# Implications of Activity-Dependent Neurite Outgrowth for Developing Neural Networks

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#### Abstract

The presence of only two basic neuronal properties - a firing threshold and activitydependent neurite outgrowth - is sufficient to cause a transient overproduction of connections in developing neural networks. This overproduction is enhanced by inhibition. Solely as the result of activity-dependent outgrowth and local cell interactions, the neuritic field of an inhibitory cell becomes smaller than that of an excitatory cell. Furthermore, a specific distribution of cell sizes is generated in the area surrounding an inhibitory cell.

# Introduction and Summary

Among the many factors influencing the ultimate structure of the nervous system, electrical activity plays a pivotal role. Many mechanisms that determine neuronal connectivity, such as neurite outgrowth, synaptogenesis and cell death, have been found to be modulated by electrical activity. For example, electrical activity of the neuron reversibly arrests neurite outgrowth (or produces retraction). High levels of activity, resulting in high intracellular calcium concentrations, cause neurites to retract, whereas low levels of activity, and consequently low calcium concentrations, allow further outgrowth [1]. As a consequence of such activity-dependent processes, a mutual influence exists between the formation of synaptic connectivity and neuronal activity, i.e., a feedback loop exists between changes in network structure and changes in network activity. This feedback loop must be expected to have major implications not only for the structure of the mature network, but also for the stages a network goes through during its development. Neurons develop their mature form and connectivity pattern under influence of both intrinsic factors and environmental factors (e.g., local cell interactions). During this development, all kinds of structural elements such as neuritic extensions and synapses are initially overproduced (so-called overshoot phenomena) [2].

In this article, we have studied the implications of activity-dependent neurite outgrowth for network formation, using a model in which initially disconnected cells organize themselves into a network under the influence of their intrinsic activity. A neuron is modelled as a neuritic field, the growth of which depends upon its own level of activity, and neurons become connected when their fields overlap. In a purely excitatory network, we have demonstrated that activity-dependent outgrowth in combination with a neuronal response function with some form of firing threshold - which gives rise to a hysteresis effect - is sufficient to cause an overshoot with respect to connectivity or synapse numbers [3]. Here we show that in the presence of inhibition, overshoot can still take place and is in fact enhanced if inhibitory cells (inh-cells) grow at the same rate as excitatory cells (exc-cells). If, on the other hand, the development of inhibition is delayed (i.e., if the outgrowth rate of inh-cells is lower than that of exc-cells) overshoot is not enhanced, while now the growth curve of the number of inhibitory connections does not exhibit overshoot. An interesting emergent property of the model is that - solely as the result of simple outgrowth rules and local cell interactions the size of the (dendritic) fields of inh-cells become always smaller than that of exc-cells, even if both types of cells have exactly the same growth properties. Furthermore, a specific distribution of cell sizes is generated in the area surrounding an inh-cell.

The results are robust and show certain similarities with findings in developing cultures of dissociated nerve cells.

## The Model

We use a distributed network, with neuron dynamics governed by (shunting model):

$$\frac{dX_i}{dt} = -\frac{X_i}{\tau} + (A - X_i) \sum_{k}^{N} W_{ik} F(X_k) - (B + X_i) \sum_{l}^{M} W_{il} F(Y_l)$$
(1)

$$\frac{dY_j}{dt} = -\frac{Y_j}{\tau} + (A - Y_j) \sum_{k}^{N} W_{jk} F(X_k) - (B + Y_j) \sum_{l}^{M} W_{jl} F(Y_l)$$
(2)

where  $X_i$  and  $Y_j$  are the membrane potentials of the N exc-cells and M inh-cells, respectively, A and -B are the saturation potentials,  $1/\tau$  determines the rate of decay, and  $W_{ik}$ ,  $W_{il}$ ,  $W_{jk}$ ,  $W_{jl}$  are the connection strengths (all  $W \ge 0$ ). All potentials are relative to the resting potential, which is set to 0. The firing-rate function, F, is taken to be the same for exc- and inh-cells, and is given by:

$$F(x) = \frac{1}{1 + e^{(\theta - x)/\alpha}}.$$
(3)

where x is the membrane potential,  $\alpha$  determines the non-linearity, and  $\theta$  is the firing threshold. The small firing rate when the membrane potential is sub-threshold can be thought of as arising from spontaneous activity. In most of the simulations we took A=B=1,  $\tau=8$ ,  $\alpha=0.10$  and  $\theta=0.5$ . The results, however, do not depend on the exact choices of the parameters. Growing cells are modelled as growing circular fields, which might be conveived of as neuritic fields. When two such fields overlap, the corresponding neurons become connected with a strength proportional to the area of overlap:

$$W_{ij} = A_{ij}c \tag{4}$$

where  $A_{ij} = A_{ji}$  is the amount of overlap ('number of synapses';  $A_{ii} = 0$ ) and c is a constant of proportionality ('synaptic strength'). Although in this way a symmetric network is built, the result do not depend on symmetry. The outgrowth of each individual cell depends upon its own level of electrical activity (in the same way for exc-and inh-cells):

$$\frac{dR_i}{dt} = \rho \left[ 1 - \frac{2}{1 + e^{(\epsilon - F(x_i))/\beta}} \right]$$
(5)

where  $R_i$  is the radius of the field of cell *i*,  $\epsilon$  is the firing-rate at which  $\frac{dR_i}{dt} = 0$ , and  $\beta$  determines the non-linearity. Eqn(5) is just a phenomenological description of Kater's hypothesis [1] that the depolarization level of the neuron influences (via Ca<sup>2+</sup> influx) its rate of outgrowth. Any other function for which  $\frac{dR_i}{dt} > 0$  at low values of  $F(x_i)$  and < 0 at high values will yield similar results.  $\rho$  is taken so small that the connectivity can be regarded as quasi-stationary on the time scale of membrane potential dynamics.



**Fig.1.** a Hysteresis. Steady state dependence  $(\frac{d\bar{X}}{dt} = 0)$  on  $\bar{W}$ . See text. b Overshoot in connectivity (C = total area of overlap  $= \frac{1}{2} \sum_{i,j}^{N} A_{ij}$ ). Simulation with c = 0.1,  $\epsilon = 0.60$ , N = 64.

## Results

#### **Excitatory** Network

Overshoot. To understand the occurrence of overshoot, first consider a purely excitatory network (i.e., M=0). For a given connectivity (W need not be symmetric) this network has convergent activation dynamics [4]; the equilibrium points are solutions of:

$$0 = -X_i/\tau + (1 - X_i) \sum_{j}^{N} W_{ij} F(X_j) \qquad \forall i$$
(6)

If the variations in  $X_i$  are small (relative to  $\bar{X}$ , the average membrane potential of the network), we find [3]:

$$0 \simeq -\bar{X}/\tau + (1 - \bar{X})\bar{W}F(\bar{X}) \tag{7}$$

Based on this approximation, the average connection strength  $\bar{W}$  can be written as a function of  $\bar{X}$ :

$$\bar{W} = \frac{X/\tau}{(1-\bar{X})F(\bar{X})} \qquad \qquad 0 \le \bar{X} < 1 \tag{8}$$

which gives the steady state  $(\frac{d\bar{X}}{dt} = 0)$  dependence on  $\bar{W}$  (Fig. 1a).

The steady states are stable on the branches ABC and DEF, and unstable on CD. This hysteresis loop (whose presence hinges upon a sigmoidal firing-rate function) underlies the emergence of overshoot. The  $R'_is$ , and therefore  $\bar{W}$ , are governed by the system itself, being under control of neuronal activity. The size of a neuritic field remains constant if  $X_i = F^{-1}(\epsilon)$ , where  $F^{-1}$  is the inverse of F. Thus, the equilibrium point of the system is the intersection point of the line  $\bar{X} = F^{-1}(\epsilon)$  with the curve of Fig. 1a. Because the activity in the network is initially low ( $\bar{W} = 0$ ),  $\frac{dR_i}{dt}$  is positive and  $\bar{W}$  increases, whereby  $\bar{X}$  follows the branch ABCuntil it reaches  $w_2$ , where it jumps to the upper branch, thus exhibiting a phase transition from quiescent to activated state. The activity in the network is then however so high, that the neuritic fields begin to retract ( $\frac{dR_i}{dt} < 0$ ) and  $\bar{W}$  to decrease, whereby  $\bar{X}$  moves along the upper branch from E to the intersection point. Thus, in order to arrive at an equilibrium point on DE, a developing network has to go through a phase in which  $\bar{W}$  is higher than in the final situation, thus exhibiting a transient overshoot in  $\bar{W}$  (Fig. 1b).

Local behaviour. In a purely excitatory network all the cells are exactly identical except for their position. Local variations in cell density, however, suffice to generate a great variability among individual cells, with respect both to their neuritic field size at equilibrium (Fig. 2)





Fig.2. Excitatory networks. a Initial network and b at equilibrium. c At equilibrium, cells on grid. d Noise on grid. e High c results in sub-networks. f Same network but with lower c.

and to their developmental course of field size and firing behaviour. Cells surrounded by a high number of neighbouring cells tend to become small since a small neuritic field will already give sufficient overlap with other cells. In contrast, relatively isolated cells must grow large neuritic fields in order to contact a sufficient number of cells. One might say that the neuritic fields adapt to the available space so as to cover it optimally.

### Mixed Network

Overshoot. In the presence of inh-cells, overshoot is enhanced (Fig. 3a). To counteract the effect of inhibition, a larger (excitatory) connectivity is necessary for the network to become activated. Also the equilibrium connectivity level must be higher. If inhibition is strong (many inh-cells or a high synaptic strength from inh-to-exc cells), the electrical activity in the network remains so low that the cells keep growing out (increasing the exc-exc and inh-exc overlap). With moderate inhibition, complicated interactions are possible between oscillatory activity and outgrowth (not shown).

Even without differences in local cell density, the presence of inhibition generates variability among individual cells, e.g., with respect to their firing behaviour. Cells that receive inhibition become activated later and retract later than cells that do not receive inhibition. For the overshoot curve this means that after the onset of network activity the average connectivity can still increase considerably (in contrast to what is found in purely excitatory networks), because there may be cells that are still growing out, while others are already retracting.

Some studies have indicated that inhibition develops later in time than excitation [5]. If the development of inhibition is delayed by giving inh-cells a lower outgrowth rate, overshoot is



Fig.3. a Overshoot in exc-exc connectivity is larger with (N = 14, M = 2) than without (N = 14, M = 0) inhibition. b Exc-exc and c exc-inh connectivity when  $\rho$  of inh-cells is lower than that of exc-cells.



Fig.4. Mixed networks at equilibrium (torus boundary conditions). a, c Central cell (dashed line) is inhibitory. b Size of neuritic field (R) against position in network of a.

not enhanced (Fig. 3b), while now the growth curve of the number of *inhibitory* connections does not exhibit overshoot (Fig. 3c), because the inh-cells develop into a network that is already electrically active.

Local behaviour. Solely as the result of activity-dependent outgrowth and local cell interactions, the neuritic field of an inh-cell becomes smaller than that of an exc-cell. To illustrate this, consider a one-dimensional string of cells with only one inh-cell in the middle (Fig. 4a). Initially, all cells have the same size. At equilibrium, however, the inh-cell becomes the smallest cell, surrounded by two large exc-cells, which, in turn, are surrounded by smaller cells, and so on. Thus, even without local variations in cell density, a great and specific variability among individual cells is generated with respect to neuritic field size. Though more complex, essentially the same situation is obtained in the two-dimensional case (Fig. 4c). Clearly, the effect of an inh-cell is not restricted to its direct neighbours, but percolates through the network, so that a particular distribution of cell sizes is imposed. The mechanism causing cell sizes to differ is as follows. Since every cell tries to adjust its neuritic field so that  $F(x_i) = \epsilon$ [eqn(5)], cells connected to an inhibitory cell must grow large neuritic fields to receive sufficient excitation. As a result, large exc-cells will surround an inh-cell, whereas the inh-cell itself can remain small because a small neuritic field will already give sufficient overlap with its large surrounding cells.

# **Conclusions and Discussion**

The presence of activity-dependent neurite outgrowth was found to have implications both for the stages a network goes through during its development and for the structure of the stable network. During development, activity-dependent neurite outgrowth in combination with a nonlinear firing-rate is sufficient to cause an overshoot with respect to connectivity or synapse numbers. This overproduction is enhanced by the presence of inhibition (if it develops simultaneously with excitation). The results are robust under: different firing-rate functions (provided they have a type of firing threshold and low but none-zero values for sub-threshold membrane potentials, i.e., spontaneous activity); variance among neurons in all parameters; different neurite outgrowth functions; the way in which connections are defined; network size and different neuron models. Our model results are similar to those found in developing tissue cultures with respect to a transient overproduction in the numerical density of synapses [6] and the existence of a transition period wherein increasing electrical activity is associated with retraction of neurites [7]. Moreover, the model responds in a similar way to suppression or intensification of electrical activity [6]. In the model, the number of inhibitory connections exhibits no overshoot if inhibition develops later in time than excitation. The observation that in tissue cultures the putative inhibitory synapses (synapses on shafts) show hardly any overshoot, while the synapses on spines - which are probably mostly excitatory - show a clear overshoot [6], would thus be in agreement with a progressive increase in the ratio of effective inhibitory to excitatory synaptic activity during development, as suggested in [5].

The distribution of neuritic field sizes emerging in the stable network show that the fields of inhibitory cells become smaller than those of excitatory cells, even if both types of cells have exactly the same growth properties. Indeed, the (dendritic) fields of most of the inhibitory cells in the cortex are smaller than those of excitatory cells [e.g., 8]. The neuritic field size of the model cells adapts to the local cell density, much in the same way as the dendritic fields of ganglion cells in the retina [9].

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