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Implications of Activity Dependent Neurite Outgrowth for Neuronal Morphology and Network Development

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Empirical studies have demonstrated that electrical activity of the neuron can directly affect neurite outgrowth. In this paper, we study the possible implications of activity-dependent neurite outgrowth for neuronal morphology and network development, 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 on its own level of activity, and neurons become connected when their fields overlap. In a purely excitatory network, we have previously demonstrated that activity-dependent outgrowth in combination with a neuronal response function with some form of firing threshold is sufficient to cause a transient overproduction (overshoot) in the number of connections or synapses.

Here we show that overshoot still takes place in a network of excitatory and inhibitory cells, and can even be enhanced. With delayed development of inhibition the growth curve of the number of *inhibitory* connections no longer exhibits overshoot. An interesting emergent property of the model is that, solely as the result of simple outgrowth rules and cell interactions, the (dendritic) fields of the inhibitory cells tend to become smaller than those of the excitatory cells, even if both type of cells have the same outgrowth properties. Other consequences of the interactions among outgrowth, excitation and inhibition are that (i) the spatial distribution of inhibitory cells becomes important in determining the level of inhibition; (ii) pruning of connections can no longer take place if the network has grown without proper electrical activity for longer than a certain critical period; (iii) inhibitory cells, by inducing outgrowth, can help to connect different parts of a structure. Further, the model predicts that excitatory cell death will be accompanied by an increased neuritic field of surviving neurons (''compensatory sprouting''). The similarities of the model with findings in developing tissue cultures of dissociated cells are extensively discussed.

1. Introduction

In the course of development, neurons become assembled into functional neural networks. Among the many factors shaping the structure of these networks, electrical activity plays a pivotal role (for reviews see Fields & Nelson, 1992; Van Ooyen, 1994): many processes that determine synaptic connectivity and neuronal form are modulated by electrical activity; for example, neurite outgrowth and growth cone behaviour (e.g. Cohan & Kater, 1986; Fields *et al*., 1990*a*; Schilling *et al*., 1991); naturally occurring cell death (e.g. Oppenheim, 1991; Ferrer *et al*., 1992); production of trophic factors (e.g. Thoenen, 1991; Lu *et al*., 1991); synaptogenesis (e.g. Constantine-Paton, 1990); secondary elimination of synapses (e.g. Purves & Lichtman, 1980; Shatz, 1990); changes in synaptic strength (e.g. Madison *et al*., 1991; Tsumoto, 1992); and functional maturation and differentiation of neurons (e.g. Spitzer, 1991; Corner & Ramakers, 1992). As a result of these activity-dependent processes, a reciprocal influence exists between the formation of neuronal form and synaptic connectivity on the one hand, and neuronal and network activity on the other. Thus, a given network may generate activity patterns which modify the organization of the

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network, leading to altered activity patterns which further modify structural or functional characteristics, and so on. This feedback loop must be expected to have major implications not only for the mature network and neurons, but also for their ontogenetic stages. In this article we address the implications of one of these activity-dependent processes; namely, neurite outgrowth.

A number of studies have demonstrated that neurotransmitters and associated electrical activity can directly affect neurite outgrowth (for review see Mattson, 1988). Electrical activity of the neuron reversibly arrests neurite outgrowth or even produces retraction (Cohan & Kater, 1986; Fields *et al*., 1990*a*; Schilling *et al*., 1991; Grumbacher-Reinert & Nicholls, 1992). Similarly, depolarizing media and neurotransmitters affect neurite outgrowth of many cell types (e.g. Sussdorf & Campenot, 1986; McCobb *et al*., 1988; Lipton & Kater, 1989; Mattson & Kater, 1989; Todd, 1992; Neely, 1993), with excitatory neurotransmitters inhibiting outgrowth and inhibitory ones stimulating outgrowth (many of the effects of neurotransmitters on outgrowth are related to their effects on electrical activity—Mattson, 1988). In cultured embryonic hippocampal pyramidal neurons, for example, the excitatory neurotransmitter glutamate causes a dose-dependent reduction in dendritic length (Mattson *et al*., 1988) (axonal outgrowth is affected at higher concentrations), which can be antagonized by the inhibitory neurotransmitter GABA or the suppression of electrical activity with anticonvulsant drugs (Mattson, 1988). Dendritic outgrowth continues when neurons are exposed to GABA plus glutamate at a concentration of glutamate that normally causes dendritic regression (Mattson *et al*., 1987). In accordance with the effect of neurotransmitters, the outgrowth rates of both the axon and the dendrites are reduced when pyramidal neurons are exposed to culture media that cause membrane depolarization (Mattson *et al*., 1988). The outgrowth of inhibitory neurons may be likewise activity-dependent: Sanes & Takacs (1993) show that inhibitory neurons in the central auditory system fail to restrict their arborizations when neural activity is decreased.

The morphological responses to neurotransmitters and electrical activity are mediated by changes in intracellular calcium levels (Cohan *et al*., 1987; Kater *et al*., 1988; Mattson, 1988; Fields *et al*., 1990*b*; Kater *et al*., 1990; Kater & Guthrie, 1990; Kater & Mills, 1991). Voltage-sensitive calcium channels in the cell membrane open in response to depolarization and thus allow calcium influx to the cytoplasm (e.g. Kudo *et al*., 1987). Thus, high levels of activity, resulting in high intracellular calcium concentrations, would cause neurites to retract, whereas low levels of activity and consequently low calcium concentrations, would allow further outgrowth (Kater *et al*., 1990). In fact, a mechanism linking electrical activity, calcium and cytoarchitecture appears to be a universal property of various cell types (Mattson, 1988).

From these studies on neurite outgrowth, the realization is growing that electrical activity and neurotransmitters are not only involved in information coding, but may also play important roles in shaping neuronal form and in defining the structure of the networks in which they operate (Mattson, 1988; Lipton & Kater, 1989). Neuronal morphology results from the genetic potentialities along with environmental inputs (e.g. local cell interactions: Johnson *et al*., 1989; Clendening & Hume, 1990). To quote Mattson, 1988 (p. 207): "Excitatory and inhibitory neurotransmitters can interact to yield a net effect on neuronal morphology. In the intact nervous system a balance between these neurotransmitter inputs is probably important in maintaining circuits." The purpose of this article is to try to make more explicit, by means of simulation models, the possible implications of activity-dependent outgrowth and locally interacting excitatory and inhibitory cells for neuronal morphology and network development.

Previously, we have demonstrated that activity-dependent outgrowth, in combination with a neuronal response function possessing some form of firing threshold—a property which gives rise to a hysteresis effect—is sufficient to cause a transient overproduction (i.e. "overshoot") of connections or synapses in a developing neural network made up of only excitatory cells (Van Ooyen & Van Pelt, 1994). Overshoot phenomena constitute a general feature of nervous system development, *in vivo* as well as *in vitro*, and occur with respect to, for example, number of synapses (e.g. Purves & Lichtman, 1980; O'Kusky, 1985; Lnenicka & Murphy, 1989; *in vitro*: Van Huizen *et al*., 1985, 1987*a*), number of dendrites (Miller, 1988), number of axons (e.g. Heathcote & Sargent, 1985; Schreyer & Jones, 1988), and total dendritic length (Uylings *et al*., 1990).

In the present study, we consider networks that also contain inhibitory cells. Preliminary results of this study have been reported in Van Ooyen and Van Pelt (1993).

2. The Model

For the purpose of determining how much of the behaviour and organization of the network might be the result of *interactions* among excitation, inhibition

and outgrowth, we utilize relatively simple cells which all have the same intrinsic properties (e.g. growth rate). Except for their action on the membrane potential, excitatory and inhibitory cells are identical. The initially disconnected neurons organize themselves into a network under influence of endogenous activity (there is no external input). Growing neurons are modelled as expanding neuritic fields, and the outgrowth of each neuron depends upon its own level of electrical activity. Neurons become connected when their neuritic fields overlap. The model is inspired in part by tissue cultures of dissociated cerebral cortex cells (Van Huizen, 1986; Van Huizen *et al*., 1985, 1987*a*; Ramakers *et al*., 1991). Cells in such cultures become organized into a network by neurite outgrowth and synaptogenesis without the influence of external input.

2.1. NEURON MODEL

The shunting model (Grossberg, 1988; Carpenter, 1989) is used to describe neuronal activity. In this model, excitatory inputs drive the membrane potential towards a finite maximum (or saturation potential), while inhibitory inputs drive the membrane potential towards a finite minimum.

$$\frac{dx_i}{dt} = -\frac{x_i}{\tau} + (A - x_i)\sum_k^N w_{ik} f(x_k) - (B + x_i)\sum_l^M w_{il} f(y_l)$$

$$\frac{dy_j}{dt} = -\frac{y_j}{\tau} + (A - y_j)\sum_k^N w_{jk} f(x_k) - (B + y_j)\sum_l^M w_{jl} f(y_l), \quad (1)$$

where x_i is the (mean) membrane potential of excitatory cell i, y_j is the membrane potential of inhibitory cell j, N and M are the total number of excitatory and inhibitory cells, respectively, A and $-B$ are the saturation potentials, τ is the membrane time constant, w_{ik}, w_{il}, w_{jk}, w_{jl} are the connection strengths (all $w \geqslant 0$; k and l are the indices of the excitatory and inhibitory driver cells, respectively; i and j are the indices of the excitatory and inhibitory target cells, respectively) and $f(\,)$ is the mean firing rate. All potentials are relative to the resting potential, which is set to 0. Equation (1) is tranformed to the following set of (dimensionless) equations (Carpenter, 1983):

$$\frac{dX_i}{dT} = -X_i + (1 - X_i)\sum_k^N W_{ik} F(X_k) - (H + X_i)\sum_l^M W_{il} F(Y_l)$$

$$\frac{dY_j}{dT} = -Y_j + (1 - Y_j)\sum_k^N W_{jk} F(X_k) - (H + Y_j)\sum_l^M W_{jl} F(Y_l), \quad (2)$$

where

$$X_i = \frac{x_i}{A}$$

$$Y_i = \frac{y_i}{A}$$

$$T = \frac{t}{\tau}$$

$$F(u) = \frac{f(uA)}{f_{\max}}$$

$$W_{ij} = w_{ij}\tau f_{\max}$$

$$H = \frac{B}{A} \quad (3)$$

The firing rate function F is taken to be sigmoidal:

$$F(u) = \frac{1}{1 + e^{(\theta - u)/\alpha}}, \quad (4)$$

where α determines the steepness of the function and θ represents the firing threshold. The low firing rate when the membrane potential is sub-threshold represents spontaneous activity, arising from threshold or membrane potential fluctuations (Verveen, 1960) and synaptic noise (Korn & Faber, 1987; Otmakhov *et al*., 1993; also see Siebler *et al*., 1993).

2.2. OUTGROWTH AND CONNECTIVITY

Neurons are randomly placed on a two-dimensional surface. Each neuron is given a circular "neuritic field", the radius of which is variable. When two such fields overlap, both neurons become connected with a strength proportional to the area of overlap

$$w_{ij} \equiv A_{ij} s \quad (5)$$

where $A_{ij} = A_{ji}$ is the amount of overlap ($A_{ii} = 0$) and s is a constant of proportionality. A_{ij} represents the total number of synapses formed reciprocally between neurons i and j, and s the average synaptic strength. Strength may depend on the type of connection; in the transformed equations

$$W_{ij} = A_{ij} S, \quad (6)$$

where $S = s\tau f_{\max}$. We distinguish S^{ee}, S^{ei}, S^{ie}, and S^{ii}, which are constants representing the excitatory-to-excitatory, inhibitory-to-excitatory, excitatory-to-inhibitory, and inhibitory-to-inhibitory synaptic strengths, respectively (in S^{ei}, for example, e represents the target and i the driver cell).

In this abstraction, no distinction has been made between axons and dendrites. The connections among excitatory cells and among inhibitory cells are therefore symmetric. The whole connectivity matrix **W** would be symmetric if $S^{ei} = S^{ie}$. To test whether asymmetry affects the results, we use several ways of adding extra asymmetry in the network (see Section 4).

In the model, the outgrowth of each individual neuron, whether excitatory or inhibitory, depends in an identical way upon electrical activity. Since the effect of activity on outgrowth is mediated by intracellular calcium and the firing of action potentials leads, via depolarization and voltage-sensitive calcium channels, to calcium influx (e.g. Hockberger *et al*., 1989), we take the outgrowth to be dependent upon the firing rate

$$\frac{\mathrm{d}R_i}{\mathrm{d}T} = \rho G(F(X_i)), \tag{7}$$

where R_i is the radius of the circular neuritic field of neuron i, and ρ determines the rate of outgrowth. The outgrowth function G is defined as

$$G(F(X_i)) = 1 - \frac{2}{1 + \mathrm{e}^{(\epsilon - F(X_i))/\beta}}, \tag{8}$$

where ϵ is the value of $F(X_i)$ for which $G = 0$ and β determines the steepness of the function. The function G remains in the bounded range $\langle -1, 1\rangle$. Depending on $F(X_i)$, a neuritic field will grow out ($G > 0$ when $F(X_i) < \epsilon$), retract ($G < 0$ when $F(X_i) > \epsilon$) or remain constant ($G = 0$ when $F(X_i) = \epsilon$). Equation (8) is thus simply a phenomenological description of the theory of Kater *et al*. (Kater *et al*., 1990; Kater & Guthrie, 1990). According to eqn (8), inhibition can prevent retraction of neurites by suppressing electrical activity, which is in accordance with experimental findings (see Introduction). An admittedly unrealistic property of eqn (8) is that if $F(X_i) < \epsilon$, a neuron could grow out indefinitely. We saw no need, however, to put explicit bounds on the neuritic field size, because it appears that the network itself regulates the size of its neurons under most conditions. Note, that connection strength is not directly modelled but is a function of neuritic field size.

To summarize, each neuron is described by differential equations for both the membrane potential X and the radius of the neuritic field R. In total, the model thus consists of $2(N + M)$ differential equations. The connectivity matrix $\mathbf{W}[(N + M) \times (N + M)]$ is variable and is determined by calculating the degree of overlap of the neuritic fields. The model is studied both analytically and by means of numerical solution, employing the variable time step Runge–Kutta integrator provided by Press *et al*. (1988). The simplified model (Section 3.3) is analysed using GRIND (De Boer, 1983).

2.3. PARAMETERS

In most neural tissues, there are more excitatory than inhibitory cells. In the visual cortex of mammals, approximately 20% of all neurons are GABAergic (Meinecke & Peters, 1987; Somogyi, 1993). For the hippocampus, values in the range of 5–10% have been reported (e.g. Traub, 1987). We take $M/(N + M)$ mostly in the range of 0.1–0.2. For the rest, all the parameter values are the same for excitatory and inhibitory cells. Outgrowth of neurons is on a time scale of days or weeks (Van Huizen *et al*., 1985, 1987*a*; Van Huizen, 1986; Ramakers *et al*., 1991; Schilling *et al*., 1991), so that connectivity is quasi-stationary on the time scale of membrane potential dynamics (i.e. ρ much smaller than $1/\tau$). To avoid unnecessarily slowing down the simulations, ρ is chosen as large as possible so as to maintain the quasi-stationary approximation. In most simulations, we use $\rho = 0.0001$, and start with initially disconnected cells. The value of A is often about ten times larger than B (e.g. Hodgkin & Huxley, 1952). Hence we took $H = 0.1$. As nominal values for the other parameters, we chose $\theta = 0.5$, $\alpha = 0.10$, $\beta = 0.10$ and $\epsilon = 0.60$. Sometimes torus boundary conditions are used (which will be denoted in the figure captions).

3. Results

3.1. EXCITATORY NETWORK

Before the effects of inhibition are described, we summarize the previous results in excitatory networks ($M = 0$) (Van Ooyen & Van Pelt, 1994). For a given connectivity **W** the network has convergent activation dynamics (Hirsch, 1989); the equilibrium points are solutions of

$$0 = -X_i + (1 - X_i)\sum_{k}^{N} W_{ik}F(X_k) \quad \forall\, i, \tag{9}$$

If the variations in X_i are small (relative to $\bar{X}$,

the average membrane potential of the network), we find:

$$0 \simeq -\bar{X} + (1 - \bar{X})\bar{W}F(\bar{X}). \tag{10}$$

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

$$\bar{W} = \frac{\bar{X}}{(1 - \bar{X})F(\bar{X})} \quad 0 \leqslant \bar{X} < 1, \tag{11}$$

which gives the equilibrium manifold of $\bar{X}$ ($d\bar{X}/dT = 0$) as depending on $\bar{W}$ (hysteresis loop, Fig. 1). States on CD are unstable with respect to $\bar{X}$, the others are stable. At the intersection point with the line $\bar{X} = F^{-1}(\epsilon)$ (F^{-1} is the inverse of F), $\bar{W}$ remains constant; above and below that line, it decreases and increases, respectively [see eqn (8)]. Connectivity is quasi-stationary on the time scale of membrane potential dynamics, and, starting at A, $\bar{X}$ will follow the branch ABC, until it reaches C, where it jumps to the upper branch, thus exhibiting a transition from quiescent to activated state. If the equilibrium point is on DE, $\bar{W}$ decreases again, and 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 $\bar{W}$.

3.2. MIXED NETWORK

Overshoot

Simulation shows that overshoot still takes place in the presence of inhibition (Fig. 2), and can even be enhanced. To counterbalance inhibition, a higher excitatory connectivity is necessary to reach the point at which the average connectivity starts declining. Also the excitatory connectivity level in the stable network must be higher. If inhibition is too strong (many inhibitory cells or a high value of S^{ei}) the electrical activity in the network will remain so low that the cells keep growing out (increasing both excitatory–excitatory and inhibitory–excitatory connectivity). With moderate inhibition, oscillations can occur between excitatory and inhibitory activity. (Note that activity-dependent outgrowth plays no role in their generation; they take place on the time scale of the dynamics of the membrane potential). Because neuritic field sizes are also changing under these conditions, oscillatory activity can eventually disappear as a result of connectivity changes. The network then either goes to a stable situation or, if inhibition is too strong, will increase its connectivity indefinitely (also see Section 3.3).

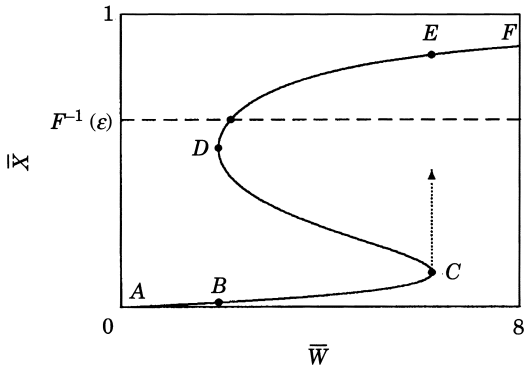

FIG. 1. Hysteresis in an excitatory network. Steady-state dependence ($d\bar{X}/dT = 0$) on $\bar{W}$ ($\bar{W} = (1/N)\Sigma_{i,k}^{N} W_{ik}$), according to eqn (11). See text, Section 3.1.

Onset of pruning

In excitatory networks with a more or less homogeneous cell density, the decline in connectivity begins shortly after the onset of network activity (Van Ooyen & Van Pelt, 1994). In mixed networks the decline in overall connectivity can be considerably delayed relative to the onset of network activity (Fig. 2). If the distribution of inhibitory cells is not strictly regular, the network may be subdivided into different parts depending on the proportion of inhibitory cells. In parts with many inhibitory cells, excitatory cells can still be growing out, while in parts with fewer inhibitory cells they are already retracting (this asynchrony in development becomes larger with stronger inhibition). For the overshoot curve this implies that average connectivity can still increase markedly after the onset of network activity.

"Critical period" for elimination of connections

Mixed networks grown under conditions in which electrical activity is blocked, thus inducing a high connectivity, do not necessarily reduce their connectivity after the block has been removed (Fig. 3). Although a restoration of activity occurs (possibly in the form of oscillations) this causes no reduction in connectivity: the average firing rate is below ϵ owing to inhibition, and connectivity will increase still further. The ability of the network to prune its connections appears to depend on the level of connectivity attained, and therefore on the time it spent under conditions of electrical silence. If this is longer than a certain critical period, elimination of connections can no longer take place (also see Section 3.3). On the other hand, blocking the activity in a normally developed network in which the process of elimination of connections have already occurred, will always result in an increase in connectivity

Compensatory sprouting

Various brain regions may lose neurons with aging (e.g. Curcio *et al.*, 1982). To study the effect of

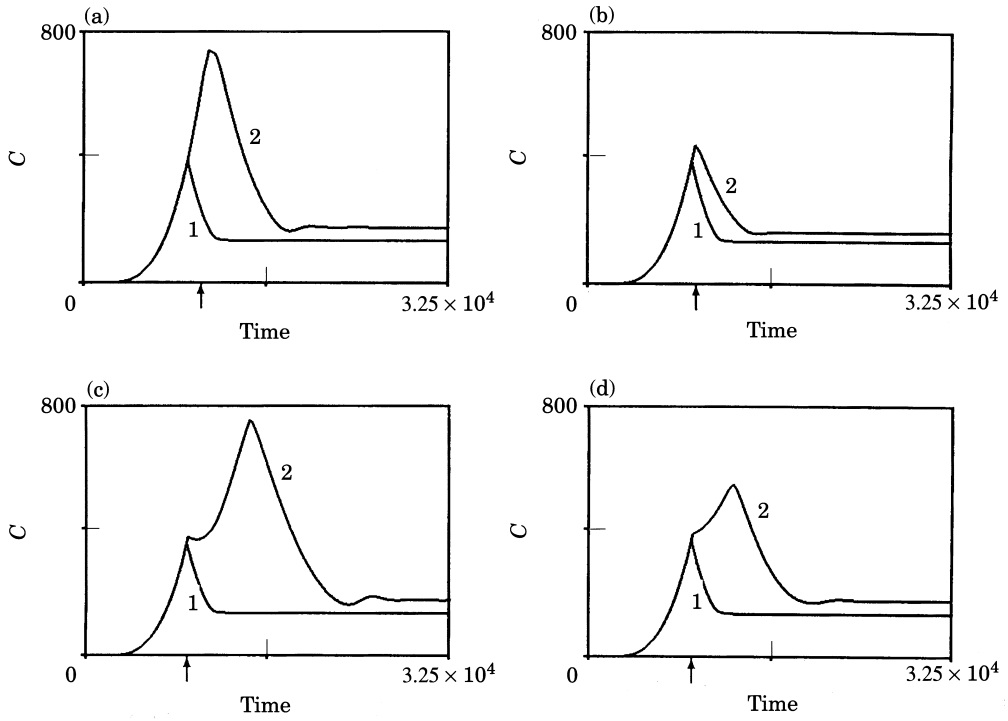

FIG. 2. Effect of inhibition on the development of connectivity. In all figures $N = 32$, $M = 4$ and $S^{ee} = S^{ie} = 0.6$. Total connectivity ($C = \Sigma_{p,q}^{N+M} A_{pq}$) in network (1) without inhibitory transmission ($S^{ei} = 0$, $S^{ii} = 0$) and (2) with inhibition ($S^{ei} \neq 0$, $S^{ii} \neq 0$). Arrows indicate the onset of network activity in the networks with inhibition. (a) $S^{ei} = 1.4$, $S^{ii} = 0.6$, (b) $S^{ei} = 1.0$, $S^{ii} = 0.6$; (c) , (d) $S^{ei} = 1.4$, $S^{ii} = 0.6$, but with diferent and less regular spatial distribution of inhibitory cells.

neuronal loss in the model, cells are progressively deleted in a mature network (each time, following the loss of some neurons, the network is allowed to stabilize). The average neuritic field size increases with the number of deleted neurons (Fig. 4). After excitatory cell loss, electrical activity decreases and cells (especially in the neighbourhood of the deleted cells) will begin to grow out until they all have the same activity level as before ($F(X_i) = \epsilon$). To compensate for the lost cells a larger neuritic (dendritic) field is necessary.

Delayed inhibition

The development of inhibition may lag that of excitation (Jackson *et al*., 1982; Barker & Harrison, 1988; Corner & Ramakers, 1992; Rörig & Grantyn, 1993). Using dissociated cell cultures from the superior colliculus of neonatal rat, Kraszewski & Grantyn (1992) show that the increasing efficacy of inhibitory synaptic transmission, observed during *in vitro* development, is primarily the result of presynaptic sprouting and a growing number of inhibitory contacts, rather than of synapse potentiation. Giving the inhibitory cells a lower outgrowth rate seems therefore a reasonable way to delay the development of inhibition in the model. Under these conditions, excitatory overshoot is not or less enhanced, while the growth curve of the number of *inhibitory* connections no longer exhibits overshoot (Fig. 5). The inhibitory cells develop into a network that has already a more or less stable electrical activity, and will therefore simply grow out until their overlap is such that $F(X_i) = \epsilon$.

The phenomenon of a critical period also occurs under delayed development of inhibition.

Network size

In excitatory networks with a low synaptic strength, cells develop into a single interconnected network whereas a high synaptic strength yields loosely connected sub-networks (Van Ooyen & Van Pelt, 1994). By inducing outgrowth, inhibitory cells increase the degree of connectivity: excitatory cells will need to grow larger neuritic fields to receive sufficient excitatory input; as a result, more cells make mutual contacts, and sub-networks that otherwise would have been relatively disconnected, now become tightly linked. For example, two disconnected, excitatory cell groups can become linked via excitatory-excitatory connections if inhibitory cells are present in one or both of the groups, or if some inhibitory cells are placed between the two groups.

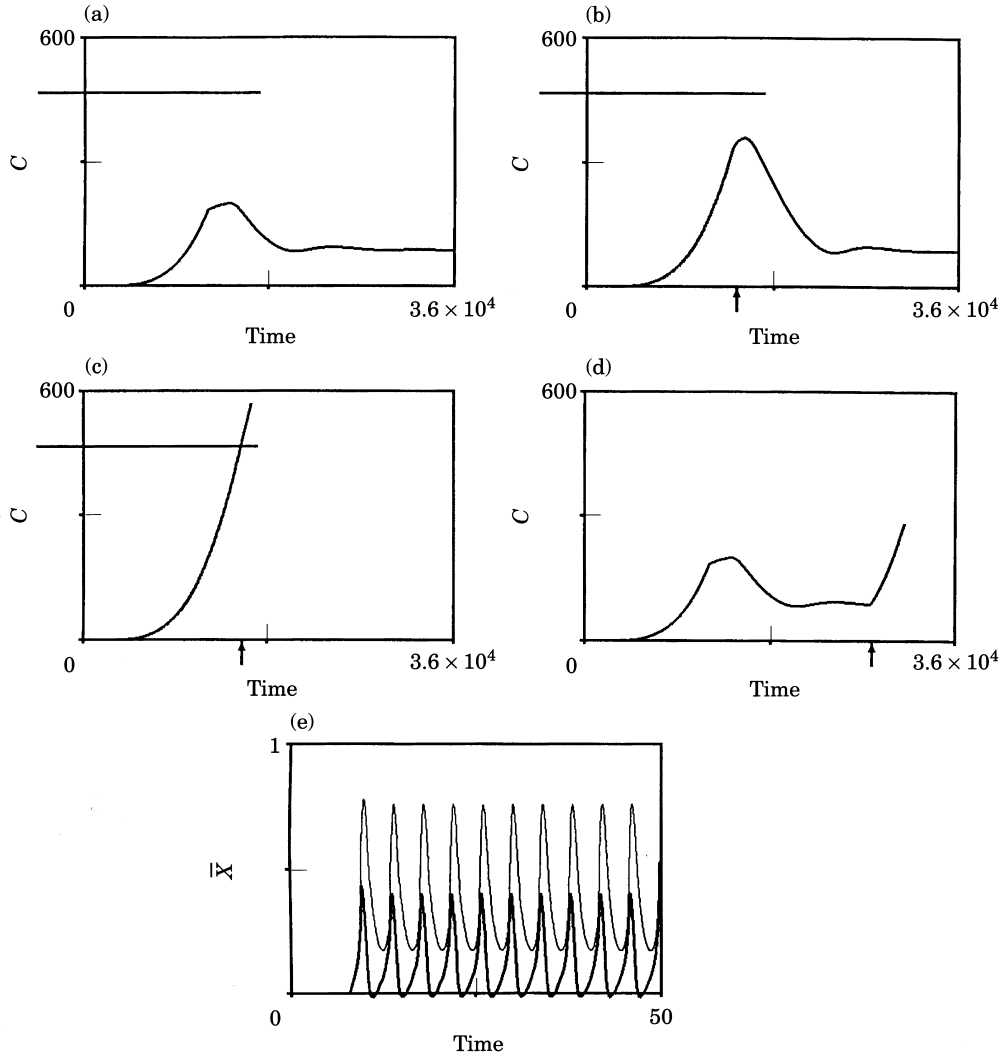

FIG. 3. Critical period for the elimination of connections. Mixed network with $N = 13$, $M = 3$, $S^{ee} = 0.6$, $S^{ei} = 1.1$, $S^{ie} = 0.6$ and $S^{ii} = 0$. C = total connectivity = $\Sigma_{p,q}^{N+M} A_{pq}$. (a) Normal development. (b), (c) The generation of electrical activity is blocked until the time indicated by the arrow. The horizontal line indicates the level of connectivity above which connectivity can no longer decrease when activity returns. Below this line activity returns without oscillations, or in the form of oscillations that gradually change (as connectivity changes) into high "constant" activity, followed by a normal decrease in activity and connectivity. Above this line, oscillations change into low "constant" activity (network becomes inhibited and cells keep growing out) as connectivity further increases. Starting at still higher connectivity values, the network comes directly in the inhibited state, without a transient oscillatory phase. (d) Activity is blocked in a normally developed network at the time indicated by the arrow. (e) The average membrane potential ($\bar{X}$) of the excitatory (thick line) and inhibitory population (thin line) just after removing the blockade in (c).

Neuritic field size

Although there are no intrinsic differences in growth properties between excitatory and inhibitory cells, their neuritic fields nevertheless differentiate. Solely as the result of simple outgrowth rules and cell interactions, the field of an inhibitory cell will tend to become smaller than that of an excitatory cell. Differences in size emerge irrespective of initial conditions (Fig. 6): inhibitory cells may initially have the same size as excitatory cells or may be introduced later, in an already well-advanced excitatory network. Let us consider a one-dimensional string of cells with one inhibitory cell (Fig. 7). During the initial period, all cells have the same size, but the moment the network becomes activated, cells will begin to become differentiated, such that the inhibitory cell ends up having the smallest neuritic field, adjacent to two large excitatory cells. The influence of an inhibitory cell is not restricted to its direct neighbours but, rather, percolates so that a characteristic distribution of cell sizes is induced. One inhibitory cell in a string of excitatory cells gives rise to a pattern of alternating small and

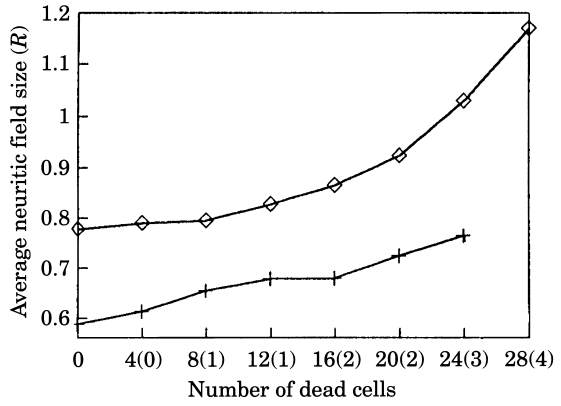

FIG. 4. Compensatory sprouting in response to cell death. Same network as in Fig. 2(a) ($N = 32$, $M = 4$) but with $S^{ee} = S^{ei} = S^{ie} = S^{ii} = 0.6$. Average field size of excitatory ($\diamond$) and inhibitory cells ($+$) against the total number of deleted cells (number of deleted inhibitory cells indicated in parentheses). Cells were deleted at random. Note that the effect of cell death becomes relatively larger as the number of remaining cells decreases.

large cells which gradually damps out (Fig. 8). A similar situation is obtained in the two-dimensional case: a kind of damping wave is generated in the region surrounding an inhibitory cell (Fig. 9). The exact form of the pattern depends also on how the cells are placed: on a grid, on a hexagonal field (not shown) or in a more randomized way. With more than one inhibitory cell, interference patterns are generated.

The mechanism causing cell sizes to differ is as follows. Each cell will attain a neuritic field size for which the input from overlapping cells is such that $F(X_i) = \epsilon$. An excitatory cell that receives inhibition needs, therefore, more excitatory input than does a cell that is not inhibited. Let a and b be two excitatory cells, with a but not b being connected to inhibitory cells. Assume that the connection strengths are such that an equilibrium exists. At equilibrium [see eqns (8) and (2)], $F(X_i) = F(Y_j) = \epsilon$, $X_i = Y_j = F^{-1}(\epsilon) \equiv \gamma$, and $\mathrm{d}X_i/\mathrm{d}T = \mathrm{d}Y_j/\mathrm{d}T = 0, \forall\, i, j$. We define [see eqn (2)] the total excitatory connectivity of a and b as $E_a = \Sigma_k^N W_{ak}$ and $E_b = \Sigma_k^N W_{bk}$, respectively, and the total inhibitory connectivity of a as $I_a = \Sigma_l^M W_{al}$ (and $I_b = 0$). Then, using eqn (2):

$$E_a = \frac{\gamma}{(1-\gamma)\epsilon} + \frac{(H+\gamma)I_a}{1-\gamma} > \frac{\gamma}{(1-\gamma)\epsilon} = E_b. \qquad (12)$$

Cell a must therefore grow a larger neuritic field than cell b (assuming a more or less homogeneous distribution of cells), in order to have sufficient overlap with other cells. As a consequence, an inhibitory cell will become surrounded by large excitatory

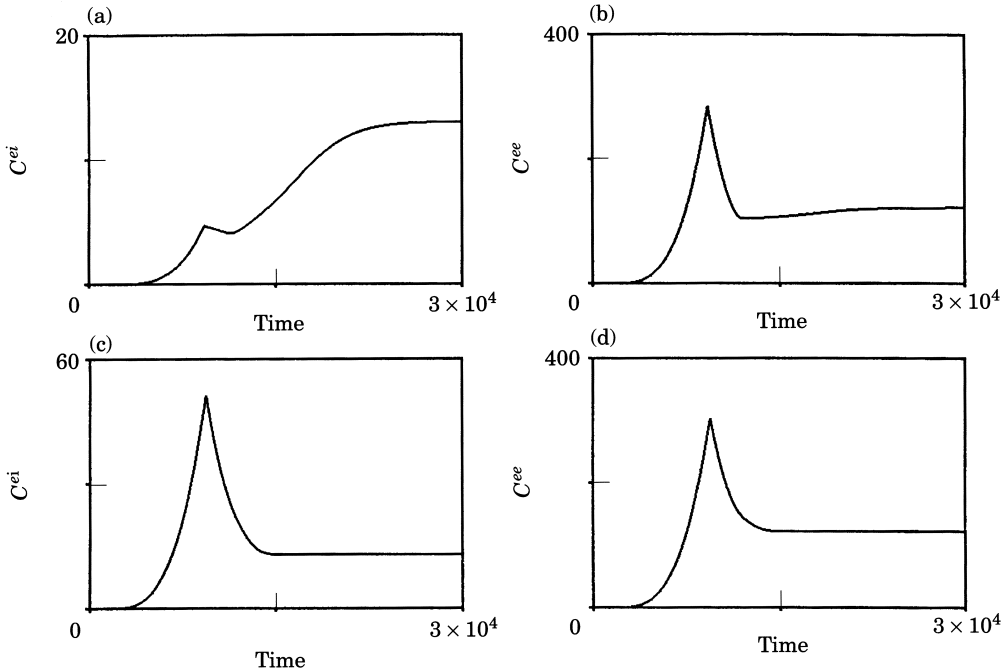

FIG. 5. Effect of delayed development of inhibition. Same network as in Fig. 4. In (a) and (b) the inhibitory cells have a lower outgrowth rate than the excitatory cells, $\rho = 0.00003$ and $\rho = 0.0001$, respectively. In (c) and (d) both type of cells have the same growth rate, $\rho = 0.0001$. In (a) and (c) the inhibitory–excitatory connectivity is shown ($C^{ei} = \Sigma_i^N \Sigma_l^M A_{il}$); (b) and (d) show excitatory–excitatory connectivity ($C^{ee} = \Sigma_{i,k}^N A_{ik}$).

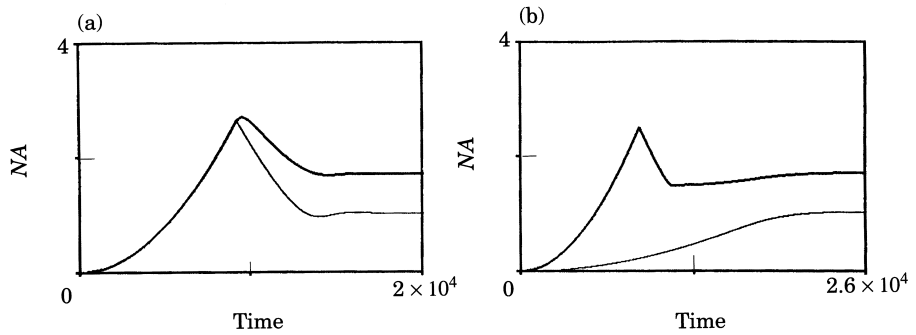

FIG. 6. The average neuritic field area (NA) of excitatory (thick lines) and inhibitory cells (thin lines). Same network as in Fig. 4, but with $S^{ei} = 1.4$ and torus boundary conditions. In (b) the inhibitory cells have a lower outgrowth rate than the excitatory cells, $\rho = 0.00003$ and $\rho = 0.0001$, respectively. Note that in (a) inhibitory cells develop without an overshoot in field size.

cells, whereas—since the same growth rules apply to inhibitory cells—the inhibitory cell itself can remain small because a small neuritic field yields sufficient overlap with its large surrounding cells. In other words, an inhibitory cell becomes small by increasing the size of its direct neighbours. If, however, an inhibitory cell is isolated, it may become larger than an excitatory cell in a dense part of the network.

The emergence of size differences does not hinge upon the exact values of the synaptic strengths: S^{ee}, S^{ei} and S^{ie} may be identical (Fig. 7) or different (Fig. 8).

Distribution of inhibitory cells

When inhibitory cells are able to contact each other, they are electrically inhibited (self-inhibition)

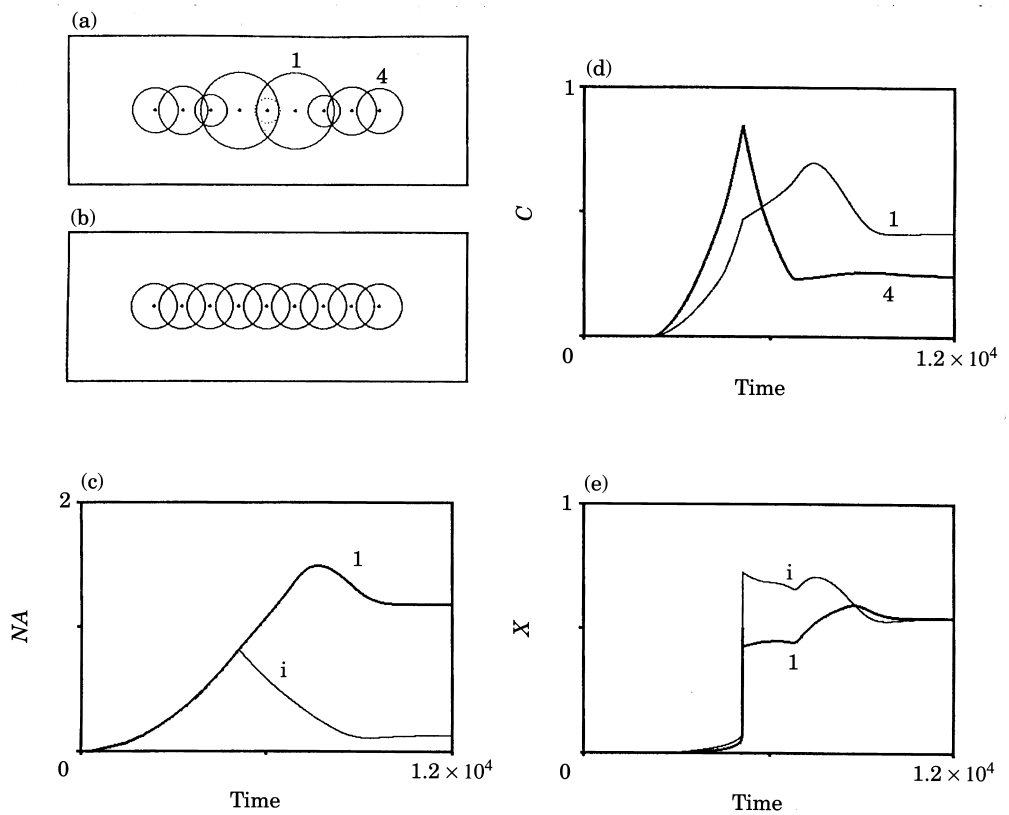

FIG. 7. Development of cell size differences. String of cells with $N = 8$, $M = 1$, $S^{ee} = S^{ei} = S^{ie} = 8{\cdot}0$, and torus boundary conditions. (a) Network at equilibrium. Central cell (dotted line) is inhibitory. (b) Same network without inhibition ($N = 9$, $M = 0$). (c) Neuritic field area (NA) of the inhibitory cell (i) and its directly neighbouring excitatory cell (cell 1). (d) The overlap of cell 1 with other excitatory cells ($\Sigma_k^N A_{1k}$), and of cell 4 with other excitatory cells ($\Sigma_k^N A_{4k}$). (e) Membrane potential of the inhibitory cell (i) and cell 1 (thick line).

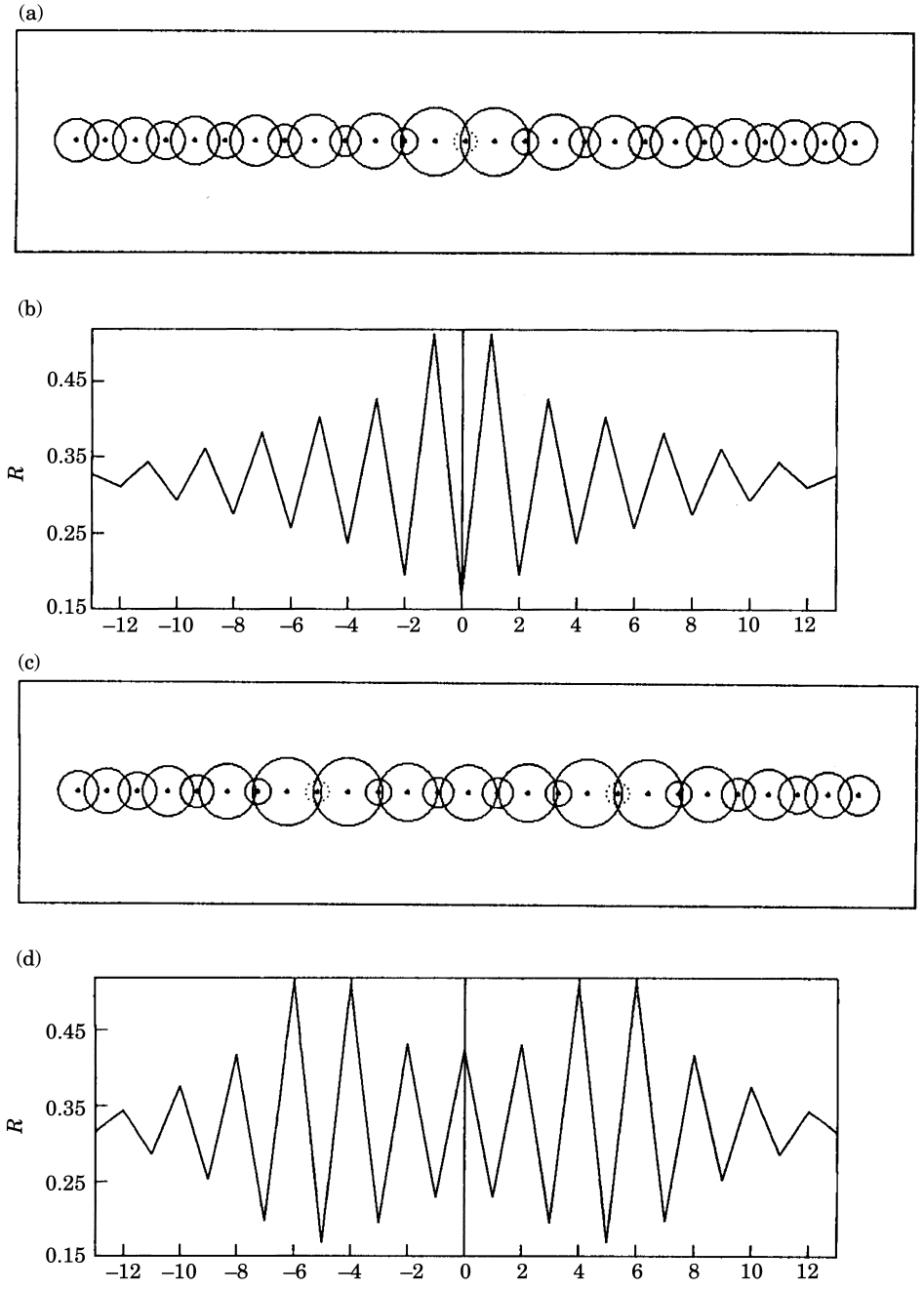

FIG. 8. Patterns imposed by inhibitory cells. $N = 26$, $M = 1$, $S^{ee} = S^{ie} = 15$, $S^{ei} = 2$, and torus boundary conditions. (a), (c) Dotted line indicate inhibitory cell. (b), (d) Radius of neuritic field (R) against position in network.

but their outgrowth will become stimulated. The ultimate level of inhibition will therefore become higher than without self-inhibition. Assume that the connection strengths are such that an equilibrium exists. At equilibrium [see eqns (8) and (2)], $F(X_i) = F(Y_j) = \epsilon$, $X_i = Y_j = F^{-1}(\epsilon) \equiv \gamma$, and $\mathrm{d}X_i/\mathrm{d}T = \mathrm{d}Y_j/\mathrm{d}T = 0$, $\forall\, i, j$. Define [see eqn (2)] the total excitatory-to-inhibitory connectivity as $W^{ie} = \Sigma_j^M \Sigma_k^N W_{jk}$, and the total inhibitory-to-inhibitory connectivity as $W^{ii} = \Sigma_j^M \Sigma_l^M W_{jl}$. Then, at equilibrium, W^{ie} in the presence of self-inhibition, $(W^{ie}|W^{ii} \neq 0)$, is larger than W^{ie} in the absence of self-inhibition $(W^{ie}|W^{ii} = 0)$:

$$(W^{ie}|W^{ii} \neq 0) = \frac{M\gamma}{(1-\gamma)\epsilon} + \frac{(H+\gamma)W^{ii}}{1-\gamma} > \frac{M\gamma}{(1-\gamma)\epsilon} = (W^{ie}|W^{ii} = 0). \quad (13)$$

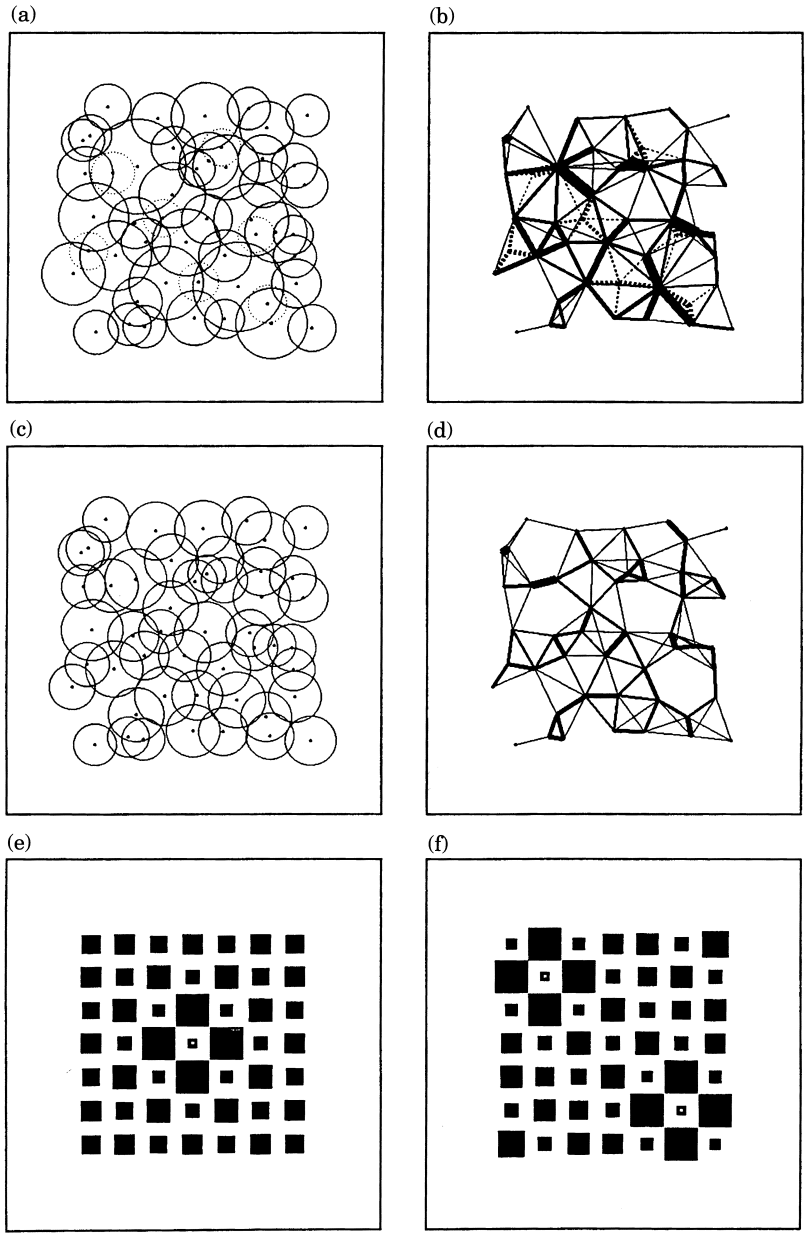

FIG. 9. Patterns imposed by inhibition. $S^{ee} = S^{ie} = 3$, $S^{ei} = 5$, $S^{ii} = 0$. Torus boundary conditions. (a) $N = 42$, $M = 7$. Cells on "noisy" grid positions. Dotted line indicate inhibitory cell. (b) Graph showing connections in network of (a). Line width is proportional to connection strength (connections that cross boundaries are not shown). Dashed line indicate connection between inhibitory and excitatory cell. (c) Same placing of cells as in, (a) but all former inhibitory cells are now excitatory ($M = 0$). (d) Graph showing connections in network of (c). (e) $N = 48$, $M = 1$. Cells on grid positions. Diameter of square is proportional to area of neuritic field. Scaled to maximum area. Cell with white dot is inhibitory. (f) Same as in (e) but with $M = 2$. Notice interference patterns.

If the connections are symmetric ($S^{ei} = S^{ie}$), $W^{ei} = \Sigma_i^N \Sigma_l^M W_{il}$ is equal to W^{ie}. In any case, W^{ei} is proportional to W^{ie} (since $A_{ij} = A_{ji}$), so that $(W^{ei}|W^{ii} \neq 0) > (W^{ei}|W^{ii} = 0)$ when $(W^{ie}|W^{ii} \neq 0) > (W^{ie}|\ W^{ii} = 0)$. To counterbalance a higher W^{ei}, the total connectivity among the excitatory cells, $W^{ee} = \Sigma_i^N \Sigma_k^N W_{ik}$, must also be higher (Fig. 10) for the network to become stable, since

$$W^{ee} = \frac{N\gamma}{(1-\gamma)\epsilon} + \frac{(H+\gamma)W^{ei}}{1-\gamma}. \tag{14}$$

In this way, not only the number of inhibitory cells is important but also their distribution. Note that long-range inhibition is obtained when inhibitory cells occur in a clustered fashion, so that they are in a position to stimulate each other's outgrowth.

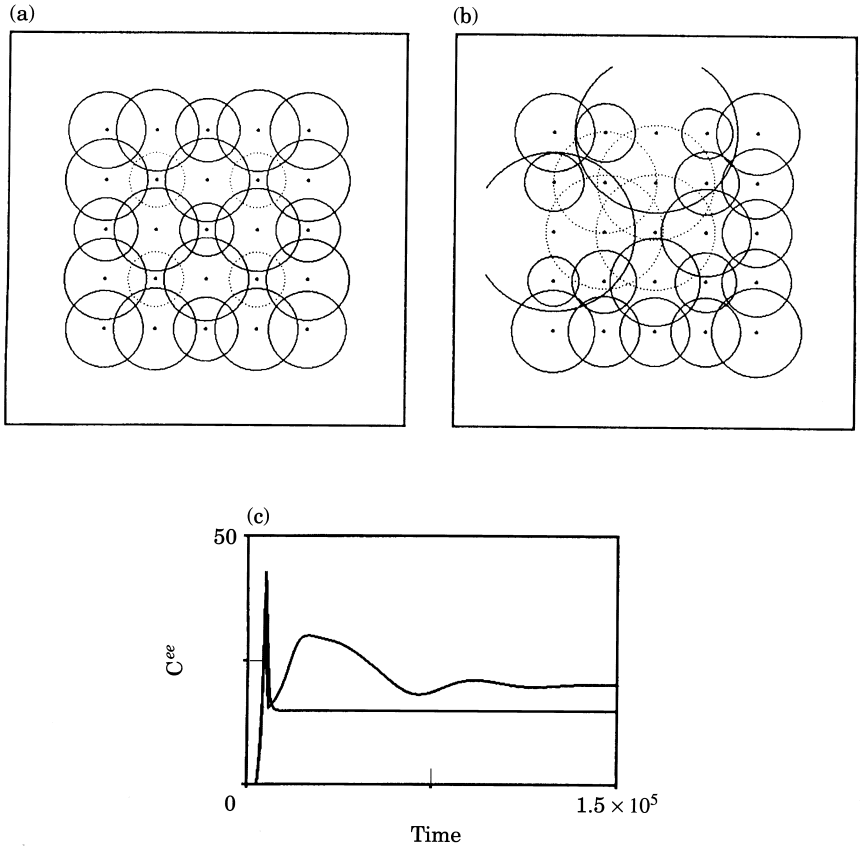

FIG. 10. Effect of distribution of inhibitory cells. Network on grid with $N = 21$, $M = 4$, $S^{ee} = S^{ie} = 3$, $S^{ei} = 1$, $S^{ii} = 2$. Dotted line indicate inhibitory cell. (a) Inhibitory cells regularly distributed. (b) Inhibitory cells clustered. (c) The connectivity from excitatory-to-excitatory ($C^{ee} = \Sigma_{i,k}^{N} A_{ik}$) cells is higher at equilibrium when cells are clustered and self-inhibition plays a role.

Differences among cells

In excitatory networks local variations in cell density suffice to generate variability among individual cells with respect to the developmental course of their field size and firing behaviour (Van Ooyen & Van Pelt, 1994). With inhibition, such variability is generated even without differences in cell density (Fig. 11). For example, cells that receive inhibition become activated later and will retract later than cells that do not receive inhibition. Outgrowth and interactions between excitation and inhibition can lead to complicated patterns of development in individual cells, since excitation increases activity but inhibits outgrowth, whereas inhibition does the opposite.

3.3. SIMPLIFIED MODEL

Aspects of the behaviour of the network can also be seen in a simplified model, which will be used to illustrate some of the effects of inhibition; it will be analysed in more detail elsewhere (Van Oss & Van Ooyen, in preparation). It consists of the following set of equations:

$$\frac{dX}{dT} = -X + (1 - X)WF(X) - (H + X)pWF(Y)$$

$$\frac{dY}{dT} = -Y + (1 - Y)pWF(X), \qquad (15)$$

where X and Y can be regarded as the average membrane potential of the excitatory and inhibitory population (provided the variations among the cells are small relative to the average values), respectively, or, alternatively, as the membrane potentials of two single cells, whereby the excitatory cell is connected to itself. We exclude self-inhibition, and assume that the connection between the excitatory and inhibitory unit is symmetrical. In the full network model, the excitatory–excitatory and excitatory–inhibitory connectivity are coupled: if an excitatory cell grows out this can lead to a larger overlap with both excitatory and inhibitory cells. This is taken account of in this model by assuming that the excitatory–inhibitory connection strength is proportional to the excitatory–excitatory connection strength (W); p also represents S^{ie} ($= S^{ei}$) of the full model. In the simplified model it is taken as a

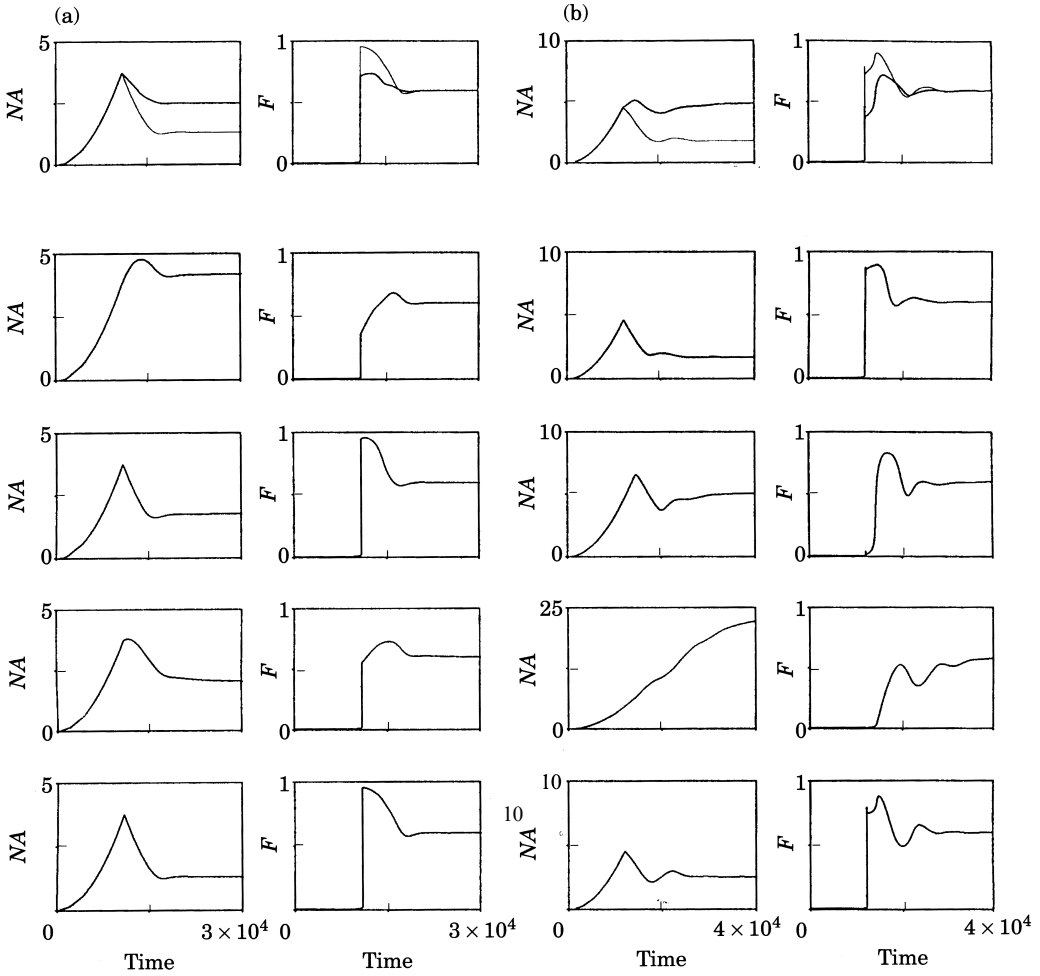

FIG. 11. Behaviour of some individual cells with respect to firing rate (F) and neuritic field area (NA) in a mixed network (a) without variation in local cell density, and (b) with variation. In the first row of (a) and (b) the average behaviour of the whole excitatory (thick lines) and inhibitory population (thin lines) are shown. The last row of (a) and (b) shows inhibitory cells. (a) Cells are placed on a hexagonal grid with torus boundary conditions. $N = 14$, $M = 2$, $S^{ee} = S^{ie} = S^{ii} = 0.6$, and $S^{ei} = 1.0$. (b) Same network as in Fig. 3.

constant. These simplifications enable us to study the equilibrium manifolds ($\mathrm{d}X/\mathrm{d}T = \mathrm{d}Y/\mathrm{d}T = 0$) as depending upon W. Activity-dependent changes of connectivity are not explicitly included in this model. In Fig. 12, the manifolds are drawn for different values of p. If inhibition is weak (p small) the manifold of X is similar to the one without inhibition. If inhibition is strong (p large) there is no hysteresis loop, and activity remains low [$< F^{-1}(\epsilon)$] for all W. If connectivity were made to change in an activity-dependent manner W would continue to increase under these conditions. For moderate inhibition strengths, network activity can be oscillatory (as in the full model) after the network has left the quiescent state, because the upper branch of the manifold can have a Hopf bifurcation at a value of W below that for which the jump occurs from quiescent to activated state. A better understanding of how a critical period for the elimination of connections can arise can also be obtained from this simplified model. For a moderate inhibition strength, there will exist a W^* such that a network grown without activity until $W > W^*$ will continue to increase W when (oscillatory) activity is allowed to return. If, on the other hand, activity returns at a smaller value of W, W will decrease. Although p must also be changing if connectivity is activity-dependent, the above argument is still valid because the general form of the manifolds do not change for small changes in p.

4. Robustness

The robustness of the results was tested under different parameter settings and some alternative formulations of the model (also see Van Ooyen & Van Pelt, 1994).

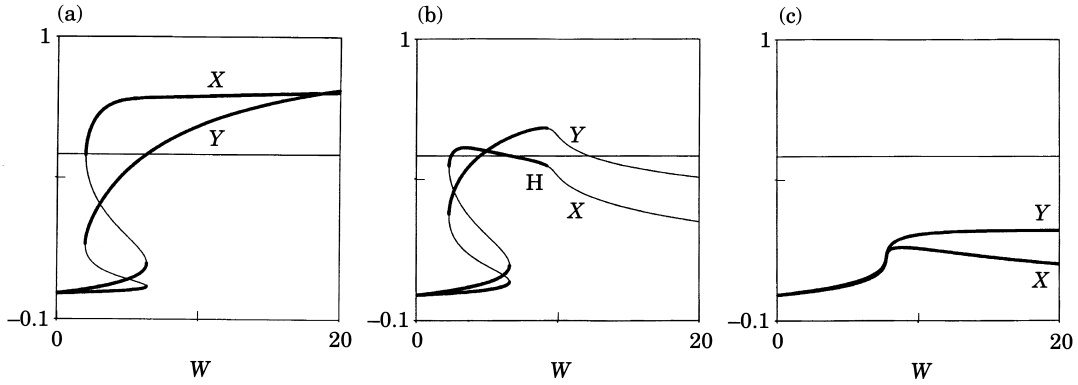

FIG. 12. Equilibrium manifolds of X and Y for different values of p [see eqn (15)]. Also drawn is the line $F^{-1}(0.6) = 0.54$. (a) $p = 0.20$. (b) $p = 0.40$. Hopf point is indicated (H). For higher W the unstable lines become stable (not shown). Also at higher W a stable and unstable branch appear (fold bifurcation). It is possible that this generates an extra stable equilibrium point in a model with growth, so that W, instead of increasing indefinitely, would stabilize at a high value. (c) $p = 0.77$.

FIRING RATE FUNCTION

With the following function exactly the same results are obtained.

$$F(u) = \begin{cases} \dfrac{1}{1 + e^{(\theta - u)/\alpha}} & \text{if } u \geqslant 0 \\ 0 \quad \text{if } u < 0 \end{cases}. \qquad (16)$$

The results are robust with respect to intrinsic differences (in α, θ and ϵ) between excitatory and inhibitory cells.

OUTGROWTH FUNCTION

The results are not dependent upon the specific outgrowth function [e.g. the value of β in eqn (8)] as long as $G > 0$ at low values of $F(X_i)$ and $G < 0$ at high values of $F(X_i)$. We are currently also studying bell-shaped functions whereby $G < 0$ for both high and low values of $F(X_i)$. This is not expected to change the main outcomes (provided that initially the activity is not so low that only retraction can take place) since only an unstable equilibrium point (with respect to outgrowth) is added. For effects of changing ϵ see Van Ooyen & Van Pelt (1994).

SYNAPTIC STRENGTH

The results do not depend on precise choices of the synaptic strengths. For example, if they are lower, the neuritic fields will grow larger, but inhibitory cells will still tend to become smaller than excitatory cells. In all simulations we took $S^{ie} = S^{ee}$; $S^{ie} > S^{ee}$ tend to enhance the size differences between excitatory and inhibitory cells, whereas $S^{ie} < S^{ee}$ does the opposite.

SATURATION POTENTIALS

Changing the value of H does not change the results, provided the synaptic strengths are changed accordingly.

ASYMMETRY AND DENDRITIC/AXONAL FIELD

With eqns (5) and (6), the connections among the excitatory and among the inhibitory cells are symmetric. One way of creating an asymmetric connectivity matrix is to draw the values of S for each separate i, j pair from uniform distributions (with means S^{ee}, S^{ei}, S^{ie} and S^{ii}); it does not affect the main findings. Another way is the use of separate axonal and dendritic fields. Let R_i^d be the radius of the dendritic field of cell i and R_i^a that of its axonal field. Equation (6) then becomes (dendritic field receives input from axonal field):

$$W_{ij} = O(R_i^d, R_j^a)S$$

$$W_{ji} = O(R_j^d, R_i^a)S, \qquad (17)$$

where $O(\,)$ gives the area of overlap. The growth of both type of fields is governed by eqn (8) whereby, in order to have axonal fields larger than dendritic fields, the growth rate of the latter is given a smaller value ($\rho^d < \rho^a$). This procedure, does not alter the general findings: inhibitory cells, for instance, still become smaller than excitatory cells [Fig. 13(a)]. Even if the axonal field of an inhibitory cell is kept at a constant (possibly large) size, its dendritic field becomes smaller than the fields of the excitatory cells [Fig. 13(b), (c)]. Note that a cell can regulate its activity only by adapting the size of its dendritic field. Excitatory cells receiving input via the (constant) axonal field of a neighbouring inhibitory cell will get a large dendritic and axonal field, so that the dendritic field of the inhibitory cell can remain small to get sufficient input. Thus it is essential that both the outgrowth of the "sending field" (axonal field, but see Discussion) of the excitatory cell, and the "receiving field" (dendritic field) of the inhibitory cell are activity-dependent.

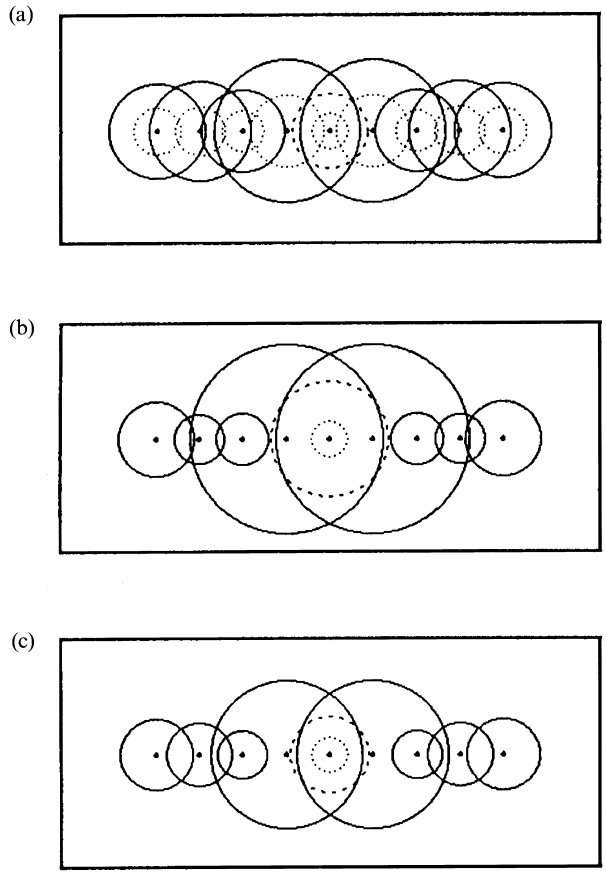

FIG. 13. Cells with separate axonal and dendritic fields. (a) String of cells with $N = 8$, $M = 1$, $S^{ee} = S^{ie} = 8.0$, $S^{ei} = 1.0$, and $\rho^a = 2\rho^d = 0.0001$. The dendritic fields are drawn with a dotted line, and the axonal field of the inhibitory cell with a dashed line. (b) Only the inhibitory cell has a separate axonal (dashed line) and dendritic field (dotted line). Its axonal field is kept at a fixed size ($R^a = 0.6$, $\rho^a = 0$); the outgrowth of its dendritic field is the same as that of the excitatory cells ($\rho = 0.0001$). $S^{ee} = S^{ie} = S^{ei} = 8.0$. (c) The same as in (b) but with $R^a = 0.4$.

5. Comparison with Empirical Data

OVERSHOOT

With respect to overshoot, the model shows similarities with developing cultures of dissociated nerve cells; namely, a transient overproduction in the numerical density of synapses in cerebral cortex cultures containing both excitatory and inhibitory neurons (Van Huizen *et al*., 1985, 1987*a*; Van Huizen, 1986) as well as the existence of a transition period wherein increasing electrical activity is associated with retraction of neurites (Schilling *et al*., 1991; also see Van Ooyen & Van Pelt, 1994). Chronically blocking inhibitory synaptic transmission in these cortex cultures does not result in a diminished overshoot. Thus, overshoot is not enhanced by inhibition, as can be the case in the model. Consistent with the effect of inhibition in the model is that cerebellar granule cells cultured in the presence of GABA exhibit a more complex network with more neurite-extending cells compared to those cultured in the absence of GABA (Hansen *et al*., 1984).

As in the model, a rather abrupt appearance of electrical activity (transition from quiescent to activated state) during development is observed in cultures of a variety of cell types: for example, hippocampal neurons (Siebler *et al*., 1993), striatal neurons (Dubinsky, 1989), spinal cord neurons (Jackson *et al*., 1982), brain-stem neurons (Corner & Crain, 1972), Purkinje cells (Schilling *et al*., 1991) and neocortex cells (Habets *et al*., 1987), and occur (as in the model) probably as the result of reaching a critical synapse density (Siebler *et al*., 1993; Schilling *et al*., 1991).

In mixed networks the decline in connectivity can be considerably delayed relative to the start of network activity [Fig. 2(c), (d)]. This is what is actually observed in neocortical cell cultures (Van Huizen *et al*., 1985): electrical activity is readily detectable after c. 12 days *in vitro*, whereas the overall decline in synapse numbers occurs only after about 18 days *in vitro* (continuing up to about 40 days *in vitro*). The decline in connectivity can occur earlier in excitatory networks than in mixed networks [Fig. 2(c), (d)]. This is in agreement with the observation in tissue culture that chronic blockade of GABAergic transmission advances the process of synapse elimination (Van Huizen *et al*., 1987*a*).

CRITICAL PERIOD

The results of the experiments done in the model with blocking of activity (see Section 3.2) show similarities with similar experiments done in cultures of dissociated cerebral cortex cells (Van Huizen *et al*., 1987*b*). If cultures in which electrical activity has been chronically blocked during development—resulting, as in the model, in an enhanced neurite outgrowth (Van Huizen & Romijn, 1987) and a prevention of synapse elimination (Van Huizen *et al*., 1985)—are then placed in control medium, no elimination of synapses occurs even over a period of several weeks, although electrical activity is restored. On the other hand, blocking the activity in normally developed cultures in which the process of synaptic elimination has already occurred results in a substantial increase in synapse density, pointing to a process of sprouting. These observations have been taken to indicate that there may exist a critical period after which electrically controlled *elimination* of connections is no longer possible (Van Huizen *et al*., 1987*b*).

COMPENSATORY SPROUTING

The model predicts that age-related excitatory cell loss (if it occurs) will be accompanied by an increased neuritic (dendritic) field of the surviving neurons. In human cortex the dendritic extent per neuron increases steadily through old age (Beull & Coleman, 1979; Coleman & Flood, 1986). It has been interpreted as a compensatory response to neuronal death (Curcio *et al*., 1982; Coleman & Flood, 1986). This is consistent with the observation that no increase of dendritic extent occurs in brain regions which do not lose neurons with age (Coleman *et al*., 1986).

DELAYED INHIBITION

The number of inhibitory connections will not exhibit overshoot if inhibition develops later than excitation. The observation that, in tissue cultures of dissociated cerebral cortex cells, the putative inhibitory synapses (synapses on shafts: Shepherd, 1990) show no pronounced overshoot during development, while the synapses on spines (which are mostly excitatory: Shepherd, 1990) do show a clear overshoot (Van Huizen *et al*., 1985), would thus be consistent with a progressive increase in the ratio of effective inhibitory to excitatory synaptic activity during development as suggested by Corner & Ramakers (1992; also see Jackson *et al*., 1982; Barker & Harrison, 1988; Rörig & Grantyn, 1993).

In the model, both inhibitory and excitatory connections will not be pruned if activity is blocked. This is in accordance with chronically silenced cultures, which show no decline in either spine or shaft synapses once the peak value has been reached (Van Huizen *et al*., 1985).

NEURITIC FIELD SIZE

The neuritic fields of inhibitory cells tend to become smaller than those of excitatory cells. In the cerebral cortex the dendritic (and axonal) fields of inhibitory neurons are indeed smaller, on the whole, than those of excitatory neurons. Two main types of neurons can be distinguished in the cerebral cortex: pyramidal cells and non-pyramidal cells (e.g. Kandel *et al*., 1991; Abeles, 1991). Pyramidal cells are excitatory and have large apical dendrites that often cross several layers; their axons are long, terminating in other areas of the cortex, and each axon has many collaterals which make synapses on neighbouring cells. The non-pyramidal cells, most of which are inhibitory, usually have smaller cell bodies, with dendritic and axonal branches that extend only locally.

6. Conclusions and Discussion

Many processes contributing to the proper development of neurons into functional networks are dependent upon electrical activity. In this study we further examined the possible consequences of activity-dependent neurite outgrowth, thereby focusing on the role of interactions among excitatory and inhibitory cells. Each cell in the model seeks to maintain its setpoint of electrical activity by means of adjusting the size of its neuritic field. This leads to a number of interesting properties:

- a transient overproduction of connections or synapses (overshoot), previously shown to occur in purely excitatory networks (Van Ooyen & Van Pelt, 1994), also occur in networks with inhibitory circuits;
- even without intrinsic growth differences between excitatory and inhibitory cells, the neuritic fields of the latter tend to become smaller;
- the distribution of inhibitory cells becomes important in determining the ultimate level of inhibition;
- in the presence of inhibition, sub-networks that otherwise would have remained disconnected can become connected;
- with a moderate level of inhibition, pruning of connections can no longer take place if the network has grown without electrical activity for longer than a certain time.

Our model can account for various (seemingly unrelated) phenomena in developing cultures of dissociated cells, to wit, (i) a sudden transition from a quiescent state to one of network activity; (ii) a transient overproduction of synapses; (iii) an enhanced neurite outgrowth and prevention of synapse elimination after chronically blocking electrical activity; (iv) different growth curves for synapses on shafts and on spines; (v) a delayed onset of the pruning phase relative to the onset of network activity; (vi) an advancement of synapse elimination after chronically blocking inhibitory transmission; (vii) a critical period for synapse elimination but not for synapse formation; and (viii) size differences between inhibitory and excitatory neurons. Experimental studies are now needed for testing the extent to which activity-dependent outgrowth indeed plays a role in the mechanisms underlying these phenomena.

This study demonstrates how, as the result of cell interactions, a differentiation with respect to size could arise between excitatory and inhibitory cells that have the same intrinsic growth properties. This

is, of course, not to say that intrinsic differences are not important, but they need not be present.

In the standard model we use neuritic fields, but the results are robust if one distinguishes between axons (''sending'') and dendrites (''receiving''). One should realize, however, that such a structure-function relation between axons and dendrites is not so simple (especially in developing neurons), considering the presence of dendro-dendritic synapses (Shepherd, 1990), bidirectional chemical synapses (Andersen, 1985), and synaptically triggered action potentials in dendrites (Regehr *et al*., 1993). If one makes a distinction, it is the *dendritic* field of inhibitory cells that become smaller than the fields of excitatory cells. This as the result of (at least) the activity-dependence of the axonal field of excitatory cells and the dendritic field of inhibitory cells (see Section 4).

The neuritic fields in the model are spatially isotropic (circle). We are currently studying the case in which each circular field is subdivided into separate neurites. Since the present study has demonstrated that activity-dependent outgrowth has considerable potential for controlling neuronal morphology, new (and possibly different) results may be expected in such a case.

A variant of the model in which neuritic field sizes are constant and (partly) overlapping, while the formation of new synapses is activity-dependent, is expected to lead to similar results: for example, excitatory cells would develop more (excitatory-to-excitatory) synapses if connected to inhibitory cells.

As in tissue cultures, the model cells have no external input. External excitatory input to a single cell will diminish its neuritic field size, whereas inhibitory input will have the opposite effect.

The model shows that for the network to develop properly it is important that the growth of connectivity be ''guided'' by electrical activity. If connectivity develops for longer than a certain period without concomitant electrical activity, pruning of exuberant connections is no longer possible. This may serve to illustrate that such a ''critical period'' need not be the result of predetermined cellular time schedules, but can arise as a result of non-linear neuron properties and cell interactions. It has been assumed here that synaptogenesis of both excitatory and inhibitory synapses progresses normally in the absence of electrical activity. There are, however, indications that the development of inhibitory synaptic transmission in cortical cell cultures is directly dependent upon the level of electrical activity (Ramakers *et al*., 1990; Corner & Ramakers, 1992).

The observation that the synapses on shafts (putative inhibitory ones) show no pronounced overshoot, together with the finding that chronic blockade of inhibition does not result in a diminished overshoot (Van Huizen *et al*., 1985), may point to a delayed development of inhibition: in the model, slower development of inhibition gives rise to precisely these effects. It would fit in with indications for a more rapid development of glutamatergic/aspartatergic (excitatory amino acids) relative to the GABAergic synaptic function in these cultures (Ramakers *et al*., 1991).

Two patterns of dendritic development have been described, a pattern of initial dendritic growth, followed by retraction and modification (overshoot), but also a monotonic pattern where dendritic arbors simply increase until their adult length is attained (e.g. Petit *et al*., 1988; Ulfhake *et al*., 1988). In the model, both patterns can be observed within one and the same network. A cell (excitatory or inhibitory) connecting to a structure that is not yet electrically active will exhibit overshoot in its growth curve, whereas a cell growing into structure that has a constant, non-zero level of activity fails to show overshoot (Van Ooyen & Van Pelt, 1994). This difference might in itself suffice to explain the existence of these two modes of dendritic development.

To explain how neuronal loss might induce dendritic proliferation in surviving neurons, Coleman & Flood (1986) suggested a mechanism whereby the death of a neuron brings about the release of a trophic factor that induces the proliferation of neighbouring dendrites. Alternatively, death of neurons could result in a reduced competition for afferent supply, which would allow dendrites to proliferate (Coleman & Flood, 1986). The mechanism emerging from the present model may provide a simpler alternative. Following excitatory cell death, the level of electrical activity drops, thus permitting outgrowth until all the cells have the same activity level as before. This mechanism is essentially the same as the one put forward by Mattson (1988). He proposes that loss of inputs as a consequence of cell death will result in a reduced availability of neurotransmitter, leading to resumed outgrowth until the dendrites encounter another terminal that releases transmitter, which will stop outgrowth.

In the model, inhibitory cells impose a structure on neighbouring excitatory cells. Interestingly, Lund *et al*. (1993) propose that inhibitory neurons may help to shape patchy and stripe-like connectivity patterns in different areas of macaque monkey cerebral cortex. They offer a conceptual model in which local circuit inhibitory basket interneurons (activated at the same time as pyramidal cells and colocalized with them) could veto pyramidal neuron connections within

either circular or stripe-like domains. The model requires the pyramidal neuron axon to "step over" a zone of inhibition. According to our model, inhibition would have exactly such an effect, since it favours outgrowth. Along the same lines, DeFelipe *et al*. (1990) suggest that the "double bouquet" cell, which is probably GABAergic, imposes a microcolumnar organization upon the cerebral cortex.

In summary, experimental results (mainly in isolated neurons) have indicated that neurotransmitters and associated electrical activity, by means of their effect on neurite outgrowth, have considerable potential for controlling the development of neuronal form and circuitry. In this study we have begun to explore this potential, and have shown that activity-dependent neurite outgrowth in a network of interacting excitatory and inhibitory cells can indeed have profound effects on both neuronal morphology and network development.

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REFERENCES

Abeles, M. (1991). *Corticonics. Neural Circuits of the Cerebral Cortex*. Cambridge: Cambridge University Press.

Andersen, P. A. (1985). Physiology of a bidirectional excitatory chemical synapse. *J. Neurophysiol*. **53,** 821–835.

Barker, J. L. & Harrison, N. L. (1988). Outward rectification of inhibitory postsynaptic currents in cultured rat hippocampal neurons. *J. Physiol*. **403,** 41–55.

Beull, S. J. & Coleman, P. D. (1979). Dendritic growth in the aged human brain and failure of growth in senile dementia. *Science* **206,** 854–856.

Carpenter, G. A. & Grossberg, S. (1983). A neural theory of circadian rhythms: the gated pacemaker. *Biol. Cybernet*. **48,** 35–59.

Carpenter, G. A. (1989) Neural network models for pattern recognition and associative memory. *Neural Networks* **2,** 243–257.

Clendening, B. & Hume, R. I. (1990). Cell interactions regulate dendritic morphology and responses to neurotransmitters in embryonic chick sympathetic preganglionic neurons *in vitro*. *J. Neurosci*. **10,** 3992–4005.

Cohan, C. S. & Kater, S. B. (1986). Suppression of neurite elongation and growth cone motility by electrical activity. *Science* **232,** 1638–1640.

Cohan, C. S., Connor, J. A. & Kater, S. B. (1987). Electrically and chemically mediated increases in intracellular calcium in neuronal growth cones. *J. Neurosci*. **7,** 3588–3599.

Coleman, P. D. & Flood, D. G. (1986). Dendritic proliferation in the aging brain as a compensatory repair mechanism. *Prog. Brain Res*. **70,** 227–237.

Coleman, P. D., Buell, S. J., Magagna, L., Flood, D. G. & Curcio, C. A. (1986). Stability of dendrites in cortical barrels of C57B1/6N mice between 4 and 45 months. *Neurobiol. Aging* **7,** 101–105.

Constantine-Paton, M. (1990). NMDA receptor as a mediator of activity-dependent synaptogenesis in the developing brain. *Cold Spring Harbor Symp. Quant. Biol*. **55,** 431–443.

Corner, M. A. & Crain, S. M. (1972). Patterns of spontaneous bioelectric activity during maturation in culture of fetal rodent medulla and spinal cord tissues. *J. Neurobiol*. **3,** 25–45.

Corner, M. A. & Ramakers, G. J. A. (1992). Spontaneous firing as an epigenetic factor in brain development—physiological consequences of chronic tetrodotoxin and picrotoxin exposure on cultured rat neocortex neurons. *Devl Brain Res*. **65,** 57–64.

Curcio, C. A., Buell, S. J. & Coleman, P. D. (1982). Morphology of the aging nervous system. In *The Aging Motor System*, *Advances in Neurogerontology*, Vol. 3 (Mortimer, J. A., Pirozzolo, F. J. & Maletta, G. J., eds), pp. 7–35. New York: Praeger.

De Boer, R. J. (1983). GRIND: Great Integrator Differential Equations. University of Utrecht, The Netherlands: Bioinformatics Group.

DeFelipe, J., Hendry, S. H. C., Hashikawa, T., Molinari, M. & Jones, E. G. (1990). A micro-columnar structure of monkey cerebral cortex revealed by immunocytochemical studies of double bouquet cell axons. *Neuroscience* **37,** 655–673.

Dubinsky, J. M. (1989). Development of inhibitory synapses among striatal neurons *in vitro*. *J. Neurosci*. **9,** 3955–3965.

Ferrer, I., Soriano, E., Del Rio, J. A., Alcantara, S. & Auladell, C. (1992). Cell death and removal in the cerebral cortex during development. *Prog. Neurobiol*. **39,** 1–43.

Fields, R. D., Neale, E. A. & Nelson, P. G. (1990*a*). Effects of patterned electrical activity on neurite outgrowth from mouse neurons. *J. Neurosci*. **10,** 2950–2964.

Fields, R. D., Guthrie, P. B., Nelson, P. G. & Kater, S. B. (1990*b*). Calcium homeostatic capacity is regulated by patterned electrical activity in growth cones of mouse DRG neurons. *Soc. Neurosci. Abstr*. **16,** 457.

Fields, R. D. & Nelson, P. G. (1992). Activity-dependent development of the vertebrate nervous system. *Int. Rev. Neurobiol*. **34,** 133–214.

Grossberg, S. (1988) Nonlinear neural networks: principles, mechanisms, and architectures. *Neural Networks* **1,** 17–61.

Grumbacher-Reinert, S. & Nicholls, J. (1992). Influence of substrate on retraction of neurites following electrical activity of leech Retzius cells in culture. *J. expl Biol*. **167,** 1–14.

Habets, A. M. M. C., Van Dongen, A. M. J., Van Huizen, F. & Corner, M. A. (1987). Spontaneous neuronal firing patterns in fetal rat cortical networks during development *in vitro*: a quantitative analysis. *Expl Brain Res*. **69,** 43–52.

Hansen, G. H., Meier, E. & Schousboe, A. (1984). GABA influences the ultrastructure composition of cerebellar granule cells during development in culture. *Int. J. Devl Neurosci*. **2,** 247–257.

Heathcote, R. D. & Sargent, P. B. (1985). Loss of supernumary axons during neuronal morphogenesis. *J. Neurosci*. **5,** 1940–1946.

Hirsch, M. W. (1989). Convergent activation dynamics in continuous time networks. *Neural Networks* **2,** 331–349.

Hockberger, P. E., Tsen, H.-Y. & Connor, J. A. (1989). Fura-2 measurements of cultured rat Purkinje neurons show dendritic localization of Ca^{2+} influx. *J. Neurosci*. **9,** 2272–2284.

Hodgkin, A. L. & Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol*. **117,** 500–544.

Jackson, M. B., Lecar, H., Brenneman, D. E., Fitzgerald, S. & Nelson, P. G. (1982), Electrical development in spinal cord cell culture. *J. Neurosci*. **2,** 1052–1061.

Johnson, M. I., Higgins, D. & Ard, M. D. (1989). Astrocytes induce dendritic development in cultured sympathetic neurons. *Devl Brain Res*. **47,** 289–292.

Kandel, E. R., Schwartz, J. H. & Jessel, T. M. (1991). *Principles of Neural Science*. London: Prentice-Hall, International.

Kater, S. B., Mattson, M. P., Cohan, C. & Connor, J. (1988). Calcium regulation of the neuronal growth cone. *Trends Neurosci*. **11,** 315–321.

Kater, S. B & Guthrie, P. B. (1990). Neuronal growth cone as an integrator of complex environmental information. *Cold Spring Harbor Symp. Quant. Biol*. **55,** 359–370.

Kater, S. B., Guthrie, P. B. & Mills, L. R. (1990). Integration by the neuronal growth cone: a continuum from neuroplasticity to neuropathology. In *Progress in Brain Research*, *Vol*. 86,

Molecular and Cellular Mechanisms of Neuronal Plasticity in Normal Aging and Alzheimer's disease (Coleman, P. D., Higgins, G. A. & Phelps, C. H., eds), pp. 117–128.

KATER, S. B. & MILLS, L. R. (1991). Regulation of growth cone behaviour by calcium. *J. Neurosci.* **11,** 891–899.

KORN, H. & FABER, D. S. (1987). Regulation and significance of probalistic release mechanisms at central synapses. In *Synaptic functions* (Edelman, W. M., Gall, W. & Cowan, W. M., eds), pp. 57–108. New York: John Wiley and Sons.

KRASZEWSKI, K. & GRANTYN, R. (1992). Development of GABAergic connections *in vitro*: increasing efficacy of synaptic transmission is not accompanied by changes in miniature currents. *J. Neurobiol.* **23,** 766–781.

KUDO, Y., ITO, K., MIYAKAWA, H., IZUMI, Y., OGURA, A. & KATO, H. (1987). Cytoplasmic calcium elevation in hippocampal granule cell induced by perforant path stimulation and L-glutamate application. *Brain Res.* **407,** 168–172.

LIPTON, S. A. & KATER, S. B. (1989). Neurotransmitter regulation of neuronal outgrowth, plasticity and survival. *Trends Neurosci.* **12,** 265–270.

LNENICKA, G. A. & MURPHY, R. K. (1989). The refinement of invertebrate synapses during development. *J. Neurobiol.* **20,** 339–355.

LU, B., YOKOYAMA, M., DREYFUS, C. F. & BLACK, I. B. (1991). Depolarizing stimuli regulate nerve growth factor gene expression in cultured hippocampal neurons. *Proc. natn. Acad. Sci. U.S.A.* **88,** 6289–6292.

LUND, J. S., YOSHIOKA, T. & LEVITT, J. B. (1993). Comparison of intrinsic connectivity in different areas of macaque monkey cerebral cortex. *Cerebral Cortex* **3,** 148–162.

MADISON, D. V., MALENKA, R. C. & NICOLL, R. A. (1991). Mechanisms underlying long-term potentiation of synaptic transmission. *A. Rev. Neurosci.* **14,** 379–397.

MATTSON, M. P., DOU, P. & KATER, S. B. (1987). Pruning of hippocampal pyramidal neuron dendritic architecture *in vitro* by glutamate and a protective effect of GABA plus diazepam. *Soc. Neurosci. Abstr.* **13,** 367.

MATTSON, M. P. (1988). Neurotransmitters in the regulation of neuronal cytoarchitecture. *Brain Res. Rev.* **13,** 179–212.

MATTSON, M. P., DOU, P. & KATER, S. B. (1988). Outgrowth-regulation actions of glutamate in isolated hippocampal pyramidal neurons. *J. Neurosci.* **8,** 2087–2100.

MATTSON, M. P. & KATER, S. B. (1989). Excitatory and inhibitory neurotransmitters in the generation and degeneration of hippocampal neuroarchitecture. *Brain Res.* **478,** 337–348.

MCCOBB, D. P., HAYDON, P. G. & KATER, S. B. (1988). Dopamine and serotonin inhibition of neurite elongation of different identified neurons. *J. Neurosci. Res.* **19,** 19–26.

MEINECKE, D. L. & PETERS, A. (1987). GABA immunoreactive neurons in rat visual cortex. *J. comp. Neurol.* **261,** 388–404.

MILLER, M. W. (1988). Development of projection and local circuit neurons in neocortex. In *Cerebral cortex. Development and Maturation of the Cerebral Cortex*, *Vol.* 7 (Peters, A. & Jones, E. G., eds), pp. 133–175. New York: Plenum Press.

NEELY, M. D. (1993). Role of substrate and calcium in neurite retraction of leech neurons following depolarization. *J. Neurosci.* **13,** 1292–1301.

O'KUSKY, J. R. (1985). Synapse elimination in the developing visual cortex: a morphometric analysis in normal and dark-reared cats. *Devl Brain. Res.* **22,** 81–91.

OPPENHEIM, R. W. (1991). Cell death during development of the nervous system. *A. Rev Neurosci.* **14,** 453–501.

OTMAKHOV, N., SHIRKE, A. M. & MALINOW, R. (1993). Measuring the impact of probabilistic transmission on neuronal output. *Neuron* **10,** 1101–1111.

PETIT, T. L., LEBOUTILLIER, J. C., GREGORIO, A. & LIBSTUG, H. (1988). The pattern of dendritic development in the cerebral cortex of the rat. *Devl Brain Res.* **41,** 209–219.

PRESS, W. H., FLANNERY, B. P., TEUKOLSKY, S. A. & VETTERLING, W. T. (1988) *Numerical Recipes in C. The Art of Scientific Computing*. Cambridge: Cambridge University Press.

PURVES, D. & LICHTMAN, J. W. (1980). Elimination of synapes in the developing nervous system. *Science* **210,** 153–157.

RAMAKERS, G. J. A., CORNER, M. A. & HABETS, A. M. M. C. (1990). Development in the absence of spontaneous bioelectric activity results in increased burst firing in cultures of dissociated cerebral cortex. *Expl Brain Res.* **79,** 157–166.

RAMAKERS, G. J. A., RAADSHEER, F. C., CORNER, M. A., RAMAEKERS, F. C. S. & VAN LEEUWEN, F. W. (1991). Development of neurons and glial cells in cerebral cortex, cultured in the presence or absence of bioelectric activity: morphological observations. *Eur. J. Neurosci.* **3,** 140–153.

REGEHR, W., KEHOE, J., ASCHER, P. & ARMSTRONG, C. (1993). Synaptically triggered ation potentials in dendrites. *Neuron* **10,** 145–151.

RÖRIG, B. & GRANTYN, R. (1993). Glutamergic and GABAergic synaptic currents in ganglion cells from isolated retinae of pigmented rats during postnatal development. *Devl Brain Res.* **74,** 98–110.

SANES, D. H. & TAKACS, C. (1993). Activity-dependent refinement of inhibitory connections. *Eur. J. Neurosci.* **5,** 570–574.

SCHILLING, K., DICKINSON, M. H., CONNOR, J. A. & MORGAN, J. I. (1991). Electrical activity in cerebellar cultures determines Purkinje cell dendritic growth patterns. *Neuron* **7,** 891–902.

SCHREYER, D. J. & JONES, E. G. (1988). Axon elimination in the developing corticospinal tract of the rat. *Devl Brain Res.* **38,** 103–119.

SHATZ, C. J. (1990). Impulse activity and the patterning of connections during CNS development. *Neuron* **5,** 745–756.

SHEPHERD, G. M. (1990). *The Synaptic Organization of the Brain*. Oxford: Oxford University Press.

SIEBLER, M., KÖLLER, H., STICHEL, C. C., MÜLLER, H. W. & FREUND, H-J (1993). Spontaneous activity and recurrent inhibition in cultured hippocampal networks. *Synapse* **14,** 206–213.

SOMOGYI, P. (1993). The GABAergic synapse in neuronal circuits of the brain. *Neurosci. Facts* **4(2),** 5–6.

SPITZER, N. C. (1991). A developmental handshake: neuronal control of ionic currents and their control of neuronal differentiation. *J. Neurobiol.* **22,** 659–763.

SUSSDORF, W. S. & CAMPENOT, R. B. (1986). Influence of the extracellular potassium environment on neurite growth in sensory neurons, spinal cord neurons and sympathetic neurons. *Devl Brain Res.* **25,** 43–52.

THOENEN, H. (1991). The changing scene of neurotrophic factors. *Trends Neurosci.* **14,** 165–170.

TRAUB, D. R., MILES, R. & WONG, R. K. S. (1987). Models of synchronized hippocampal bursts in the presence of inhibition. I. Single population events. *J. Neurophysiol.* **58,** 739–751.

TODD, R. D. (1992). Neural development is regulated by classical neurotransmitters: dopamine D2 receptor stimulation enhances neurite outgrowth. *Biol. Psychiat.* **31,** 794–807.

TSUMOTO, T. (1992). Long-term potentiation and long-term depression in the neocortex. *Prog. Neurobiol.* **39,** 209–228.

UYLINGS, H. B. M., VAN EDEN, C. G., PARNAVELAS, J. G. & KALSBEEK, A. (1990). The prenatal and postnatal development of rat cerebral cortex. In: *The Cerebral Cortex of the Rat* (Kolb, B. & Trees, R. C., eds). Cambridge, MA: MIT Press.

ULFHAKE, B., CULLHEIM, S. & FRANSON, P. (1988). Postnatal development of cat hind motoneurons. I. Changes in length, branching structure and spatial distribution of dendrites of cat triceps surae motoneurons. *J. comp. Neurol.* **278,** 69–87.

VAN OOYEN, A. & VAN PELT, J. (1993). Implications of activity-dependent neurite outgrowth for developing neural networks. In *Proceedings of ICANN'93* (Gielen, S. & Kappen, B., eds), pp. 177–182. Berlin: Springer Verlag.

VAN OOYEN, A. & VAN PELT, J. (1994). Activity-dependent outgrowth of neurons and overshoot phenomena in developing neural networks. *J. theor. Biol.* **167,** 27–43.

VAN OOYEN, A. (1994). Activity-dependent neural network development. *Network*, 401–423.

VAN HUIZEN, F. (1986). Significance of Bioelectric Activity for Synaptic Network Formation. PhD Thesis, University of Amsterdam.

VAN HUIZEN, F. & ROMIJN, H. J. (1987). Tetrodotoxin enhances initial neurite outgrowth from fetal rat cerebral cortex cell *in vitro*. *Brain Res*. **408,** 271–274.

VAN HUIZEN, F., ROMIJN, H. J. & HABETS, A. M. M. C. (1985). Synaptogenesis in rat cerebral cortex is affected during chronic blockade of spontaneous bioelectric activity by tetrodotoxin. *Devl Brain Res*. **19,** 67–80.

VAN HUIZEN, F., ROMIJN, H. J., HABETS, A. M. M. C. & VAN DEN HOOFF, P. (1987*a*). Accelerated neural network formation in rat cerebral cortex cultures chronically disinhibited with picrotoxin. *Expl Neurol*. **97,** 280–288.

VAN HUIZEN, F., ROMIJN, H. J. & CORNER, M. A. (1987*b*). Indications for a critical period for synapse elimination in developing rat cerebral cortex cultures. *Devl Brain Res*. **13,** 1–6.

VERVEEN, A. A. (1960). On the fluctuations of threshold of the nerve fibre. In *Structure and Function of the Cerebral Cortex* (Tower, D. B. & Schade, J. P., eds). Amsterdam: Elsevier.