CHAPTER 23

Growth cone dynamics and activity-dependent processes in neuronal network development

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Introduction

Neuromorphogenesis and network formation proceed via the dynamic behavior of growth cones which mediate neuritic elongation, retraction, redirection and synapse formation. Mature neurons have attained their morphological characteristics as result of this dynamic process. For studying the determinants of neuronal shape it is therefore crucial to understand how shape characteristics emerge from properties of this dynamic process. Among the many processes involved in growth cone dynamics are the exploratory interactions of filopodia with the local environment (e.g., Kater et al., 1994), receptor mediated transmembrane signalling to cytoplasmic regulatory systems (e.g., Letourneau et al., 1994) and the formation of the cytoskeleton in the elongating neurite, comprising the polymerization of tubulin into microtubules (e.g., Black, 1994). This chapter focuses on the emergence of shape characteristics in dendritic branching patterns, and on the impact of activity-dependent processes in neurite outgrowth and network formation. The role of spontaneous activity in the formation of cultured neuronal networks is emphasized. Mathematical models are shown to be crucial tools for studying these subjects by making it possible to examine rigorously hypothesized mechanisms at the growth cone level for their consequences on emergent neuronal morphological and network properties. This paper aims to illustrate the fruitfulness of such an integrated theoretical and empirical approach. Finally, it is emphasized how activity-dependent mechanisms, serving primarily a homeostatic goal, underlie many emergent structural and functional neural network properties.

Random branching and topological variability in dendritic trees

During dendritic development growth cones migrate and branch. On branching, a growth cone splits into two, which continue to grow each on its own. After a number of such events a branching pattern has been formed, with a topological structure determined by the particular sequence with which the growth cones have split. Fig. 1 shows schematically how two different branching sequences result in different topological structures (tree types).

We have studied the growth of branching patterns mathematically by assuming a random process for the selection of which of the existing segments will branch (Van Pelt et al., 1992). We assumed that the probability of each segment in the tree to be selected depends on the type (i.e., intermediate or terminal segment) and on the centrifugal order of the segment (i.e., the number of segments on the path from the root to the segment). Then, each sequence of branching events is the result of random choices according to these probabilities. Each sequence, resulting in a particular tree type, therefore has a certain probability of occurring. The tree types, obtained after as many such sequences, thus will occur with probabilities
that depend on the values of the two model parameters. The growth model includes both the random terminal and the random segmental growth mode.

The variation in tree types can numerically be dealt with by the use of an index to characterize the topological structure. We used the tree-asymmetry index, defined as the mean relative difference in the number of terminal segments between the two subtrees at a branch point. It has a value of zero for strict symmetrical trees, when at each branch point the two subtrees have an equal number of terminal segments, and a value close to one for asymmetrical trees, when one subtree at each branch point consists of only one (terminal) segment. The expected value for the tree-asymmetry index for the random terminal growth mode is 0.46.

The tree-asymmetry index of reconstructed trees can easily be calculated by counting the number of terminal segments in all subtree pairs. Based on Golgi-stained material, mean (SD) values for the asymmetry index were found to be 0.49 (0.02) for rat Purkinje cell dendritic trees, 0.38 (0.22) for rat visual cortex pyramidal cell basal dendrites and 0.43 (0.26) for multipolar non-pyramidal dendrites (Van Pelt et al., 1992). Dityatev et al. (1995) found mean (SD) values in the range of 0.29 (0.24)–0.46 (0.13) for motoneuronal dendrites from the rat, cat and frog.

Most dendrites are thus slightly more symmetrical than predicted by the random terminal growth mode. Trees with greater symmetry are generated when the branch probability for terminal segments decreases with increasing centrifugal order. The analyzed Purkinje cells were slightly less symmetrical than predicted by the random terminal growth mode. Such trees can be generated by assuming either a slight increase in terminal segment branch probability with increasing centrifugal order or a small branching probability for intermediate segments. In all cases the model parameters could be adapted so that the observed mean asymmetry could be accurately reproduced. The most important finding, however, was that the standard deviation predicted by the model was similar to the empirically observed one. This finding demonstrates that the random branching assumption in the growth model is sufficient to explain the topological variability in dendritic shapes.

During outgrowth, transient phases of growth cone retraction may occur. Although the topological growth model does not consider pruning, it has been shown that the relationship between growth mode and final topological variability is not affected when random pruning is part of the growth process (Van Pelt and Verwer, 1984). The conclusion is that the natural variability in the topology of dendritic branching patterns is consistent with the assumption of random branching growth cones.

The processes in a growth cone which precede a branching event are not yet well understood. Wessels and Nuttall (1978) were able to induce branching by lifting the filopodia at the leading edge of a growth cone, leaving adherent filopodia only at the sides. Branching is thought to be based on an interplay between the filopodia and the microtubules in a given neurite (Letourneau et al., 1986), whereby branching occurs when the filopodia direct the microtubules in the central body of the growth cones into distinct groups. Van Veen (1993) has studied quantitatively the putative role of the filopodial angular distribution in the initiation of branching. In a tissue culture system of chick dorsal root ganglion cells he showed that the branch probability of a growth cone progressively

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Fig. 1. Growth of a branching pattern depicted as a sequence of branching events. At each growth step, one of the terminal segments is randomly selected for branching according to a uniform probability distribution (random terminal growth). The two random sequences result in different tree types. The tree types are plotted in a standardized way, with the largest subtree at a branch point to be the right one.
increases with increasing sidewards, relative to forward orientation of the filopodia. Filopodia are highly motile parts of a growth cone. They explore the local environment and adhere to the substrate. The motile character of filopodia implicates continuing fluctuations in both the filopodial orientations and the growth cone's response to local cues. Because the actual behavior of a growth cone depends on so many factors, its behavior can best be described as a stochastic process. When local cues dominate in the induction of branching, it may be expected that growth cones will branch largely independent of one another. The natural topological variability observed in dendritic trees is in agreement with this hypothesis.

**Growth-cone migration and segment length characteristics in dendritic trees: rate-limiting factors in microtubule formation – a hypothesis**

Branching at terminal segments may proceed by splitting of growth cones at the tips, but also by the outgrowth of a growth cone located somewhere along a terminal segment. Such a growth cone could have been the 'dormant' sister of the growth cone at the tip of the segment with a delayed outgrowth, as observed by Bray (1973). Such a delay can result in a reduced length of the "delayed formed" terminal segment. In a study of segment lengths in rat Purkinje cell dendritic trees, Woldenberg et al. (1993) found that in pairs of terminal segments, arising from the same branch point, the shortest and the longest segments formed distinct length distributions. The difference in mean lengths was larger than could be explained simply by a selection of shorter and longer segments originating from a single length distribution. This unexpected statistical finding suggests that an unequal development of paired terminal branches is in fact a common phenomenon in Purkinje cell dendritic trees.

The lengths of segments in dendritic trees strongly depend on their type and their topological position within the tree. Terminal segments are on the average longer than intermediate ones (Uylings et al., 1986), and they decrease in length with increasing centrifugal order, whereas intermediate segments show only minor changes in length throughout the dendritic tree (Uylings, 1978). Additionally it is shown that during dendritic development the mean length of intermediate segments remains constant, while mean terminal segment length increases steadily (e.g. Uylings et al., 1994). Van Veen and Van Pelt (1993) have shown that two simple assumptions for neurite outgrowth, viz. a constant elongation rate and a random (Poisson) process for the occurrences of branch points, are sufficient for qualitatively explaining such characteristic features. The main point is that intermediate segments are formed by the actual occurrences of branching events, and no longer elongate during the subsequent period, while terminal segments can continue to elongate up to the time of observation. Terminal segments of lower order have, in fact, developed for a longer period than have terminal segments of higher order.

The neuritic cytoskeleton contains microtubules which form bundles running from the cell body through the neurites into the growth cone. Individual microtubules may start and end all along this path (e.g., Bray and Bartlett Bunge, 1981; Black, 1994). Migration of growth cones and neuritic elongation proceed by elongation of these microtubule bundles. The assembly of tubulin takes place at the ends of microtubules in a highly dynamic pattern. Periods of tubulin assembly into the polymer (elongation) alternates with periods of disassembly (retraction), a behavior called 'dynamic instability' (e.g., Horio and Hatani, 1986). A crucial parameter in this process is the concentration of free tubulin, with higher tubulin concentration increasing growth rate and growth-state lifetime and decreasing shortening rate and shortening-state life time (Martin et al., 1993). The local tubulin concentration will depend on the tubulin flux through the neurite and the assembly/disassembly rates. The local concentration fluctuations caused by the dynamic instability may therefore have substantial impact on this behavior itself. Neuritic elongation may exclusively proceed by net assembly at the endings of the microtubules in the growth cones (Lim et al., 1990) but also by translocation of the microtubule bundle (Black, 1994). In a model study of neuritic elongation and
microtubule dynamics, Van Veen and Van Pelt (1994) assumed an assembly rate proportional to the tubulin concentration, and an invariant disassembly rate determined by the structure of the microtubules. They showed that under these conditions the system will evolve towards a tubulin concentration for which assembly and disassembly rates are in equilibrium. In the long run, the average outgrowth rate is determined by the average tubulin flux towards the growth cone. A remarkable finding was that small differences in assembly/disassembly rates (and, thus, in equilibrium tubulin concentrations) resulted in competition for free tubulin between growth cones sharing the same pool of free tubulin at a given branch point. Under such conditions, the faster growing growth cone forces the concentration in the slower one to such a low level that it can not grow out. Such a mechanism would explain the phenomenon of ‘dormant’ growth cones. No experimental studies have as yet been performed to test this hypothesis. Additionally, the model revealed that, when the tubulin production in the cell body and flux through the neurites does not keep pace with an increasing number of growth cones, the elongation rates will slow down accordingly.

Implications of activity-dependent neurite outgrowth for neuronal morphogenesis and network formation

The elongation of neurites is modulated by, among other things, the calcium concentration in the growth cone: the overall movement of the growth cone is driven by the assembly and disassembly of microtubules, which are Ca²⁺ dependent (e.g., Kater et al., 1988; Schilstra et al., 1991). All factors that change the intracellular calcium concentration ([Ca²⁺]ₗ) are thus potentially able to affect neurite outgrowth. Synaptic input, for example, can alter ([Ca²⁺]ₗ) via Ca²⁺ influx through voltage-sensitive and receptor linked Ca²⁺ channels, which open upon membrane depolarization as a result of excitatory inputs and nerve impulses. The empirical observations concerning the relationship between Ca²⁺, c.q. electrical activity, and outgrowth are summarized in the Ca²⁺ theory of neurite outgrowth (e.g., Kater et al., 1988, 1990; Kater and Guthrie, 1990), which states that low [Ca²⁺], at the growth cone (c.q. a low level of electrical activity of the cell) stimulates outgrowth, higher concentrations cause a cessation of outgrowth, and still higher concentrations (c.q. a high level of electrical activity of the cell) lead to regression of neurites. Since outgrowth is also blocked if [Ca²⁺]ₗ is too low, there appears in fact to be an optimal level.

Hentschel and Fine (1996) have studied Ca²⁺-dependent neurite outgrowth in models of isolated, single cells. They have demonstrated that growth under the control of Ca²⁺ leads to the emergence of dendritic forms from initially spherical cells, using an outgrowth rule in accordance with the theory of Kater et al. (1988, 1990): the local outgrowth rate increases as the local Ca²⁺ concentration close to the internal surface of the membrane rises to some optimum value, above which it decreases and becomes negative at still higher levels. Essential in the process of neurite formation in their model are the differences in Ca²⁺ concentration that emerge at concavities and convexities in the membrane (due to differences in surface to volume ratio) and the positive feedback loop between Ca²⁺ influx and submembrane Ca²⁺ concentrations.

The previous model study considered outgrowth in isolated neurons. A neuron never grows in isolation, however, but in interaction with its environment (including other cells). Electrical activity, resulting from synaptic interactions with other cells, can modulate neurite outgrowth via Ca²⁺ 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 have not previously been made explicit.

In a number of studies we have begun to explore these implications (Van Ooyen, 1994; Van Ooyen and Van Pelt, 1994, 1996; Van Ooyen et al., 1995; Van Oss and Van Ooyen, 1995), using models in which neurons (both excitatory and inhibitory) are capable of interacting electrically. In this model, there is no external input to the network (thus mimicking the natural situation in the
earliest phase of network formation) and, in the absence of synaptic connections, each cell has a very low “spontaneous” firing rate (also see Corner, 1994). A neuron is modelled as having one neuritic field, representing axonal as well as dendritic extensions, and having a membrane potential dependent sigmoidal firing rate function. The initially disconnected neurons start interacting electrically as soon as they become connected to other neurons, viz., when their neuritic fields overlap. The overlap thus represents axo-dendritic as well as dendro-dendritic interactions. Since the influx of Ca²⁺ is dependent upon a cell’s level of electrical activity (membrane potential, firing rate), the growth of such a neuritic field is taken to depend upon the neuron’s own activity level, in accordance with the theory of Kater et al. (1988, 1990). Thus, when the neuron’s activity is higher than a given value (which will henceforth be called \( \varepsilon \); it acts as a homeostatic “setpoint” of activity) it will retract its neuritic field, as a result of which its connectivity with other cells decreases. When activity is lower than \( \varepsilon \), the neuritic field will be extended, as a result of which the cell’s connectivity with other cells increases. (Not included in these models (but see below) is the observation that outgrowth is blocked also when the level of electrical activity is too low.) The interactions between activity and outgrowth are thus reciprocal: electrical activity influences outgrowth, while outgrowth, in turn, alters the network’s internal connectivity and, consequently, the overall level of electrical activity. A number of interesting properties arise as a result of this reciprocity.

**Overshoot**

A general feature of nervous system development is that virtually all elements show an initial overproduction (so-called overshoot phenomena; for references see Van Ooyen and Van Pelt, 1994). In our model networks, we found such an overshoot with respect to connection strength (Fig. 2). Initially, the neurons are disconnected and have a low level of “spontaneous” electrical activity, as a result of which they grow out and establish connections with other cells. When the excitatory connectivity has become sufficiently high, this “spontaneous” activity triggers generalized network activity. The electrical activity can then become so high that the neuritic fields start retracting. As a result, connectivity and electrical activity again decrease, until a level of activity is reached at which neither retraction nor outgrowth takes place (viz., \( \varepsilon \)). Thus, a developing network has to go through a phase in which the connectivity is higher than in the final, stable situation. This is the consequence of a hysteresis relationship existing between network connectivity and activity, meaning simply that a higher connectivity is needed to trigger generalized activity in a quiescent network than to sustain it once the network has been activated.

With respect to the development of activity and connectivity, the model shows similarities with developing in vitro cultures of dissociated cells: an initial phase of neurite outgrowth and synapse formation while activity is low, a rather abrupt onset of network activity when the synapse density reaches a critical value, followed by a phase of neurite retraction (Purkinje cells in cerebellar cultures: Schilling et al., 1991) and/or elimination of synapses (cerebral cortex cells: van Huizen et al., 1985, 1987a; van Huizen, 1986). As in the simulation model, these latter cultures show a transient overproduction (overshoot) of synapses during development (Fig. 3, control). Chronic blockade of electrical activity (Fig. 3, TTX) results, as in the model, in an absence of synapse elimination, while chronic blockade of inhibitory transmission leads to an advancement of synapse elimination (Fig. 3, PTX).

In the previous studies, the value of \( \varepsilon \) was taken

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**Fig. 2. Overshoot in connectivity (C) in a purely excitatory network. After Van Ooyen and Van Pelt (1994).**
the same for all neurons. As empirical studies have indicated that different classes of neurons react differently to activity (Guthrie et al., 1988; Kater et al., 1988, 1990), we have also examined networks in which ε is allowed to vary among neurons (Van Ooyen and Van Pelt, 1996). An overshoot in connectivity occurs even under these conditions, albeit that in the final state the level of connectivity can oscillate to a variable extent. Overshoot still takes place in the presence of inhibitory cells, and can even be enhanced. In excitatory networks, the decline in connectivity begins shortly after the onset of network activity, whereas in mixed networks (containing both excitatory and inhibitory cells) the decline in overall connectivity can be considerably delayed relative to the onset of network activity. This is what was actually observed in rat neocortical cell cultures with respect to the decline in the number of synapses from their transient peak level (Van Huizen et al., 1985).

If the development of inhibition is delayed with respect to excitation, the growth curve for the number of inhibitory connections fails to exhibit an overshoot. In tissue cultures the putative inhibitory synapses (those on shafts: Shepherd, 1990) show no pronounced overshoot during development, while the synapses on spines (which are mostly excitatory: Shepherd, 1990) do show such an overshoot (Van Huizen et al., 1985). This observation would thus be consistent with indications that there is a progressive increase in the ratio of inhibitory to excitatory synaptic activity during development (see Corner, 1994).

Multistability and periodic behavior in mixed networks

Under all initial conditions purely excitatory networks develop to the same end state (attractor). Mixed networks, however, do not necessarily do so. In a network with a moderate level of inhibition and an initial average connection strength that is larger than a critical value (e.g., brought about by blocking electrical activity for a certain time) connectivity will not be reduced to the normal (low) equilibrium value but will continue to increase, instead. A similar phenomenon has been observed in developing cultures of dissociated cortex cells: cultures which were chronically deprived of electrical activity (resulting in enhanced
neurite outgrowth, with no subsequent elimination of synapses) for longer than a certain period, failed to eliminate their excess synapses when activity was eventually allowed to return (Van Huizen et al., 1987b). This has been taken to indicate that there exists a ‘critical’, or ‘sensitive’, period after which the cells can no longer prune their connections. Our simulations, however, suggest the alternative that, although the intrinsic properties of the cells do not change over time, the interactions between excitation, inhibition and outgrowth are in themselves capable of generating similar phenomena.

To further study these phenomena, we used a number of simplified models (Van Oss and Van Ooyen, 1995). In these models there is, in most cases, a point attractor (attractor A) at a low connectivity level and a limit cycle attractor at a high connectivity level (attractor B). Under the usual initial conditions (namely, a low level of connectivity) the system ends up in attractor A, whereas a high initial connectivity will cause the system to end up in attractor B. Attractor B is interpretable as a “pathological” state: the limit cycle has large and fast oscillations in electrical activity (“epileptiform”). In tissue culture, the cells have also enhanced “bursting” patterns following chronic blockade of electrical activity (see following section). An interesting model result is that the higher the (initial) level of inhibition during development, the more likely the system is to end up in attractor B. This is in agreement with a number of recent experiments on induced hypoxic-ischemic encephalopathy (HIE, i.e., brain damage as a result of lack of oxygen), which in rat pups can lead to permanent epileptiform activity later on in adulthood (Romijn et al., 1994). Such epileptiform activity was not the result of a preferential loss of inhibitory elements following HIE (Romijn et al., 1992, 1993); indeed, there was a preferential survival of inhibitory elements. As in the model, the relatively high level of inhibition might have contributed to the development of an epileptiform state. During the development of the hippocampus, the normally inhibitory neurotransmitter GABA initially works in an excitatory fashion (Cherubini et al., 1991), which might have the result of reducing the risk of pathological development.

Differences among cells

The neuritic field size adapts to the local cell density, resulting in small fields in dense areas and larger ones in sparse areas. Local variations in cell density generate also large variations in developmental time course of field size and firing behavior. For example, some cells show a clear overshoot, while others simply increase until equilibrium is attained. These two patterns have also been described in the literature (e.g., Petit et al., 1988; Ulfhake et al., 1988).

Compensatory sprouting

Excitatory cell death in the model will be accompanied by an increased neuritic field of the surviving neurons. In human cortex the total amount of dendrites per neuron increases steadily through old age (Beull and Coleman, 1979; Coleman and Flood, 1986), which has been interpreted as a compensatory response to neuronal death (Curcio et al., 1982; Coleman and Flood, 1986).

Network size

In excitatory networks with a relatively low synaptic strength, cells develop into a single interconnected network, whereas higher synaptic strengths tend to yield a system containing several loosely connected sub-networks. By inducing outgrowth, inhibitory cells serve to increase the degree of excitatory connectivity; cells will need to make more contacts in order to receive sufficient excitatory input. In this way, sub-networks that otherwise would have remained disconnected can become connected in the presence of inhibition. It would be worthwhile to see whether this can also be observed experimentally.

Importance of the spatial distribution of inhibitory cells

When inhibitory cells make contact to each other, they become electrically inhibited but their outgrowth will thereby be stimulated. The ultimate
level of inhibition will therefore become higher. As a result of outgrowth, therefore, not only the number of inhibitory cells but also their spatial distribution is important for determining the organization of the network.

**Differentiation between excitatory and inhibitory cells**

Although, in the model, there are no intrinsic differences in growth properties between excitatory and inhibitory cells, their neuritic fields will nevertheless become different. The neuritic field of inhibitory cells will in general become smaller than that of excitatory cells. In the cerebral cortex, the dendritic (and axonal) fields of inhibitory neurons (non-pyramidal) are indeed smaller, on the whole, than those of excitatory neurons (pyramidal) (e.g., Abeles, 1991; Kandel et al., 1991). In order to receive sufficient excitation, a model cell connected to an inhibitory cell grows a larger field than does one that is not inhibited. An inhibitory cell can therefore remain small because it will become surrounded by large excitatory cells. These, in turn, will become surrounded by relatively small cells, and so on. Thus, an inhibitory cell can impose a characteristic spatial pattern of neuritic field sizes on the surrounding 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.

**Extended growth function**

With a growth function where neurite retraction takes place also when neuronal activity falls below a critical value, much the same results are obtained as listed above, provided that the initial activity of the neurons is sufficiently high to allow outgrowth. In summary, these model studies have shown that all the above mentioned, seemingly unrelated observations and phenomena may in fact be interrelated, all of them being the consequence of activity-dependent neurite outgrowth. Spontaneous neuronal activity, in combination with activity-dependent neurite outgrowth, thus has considerable potential for controlling the development of neuronal form and network circuitry.

The properties of receptors and ion channels are not fixed, but can also be modified under influence of electrical activity. Lobster stomatogastric ganglion neurons, for example, have been found to regulate their conductances so as to maintain stable activity patterns (e.g., Turrigiano et al., 1994). Abbott et al. (1993), LeMasson et al. (1993) and Abbott and LeMasson (1993) have developed model neurons in which the maximal conductance of each ionic current is not fixed, as in most models, but is regulated by the neuron's own activity, with \([Ca^{2+}]_i\), as an indicator of activity levels. The conductances react to \([Ca^{2+}]_i\) in such a way that a negative feedback loop exists from activity to current strength, which stabilizes the activity of the neuron. Thus, when the amount of extracellular potassium in the model is increased so that an isolated, periodic bursting neuron goes into a fast, tonic firing mode, the resulting increase in electrical activity and, consequently, \([Ca^{2+}]_i\), causes a readjustment of the ion channels so that the initial behavior is restored (although possibly with a different set of conductances). This type of regulation also causes the intrinsic properties to shift in response to the presence of other neurons. The coupling between two identical bursters induces the neurons so as 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 the conductances change locally, a realistic pattern of membrane conductances (viz., 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.

**Physiological role of spontaneous bioelectric activity in network formation**

As discussed in the previous section, generalized network activity is triggered by "spontaneously" active cells when the excitatory connectivity has become sufficiently high. At this critical point, the network may show long-lasting transients of ac-
tivity (Van Ooyen et al., 1992). Tissue culture systems, however, show a relatively brief and highly stereotyped pattern of neuronal discharges, separated by variable epochs of electrical silence (see Fig. 4; Corner, 1994).

This feature of the living system can be reproduced in network models by adding a cumulative, activity dependent, "refractoriness" to the functional properties of the model neurons (Kowalski, 1992; Van Ooyen et al., 1992). In addition, the inclusion of inhibitory interactions produces variable, sometimes localized, bursts of activity (Kowalski, 1992) which strikingly mimick changes observed in neuronal networks in vitro (from "phasic" to "burst" firing) as synaptic inhibition becomes added in the course of maturation (Corner and Ramakers, 1992).

Intrinsically generated bioelectric discharges have proven to exert a considerable influence on neural network ontogeny, at least under in vitro conditions. Neuronal survival, for instance, is greatly reduced in a wide variety of CNS regions if their physiological activity is chronically suppressed. In addition, "exuberant" non-selective excitatory connections are formed by sensory ganglion afferent fibers within the cord under such conditions, but these projections are relatively ineffective in triggering polysynaptic activity within the spinal network (for review, see Corner, 1994). This function-dependent "pruning" of excess afferent fibers bears a certain resemblance to the formation of ocular dominance columns in the visual system (e.g., Miller, 1994). In the spinal cord experiments, however, it is the target rather than the source neurons which generate the required activity, and it will be of great interest now to investigate whether this principle also applies at higher levels of the central nervous system.

As mentioned in the previous section, cerebral cortex tissue in vitro shows a similar failure for the initially over-abundant excitatory synapses to be pruned back to their mature level in the chronic

![Graphs showing neuronal activity](image_url)

Fig. 4. Samples of spontaneous "bursting" events recorded in different rat neocortex explants cultured in vitro. Left, typical slow-wave events (sweeps triggered by the initial negative wave) showing spike-associated after potentials. Right, intracellular recordings showing (above) a large "paroxysmal depolarizing shift" associated with transient spike inactivation, and (below) a train of afterpotentials following the initial deflection after a long delay (from Corner, 1994).
absence of neuronal discharges (Van Huizen et al., 1987a), even if the activity is restored after the chronic blockade (Van Huizen et al., 1987b). In addition, bioelectric activity patterns are present under such conditions which reflect an abnormal balance between effective excitatory and inhibitory synaptic drive within the network, and these epileptiform patterns appear too early in development to be fully explained by the cytomorphological abnormalities (Corner and Ramakers, 1992). It appears that, in addition, inhibitory neuronal mechanisms fail to mature fully in the absence of functional stimulation (Ramakers et al., 1994), thus leaving the tissue in a physiologically primitive condition, which can be mimicked in normal cultures by experimentally disinhibiting them using GABA receptor blockers (see Corner, 1994). Such coupling of inhibitory maturation to ongoing activity levels would serve as a homeostatic developmental mechanism for guaranteeing that network excitability at maturity falls within an adaptive range. Early onset of spontaneous action potential discharges, then, would make it possible for this ‘tuning’ process to play a role throughout the entire period of neuronal network formation.

More recent experiments, involving “organotypic” instead of dissociated neural tissue in which glutamatergic neurotransmission has been chronically blocked, have confirmed the importance of critical levels of excitatory synaptic drive for proper physiological development.

Combined treatment with DNQX plus APV was able to virtually eliminate all ongoing “spiking” activity throughout the treatment period lasting up to 3 weeks; full-scale spiking recommenced within minutes after return to normal growth medium. APV alone, in contrast, caused a profound transient depression with the return of spontaneous firing starting about 1 h after the onset of treatment, and recovering to control levels within 24 h (where it remained throughout the rest of the treatment period).

Excessive bursting activity appears as a result of such treatment (Fig. 5); interestingly, also when the NMDA receptors alone have been blocked, despite the fact that spontaneous firing is reduced only transiently in that case (Fig. 6).

Presumably, calcium entry during action potential generation (see Kater et al., 1988, 1994) is insufficient to assure full functional maturation, at least under the given experimental conditions, and thus needs to be supplemented by entry via NMDA channels. The fact that, within hours, the non-NMDA receptors on developing cortical neurons are capable of taking over the function of chronically blocked NMDA receptors (see Corner 1994) might seem to imply that the former “up-regulate” under such conditions, thus restoring network excitability, e.g. spontaneous firing, to its original level. This conclusion would be premature, however, since a picrotoxin-induced blockade of GABA receptors is also capable, within minutes, of reversing the effect of acute NMDA recep-

![Fig. 5. Effects of chronic NMDA receptor blockade (APV group) or total suppression of spontaneous neuronal firing (APV/DNQX group) for 2 (left) and 3 (right) weeks in vitro on mean firing rates (spikes/s) and incidence of afterdischarges (bursts/min). **P < 0.025; ***P < 0.01 for the differences from the respective control group (Mann–Whitney U-test; median values and 50% ranges are plotted.](image-url)
tor blockade (unpublished observations). The actual contribution of this mechanism to the observed recovery will need to be experimentally established but, in view of the crucial importance of well-balanced excitatory and inhibitory synaptic activities for optimal function of the nervous system, it is tempting to postulate the existence of a broad spectrum of synergistic “neuroplastic” mechanisms for ensuring that a good balance is eventually achieved. In any event, “down-regulation” of GABA receptors or release cannot constitute the full explanation for the APV induced “hyperexcitability syndrome”, since exposure of control (untreated) cultures to picrotoxin after 3 weeks in vitro failed to mimic the effects of chronic NMDA receptor blockade (unpublished observations).

The theoretical implications of these findings, should they be generalizable to the intact organism, can hardly be overemphasized. They imply no less than that a hitherto unsuspected phase of function-dependent development exists, one which depends upon the nervous system’s endogenous physiological activity rather than upon responses to sensory stimulation. In view of its extremely early onset, we propose to designate this as the PREMATURE period of “neuro-plasticity”, in order to distinguish it from the later IMMATURE period in which environmental stimuli act permissively to fine-tune neural projections such as ocular dominance columns (Miller, 1994), as well as from the MATURE period characterized by the instructive, anatomically elusive, “imprinting” phenomena with which we are familiar as learning and memory. Both of these latter neuroplasticity phases may in fact even depend upon the persistence into later life of the “premature” one, e.g., as a biorhythmic priming mechanism for selective retention of information about the environment (e.g., Singer, 1985).

Summary

Many structural and functional properties of neuronal networks find their origin in the dynamic behavior of growth cones during development. The variation in dendritic morphologies can be traced back to random branching of growth cones. Segment length characteristics arise under random branching and steady growth cone propagation. Delayed outgrowth, as a result of competition between growth cones after splitting, is hypothesized to explain different lengths of paired terminal segments in Purkinje cells.

The implications of activity-dependent neurite outgrowth were studied using an outgrowth function based on the theory of Kater et al. (1988, 1990). This theory embodies a homeostatic principle, according to which a neuron adapts its neuritic field so as to maintain a certain level of bioelectric activity. It is shown that such homeostasis has many implications for neumorphogenesis and network formation, as it may underlie phenomena such as overshoot during development, size differences among cells, differentiation between excitatory and inhibitory cells and compensatory sprouting.

Finally, function-dependent regulation of development involves physiological as well as morphological variables. For instance, activity-dependent regulation of ionic conductances such as
to stabilize functional activity can result in a differ-
entiation of certain neurons into, respectively,
bursting and regular firing sub-types (Abbott et al.,
1993; LeMasson et al., 1993). Similarly, the
GABAergic phenotype comes fully to expression in
hindbrain (cerebellar) and forebrain (neocortical)
networks only if the level of ongoing excitatory activity
during development is sufficiently high, whereas chronically intensified activity leads to a compensatory hypertrophy of inhibitory mechanisms (for review, see Corner 1994).
Many of these results could only have been obtained
by the use of mathematical models which allow rigorous analysis of the consequences of basic assumptions in the dynamics of neurite outgrowth. All in all, the findings further emphasize the role of spontaneous bioelectric activity during early development in neuronal network formation, the importance of which was first established in cultures of developing neural tissue.

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