NEUROGENESIS: FROM EXPERIMENTAL DATA TO SYSTEM DYNAMICS MODEL

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Abstract

Formation of the cerebral cortex is one of the most complex processes of the mammal development. It has been postulated that time of origin of particular cortical neurons along with their position within proliferative population determine majority of their properties within mature brain. Also, during early postnatal life, substantial reduction of neuron number occurs as the consequence of programmed cell death that further modifies structure and function of maturing brain. In this paper we present the first attempt, at least according to the thorough search through available literature, to integrate available experimental data on dynamics of cerebral cortex neuron production, cortical stratification, and postnatal programmed cell death within cerebral cortex into single system dynamics simulation model. Stella Research modeling software was employed to build up a model and perform simulation experiments. Results of the simulation experiments confirmed that parameters of cell cycle kinetics of the pseudostratified ventricular epithelia (PVE), particulary 'q factor' (proportion of asymmetric mitoses within proliferative population during neurogenetic interval that give birth to young postmitotic neurons) fundamentally influence final amount of cerebral cortex neurons. Model enables effortless expansion by incorporation of other proliferative populations that colonize cerebral cortex or rise from PVE. Hypothesis testing in the course of experiments on animal models is additional possible use of this model.

Keywords: System dynamics, modeling, computer simulation, neurogenesis, cerebral cortex development.

Presenting Author's biography

Jadranka Božikov graduated in Mathematics and earned MSc and PhD degree in field of Biomedicine and Health Sciences at Zagreb University. Since 1978 she works in Department for Medical Statistics, Epidemiology and Medical Informatics at Andrija Štampar School of Public Health, Medical School, University of Zagreb, currently as associated professor. She introduced simulation modeling methods and applications as teaching subject for medical students and graduates and supervised several MSc theses obtained by young researchers who employed system dynamics approach and continuous simulation techniques in their investigation of the phenomena in medicine and public health. URL: www.snz.hr/~jbozikov.

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1 Introduction

Neurogenesis (NG) stands for generation of neurons of the cortical plate (CP) of the forebrain. It includes quantitative processes of proliferation, migration (quantitative in sense of cortical stratification/ distribution), and programmed cell death (PCD). Those processes have been thoroughly studied on mouse model through the series of experiments by Takahashi, Nowakowski, Caviness et al.

Vast majority of cortical neurons are born within pseudostratified ventricular epithelia (PVE) that lines telencephalic vesicles during prenatal development of the brain [1,2]. Period of time when neurons are born is called neurogenetic interval (NI). Prior to NI there are only symmetric mitoses that further populate PVE. NI starts when asymmetric mitoses appear in PVE [1,3,4]. Asymmetric mitosis gives birth to one young postmitotic neuron (PN), while remaining cell stays within PVE and keep on proliferate. Proportion of asymmetric mitoses rises throughout NI. NI ends with symmetric mitosis when both daughter cells become PN, and PVE is emptied. In the future area 1 of the mouse cortex NI begins on 11th embryonal day (E11) and ends in the first half of E17 [5,6]. Note that E0 is time of conception.

Takahashi et al. postulated that final number of neurons originated form PVE depends on four parameters: 1) size of the proliferative population at the onset of NI; 2) percentage of cells within proliferative population that actually proliferate (growth fraction – GF); 3) number of cell cycles executed by proliferative population; and 4) proportion of mitosis throughout NI that give birth to young postmitotic neurons (q factor) [7]. They also proposed simple mathematical model of NG within PVE and predicted that 11 cell cycles are executed by proliferative population during NI [8]. It is important to know that population of the PVE proliferates synchronous [9]. That means that all cells within the population that are in matching phase of the cell cycle have the same duration of cell cycle.

CP of the mammal neocortex has 6 layers. It has been known for a while that laminar position of neurons within CP depends on time of their origin [10,11,12,13]. Earlier born neurons are positioned in deeper cortical layers. Cortical distribution of PN in area 1 at 22nd postnatal day (P22) in dependence of time of their origin within murine neocortex has been quantitatively measured for neurons born on E11 through E16 [14].

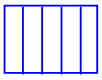
PN reaches their place by climbing the radial glial cells that stretch from telencephalic vesicle to the brain surface [15]. That process is called radial migration. It lasts, as it has been experimentally estimated, for 60 hours in mouse brain on E14 [16].

Rearrangement of neuronal density and reduction of number of cortical neurons is postnatally regulated by PCD (apoptosis) [17,18]. Amount of postnatal PCD within cortical plate of the mouse have been estimated for time of birth (P0), P4, P8, and P14 [19]. Dying cells have been counted by TUNEL labeling method that makes them visible for approximately 2.5 hours.

Although it has been initially developed for production/economic system modeling, system dynamics method has been found suitable for modeling cell proliferation and has been vastly used in study of carcinogenesis. On the other hand there are few papers in available literature that deals with developmental processes.

This method deals with compartments (*stocks*) that accumulate materials, or in this case cells, and *flows* of materials among them (Tab. 1). There are several stock subtypes, but only two were found suitable: *reservoir* and *conveyor*. Variables that determine the way the model performs could be contained by stocks and flows or could be defined within third component of the model: *converter*. Data but not materials are carried among mentioned components by *conveyors*. Filling and drain of the stocks from and to external source is shown by *cloud*, which is not really a separate component but just schematic drawing.

Tab. 1 Components of the system dynamics model in Stella 8.0 software.

Component name and symbol	Component description
Reservoir 	• Contains materials • Delay of first order • Multiple inputs and outputs
Conveyor 	• Contains materials for a defined period of time • Multiple inputs • One output + one 'leak'
Converter 	• Contains informations but not materials • Auxiliary variables about the system
Flow 	• Flow of materials between materials containing components
Connector 	• Flow of informations between components

Development of the model starts with drawing of the components on the dashboard and their logical connection in sense of materials and information flows. Particular component is then characterized by

definition of parameters that influence their behavior in the model.

We developed system dynamics model of the NG in mouse neocortex that integrates available experimental data. Selection of the most suitable stock type for the model of the proliferation within PVE was firstly done. Models of the PVE and postnatal apoptosis within CP were then created. Finally, overall model of the neocortical neurogenesis, along with migration, cortical distribution and PCD was built and tested. Simulation experiments were run in order to establish models stability, validity and dependence on initial parameters.

2 Material and methods

Following experimental data were used for PVE and CP at future area 1 of the murine neocortex: 1) cell cycle duration [20,21]; 2) q factor [21,22]; 3) growth fraction [20]– all of them are parameters of cell cycle kinetics (Tab.2); 4) duration of migration [14]; 5) distribution of young postmitotic neurons within CP as function of their time of birth (Tab. 3) [16]; and 6) proportion of cells that undergoes programmed cell death within CP in early postnatal life (Tab. 4) [19]. Proportions of dying cells within CP of the area 1 for P21 and P22 have been estimated to be about 0.015 (Nowakowski RS - personal communication).

Tab. 2 Parameters of the cell cycle kinetics of the PVE during NI within future area 1 of the mouse cerebral cortex (T_{CC}-duration of the cell cycle; T_{G1S}-duration of G1 and S phases of the cell cycle; T_{G2M}-duration of G2 and M phases of the cell cycle; qF-q factor; GF-growth fraction).

	E11	E12	E13	E14	E15	E16
T_{CC}	8.1	10.2	11.4	15.1	17.5	18.4
T_{G1S}	6.1	8.2	9.4	13.1	15.5	16.4
T_{G2M}	2	2	2	2	2	2
qF	N/A	0.11	0.19	0.36	0.67	0.79
GF	1	1	1	1	1	1

Tab. 3 Estimated probabilities for PN born throughout NI that determines mature neurons position within cortical layers of the area 1 of the mouse cerebral cortex on P22 (I through VI-cortical layers).

	E11	E12	E13	E14	E15	E16
I	0.000	0.000	0.000	0.000	0.004	0.013
II/III	0.000	0.000	0.012	0.000	0.212	0.827
IV	0.000	0.000	0.020	0.018	0.712	0.124
V	0.000	0.049	0.058	0.335	0.051	0.017
VI	0.000	0.951	0.911	0.647	0.022	0.019

Models were developed and tested with system dynamics software Stella 8.0 for Windows XP operating system [23].

Stock selection was made by simple simulation model of the cell proliferation. Cells were symmetrically divided through 100 hours with T_{CC} equaled 10 hours (T_{G1S} 8, and T_{G2M} 2 hours).

Tab. 4 Proportions of dying cells (TUNEL labeling index – TUNEL LI) within cortical layers of the area 1 of the mouse cerebral cortex in early postnatal life.

	P0	P4	P8	P14
I	0.071	0.070	0.045	0.593
II/III	0	1.035	0.197	0.082
IV	0.011	0.481	0.122	0.125
V	0	0.076	0.143	0.085
VI	0.139	0.169	0.137	0.082

Simulation experiments with integral model, as well as with models of the PVE and CP, were conducted with starting population of $1*10^3$ cells, equally distributed throughout cell cycle, for the following time frames: from E11 to P22 (integral), from E11 to P2 (PVE), and from P0 to P22 (or reverse) (CP).

Euler's integration method was used; with integration time (DT) equaled 1/10 hours. Duration of the cell cycle, cortical distribution of young postmitotic neurons, and proportion of dying cells were implemented as table-vice-linear function with linear interpolation. Growth fraction and duration of migration were constant. Q factor was estimated by polynomial fit that was obtained by Microsoft Excel 2003 software.

3 Results

In order to establish the most suitable stock, models of the cell population proliferation implemented by *reservoir* and *conveyor* was built and tested with simulation experiments (Fig. 1). Each model contains one *stock*. When certain amount of cells exit the cell cycle doubled number of cell enters the stock therefore simulating mitosis (Fig. 2).

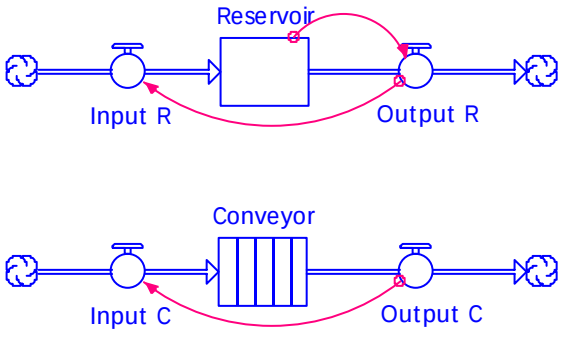


Fig. 1 Models of the proliferative populations implemented with *reservoir* and *conveyor*.

The most suitable stock subtype was shown to be *conveyor*. Starting population implemented with *conveyor* increased from 10^3 cells to expected $1.024*10^6$, through 10 cell cycles. In the same time population implemented by *reservoir* expanded to about $20.959*10^6$ cells. Expected number of cells was simply calculated by following formula:

$$N = N_0 * 2^k$$

where N_0 is number of cells of the starting population, and k is number of divisions (mitoses) executed by starting population.

*Conveyor(t) = Conveyor(t - dt) + (Input_C - Output_C) * dt*
INIT Conveyor = 1000
TRANSIT TIME = 10
INFLOW LIMIT = INF
CAPACITY = INF
INFLOWS:
*Input_C = 2*Output_C*
OUTFLOWS:
Output_C = CONVEYOR OUTFLOW
*Reservoir(t) = Reservoir(t - dt) + (Input_R - Output_R) * dt*
INIT Reservoir = 1000
INFLOWS:
*Input_R = 2*Output_R*
OUTFLOWS:
Output_R = Reservoir/10

Fig. 2 Equations layer of models of the proliferative populations.

Amount of *conveyors* connected serially, with total duration of cell cycle remained the same, did not influence dynamics of cell proliferation model. That enabled division of the modeled population of the PVE on two subsets that contained cells in G1 and S phases, and G2 and M phases of the cell cycle, respectively (Fig. 3).

Transit time of the G2M was set to be constant within the stock, while transit time of the G1S was set by *Tcc PVE* converter where T_{cc} was decreased by 2 hours (duration of G2M). Converter *Q LIMIT* served to keep values of q factor in the range from 0 to 1. Starting population was distributed between compartments proportionally to the durations of respective cell cycle phases. Cells exiting *G2M* compartment were divided in two flows in proportion q factor : (1-q factor). One representing PN (*TO CP*) that leads cells to the CP, and another (*TO G1S*) that turns back remained cell through the next cell cycle. Duplication of cell count occurs within the flows (half shaded valves)

According to results of simulation experiments, NI lasted 150 hours, from E11+4.9h to E17+10.9h. Each cell of the starting population of the PVE at the beginning of NI gave rise to ~145 postmitotic neurons. Population of the PVE was largest at E14+13.5h. During NI, cells of the PVE executed 11 cell cycles if they just entered G1 phase at the onset of neurogenetic interval, but only 10 cycles if they were anywhere in the cell cycle at least 1.6 hours away from the start of G1 phase.

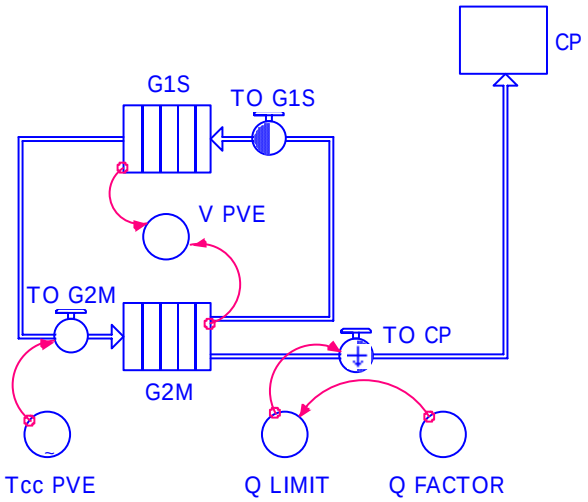


Fig. 3 Model of the PVE.

Since cortical distribution of neurons born during NI has been experimentally established for P22, submodel of the PCD was used to estimate their distribution on P0 (Fig. 4 – violet field). Submodel contain five compartments, each enclosing cells of one layer, except for second and third layer that were modeled jointly (in animal experiments there has not been possible to divide them with certainty). From each compartment cells were subtracted proportionally to the TUNEL LI divided by 2.5 (time of dying cell visibility). Simulation experiments was conducted for 10^3 neurons, which were born on each embryonal day of NI, finally positioned within cortical layers on P22. Simulations were executed in reverse timetable from P22 to P0, so that died cells were added to compartments. Then, probabilities for PN born throughout NI to end in each cortical layer on P0 were recalculated (Tab. 5).

Tab. 5 Calculated probabilities for PN born throughout NI to become a part of each cortical layer on P0.

	E11	E12	E13	E14	E15	E16
I	0.000	0.000	0.000	0.000	0.004	0.012
II/III	0.000	0.000	0.017	0.000	0.212	0.859
IV	0.000	0.000	0.022	0.021	0.712	0.103
V	0.000	0.046	0.054	0.322	0.041	0.012
VI	0.000	0.954	0.907	0.658	0.019	0.014

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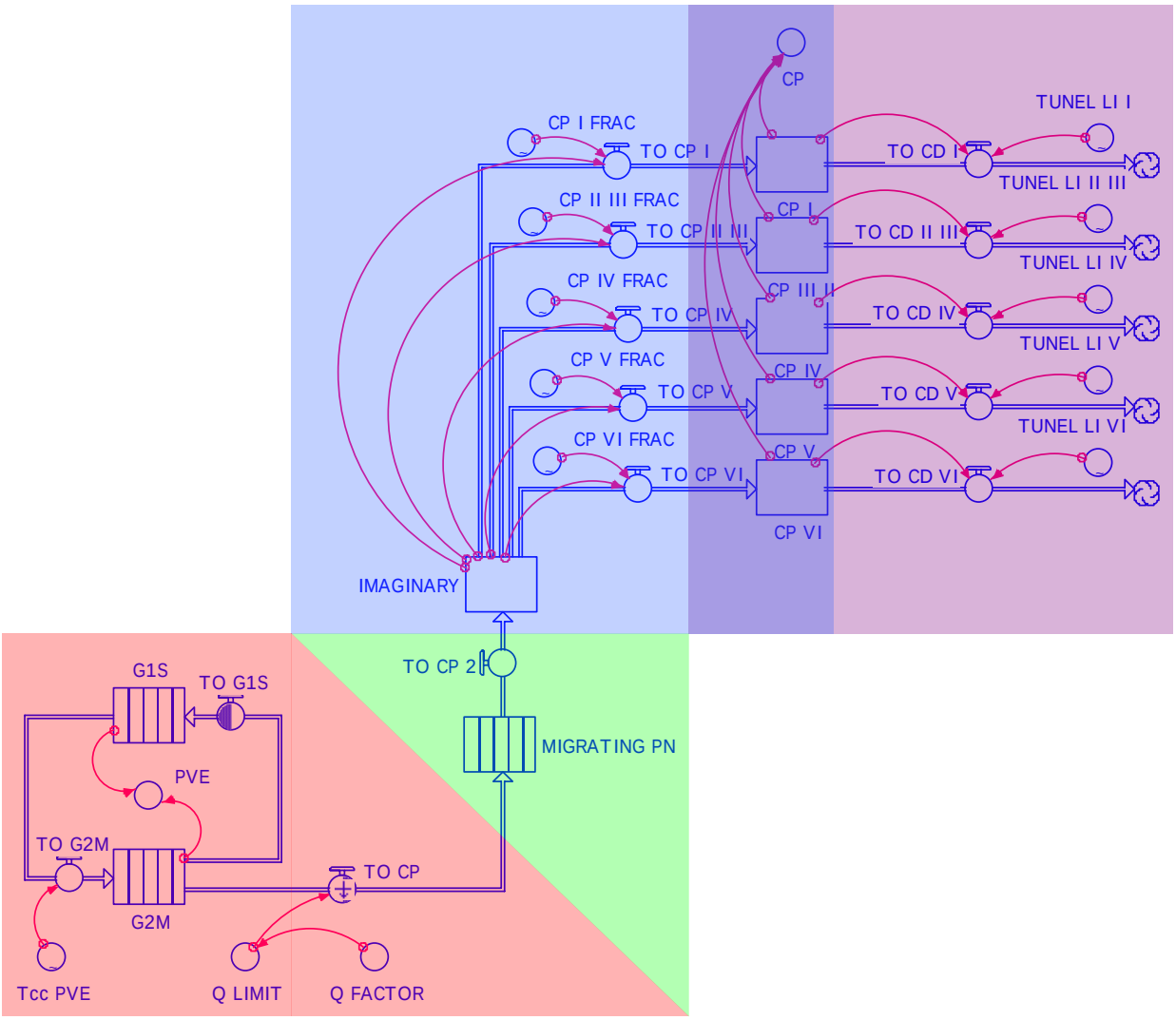

Fig. 4 Integral model of the neurogenesis with submodels: PVE (red), migration (green), cortical distribution (blue), and PCD (violet); dark violet is overlap between PCD and cortical distribution submodels.

Migration of PN was modeled with single *conveyor* where cells were held for 60 hours after they entered it (Fig. 4 – green field).

Cortical distribution was modeled with additional imaginary *reservoir* because it enabled more than two outputs (Fig. 4 – blue field). Its sole purpose was to hold PN for one DT interval. Afterward they are distributed to each cortical layer in amount proportional to calculated probabilities (Tab. 5).

In simulation experiment without PCD submodel, cortical distribution of PN by layers was as follows (in %): VI-23.1; V-9.0; IV-26.4; III/II-41; I-0.6. Simulation experiment with PCD yielded somewhat different distribution at P0 (in %: VI-25.2; V-9.8; IV-28; III/II-36.5; I-0.5) with 9.1% of cells still migrating.

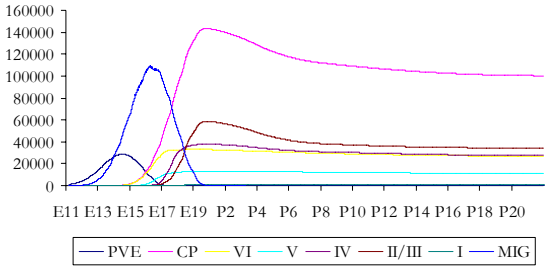

Fig. 5 Dynamics of cells amount in modeled populations during NI and early postnatal life obtained with simulation experiment on integral model of neurogenesis within area 1 of the mouse cerebral cortex.

According to the result of simulation experiment on integral model, each cell of the initial population within PVE at the onset of NI gave rise to ~100 cells

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on P22. Overall reduction of cortical neuron number in the early postnatal life in simulation experiment, compared to result without PCD, was 28.0% for period P0-P14, and 30.7% for period P0-P22. Reduction by layers for period P0-P14 was as follows (in %): VI – 16.6; V-11.5; IV-24.5; III/II-40.3; I-20.7. Final number of PN linearly depended on the size of the founder population within PVE.

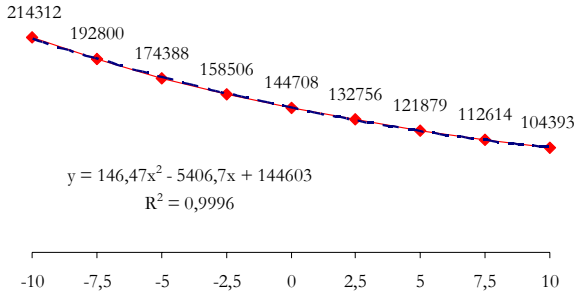

Fig. 6 Final neuron number as a function of cell cycle duration (changed in a manner described in the text).

In order to establish its influence on final neuron number, duration of the cell cycle was varied linearly throughout NI, by adding or subtracting 2.5, 5, 7.5, and 10% of its experimentally estimated value. That showed reverse polynomial (2nd order) dependence of the final neuron number on the length of the cell cycle (Fig. 6).

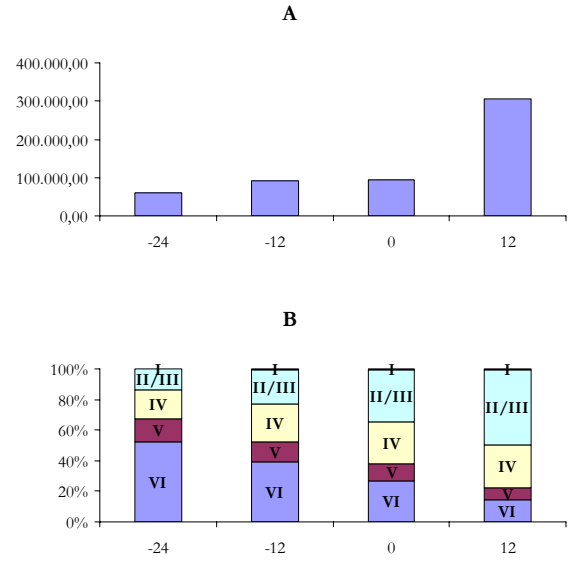

Fig. 7 Final number of cortical neurons (A), and their relative cortical distribution (B) as a function of q factor (changed in a manner described in the text).

Q factor was implemented by polynomial fit of experimentally estimated values. Alteration of q-factor was made by constructing the family of curves with the same 'starting' end 'ending' points (points where q factor crosses values 0 and 1 respectively). Point where q factor reach 0.5 was shifted by 12 hours interval before or after fitted one. Those changes considerably influenced not only final neuron number but their cortical distribution as well (Fig. 7).

4 Conclusion

Model was shown to be stable for given parameters providing repeatable results in each performed experiment. There was no nonlinearity noticed within the model.

Results of simulation experiments were in accordance to experimentally observed facts for onset of NI, its duration, time of maximal size of PVE, and persistent migration of PN during early postnatal life. Final neuron number within CP obtained with the model of the PVE was comparable with results obtained by Takahashi et al. On the other hand, their simplification of cell cycle dynamics by 11 cell cycle executed by starting population was shown wrong, because ~80% of the starting population execute 10 cell cycles only.

Cortical distribution of PN at P14 and postnatal cell death within CP showed differences when compared with previously published data that might be attributed to the numerical estimation methods used and populations of neurons that contribute to the cortical neuron number but were not included in the model (SPP-secondary proliferative population, GE-ganglionic eminence, ML-marginal layer, and SP-subplate) [19,24,25,26,27].

Model enables further expansion by incorporation of other previously mentioned proliferative populations as soon as there would be experimental data on their cell cycle dynamics available. Dynamics of SPP could already be studied with modest alterations of this model, though just few data have currently been revealed [24].

This model also enables hypothesis testing such as one on great amount of PCD within PVE during NI [28,29]. Although neuroscientists in majority disagreed with it, there is no firm proof yet, mathematical or experimental [30].

By modification of single parameter of the integral model, namely q factor, it was possible to gain substantial difference among final neurons number and their cortical distribution. Modifications of the cell cycle duration mostly affected final cortical neurons number. Knowing that, it could be confirmed that major shifts in cerebral structure and function among mammal species as well as differences among various cortical areas of the single species, might evolutionary be possible simply by alteration of cellular processes that orchestrates ratio of production of PN during NI and duration of proliferative populations cell cycle [1,7,31].

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System dynamics was found suitable for simulation modeling of quantitative developmental cellular processes. Cell cycle dynamics should be modeled by utilization of *conveyer* stock type since it reproduces exact cell cycle dynamics. Method enables integration of variety available experimental data into single model.

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