

A GENE NETWORK APPROACH TO MODELING EARLY NEUROGENESIS IN DROSOPHILA

G. MARNELLOS

*Sloan Center for Theoretical Neurobiology,
The Salk Institute, San Diego, CA 92186, USA*

E. MJOLSNES^a

*Department of Computer Science and Engineering
and Institute for Neural Computation, UCSD, La Jolla, CA 92093, USA*

We have produced a model of genetic regulation to simulate how neuroblasts and sensory organ precursor (SOP) cells differentiate from proneural clusters of equivalent cells. Parameters of the model (mainly gene interaction strengths) are optimized in order to fit schematic patterns of expression, drawn from the literature, of genes that are involved in this process of cell fate specification. The model provides suggestions about the role of lateral signalling in neurogenesis and yields specific and testable predictions about the timing and position of appearance of neuroblasts and SOPs within proneural clusters, and about the dynamics of gene expression in individual cells. Experimental testing of these predictions and fits to more accurate quantitative data will help determine which set of model parameters can best describe early neurogenesis.

1 Introduction

In *Drosophila*, neuroblasts and sensory organ precursor (SOP) cells differentiate from epithelia to give rise to the central nervous system in the fly embryo and to epidermal sensory organs in the peripheral nervous system of the adult fly, respectively. Neuroblasts are neural precursor cells that divide to form neurons and glia; they segregate from the ventral neuroectoderm of the insect embryo in a regular segmental pattern¹. SOPs appear at stereotypical positions on imaginal discs of fly larvae and divide to produce a neuron and three other cells that form *Drosophila*'s sensory organs, like the bristles on its thorax².

Neuroblasts and SOPs differentiate from apparently equivalent clusters of cells expressing genes of the *achaete-scute* complex, so called *proneural* genes. Eventually only one cell from each proneural cluster retains proneural gene expression and becomes a neuroblast or SOP, in a process referred to as *cluster resolution*. Other genes involved in this specification of cell fate are also expressed in characteristic spatiotemporal patterns, including *hairy* and genes of the *Enhancer-of-split* complex which tend to restrict proneural clusters and

^aPresent address: Machine Learning Systems Group, Jet Propulsion Laboratory, 4800 Oak Grove Drive, Pasadena, CA 91109, USA.

favor the undifferentiated, epithelial state and which we refer to as *epithelial* genes. Genetic, molecular and ablation studies have pointed to a lateral signalling interaction between adjacent cells, through which the neural fate is promoted in the future neuroblasts and SOPs and suppressed in other cells^{3,4}.

Despite the number of empirical observations that have been gathered, several features of this system remain unexplained: a precise characterization of lateral signalling is still lacking; we do not understand dynamical aspects of the system, for example, whether and how the shape and size of proneural clusters determine how cluster resolution proceeds; it is not clear what the role of neuroblast delamination is in cluster resolution in the fly embryo.

To address questions like these and guide further experiments, we have constructed a computational model to simulate neuroblast and SOP differentiation, based on the modeling framework described in Mjolsness *et al.*⁵: we have used recurrent neural nets to represent gene interactions as well as other regulatory and signalling events. The same framework has been previously used to simulate gene expression patterns in the *Drosophila* blastoderm^{6,7}.

2 Model

In our model, cells are represented as overlapping cylinders in a 2-dimensional hexagonal lattice; the extent of overlap determines the strength of interaction between neighboring cells (see Fig. 1). Cells in the model express a small number of genes corresponding to genes that are involved in neuroblast and SOP differentiation. In the work presented here, we have used networks of two genes to fit expression patterns corresponding in broad terms to proneural genes, on one hand, and to epithelial genes, on the other.

Genes interact as nodes in recurrent neural nets, with connection weights depending on the kind of the interaction: we allow two kinds of interaction, an intracellular and a lateral signalling one (which respectively correspond to one-cell and two-cell continuous rules of Mjolsness *et al.*⁵). A gene a sums inputs from genes in the same cell or in neighboring cells at time t according to the following equation (cf. Eq. 22 of Mjolsness *et al.*⁵)

$$u_a(t) = \sum_b T_{ab} v_b(t) + \sum_{i \in N} \Lambda^i \sum_b \hat{T}_{ab} \hat{v}_b^i(t) \quad (1)$$

where T is the matrix of gene interactions and the $v_b(t)$ gene product concentrations within the cell; \hat{T} is the matrix of gene interactions with neighboring cells and the $\hat{v}_b^i(t)$ product concentrations in neighboring cell i ; N the set of neighboring cells (the neighborhood of a cell consists of the six surrounding cells); and Λ^i a factor depending on the overlap of the cell with neighboring

cell i , as measured for instance by the common chord of the bases of the two adjacent cylinders (see Fig. 1). Concentration $v_a(t)$ of the product of gene a then changes according to (cf. Eq. 2 of Mjolsness *et al.*⁵)

$$\frac{dv_a}{dt} = R_a g(u_a(t) + h_a) - \lambda_a v_a(t) \quad (2)$$

where $u_a(t)$ is the linear sum of Eq. 1, g a sigmoid function, R_a the rate of production of gene a 's product, h_a the threshold of activation of gene a and λ_a the rate of decay of gene a product.

The model also includes detachment of cells from the epithelium, *delamination*, perpendicular to the plane of the epithelium — neuroblasts are known to delaminate as they differentiate. Cell delamination is directly controlled by genes within a cell and only indirectly by neighboring cells; there are two parameters that determine how strongly each gene promotes or suppresses delamination, as well as another two that determine delamination rate and a threshold for delamination to be activated. Delamination changes the area of apposition of neighboring cells, i.e. changes the factors Λ^i in Eq. 1 above and therefore modulates the strength of interaction between neighboring cells.

We optimize gene interaction strengths (and other parameters in the equations above) to fit gene expression patterns; the cost function optimized is

$$E = \sum_{cells, genes, times} (v_{aMODEL}^i(t) - v_{aDATA}^i(t))^2, \quad (3)$$

which is the squared difference between gene product concentrations in the model and those in the dataset, summed over all cells and over all gene products and times for which data is available. We have used a stochastic algorithm, simulated annealing, for this optimization. For more details on the model and the optimization method used see Marnellos (1997)⁸.

3 Simulation Results

3.1 Design of optimization and test runs

The gene expression datasets we optimize on, the *training* datasets, are adapted from schematic results described in the experimental literature^{9,10,11}; they specify the initial pattern of concentrations of the gene products, i.e. the proneural clusters, and the desired final pattern when the proneural clusters have resolved to single cells expressing the proneural gene at high levels; it is left to the optimization to find the right model parameters so that the system develops from the initial state to the desired final one (see Fig. 1). All cells

in a proneural cluster have initially the same gene expression levels. The size and cluster arrangement of the training datasets do not have any particular biological significance; the datasets have been designed in such a way as to keep the number of cells low while including as many 7-cell symmetrical clusters as possible, since optimization is very expensive computationally and so optimization runs on datasets with more cells than we have used would be impractical: it could take more than a week on an IBM PowerPC or an SGI Indigo and might also get stuck at a greater number of bad local optima.

Apart from the training dataset illustrated in Fig. 1, in runs with cell delamination we have used another identical dataset (not shown) which additionally contains information about the delamination of the cells at the desired final state, when the prospective neuroblasts have fully delaminated, while all the other cells are in their initial positions in the epithelium.

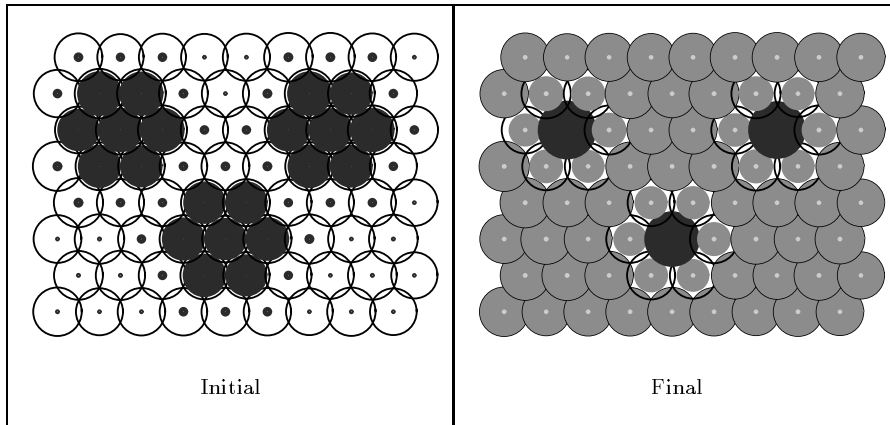


Figure 1: Cells are modeled as cylinders in a hexagonal lattice (viewed here from above). Gene expression is represented by disks, proneural expression in dark gray and epithelial in medium gray; disk radius is proportional to level of expression. This figure shows the training dataset: on the left, the initial concentrations of the gene products — there is only proneural gene expression in three symmetrical clusters; on the right, the desired final pattern of gene expression — proneural expression is retained only in the central cell of each cluster, the future neuroblast or SOP, whereas all other cells express the epithelial gene.

We have mainly focused on optimizing gene interaction strengths, i.e. the T and \hat{T} matrices of Eq. 1, and not so much other parameters of the model. An illustration of the optimization run designs we have used appears below; there are five optimized parameters, four of them for gene interaction strengths within a cell and one for a gene interaction across cells; columns in these matrices are for input genes and rows for genes affected (empty boxes signify zero

interaction strength. i.e. no interaction):

Intracellular Interactions			Lateral Signalling Interactions		
	<i>Proneural</i>	<i>Epithelial</i>		<i>Proneural</i>	<i>Epithelial</i>
<i>Proneural</i>	0.283	-0.015	<i>Proneural</i>		-10.1
<i>Epithelial</i>	-0.555	9.74	<i>Epithelial</i>		

The simulation corresponding to the solution above appears in Fig. 2.

We have tried to limit the number of parameters we optimize on, in order to avoid overfitting our rather small datasets. Successful optimization runs have yielded solutions that not only perform well on the training datasets but also work for larger test datasets similar to the training ones but with a greater number of 7-cell symmetrical clusters in various spatial arrangements (not shown). This indicates that optimization does not just find parameter values that only work for the specific size and cluster arrangement of the training dataset, but rather extracts rules for resolving clusters.

Since optimization on various sets of model parameters leads to solutions that work well on the training dataset, in order to further evaluate these solutions and determine which are robust and can best describe the biological system under consideration, we have also run these solutions on *test* datasets to see how they perform on novel proneural clusters. Test datasets may of course contain many more cells than training ones (since we do not optimize on them), so we have used datasets with several clusters of various shapes and sizes like the one in Fig. 3. The test datasets could in principle have been used as training datasets, if it were not for the practical considerations mentioned above. Test runs have provided suggestions about the roles of different model parameters.

3.2 Lateral interactions and cell delamination

Our results show that cell-cell interactions involving just the immediate neighborhood of any given cell can bring about cluster resolution, without the need of other longer range processes like diffusion, i.e. lateral signalling interactions are sufficient for cluster resolution. They also appear to be crucial for cluster resolution, since optimization has not come up with any solutions, when lateral interactions are not included and the proneural cluster cells are all equivalent; only when prospective neuroblasts or SOPs are marked with differences in gene expression levels, does optimization find solutions that do not require lateral signalling. Moreover, for some solutions, like the one in Fig. 2, when lateral interactions are abolished, then clusters do not resolve but all cells in them retain proneural gene expression. This parallels the effect of the so called *neu-*

rogenic mutations in the real biological system; these mutations disrupt lateral communication between cells and lead to overproduction of neurons^{3,4}.

Which lateral signalling interactions are allowed also plays an important role in whether optimization can fit the training dataset in a satisfactory way or not. For example, if no Epithelial-to-Proneural or Epithelial-to-Epithelial lateral signalling interactions are allowed (i.e. if both entries in the right column of the of the lateral signalling matrix are zero), then our optimization runs on the remaining interaction strengths have not produced values that lead to cluster resolution — and of course the same is true when no lateral interactions at all are allowed; whereas, if only the Epithelial-to-Proneural interaction of the lateral matrix is allowed, with the same total number of optimized parameters, then optimization can readily yield values leading to cluster resolution (as mentioned above and shown in Fig. 2). This suggests that some lateral gene interactions are more important than others in producing cluster resolution.

If all four lateral signalling interactions are allowed, then, apart from fitting the training data with the 7-cell symmetrical clusters, optimization solutions can successfully resolve bigger or smaller asymmetrical clusters in test datasets (not shown), even though, as mentioned above, some of these interactions are not necessary for the resolution of training dataset clusters. This suggests that, although Proneural-to-Proneural and Proneural-to-Epithelial lateral interactions may not be sufficient on their own to bring about cluster resolution, they may still enable lateral signalling to resolve a larger range of cluster shapes. Very large clusters, like the one on the right in Fig. 3, still do not resolve.

If the delamination parameters are optimized on together with four lateral signalling and four intracellular gene interaction parameters, then optimization solutions like the one in Fig. 3 can resolve an even larger variety of shapes and sizes of clusters in test datasets, that cannot be resolved readily without delamination; however, if lateral interactions in the solution of Fig. 3 are abolished, all cluster cells loose proneural gene expression, unlike what happens with the solution of Fig. 2.

3.3 Dynamics of cluster resolution

The degree of encirclement of a cell in a proneural cluster by other cells in the cluster can determine which cell becomes the neuroblast or SOP, as is shown in Figs. 2 and 3. Smaller clusters generally resolve faster than larger ones and proneural and epithelial gene expression changes at different rates depending on cell position in a cluster, even for cells that adopt the same fate. Furthermore, from simulations both with and without cell delamination, it becomes apparent that lateral effects propagate further than one cell, as

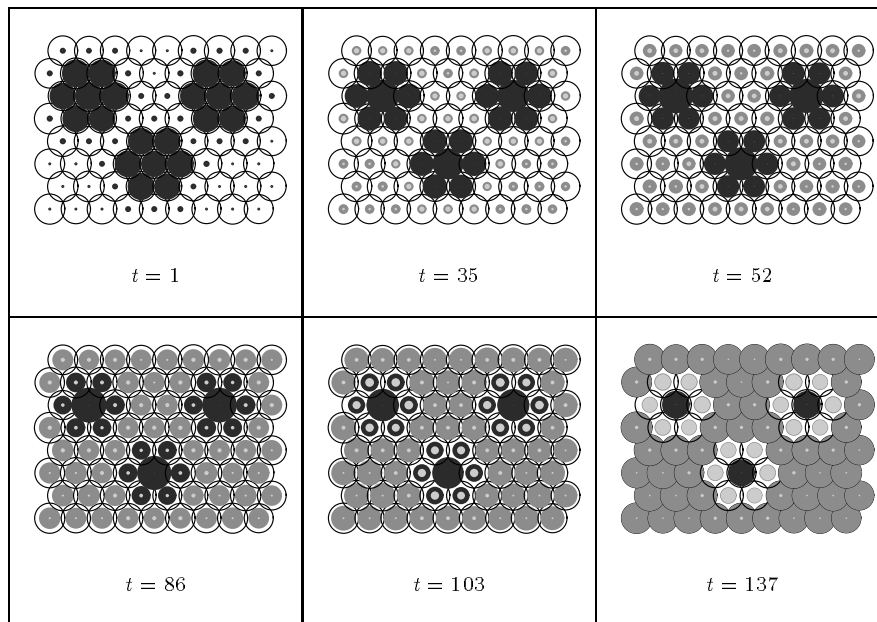


Figure 2: Computer simulation of neuroblast and SOP differentiation in a two-gene model. From left to right and from top to bottom, different time frames of the evolution of gene product concentrations. In the initial state, clusters of cells express the same amount of proneural protein. As the run proceeds, the concentration of epithelial protein rises in most cells, except for the segregating neuroblasts and SOPs, which retain high levels of proneural protein. Light gray indicates overlap of proneural and epithelial proteins; other conventions as in Fig. 1.

is indicated by the fact that gene expression in a group, for instance, of five cells changes in a particular way when this group of cells is a separate cluster (in this case one cell from the group becomes a neuroblast) and in a different way when such a group is part of a bigger cluster (when all cells in the group may adopt the epithelial fate); see Fig. 3. It is also interesting to note, that in all the simulations we have run, irrespective of the number of optimized interaction strengths, clusters of four equivalent cells do not resolve but all cells in the cluster remain epithelial. These observations are predictions of the model, since they have not been built into the model in any way.

In order to further study the dynamics of cluster resolution in our simulations, we have sketched the phase portraits, at successive points in time, of solutions obtained through optimization. For each point in time, we have

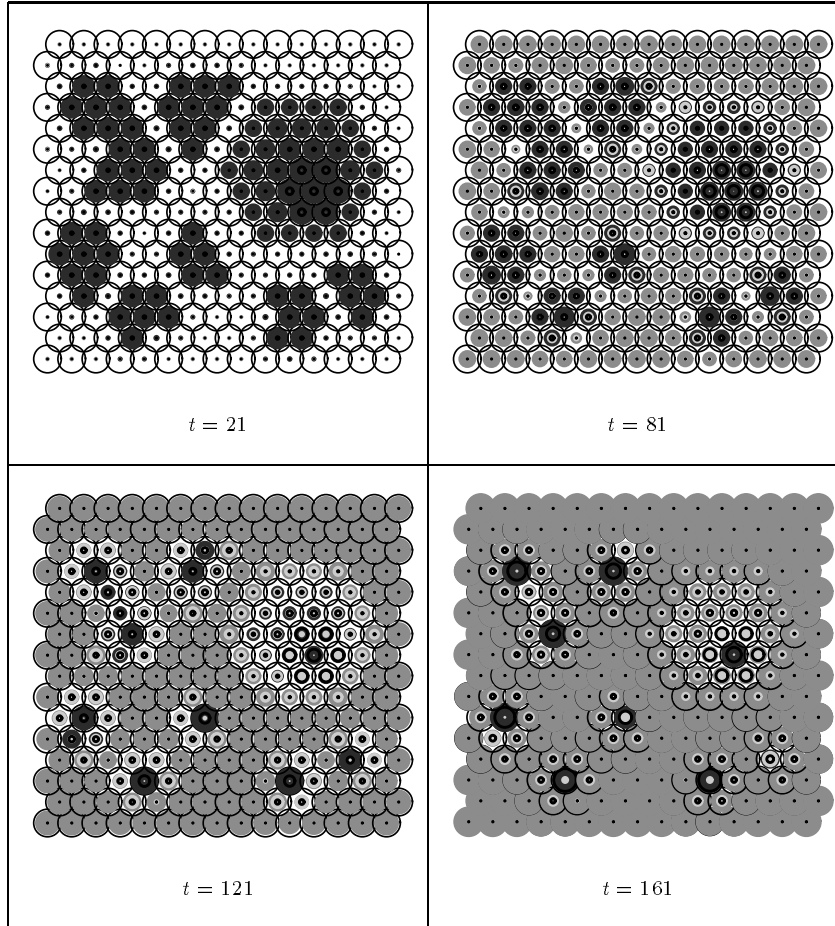


Figure 3: Simulation with cell delamination and full lateral interactions. Same conventions as in Fig. 2, with the addition that cell delamination is represented by the thicker inner circle in the middle of the cells; the greater the radius of this circle, the further a cell has delaminated. Note that the large cluster on the right resolves successfully.

plotted the direction and magnitude of the change in gene product levels in a particular cell, given any value for the current product levels (see Fig. 4). This essentially shows how a cell would respond (in terms of modulation of its gene expression) if we altered (increased or decreased) the levels of its gene products. If there are no lateral interactions the phase portrait of proneural versus epithelial gene expression levels does not change, of course, over time and is the same for all cells. By contrast, when there are lateral interactions, each cell has a different phase portrait that changes in time, depending on cell position (as in the example of Fig. 4). The phase portrait can be thought to represent the epigenetic landscape that each cell finds itself in; this is a dynamic landscape that changes depending on the strength of lateral interactions (as well as on the geometry of the tissue and on intracellular interactions).

4 Discussion

4.1 Implications of the model

In this work we have applied the framework developed in Mjolsness *et al.*⁵ to model early neurogenesis in *Drosophila*. Previous models of this process have looked at how neuroblasts or SOPs might emerge from homogeneous epithelia either through long range interactions, like diffusion of a substance¹², or through lateral inhibition^{13,14}; these models have used a different approach from ours, in that they have only considered cell-cell interactions and have not explicitly included regulatory gene interactions or attempted to model known patterns of gene expression.

Despite the relative simplicity of our model, the simulations described in this paper have allowed us to look closer at questions like the role of lateral signalling and cell delamination in neuroblast and SOP differentiation and have provided a tool to study the dynamics of proneural cluster resolution. Optimization of parameters in the model to fit schematic gene expression datasets has come up with solutions that are also robust to perturbations, e.g. can resolve novel clusters of different sizes and shapes.

Our simulations have allowed us to conclude that lateral interactions with only the immediate neighborhood of a cell are sufficient for cluster resolution; that, when proneural cluster cells are equivalent, such interactions are important for cluster resolution; that different lateral interactions may have different roles in bringing about cluster resolution; and that lateral effects can propagate further than a cell's immediate neighborhood through a cell-to-cell relay.

The model has produced further predictions about how cluster resolution proceeds: smaller clusters generally resolve faster than larger ones; gene ex-

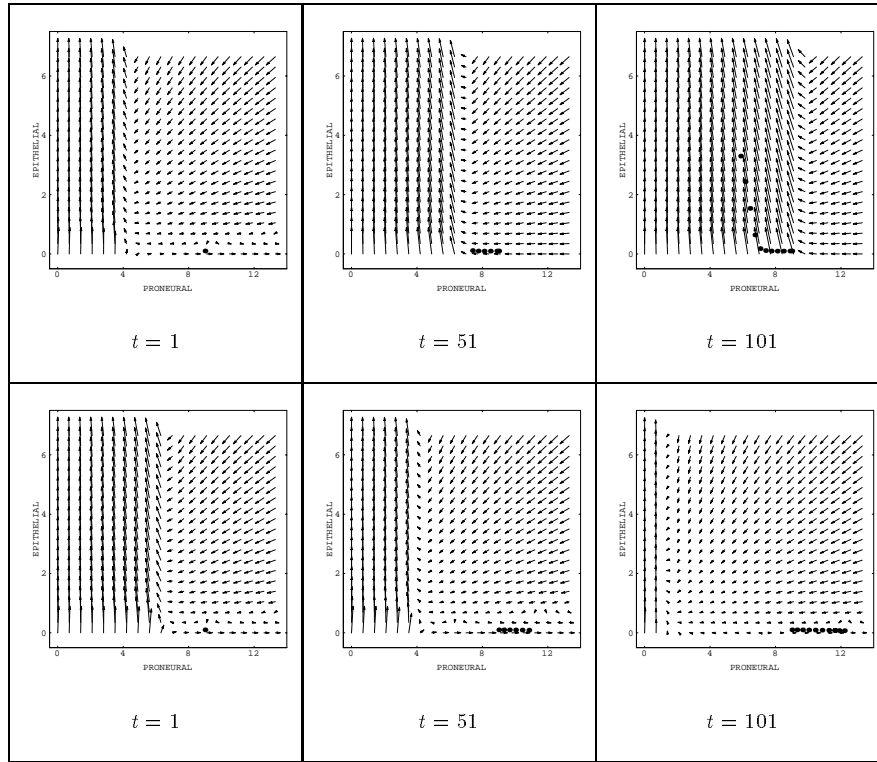


Figure 4: Phase-portraits in the Epithelial-vs-Proneural plane for two cells from the same 7-cell symmetrical cluster at three successive stages in time; this is from a simulation with cell delamination and full lateral interactions. The dots indicate the trajectory of the cell. Top row: phase-portraits for a cell at the cluster periphery; the cell starts at high proneural and low epithelial levels in the left-most panel but soon the dynamic landscape changes and forces the cell to move to the epithelial attractor towards the top left in the following panels; note that by the time-point of the middle panel the cell is destined to the epithelial fate despite its almost zero epithelial gene expression levels, since, even if its proneural gene expression level were almost doubled at this point, it would still move towards the epithelial attractor. Bottom row: phase-portraits for a cell at the cluster center; this cell starts at the same point in the phase plane as the peripheral cell in the top row, but the phase-portrait at the start already looks different from that of the peripheral cell and changes in a different way over time; note that even a small increase in epithelial expression would push this cell towards the epithelial attractor to the left, otherwise the cell moves towards the neural attractor to the bottom right.

pression changes at different rates depending on cell position in the proneural cluster, even for cells that will eventually adopt the same fate; the degree of encirclement of a cell in a proneural cluster by other cluster cells can specify which cell becomes the neuroblast or SOP (namely the most encircled cell), especially in smaller clusters. The analysis of gene interaction dynamics is also a source of very specific and quantitative predictions about how cells would respond to externally imposed changes in their gene product levels; for example, such analysis can show that a cell may be strongly committed to a particular fate even when its gene expression levels provide no indication of that (see Fig. 4); such predictions are now testable in *Drosophila* where manipulation of gene expression in individual cells is possible⁵.

The results of the simulations have not provided clear answers about the role of cell delamination; although delamination does seem to facilitate the resolution of large clusters, abolishing lateral interactions in solutions that include delamination does not lead to all cells in a cluster retaining proneural gene expression (the *neurogenic* phenotype), as would be expected from experimental observations. However, our results do indicate that modulation of the strength of lateral, cell-cell interactions, either through delamination or perhaps through changes in the activity of cell-membrane signalling molecules, can be crucial for cluster resolution.

Experimental testing of the various predictions of the model as well as further fits to more quantitative datasets of higher temporal resolution will help us determine which sets of model parameters are more biologically plausible and better suited for modeling neurogenesis in *Drosophila*.

4.2 Model extensions and further considerations

An obvious way to extend this model is to include more genes in the regulatory circuit. A specific extension would be to focus on how lateral signalling is mediated: introducing gene products at the cell membrane, like receptors and ligands, in order to gate gene interactions across cells, would be biologically more realistic, since Notch, a receptor, and Delta, its ligand, are known to mediate lateral communication in the biological system¹⁶; this would produce more detailed predictions about the functions of lateral signalling.

The kinds of questions that can be posed with the model described in this paper are not only of relevance to neurogenesis in *Drosophila* but are common to many developing organisms, especially in view of the fact that homologues to genes involved in *Drosophila* neurogenesis have been isolated in many species from worms to mammals participating in a variety of developmental processes^{16,17}. In vertebrate neurogenesis such homologues act in ways

similar to those of the *Drosophila* genes to regulate the number of neurons generated; one would therefore expect that a theoretical and empirical understanding of *Drosophila* neurogenesis would provide insights into neurogenesis in higher vertebrates.

A limitation of the approach described in this paper may be the fitting method used: stochastic optimization methods available (like the simulated annealing one we have used) do not guarantee convergence to a global optimum in reasonably short amounts of time, and so our approach may not readily scale up to much larger numbers of optimized parameters. However, issues in global optimization are the object of intense study because of their importance in diverse problems in science and engineering, and therefore it is still possible that faster methods will become available.

Acknowledgements

We wish to thank Anne Bang, Chris Kintner, Jim Posakony and John Thomas for discussions, and Larry Carter (UCSD and SDSC) and the Yale Center for Parallel Supercomputing for use of their computers.

References

1. C. Doe and C. Goodman, *Developmental Biology* **111**, 193 (1985).
2. V. Hartenstein and J. Posakony, *Development* **107**, 389 (1989).
3. S. Artavanis-Tsakonas and P. Simpson, *Trends in Genetics* **7**, 403 (1991).
4. S. Campuzano and J. Modollel, *Trends in Genetics* **8**, 202 (1992).
5. E. Mjolsness *et al.*, *Journal of Theoretical Biology* **152**, 429 (1991).
6. J. Reinitz *et al.*, *Journal of Experimental Zoology* **271**, 47 (1995).
7. J. Reinitz and D. Sharp, *Mechanisms of Development* **49**, 133 (1995).
8. G. Marnellos, *Gene Network Models Applied to Questions in Development and Evolution*, Ph.D. thesis, Yale University (1997).
9. P. Cubas *et al.*, *Genes and Development* **5**, 996 (1991).
10. J. Skeath and S. Carroll, *Development* **114**, 939 (1992).
11. B. Jennings *et al.*, *Development* **120**, 3537 (1994).
12. J. Richelle and A. Ghysen, *Developmental Biology* **70**, 418 (1979).
13. M. Tanemura *et al.*, *Journal of Theoretical Biology* **153**, 287 (1991).
14. J. Collier *et al.*, *Journal of Theoretical Biology* **183**, 429 (1996).
15. M. Halfon *et al.*, in *Abstracts of the 37th Annual Drosophila Research Conference*, 46, The Genetics Society of America (1996).
16. S. Artavanis-Tsakonas *et al.*, *Science* **268**, 225 (1995).
17. J. Lewis, *Current Opinion in Neurobiology* **6**, 3 (1996).