists of approximately 1000 lines of code. Separate modules are provided for maintaining information regarding each cell, controlling the simulation parameters (time between divisions, probability of a cell being proliferative), creating and displaying plots and simulation output, and controlling cell migration, layer assignment, and collision-avoidance.

4 Results

We have produced a simulation program, SimCortex, that models development of the cerebral cortex in mouse from E11 to E17, the neurogenetic period. Here we show images of a typical section of cortex produced by the simulation, and compare simulation results with experimental data. The images shown were produced from 5 founder cells; the final section of cortex comprised about 1000 cells.

Figures 1 and 2 show synthesized sections of cortex at days E13 and E17. The cells are colored according to the layers they will populate in the mature cortex; these layers would not be morphologically distinguishable in the cortical plate at this time in development. The boundaries between layers are not smooth due to the simplicity of our migration model and the lack of consideration of boundary effects. These issues will be addressed in future versions of the software. Figure 3 shows a completed simulation with a clone of cells arising from one founder cell highlighted in white. Figures 4 through 6 show a number of the plots available to users during and after the simulation.

The number of cells generated over time shows a smooth growth curve, as would be expected (Fig. 4). According to Takahashi and colleagues, each founder cell in the simulation should generate approximately 140 progeny, given the values of Q they calculate [13]. In our simulations, the number of progeny per original progenitor ranged from 150 to 250. Takahashi and colleagues calculate that small changes in the exact curve used to describe how Q changes over the neurogenetic interval can result in large differences in the number of progeny per progenitor [20]; we have also found this to be true using our simulation. Thus, our numbers are in reasonable agreement. The proportions of cells in each layer are also in general agreement with Caviness et al (Fig. 5; [21]). In particular, the general trend that the largest number of cells migrate to layer IV, with fewer cells in both deeper and more superficial layers, is preserved by the simulation.

Clones of cells arising from a single founder cell are distributed among all the layers (Fig. 3), as would be expected at this early developmental time point [15]. Because the current simulation only allows examination of clones arising from founder cells, it is not yet possible to assess whether clones generated from progenitors at later times in the simulation correspond to the patterns produced experimentally by labeling progenitors with retroviral vectors at later times in the neurogenetic period. In addition, a number of aspects of cortical development are not yet implemented in the model, including glial cell generation, cell death, non-radial migration, and changes in progenitor potential; all of these factors will have a profound effect on cell types, numbers, and distribution in clones.

In the simulation, progenitor cells give rise to two daughter cells that have either the same fate (two progenitors or two post-mitotic neurons) or different fates (one progenitor and one post-mitotic neuron). Consistent with Chenn and McConnell's hypothesis [14], we have termed these vertical and horizontal divisions, respectively. The percentages of vertical and horizontal divisions generated by the simulation were roughly consistent with those imaged experimentally in cortical slices *in vitro* (Fig. 6; [14]). Since the data was from experiments carried out in the ferret, and was not available for the second half of the neurogenetic interval, it is not yet possible to test this hypothesis more rigorously with our simulation.

5 Discussion

We have generated a computational simulation and visualization of early development of the mouse cerebral cortex. In this initial version of the simulation, we have emphasized providing a web-based model that is readily available to users, and that is easily modifiable to permit users to customize it to their needs. The simulation generates a recognizable section of cortex, with a number of features that are in general agreement with the experimental literature; however, it is clearly over-simplified in many respects. Our hope is that by making a simple version of the model available to the research community, we will receive input on desirable features for future versions.

This program has several similarities to the *Biological Toolbox* [3]. In particular, we are modeling individual cell behavior (division, migration, categorization), generating animations of the simulations, and providing web access. Our model, however, is specific to the developing cortex, which allows it to incorporate specialized properties of this tissue (e.g., migration patterns of cells; changes in behavior of cells by birthdate). Rather than being rule-based, we use several time-varying functions and random number generation to control the simulation. Also, as SimCortex is implemented in Java, the simulation runs on the user's system rather than on a remote host. While the Biological Toolbox generates a single MPEG animation based on the specification of parameters and rules, SimCortex permits user interaction during the simulation, allowing users to retrieve information about individual cells and plot a variety of information extracted from the simulation.

We generated data on numbers of vertical and horizontal divisions assuming that each daughter cell from a division would become post-mitotic independently, with probability Q. While this was a convenient first approximation for purposes of the simulation, the biological situation is more complex. In a horizontal division, for example, it appears that the asymmetric distribution of cellular components may cause one daughter cell to become a post-mitotic neuron, while the other continues to proliferate; thus, the fates of the daughter cells would not be independent of one another [14]. Takahashi and colleagues suggest the intriguing possibility that whether daughter cells make their "Q vs P choice" independently or not may depend on the type of neurons that are produced by the division [22, 23]. Daughter cells producing interneurons, whose properties do not vary dramatically across layers, may make their choice independently, while daughters producing projection neurons, whose properties vary in different layers, may always choose the same fate. Post-mitotic neurons have been observed leaving the PVE at two different rates [22]; Takahashi and colleagues suggest these two populations may correspond to the two different classes of neurons. Further refinements to the simulation should allow the testing of this type of model.

In future versions of SimCortex, we plan to model migration as motion along radial glial cell guides, rather than simply as outward trajectories. This will alleviate the need to perform collision avoidance, which should dramatically increase the speed of the simulation. We also plan to model non-radial migration, which has been observed experimentally in a minority of migrating cells [24]. We will investigate ways to make our assignment of cells to layers more realistic; for example, we will incorporate a probabilistic assignment of cells to layers rather than discrete steps. In addition, we will include the production of glial cells and the neurons of Layer 1, and we will allow cell death to occur. Edge effects resulting in a somewhat 'bumpy' cortex will be addressed. It is known that cell-cell interactions (e.g., [25]) as well as changes intrinsic to cells (e.g., [26, 27]) are important in the generation of the cortex; eventually, we would also like to include such effects in the simulation.

Much of the data needed for making these improvements is incomplete or controversial; for example, the amount of cell death that occurs during the formation of the cortex is not clear (for review, see [28]). For this reason, future versions of the software will allow more user control of the simulation. For example, the amount and timing of cell death will be under the user's control, as will specification of the variation of Q with time. This feature will allow users to test their own hypotheses about cortical development; for instance, what amounts and distribution of cell death would generate clones that are consistent with experimental data? Would the rate of death need to vary to generate reasonable distributions of cells to the various cortical layers? Clearly, a chalkboard will not suffice to provide answers to these questions.

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Day	G1 Phase		S Phase		G2+M Phases	
	mean	2*dev.	mean	2*dev.	mean	2*dev.
E11	3.2	0.6	2.8	0.0	2.0	0.0
E12	3.3	0.7	4.9	0.0	2.0	0.0
E13	5.5	1.1	3.9	0.0	2.0	0.0
E14	9.3	1.9	3.8	0.0	2.0	0.0
E15	11.8	2.4	3.7	0.0	2.0	0.0
E16	12.4	2.5	4.0	0.0	2.0	0.0
E17	13.0	2.6	4.3	0.0	2.0	0.0

Table 1: Cell phase times (mean and 2* standard deviation) for different days.

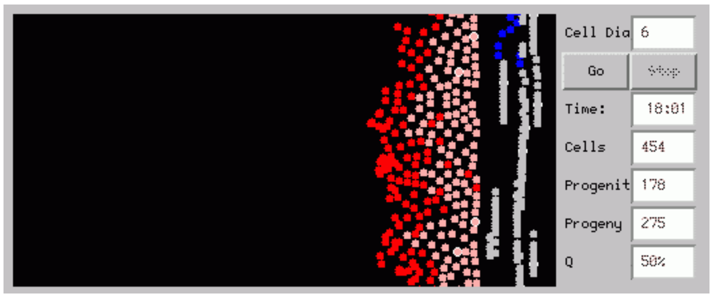

Figure 1: Simulation in progress. Progenitor cells in the PVE are on right. Cells that will constitute layers VI and V in the mature cortex have already migrated into the cortical plate. Cells that will constitute layer IV are just beginning to migrate.

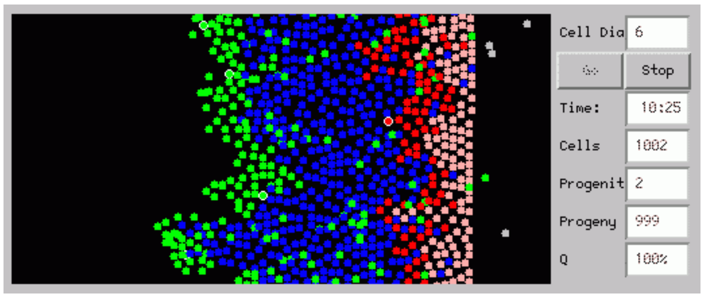

Figure 2: Simulation in final day. Note some cells percolating through earlier born neurons to reach their more superficial destinations.

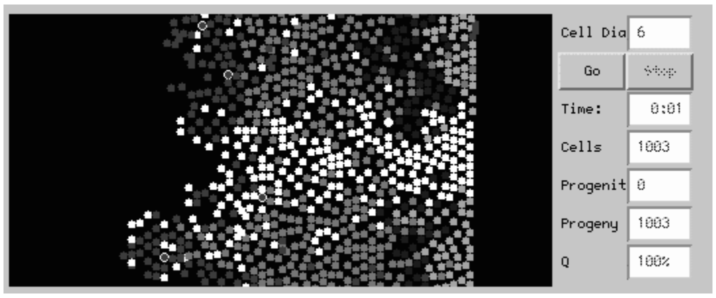

Figure 3: Clone (highlighted) arising from one of the founder cells. Layer colors have been adjusted to emphasize clones.

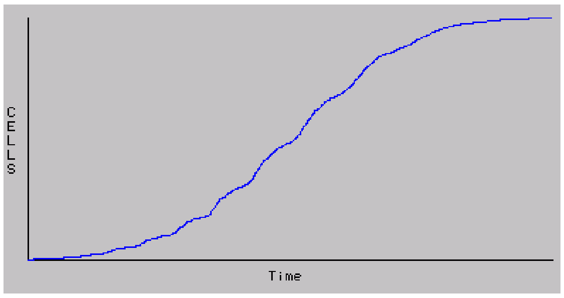

Figure 4: Plot of number of cells over time. Bumpiness is due to the small number of founder cells used (5).

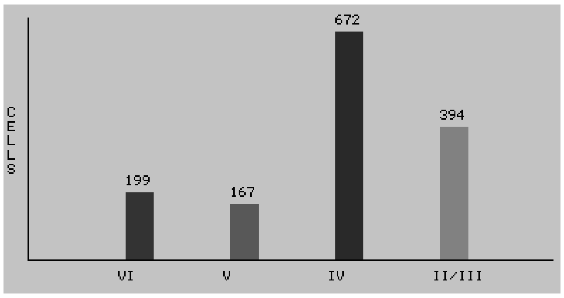

Figure 5: Bar graph of layer populations at end of simulation.

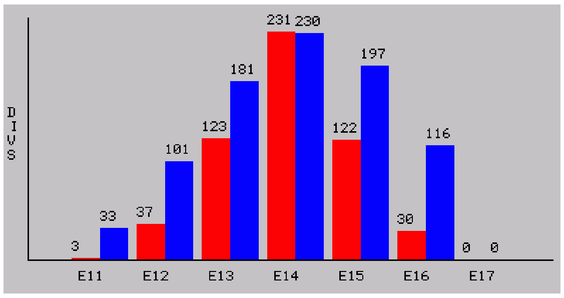

Figure 6: Graph of Horizontal and vertical divisions by simulation day.