Chapter 1

General Introduction: Activity-Dependent Neural Network Development

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Electrical activity plays a pivotal role in the development of neurons into functional neural networks. Besides changes in synaptic strength, many other processes are activity-dependent. They influence neuronal function and connectivity, on multiple time scales as well as on multiple levels of specificity, both during development and in adulthood. A number of these processes, including neurite outgrowth, are reviewed here, together with some simulation models. In the next chapters of this thesis, the consequences of activity-dependent neurite outgrowth for network development are explored by means of simulation models.

1.1 Introduction

In the course of development neurons become assembled into functional neural networks. Electrical activity plays a central role in this process. Most studies, theoretical as well as empirical, have largely focussed on activity-dependent changes in synaptic efficacy. Many other processes that determine connectivity and neuronal function are, however, also modulated by electrical activity: e.g., naturally occurring cell death, trophic factor production and responsiveness, number and effectiveness of transmitter receptors, neurite outgrowth and growth cone behaviour, neuronal morphology, gene expression and differentiation of neurons, neuron-glia interactions, synaptogenesis and secondary elimination of synapses. A review of these activity-dependent processes is given in this chapter.

As a result of these processes, a reciprocal influence or feedback loop (a characteristic of self-organizing systems) exists between the development of neuronal form, function and connectivity on the one hand, and neuronal and network activity on the other hand (also see Van Pelt *et al.*, 1994). A given network may generate activity patterns which modify the organization of the network, leading to altered activity patterns which may further modify structural or functional characteristics, and so on (e.g., Von der Malsburg & Singer, 1988). Furthermore, in the initial stages of development, activity patterns that are not evoked by external input (endogenous or 'spontaneous' activities: see Corner, 1990) play an important role, as has been demonstrated in a considerable number of cell and tissue culture experiments (for review see Corner 1994).

A developing network is thus a dynamic system in which the structure, number of elements, and functional characteristics of the elements are modifiable, in part being under control of the system's own electrical activity. The interactions between electrical activity and developmental processes are expected to have important implications for the emergence of network organization. In this thesis we focus on one of these interactions, namely that between electrical activity and neurite outgrowth.

Empirical studies have demonstrated that high levels of neuronal electrical activity cause neurites to retract, whereas low levels allow further outgrowth. Although it has been realized that activity-dependent neurite outgrowth could have considerable potential for controlling neuronal form and circuitry, the possible implications have not previously been made explicit. The aim of this thesis is to explore these implications by means of mathematical simulation models.

The organization of this chapter is as follows. In Sections 1.2, 1.3, 1.4 and 1.5 is described how various developmental processes depend upon electrical activity, in order to situate neurite-outgrowth within a broader context. In Section 1.6 the empirical studies on neurite outgrowth are reviewed, and in Section 1.7 the model studies. Section 1.8 contains the outline of this thesis. A summary and discussion of this chapter is found in Section 1.9.

1.2 Cell Death and Trophic Factors

During development, up to about twice as many neurons are produced in many structures as will survive into adulthood (e.g., Oppenheim, 1991). This process of overproduction followed by death occurs in regions of the peripheral as well as central nervous system. The survival of neurons depends to a great extent on whether or not they have obtained adequate amounts of specific neurotrophic factors (Purves, 1988; Oppenheim, 1991), which seem to act by suppressing an intrinsic cell suicide mechanism (Johnson & Deckwerth, 1993). The survival requirements of developing neurons include factors that are produced by their target cells. These are taken up through the synaptic membrane of presynaptic cells, and reach the soma by retrograde axoplasmic transport. In addition to affecting survival, trophic influences can affect axonal and dendritic arborization, the production and choice of neurotransmitter, the expression of receptors, and a variety of electrical membrane properties, both in development and in adulthood (for references see Clarke, 1991).

The synthesis of neurotrophic factors is regulated by electrical activity. For example, the synthesis of the neurotrophins NGF (nerve growth factor) and BDNF (brain derived neurotrophic factor) by neurons in the central nervous system is up-regulated via the excitatory neurotransmitter glutamate and down-regulated via the inhibitory neurotransmitter GABA (Zafra *et al.*, 1990; Lu *et al.*, 1991; Thoenen, 1991). The responsiveness to trophic stimulation may also be regulated by electrical activity, since depolarization increases the expression of the receptors for neurotrophic factors (Black, 1993). Moreover, different excitatory receptor subtypes mediate trophic or *regressive* influence: stimulation of ionotropic receptors (see Section 1.3) decreases survival, whereas stimulation of metabotropic receptors is necessary for survival (Black, 1993; for a review on the trophic effects of excitatory neurotransmitters and depolarization, see Balázs *et al.*, 1992). Electrical activity and trophic factors may interact via levels of intracellular calcium ($[Ca^{2+}]_{in}$). Depolarization causes Ca^{2+} entry, and neurons that have low $[Ca^{2+}]_{in}$ are more dependent on trophic factors than those with high $[Ca^{2+}]_{in}$ (Koike & Tanaka, 1991). The expression of endogenous BDNF, which enhances cell survival, is increased by the activation of voltage-sensitive Ca^{2+} channels (Ghosh *et al.*, 1994). At very high $[Ca^{2+}]_{in}$, however, neuronal death is increased. In connection with these observations, the 'calcium set-point hypothesis' of neuronal survival and dependence on neurotrophic factors has been proposed (Franklin & Johnson, 1992), which states that (i) at $[Ca^{2+}]_{in}$ substantially below resting levels, neurons do not survive, even in the presence of neurotrophic factors; (ii) at normal $[Ca^{2+}]_{in}$ neurons survive only if adequate amounts of trophic factors are available; (iii) at modestly elevated $[Ca^{2+}]_{in}$ neurons will survive even in the absence of trophic factors; and (iv) at very high $[Ca^{2+}]_{in}$ neuronal death is increased. Finally, both the uptake of trophic substances and the transport through axons might themselves be

activity-dependent, the former also via the activity-dependent regulation of neurite outgrowth and branching patterns (see Section 1.6), which influences the number of synapses and thereby the attainment of trophic substances.

In addition to the mechanisms described above, by which the electrical activity in afferent nerve fibres modulates the effects of retrogradely transported neurotrophic factors, the afferents themselves may also produce specific neurotrophic factors that are anterogradely transported through their axons and synaptically released, possibly in an activity-dependent manner (Linden, 1994).

The neurotrophic factors that are retrogradely transported through an axon may affect not only the parent neuron but also the synapses impinging on it. Cutting axons can cause withdrawal of synapses from the damaged cells (for references see Clarke, 1991). Neurons deprived of retrograde trophic support will then in turn fail to provide trophic support to the neurons one stage further upstream. Similarly, anterograde trophic effects might be transmitted further downstream. Thus, there could exist a network of trophic interactions comparable in complexity to neural networks based on electrical activity, with both systems reciprocally influencing each other at multiple levels (Clarke, 1991).

The roles of trophic interactions and neuronal death are most pronounced during development: establishment of neuronal circuits, formation of morphogenetic patterns, control of neuronal numbers, matching the number of presynaptic and postsynaptic cells, and eliminating wrongly connected cells (Oppenheim *et al.*, 1992; Raff, 1992). The rapid regulation of neurotrophic substances by neuronal activity, however, allowing the conversion of short-term signaling into long-term changes, suggests that their function is much more than merely regulating neuronal survival during development (Thoenen, 1991; also see Lindsay *et al.*, 1994). Trophic interactions may also be involved in memory and learning in adulthood (Thoenen, 1991; Black 1993; Tancredi *et al.*, 1993).

1.2.1 Models

Understanding the implications of trophic interactions at the network level requires simulation models (Clarke, 1991). In Galli-Resta & Resta (1992) a model for the regulation of cell death has been developed. Cells are generated according to a specific scheme and die around a certain age unless trophic influence saves them. By assuming that trophic interactions between connected structures are mutual, i.e., trophic factors are supplied from the target to the input cells and vice versa, apparently conflicting experimental findings with respect to the regulation of target and input size could be reconciled. The model predicts both outcomes that support the size-matching hypothesis (i.e., a linear relationship between target and input size) and outcomes that strongly deviate from this linear relationship.

Trophic interactions are used in a model of the establishment of connec-

tions between motor neurons and muscle fibres (Rasmussen & Willshaw, 1993: a revised model of that of Bennett & Robinson, 1989). During development of the nervous system, the axons of motor neurons branch into a number of terminals, so that each muscle fibre is connected by terminals from several different neurons. Withdrawal of terminals takes then place until each fibre is innervated by a single axon. In the model, competition for a postsynaptic (trophic) substance produced by each fibre together with competition for a presynaptic substance lead to such a single innervation pattern, as well as to a maximal number of terminals that a neuron can maintain (there is no neuronal death nor effects of activity). Intrinsic withdrawal, as observed in some experiments, appears as a side effect of the competitive mechanism. For related models, see references in Rasmussen & Willshaw (1993).

Trophic interactions are also used in a model of the mapping of neurons from spinal segments onto targets in the periphery (Liestøl *et al.*, 1993). In the model, each postsynaptic cell produces a limited amount of trophic factor so that more connections onto a cell leads to a reduced survival chance of each connection. An increase in the number of connections of a given presynaptic cell also decreases the survival chance of each connection. Together with chemoaffinity (no activity-dependent processes are assumed) these interactions generate an innervation pattern resembling that of the biological system. In the model, the final pattern is sensitive to the initial distribution of presynaptic fibres onto their targets. This sensitivity is markedly reduced if neurons are allowed to die depending on the sum of the survival chances of their connections.

In a model for the self-organization of neural assemblies into clusters of cells with characteristic, input-related responses, synapses degenerate unless they receive sufficient trophic factor. This factor is distributed from postsynaptic cells under an activity-dependent Hebb-like rule (Kerszberg *et al.*, 1992). A pattern of connections emerges with short-range excitation and longer-range inhibition. Synaptic stabilization based on post- and presynaptic trophic factors is also used in a model of the self-organization of cortical maps (Tanaka, 1990).

In developing neural networks, the number of elements of the system itself changes, as new cells are added and others may die. This could lead to novel dynamics. In Kaneko (1994) and Kaneko & Yomo (1994) systems with a growing number of elements are considered. Cells compete for a resource term which is supplied from the environment. The ability to obtain this depends on the internal state of the cell, and cells divide and die depending on their state. During development, simultaneous death of many cells is seen (due to *interaction* among cells) resembling 'programmed' cell death in many biological systems.

Neuronal death and pruning of connections may have conceptual links with structural plasticity in artificial neural networks in which, in contrast to conventional learning algorithms, the architecture of the network itself (number of elements, pattern of connections) varies during learning, e.g. in Koho-

nen networks (Fritzke, 1991) and feedforward networks (Refenes & Vithlani, 1991; Hirose *et al.*, 1991; Romaniuk & Hall, 1993; Odri *et al.*, 1993; Bartlett, 1994; Nabhan & Zomaya, 1994; Fahner & Eckmiller, 1994).

1.3 Receptors and Ion Channels

The roles of ion channels and neurotransmitter receptors in mediating rapid electrical signalling have been appreciated for a long time. More recently it has been recognized that they are also involved in much slower processes. Via neurotransmitter release and receptor actions, electrical activity can (slowly) modulate itself, and can even influence neuronal differentiation.

Transmitter receptors can be divided into two families according to how the receptor and effector function (i.e., gating ion channels) are coupled (Schwartz & Kandel, 1991). In the family of gated ion channel or ionotropic receptors, the recognition site and channel are different parts of the same protein. This family includes several glutamate receptor types (AMPA, kainate, NMDA) and the GABA receptors, which mediate neuronal excitation and inhibition, respectively. The family of G-protein coupled or metabotropic receptors contains the serotonin, dopamine and neuropeptide receptors, but also one of the glutamate receptor types (Schoepp *et al.*, 1990). Here, recognition is carried out by a separate molecule that is coupled via a G-protein to an effector molecule, which typically produces a diffusible second messenger (e.g., cAMP). This second messenger in turn triggers changes in a variety of proteins.

Second messengers can induce the opening or closure of ion channels (in some instances the G-protein can act directly on an ion channel), thus modulating the signalling properties of the cell. These changes, which depend on transiently elevated concentrations of second messenger, last from seconds to minutes, usually much longer than the changes in membrane potential produced by ionotropic receptors. As second messengers can diffuse intracellularly, they can affect channels in distant parts of the cell (also see Kasai & Petersen, 1994).

Another class of proteins upon which second messengers act are neurotransmitter receptors (of both families, so that interactions between ionotropic and metabotropic receptors - e.g., different types of glutamate receptors - are possible). In this way, a receptor can regulate its own effectiveness, as well as that of a receptor for another neurotransmitter. For example, after prolonged exposure to its own transmitter, a receptor can become desensitized. Also the number of receptors can be regulated, usually on a time scale of hours (for references see Shaw *et al.*, 1989) with down-regulation following agonist stimulation or increases in electrical activity, and up-regulation following antagonist treatment or decreases in electrical activity. This type of regulation is not restricted to G-protein coupled receptors. In adult rat neocortex, for example, an increase in electrical activity or agonist stimulation decreases the

number of AMPA receptors (a type of glutamate receptors) while increasing the number of GABA receptors (Shaw & Scarth, 1991; Shaw & Lanius, 1992; for the effects of depolarization on AMPA receptors in cerebellar granule cells, see Condorelli *et al.*, 1993). Such control of receptor number and sensitivity may be regarded as a form of homeostasis of neuronal activity (also see Turrigiano *et al.*, 1994).

Through second messenger systems, electrical activity can act on proteins that regulate the expression of genes (Armstrong & Montminy, 1993), including genes related to channels and receptors. This can cause more enduring effects in neuronal excitability lasting days or weeks, as well as persistent changes in cellular function (neuronal differentiation). Stimulation of gene expression often occurs via Ca^{2+} dependent mechanisms. Changes in $[Ca^{2+}]_{in}$ can occur either through second messengers mobilizing Ca^{2+} ions from intracellular stores, or directly via Ca^{2+} flux through NMDA channels and voltage-sensitive Ca^{2+} channels which open upon membrane depolarization (in this way, the activity of ionotropic receptors can also lead to long-lasting changes). Already the early differentiation of neurons is dependent upon neurotransmitter actions and membrane polarization (for references see Harris, 1981). Ion flux in immature cells triggers later steps of development, including developmental changes in ion channels and receptors (Spitzer, 1991). For example, Ca^{2+} influx elicited by depolarization influences neurotransmitter levels, the choice of neurotransmitter phenotype and properties of potassium channels (for references see Spitzer, 1991). Electrical activity may be crucial for the full maturation of inhibitory transmission (in cultured rat neocortex: Corner & Ramakers, 1992; in mouse cerebellar cultures: Seil & Drake-Baumann, 1994). In cultured cerebellar granule cells, glutamate stimulates GABA receptor mRNA expression, whereas treatment with NMDA receptor antagonists does the opposite (Memo *et al.*, 1991). In adult monkey visual cortex, GABA and its receptor are regulated, possibly through changes in gene expression, in an activity-dependent manner, with down-regulation following afferent deprivation (Jones, 1993). Thus, both during development and in adulthood, levels of electrical activity are likely to control the balance between excitation and inhibition.

To summarize, the neuron does not have a static set of properties, even in adulthood, but changes under influence of its own activity. These interactions are reciprocal: electrical activity depends on ion channels and receptors, the properties of which, in turn, can be modified by electrical activity. This loop operates on various time scales, not only during development, but also during the functioning of the adult brain.

1.3.1 Models

To study what impact such a feedback loop could have, Abbott *et al.* (1993), LeMasson *et al.* (1993) and Abbot & LeMasson (1993) have developed model neurons (single as well as multi-compartmental) in which the maximal con-

ductance $\bar{g}_j$ of each ionic current is not fixed, as in most models, but dynamic and regulated by the neuron's own activity, with $[Ca^{2+}]_{in}$ as an indicator of activity levels. The behaviour of $\bar{g}_j$ obeys

$$\tau_j \frac{d\bar{g}_j}{dt} = f_j([Ca^{2+}]_{in}) - \bar{g}_j, \tag{1.1}$$

where τ_j is much larger (minutes to hours) than the time constants of the other processes in the neuron; $f_j([Ca^{2+}]_{in})$ is the steady-state value of $\bar{g}_j$, which depends sigmoidally on the calcium concentration:

$$f_j([Ca^{2+}]) = \frac{G_j}{1 + e^{s([Ca^{2+}]-C_T)/\Delta}}, \tag{1.2}$$

where G_j, C_T and Δ are parameters determining the form of the sigmoid, and s is $+1$ for inward and -1 for outward currents, so that a negative feedback loop exists from activity to current strength. This regulation scheme stabilizes the activity of the neuron (lobster stomatogastric ganglion neurons have indeed been found to regulate their conductances in order to maintain stable activity patterns: Turrigiano *et al.*, 1994). If, for example, the amount of extracellular potassium is increased so that an isolated, periodic bursting neuron (single compartment) goes into a fast, tonic firing mode, the resulting increase in electrical activity and $[Ca^{2+}]_{in}$ will, via Eqs (1.1) and (1.2), readjust the maximal conductances so that the initial behaviour is restored (although possibly with a completely different set of conductances). This type of regulation also causes the intrinsic properties to shift in response to external stimulation or the presence of other neurons. An example of the latter is given by a network of two neurons, which, when uncoupled, are identical bursters. When they are symmetrically coupled, however, their activity is no longer identical. The coupling shifts their intrinsic properties: the behaviour of one of the neurons, when again uncoupled, has changed from bursting to tonic firing. Thus, the coupling has spontaneously differentiated the neurons to form a circuit in which one acts as a pacemaker and the other as a follower. In a multi-compartmental version of the model, with soma, axon and dendritic tree, in which Eqs (1.1) and (1.2) act locally, a realistic pattern of membrane conductances (i.e., the strongest sodium current near the soma, intermediate along the axon, and smallest on the dendritic tree) spontaneously arises when the dendritic tree is randomly stimulated with excitatory inputs. This pattern emerges purely as a result of the morphology of the neuron.

In Bell (1991) the ion channels in dendritic compartments are also treated more dynamically: during learning, the conductances are changed so as to optimize an objective function of local current flow. It is shown this gives rise to motion sensitive receptive fields. Interestingly, Zohary *et al.* (1994) have found that the increase in perceptual sensitivity of monkeys practising a motion detection task is accompanied by an increase in sensitivity of directionally selective neurons.

Taken together, this type of dynamic regulation of intrinsic properties may play an important role in network adaptation and development. Along with activity-dependent synaptic plasticity, the existence of activity-dependent neuronal properties suggests that plasticity at 'nodes' (i.e., neurons) may be an important element in network learning (Abbott *et al.*, 1993; and references therein).

Using globally coupled networks of chaotic elements, Kaneko (1994) has studied such plasticity: synaptic change is not assigned to the interaction between i and j, but to the coupling w_i at node i, so that the coupling strength, for a given i, takes the same value for every j. Given an update rule for w_i, common inputs, applied to a subset of elements, increase the degree of synchronization of oscillations of these elements, which persists even if the inputs are removed. Thus, clustering according to inputs is obtained.

Tawel (1992) has studied a neuron that adaptively participates in the learning process of a feedforward back propagation network. During learning, both the synaptic weights and the steepness of the sigmoidal activation function of the individual neurons are optimized, so that each neuron in the end attains its own characteristic activation function (stimulus-response properties of individual neurons in the adult cortex can indeed be modified: see references in Zohary *et al.*, 1994). The method appears to reduce the learning time significantly.

Another intrinsic property that can improve learning is the decrease in neuronal activity when a neuron is repeatedly stimulated (i.e., habituation or adaptation, e.g., through desensitization of receptors). It was implemented in the Neocognitron (Van Ooyen & Nienhuis, 1993), which is a multi-layered feedforward network for visual pattern recognition (Fukushima, 1980). To train this network, a type of competitive learning is used. After learning is completed, the model has a hierarchical structure in which simple features of the input patterns are combined step by step into more complicated features. The cells in the highest layer will each respond to only one input pattern (if learning was successful). Habituation improves pattern discrimination because cells will become less responsive to the more frequent features, so that circuits for detecting them will not be reinforced. Circuits for relatively infrequent features (i.e., discriminating features) develop preferentially. Without habituation, features shared by different patterns are preferentially learned.

Neuromodulators (e.g., neuropeptides and hormones; see Section 1.3) modify the intrinsic properties of neurons (e.g., Kaczmarek & Levitan, 1987). In Coolen *et al.* (1993) a simplified form of neuromodulation is implemented in Ising-spin neural networks: a modulator-specific subset of neurons is prevented from transmitting signals. Neuromodulation, which is present both during learning and recall, enables associative memories to perform selective pattern reconstruction. The similarity of the modulation setting at the moment the patterns were learned with that present during the recall phase determines which of the stored patterns will be selected as candidates for reconstruction.

The modulatory system need not be separate from the system it modulates (i.e., intrinsic neuromodulation). In molluscs, it has been found that neurons within a central pattern generator circuit can, by their own activity, dynamically modulate synaptic strengths within that same circuit during its normal operation (Katz *et al.*, 1994).

1.4 Synapses

The efficacy of synaptic transmission in existing connections can be modulated by electrical activity. Hebb (1949) has proposed that coinciding pre- and postsynaptic activity leads to changes in synaptic strength. In most neural network models some form of Hebbian learning is used (see e.g., Brown *et al.*, 1990). Synaptic mechanisms resembling Hebbian learning (i.e., long-term synaptic potentiation or LTP) are found in the hippocampus, and probably in many other areas. The Hebbian properties of LTP derive from the properties of the NMDA glutamate receptor and its associated Ca^{2+} channel: Ca^{2+} influx requires the coincidence of receptor activation by presynaptically released glutamate and depolarization of the postsynaptic membrane to expel Mg^{2+} that normally blocks the channel at resting potential. If LTP involves a presynaptic increase in neurotransmitter release, some retrograde signal from post- to presynaptic neuron is necessary. Diffusible messengers such as arachidonic acid, nitric oxide and carbon monoxide have been implicated (Hawkins *et al.*, 1993; Schuman & Madison, 1994b). As such signals can diffuse widely, not only the synapses where LTP is induced, but also those of nearby neurons would be strengthened. Indeed, when LTP is triggered in a single synapse by simultaneously stimulating both the pre- and postsynaptic neuron, synapses on neighbouring neurons are strengthened as well (Bonhoeffer *et al.*, 1989; Schuman & Madison, 1994a). Thus, LTP may not be as specific as had previously been thought (Barinaga, 1994), which may have important consequences for learning and memory (also see Montague *et al.*, 1991).

Synaptogenesis and elimination of synapses are controlled by electrical activity (e.g., Purves & Lichtman, 1980; Constantine-Paton, 1990; Shatz, 1990). For further information on synaptic plasticity, the reader is referred to the review of Fields & Nelson (1992). Many models of activity-dependent development deal with the emergence of ocular dominance columns (e.g., Von der Malsburg, 1979; Swindale, 1980; Miller *et al.*, 1989; Obermayer *et al.*, 1990; Goodhill & Willshaw, 1990).

1.5 Glial Cells

The central nervous system is composed of an intimately associated network of neurons and astrocytes (a type of glial cells). Although glial cells

outnumber neurons, relatively little attention has been paid to their function in development and information processing. Only recently have studies suggested that their function may not be limited to the rather passive role of providing structural and trophic support. Astrocytes possess functional neurotransmitter receptors, and hence are potentially capable of monitoring neuronal activity. They can also directly influence neuronal activity (Nedergaard, 1994) and may be involved in non-vesicular release of neurotransmitters (Attwell *et al.*, 1993). Müller (1992) gives a review of the evidence for glial participation in activity-dependent plasticity.

1.6 Neurite Outgrowth

During early development neurons attain their characteristic morphology of branched axons and dendrites [the conventional view is that dendrites always conduct information towards the soma and axons away; in fact, neurons have a more varied repertoire: see e.g., Andersen (1985), Shepherd (1990; 1991), Regehr *et al.* (1993), Stuart & Sakmann (1994)]. Around the cell body, protrusions of the cell membrane emerge which later develop into neurites (neurite is a general term for both axon and dendrite). The one with the highest outgrowth rate usually becomes the axon, while the others turn into dendrites (Sargent, 1989; Craig & Banker, 1994). At the tip of a growing neurite is a highly specialized motile structure, the growth cone, which typically consists of a flat lamellipodium and numerous filopodial extensions. It is capable of path finding, elongating, retracting, branching and initiating synaptogenesis. Neurite outgrowth and growth cone behaviour are therefore essential to the development of neuronal morphology and the pattern of synaptic connections. Apart from providing space for other cells to synapse upon, dendritic and axonal trees influence the current flow through the cell and, thereby, its electrical properties.

1.6.1 Neurite Outgrowth, Electrical Activity and Neurotransmitters

Neuronal morphology results from the genetic potentialities along with environmental inputs such as growth factors, substrate adhesion molecules and local cell interactions (for the latter see e.g., Gao *et al.*, 1991; Baptista *et al.*, 1994). Numerous studies have demonstrated that electrical activity and neurotransmitters can directly affect neurite outgrowth and neuronal morphology. Neurotransmitters could play important roles, therefore, not only in information coding but also in defining the structure of the networks in which they operate (Mattson, 1988).

High levels of electrical activity of the neuron reversibly arrests neurite outgrowth or even produces retraction, whereas low levels allow further outgrowth (Cohan & Kater, 1986; Fields *et al.*, 1990a; 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; Neely, 1993) with, in general, excitatory neurotransmitters inhibiting outgrowth and inhibitory ones antagonizing the effects of excitatory neurotransmitters. In cultured hippocampal pyramidal neurons, for example, glutamate causes a dose-dependent reduction in dendritic length (Mattson *et al.*, 1988) (axonal outgrowth is also affected, but at higher concentrations) which can be antagonized by GABA or the by 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, 1988). Excess inhibition, however, suppresses outgrowth. When GABA and its potentiator diazepam are added, the outgrowth of both the axon and dendrites is suppressed. This supports the notion that an optimal level of electrical activity is required for neurite outgrowth. Moreover, it may be not merely the frequency of impulses that is important, but also its pattern. In mouse sensory neurons, phasic stimulation is more effective in inhibiting neurite outgrowth than is stimulation with the same number of impulses at a constant frequency (Fields *et al.*, 1990a).

The influences of electrical activity and neurotransmitters act on the level of both single neurite and whole cell. Specific cues such as neurotransmitters could act locally on individual growth cones, e.g., local application of glutamate to individual dendrites results in local dendritic regression (Mattson *et al.*, 1988), whereas action potentials may simultaneously regulate the behaviour of all the growth cones and neurites of a given neuron (Kater & Guthrie, 1990; Cohan & Kater, 1986; also see Stuart & Sakmann, 1994). The conditions under which neurites grow out may be different for different neurons (Kater *et al.*, 1990). Even different neurites of the same neuron, e.g., axons and dendrites, can have different growth properties (Kater & Guthrie, 1990).

In addition to the effects described above, neurotransmitters can regulate the direction of neurite outgrowth (Zheng *et al.*, 1994; Smith 1994). Neurites growing from cultured Xenopus neurons turn towards an acetylcholine (ACh) source. They are able to detect a gradient in neurotransmitter concentration, a phenomenon whereby Ca^{2+} in the growth cone seems to play a crucial role. Besides detecting neurotransmitter molecules, growth cones are also capable of releasing them (Young & Poo, 1983), a characteristic they share with the presynaptic terminal.

Neurite outgrowth, growth cone motility, shape and direction of outgrowth are also regulated by trophic factors such as NGF (e.g., Jacobson, 1991). Applied electric fields, too, influence nerve growth, with respect both to branching (McCaig, 1990a) and to the rate of elongation (McCaig, 1990b). Nitric oxide (NO), an endogenously produced free-radical gas, which has been implicated as an intercellular messenger subserving long-term potentiation

(LTP) (see Section 1.4), reversibly causes growth cone collapse and neurite retraction (Hess *et al.*, 1993). It may play a role in activity-dependent processes, as its production depends upon Ca^{2+} (Schuman & Madison, 1994b).

Taken together, these data show that the growth cone is a structure capable of integrating multiple cues, a process comparable to the classical integration of multiple synaptic inputs (Kater & Guthrie, 1990).

1.6.2 Calcium

The morphological responses to neurotransmitters and electrical activity are most likely mediated by changes in $[Ca^{2+}]_{in}$ (Cohan *et al.*, 1987; Kater *et al.*, 1988; Mattson, 1988; Kater *et al.*, 1990; Kater & Guthrie, 1990; Kater & Mills, 1991). Depolarization leads to Ca^{2+} entry, and a number of aspects of growth cone motility, such as assembly and disassembly of microtubules, are thought to be regulated by Ca^{2+} (see e.g., Schilstra *et al.*, 1991). In connection with these observations, the Ca^{2+} theory of neurite outgrowth has been proposed (e.g., Kater *et al.*, 1988; Kater *et al.*, 1990; Kater & Guthrie, 1990), which states that low $[Ca^{2+}]_{in}$ at the growth cone stimulates outgrowth, higher concentrations cause a cessation of outgrowth, and still higher concentrations lead to regression of neurites. Since outgrowth is also blocked if $[Ca^{2+}]_{in}$ is too low, there appears in fact to be an optimal level. Differences in reaction among cells and neurites may be attributable to differing Ca^{2+} regulating characteristics.

1.6.3 Morphology and Polarity

Electrical activity determines not only whether or not a neuron grows out, but also the morphology of its growth cones. In addition to blocking outgrowth, electrical stimulation decreases the number of filopodia and causes the lamellipodium to retract (Cohan & Kater, 1986). Since the form of the growth cone can affect branching (also see Van Veen & Van Pelt, 1992), electrical activity and neurotransmitters can regulate the branching pattern of the dendritic and axonal tree (Brewer & Cotman, 1989; Kater *et al.*, 1990). During normal development, neurotransmitters released by afferent axons could alter the dendritic morphology of growing neurons. For example, in a system where hippocampal pyramidal cells are grown on a mat of axons from entorhinal cortex, glutamate released from entorhinal axons inhibits dendritic outgrowth in pyramidal cells (Mattson, 1988).

Neurotransmitters may orient the site of axon formation (Mattson *et al.*, 1990). Early in development, hippocampal neurons extend several short neurites (Dotti *et al.*, 1988) which, at the time they first appear, cannot be specified as either axonal or dendritic. The length of these initial neurites appears to determine which one will become the axon. In experiments in which the axon is transected at various distances from the soma, the longest neurite remaining after transection usually becomes the axon, regardless of

whether it was previously an axon or a dendrite (Goslin & Banker, 1989). A similar process may be involved during normal development: when one of the neurites by chance exceeds the others by a critical length, it becomes specified as the axon (Goslin & Banker, 1989). Neurotransmitters, electrical activity and other factors that regulate neurite length might therefore play a role in the differentiation of neurites into axons and dendrites (i.e., cellular polarity).

The acquisition of axonal characteristics is correlated with the selective segregation of the protein GAP-43 to the growth cone of a single neurite (Goslin & Banker, 1990). It may be a candidate for the regulatory protein in the conceptual model of Goslin & Banker (1990), which states that the rate of neurite elongation depends on the concentration of some protein whose distribution is in turn determined by length. The transport of GAP-43 to the growth cone is an active process and therefore largely independent of neurite length, while the return to the soma might be a diffusion process, so that, according to this hypothesis, the growth cone of the longest neurite would receive more protein, and the neurite would grow longer still. Interestingly, GAP-43 is sensitive to changes in membrane polarization (Dekker *et al.*, 1989) and in Ca^{2+} levels (Aloyo *et al.*, 1983); GAP-43 could modulate Ca^{2+} signals in growth cones and synapses, and regulate membrane and cytoskeletal assembly (for references see Fields & Nelson, 1992). Direct indications that Ca^{2+} plays a role in the formation of axon-dendrite polarity have been obtained by Mattson *et al.* (1990). A *localized* influx of Ca^{2+} suppresses axon formation, in neurons that have not yet established their polarity, as well as in neurons from which the axon is transected at short distances from the soma (which causes Ca^{2+} to enter). When a Ca^{2+} gradient is present in the neuron, the axon will not form where the Ca^{2+} concentration is highest. In this way, a local encounter with neurotransmitter (or any other factor causing a local increase in Ca^{2+}) could influence the site at which an axon will be formed. It may be that a neurite becomes an axon just because it has a low $[Ca^{2+}]_{in}$ and therefore grows more rapidly than the other neurites (also see Mattson *et al.*, 1990). Indeed, $[Ca^{2+}]_{in}$ is lower in axons than in dendrites (Guthrie *et al.*, 1988).

1.6.4 Adhesion Molecules

Adhesive and repulsive interactions among cell surface molecules (e.g., on growth cones and neurites), and among cell surface molecules and molecules in the extracellular matrix also participate in growth cone behaviour (e.g., Letourneau, 1991), neurite extension, retraction and guidance, as well as in neurite fasciculation and neuronal migration (Jessell, 1991). The adhesion molecules of the immunoglobulin superfamily (e.g., neural cell adhesion molecule: N-CAM), cadherins (e.g., N-cadherin) and integrins promote neurite outgrowth; N-CAM and cadherins are involved in cell-cell adhesion, whereas integrins mediate adhesion of cells to glycoproteins (e.g.,

fibronectin and laminin) in the extracellular matrix. Not all the actions of these molecules, however, involve adhesion (Schwab *et al.*, 1993). For example, myelin, tenascin and cytotactin induce an avoidance reaction or neurite retraction (e.g., Edelman & Cunningham, 1990). Most of these interactions are probably established in an activity-independent fashion (Smith, 1994). However, in the neuromuscular junction it has been found that blockade of activity increases the level of axonal polysialic acid (PSA: the major glycoprotein on N-CAM modulating adhesiveness) resulting in axonal defasciculation and increased branching (Landmesser *et al.*, 1990). Since PSA is widely expressed in the developing nervous system, this form of activity-dependent axon behaviour may be more general (Hockfield & Kalb, 1993).

1.6.5 The Mature System

Neurotransmitters are also likely to play important roles in plastic changes in neuronal morphology during adulthood (Mattson, 1988). There are growing indications that the adult brain is not structurally static, but rather in a state of continual morphological change (for references see Mattson, 1988; Purves *et al.*, 1986). This is to be contrasted to the view that neural changes are encoded in functional alterations of synaptic networks that are anatomically fixed (see Purves & Voyvodic, 1987). For example, dendrites of individual neurons in the superior cervical ganglion of young adult mice are, when followed over intervals of up to three months, subject to continual change: some branches retract, others elongate, while still others appear to be newly formed (Purves *et al.*, 1986). This implies that the synaptic connections made onto these cells must also undergo substantial rearrangements. The dendritic tree of pyramidal cells in the visual cortex of adult rats changes in response to changes in environmental conditions (Uylings *et al.*, 1978). The dendritic extent per neuron in the human cortex may increase 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).

Polarity in mature neurons displays a surprising degree of plasticity. Axotomy or deafferentation leads to profound changes in polarity, and membranes that normally are postsynaptic can form presynaptic specializations (for references see Craig & Banker, 1994). Mechanisms involved in polarity thus continue to operate in adulthood (Craig & Banker, 1994). Alterations in these mechanisms may contribute to the pathology of some neurological diseases. The reorganization of the microtubule system appears to be an important pathological feature of Alzheimer's disease (for references see Craig & Banker, 1994). Neuronal damage and death in Alzheimer's disease may result from excessive rises in $[Ca^{2+}]_{in}$ (Mattson, 1992; Mattson *et al.*, 1993).

To summarize, electrical activity and neurotransmitters influence neurite outgrowth in a variety of ways during development. These influences also provide mechanisms for plasticity in adulthood (Kater & Guthrie, 1990).

1.7 Models of Neurite Outgrowth

Hentschel & Fine (1994a, b) have demonstrated, using models of isolated, single cells, that growth under the control of diffusible factors such as Ca^{2+} can lead to the emergence of dendritic forms from initially spherical cells. Local outgrowth and retraction of the cell membrane are taken to depend upon the local concentration of Ca^{2+} close to the internal surface of the membrane:

$$V(s) = aC(s)^{\alpha} - bC(s)^{\beta}, \tag{1.3}$$

where $V(s)$ is the growth velocity normal to the surface at point s, $C(s)$ is the submembrane Ca^{2+} concentration at that point, a and b are the rate constants for outgrowth and retraction, respectively, and α and β reflect the cooperativety of the dependence on Ca^{2+} (set at 1 and 2, respectively); Eq. (1.3) is a phenomenological description of the theory of Kater *et al.*, in that the outgrowth rate increases as the Ca^{2+} concentration rises to some optimum value, above which it decreases and even becomes negative at still higher levels. The local Ca^{2+} concentration results from influx and active extrusion. At spontaneously occurring convexities in the membrane, the local concentration will become larger than at concavities, because of the larger surface to volume ratio. These protrusions of the membrane will not decay because of the existence of a positive feedback loop: Ca^{2+} influx increases with increased submembrane Ca^{2+} concentrations, as a consequence of the presence of voltage-sensitive Ca^{2+} channels and the influence of Ca^{2+} on the membrane potential. This leads, if the growth rate has a positive dependence upon the Ca^{2+} concentration, to continued outgrowth and branching. The development in the model resembles that of living neurons: outgrowth starts with broad and irregular as well as with short and fine extensions of the membrane (lamellipodia and filopodia, respectively) followed by the emergence of distinct processes (neurites) which spontaneously form enlargements at the top (growth cones). The extension of these processes is often punctuated by periods of retraction. Growth under reduced electrical excitability (which affects the Ca^{2+} permeability) results in longer, thinner neurites. Similar changes are seen in cultured cerebellar Purkinje cells when electrical activity is blocked by TTX (Schilling *et al.*, 1991). Increasing the (voltage-independent) Ca^{2+} permeability leads to more compact dendrites with broader growth cones.

In the model of Hentschel & Fine (1994a, b) the effects of cytoskeleton elements such as microtubules, which form a rigid, continuous internal core within the neurite, are not explicitly taken into account. In Martin *et al.* (1993) a simulation model is presented of the polymerization of individual microtubules. The model can successfully simulate the so-called dynamic instability of microtubules: the transition of an individual microtubule, apparently at random, between extended periods of slow growth and brief periods of rapid shortening.

In Van Veen & Van Pelt (1994) neurite elongation is described as resulting from polymerization of microtubules, with a constant production of tubulin at the soma and a diffusion-based transport to the growth cone. At the growth cone polymerization takes place with a tubulin concentration-dependent assembly rate and a fixed disassembly rate. This leads to a constant elongation rate in a cell with a single neurite. When more neurites are present, also transient or complete retraction of segments can occur if assembly/disassembly rate constants differ among the neurites. In large trees, the maximal number of sustainable terminal segments is positively related to the production rate of tubulin. Although not implemented in the model, the (dis)assembly rate is dependent upon Ca^{2+} (for references see Van Veen & Van Pelt, 1994). So is the morphology of the growth cone (see Section 1.6.3). The latter affects branching events (see Van Veen & Van Pelt, 1992), the occurrence of which is probably random in time: a growth model which assumes that a tree grows via a sequence of random branching events (one at a time) is sufficient to explain the observed variance in dendritic trees (Van Pelt *et al.*, 1992).

1.8 This Thesis

All the model studies in literature, as described in the previous section, have considered outgrowth in isolated neurons. A neuron never grows in isolation, however, but in interaction with its environment (including other cells). As described in Sections 1.6.1 and 1.6.2, electrical activity, resulting from synaptic interactions with other cells, can modulate neurite outgrowth, via Ca^{2+} influx elicited by membrane depolarization. Although it has been realized that this could have considerable potential for controlling neuronal form and circuitry (Mattson, 1988), the possible implications of activity-dependent neurite outgrowth have not previously been made explicit. The aim of this thesis is to explore the implications of activity-dependent neurite outgrowth for neuronal morphology and network development. To this end we use mathematical simulation models of electrically interacting cells whose outgrowth depends on their level of electrical activity.

1.8.1 Model Approach

In this thesis mainly a 'bottom-up' approach (Hogeweg & Hesper, 1986; Hogeweg, 1992; Huynen, 1993) is used: models are not directed at producing any particular, *a priori* defined behaviour, but are built in order to study how a given set of interesting properties of the elements of the model (e.g., neurons) would affect the behaviour of the whole system. To predict this behaviour, human intuition often fails utterly: a collection of elements with even simple interactions can lead to extremely complex behaviours and structures. Model studies can help by (i) forcing one to explicitly define the processes and interactions of interest, (ii) allowing rigorous predictions of the

consequences of such processes, and (iii) providing us with new concepts and relationships. For instance, model studies can demonstrate that the interactions needed for generating complex behaviours and structures can be far simpler than we ever expected. Or they can show that seemingly unrelated observations are in fact aspects of the same underlying process. An illustration of this last point is found in this thesis. Without modelling it would have been very difficult to conceive that, for example, a transient overproduction of connections during development (for a biological background on this so-called overshoot phenomenon, the reader is referred to Chapter 2), 'critical' periods for pruning of connections (see Chapters 5 and 6), differential growth curves for excitatory and inhibitory connections, compensatory sprouting following cell death and differentiation in cell sizes (see Chapter 5), all could be based upon activity-dependent neurite outgrowth. Stronger still, without modelling one would not even have thought of building a model that could simultaneously account for all these phenomena.

By varying the parameter settings or the structure of the model one can study the extent to which given model phenomena are robust. In this way, the interactions and relationships that are essential for their generation can be pinpointed. One can, then, look to see whether these essentials also occur in other models that may have been formulated in a completely different way (see Chapter 8), thus further testing the generality of the findings.

Observations and relationships that are valid in the model systems can then be compared with those valid in living systems (such as, in our case, neural tissue cultures and the intact brain itself). This procedure enables us to make well-founded suggestions for experiments, the results of which can, in turn, lead to new models. In this way, experimental and theoretical biology can reinforce one another, leading to ever more profound insights into the systems we have chosen to investigate.

1.8.2 Outline of Thesis

The empirical studies on neurite outgrowth, as reviewed in Sections 1.6.1 and 1.6.2, have shown that high levels of neuronal electrical activity cause neurites to retract, whereas low levels allow further outgrowth. To explore the implications of activity-dependent neurite outgrowth, we endow model neurons (both excitatory and inhibitory) with such plasticity, and study how this would affect the behaviour of a group of interacting neurons. In individual neurons, the dependence of outgrowth on electrical activity is such that each neuron attempts to reach, by means of adjusting the size of its neuritic field, that level of electrical activity at which the size of its field, c.q., its connectivity, no longer changes (this homeostatic 'setpoint' of activity will be called ϵ in the sequel). When the neuron's activity is higher than ϵ it retracts its neuritic field (as a result of which its connectivity with other cells decreases), and when it is lower than ϵ it extends its neuritic field (as a result of which its connectivity with other cells increases).

In Chapter 2 we study such activity-dependent outgrowth in purely excitatory networks, and show that one of the implications is a transient overproduction with respect to connection strength during development ('overshoot'). Overshoot phenomena with respect to many structural elements (e.g., synapses) constitute a widespread feature of nervous system development, *in vivo* as well as *in vitro*.

In most of our studies the value of ϵ is taken to be the same for all neurons. In Chapter 3 we study a purely excitatory network in which ϵ is allowed to vary among neurons, which can lead to complex periodic behaviour, in both electrical activity and connectivity. Regular oscillations occur if most of the cells have an ϵ value in the oscillatory range. The time scale of these oscillations is determined by the time scale of the slow process, i.e., neurite outgrowth (but any other cellular property that adapts slowly to electrical activity would lead to similar results). Fluctuations in firing rate on the order of several minutes, or even slower, have indeed been observed in tissue cultures of cerebral cortex cells, as well as *in vivo*.

In Chapter 4 we show that, even without such slow intrinsic processes, long periods of high network activity can alternate with long periods of low activity.

In Chapter 5 we study activity-dependent outgrowth in networks that also contain inhibitory cells. We show (among other things) that, although there are no intrinsic differences between excitatory and inhibitory cells with respect to growth properties, their neuritic fields nevertheless become different in size. In the cerebral cortex, the dendritic and axonal fields of inhibitory neurons are indeed smaller, on the whole, than those of excitatory neurons.

Another effect of inhibition is that, in contrast to purely excitatory networks, mixed networks do not always go to the same end state or attractor (with respect to pattern of electrical activity and average connection strength) under all initial conditions. A similar phenomenon has been observed in tissue cultures of cerebral cortex cells ('critical' periods). In Chapter 6 we study this phenomenon in more detail using a few simplified models.

In Chapter 7 we study a growth function in which neurite retraction occurs not only when neuronal activity is above ϵ, but also when it is below a second critical value. Growth of connectivity and overshoot occur in those parts of the network in which the activity level is above this latter value.

1.9 Summary and Discussion

Many seemingly unrelated processes in the development of the nervous system interact through their dependence on neurotransmitter mediated signals (in many cases with Ca^{2+} as underlying messenger)(also see Harris, 1981). This is of importance for development, but also for learning and memory in the mature state (see e.g., Stryker, 1990), as many of these processes remain operative in the adult brain. Activity-dependent plasticity operates on multiple

levels of specificity, ranging from single connections to whole cells, i.e., from synapses, populations of synapses, neurites and branching patterns to intrinsic neuronal properties and cell death. In this thesis activity-dependent plasticity with respect to neurite outgrowth is studied. Electrical activity (fast dynamics) changes neuronal form, function and connectivity also on multiple time scales (slow dynamics): from minutes (e.g., modulating channels), hours (e.g., number and effectiveness of receptors) to days or weeks (e.g., gene expression and neurite outgrowth) and persistently (neuronal differentiation). Thus, in contrast to conventional neural network models in which the structure and elements are fixed and only the parameters of the model (e.g., connection strength) can change, the nervous system is a more truly variable system in which the number of the constituting elements as well as their properties (e.g., morphology and electrical characteristics) and interactions are subject to change (also see Hogeweg & Hesper, 1986). This provides possible mechanisms for learning beyond changes in synaptic strength.

A generic connectionist model is a graph consisting of nodes (e.g., neurons) and edges or links (e.g., synaptic connections) between them (Farmer, 1990). Learning in neural network models occurs via changes in connection strength in such a way that all the connections to a given node can be modified independently ('edge learning'). This is fundamentally different in connectionist models of the immune system (B-cell immune networks, e.g., De Boer & Perelson, 1991), where the only means of modifying connection strength is by changing a node parameter (i.e., lymphocyte concentration; lymphocyte types are here the nodes of the network), so that in fact all the connection strengths to a given node change simultaneously ('node learning'). However, as we have seen, many activity-dependent properties in neurons also seem to operate either on the node level or on levels intermediate between node and edge (also see Stuart & Sakmann (1994), who suggest that propagation of somatic action potentials into the dendritic tree may be important for changes in synaptic strength - that is, in a nodal way). This suggests that some form of node learning may also be important for the functioning of neural networks (also see Abbott *et al.*, 1993), although as yet most models have individual neurons that do not adaptively participate in the learning process (but see references in Section 1.3.1, and so-called weightless neural networks, e.g., Aleksander, 1991; also see Finkel and Edelman (1985), who have a node rule to modify the amount of neurotransmitter release: all the terminals of the presynaptic neuron are influenced simultaneously).

The feedback mechanisms in a neuron (e.g., regulation of receptor number and sensitivity, neurite outgrowth) seem to contribute to the homeostasis of neuronal activity (in contrast to Hebbian synaptic potentiation, which acts to modify activity) or, more generally, to the optimization of some locally defined condition. Are neurons essentially pursuing their own goals, and must learning and memory be viewed as emerging from many neurons simultaneously striving to support their own needs? (Klopf, 1982). Since no agreement exists on what constitutes information processing in neural cells (Kohonen,

1992), and many neuronal properties seem to have no place in existing neural network models, it is worthwhile to pursue several fundamentally different models (e.g., adaptive neurons, node learning).