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Dynamic mechanisms for bundling and guidance during neural network formation☆

H.G.E. Hentschel[a,*], A. van Ooyen[b]

[a] *Department of Physics, Emory University, Rollins Research Center, 1510 Clifton Road, Atlanta GA 30322, USA*

[b] *Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands*

Abstract

The dynamic mechanisms by which self-organizing circuits form in a developing nervous system remain to be elucidated. It is clear, however, that diffusible chemoattractants and chemorepellants as well as contact attraction and contact repulsion have been implicated in the establishment of connections between neurons and their targets. We will describe simulations based on the known biology of the whole sequence of events from the bundling of axons, guidance, pathfinding, to debundling and the final innervation of individual targets.

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1. Introduction

Nonlinear dynamics has influenced the development of many areas of science, and nowhere are the effects of nonlinearity more important than in the mechanisms underlying biological development and signalling. This is especially true in the area of neuroscience and the formation of the self-organizing circuits which are the developing nervous system.

The development of a biological neural network involves neurons sending out axons, which need to migrate to specified target cells; self-wiring of functioning neuronal

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* Corresponding author. Tel.: +1-404-727-0764; fax: +1-404-727-0873.

E-mail address: phshgeh@physics.emory.edu (H.G.E. Hentschel).

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circuits occurs [1]. Several dynamic mechanisms appear to be involved in guiding migrating axons to their targets. These include the diffusion of chemoattractant molecules through the extracellular space from the target [2,3] creating a gradient of increasing concentration toward the target, which the growth cone, a protrusion at the tip of a developing axon with fine hair-like processes called filopodia, can sense and follow [4,5]. Diffusible chemorepellants, secreted by tissues the axons need to grow away from, can also be used to guide axons [2,6]. In addition to diffusible factors, molecules in the extracellular matrix and molecules on the surface of cells such as cell adhesion molecules (CAM) can also attract and repel axons, the so-called contact attraction and contact repulsion, respectively [2,7].

Growing axons often form bundles or fascicles, a process called fasciculation [7–9]. Though contact attraction mediated by CAMs on the surface of axons have been implicated [9,10] and can clearly serve to keep axons together, there remains the question of how axons come together in the first place. One possibility is that the random movements of individual axons are sufficient; another suggestion [2] is that contact repulsive signals from surrounding cells push the axons together; a third possibility is that axons are attracted by diffusible molecules which they themselves secrete [11,12] or are attracted by traction-like forces exerted by the filopodia on each other when axons approach close enough [13].

The individual axons and bundles, then, need to be guided to their targets. Upon reaching the target region, the axons in the bundle must steer away from each other in order to innervate their own specific targets, a process called defasciculation.

2. Models of axon guidance and bundling

Many possible mechanisms may underlie axon guidance, bundling, and debundling. Some of these mechanisms may have a strong genetic component, while others may have a stronger physical component. In this paper we consider some mechanisms of the latter category, viz. diffusion and contact interaction. In simulations of two specific models, we study the consequences of these dynamic mechanisms for growth. These models should be regarded as limiting cases, and we may suppose that depending on the detailed biology, aspects of both models describe the growth.

To investigate diffusive mechanisms involved in axon guidance, we need to consider axon migration in a diffusive chemoattractant which is released by the target and whose gradients the growth cones can sense; while for bundling to occur, there need to be attractive interactions between the growing fibers, which can be due to both contact adhesion and to diffusive chemoattractants released by the migrating growth cones themselves. Chemoattractants will naturally degrade in the extracellular space, and axon-derived chemoattractants can, therefore, also mimic the attractive forces due to the filopodia [13] which will extend and retract over some finite length scale (the growth cone ≈ 10 μm, the extensions up to ≈ 100 μm).

Consider, therefore, the interaction of two types of diffusible molecules: (1) a chemoattractant, which is released by the target cells at rate J_{target}, has diffusion constant D_{target}, and spatial concentration $\rho_{target}(\mathbf{x})$; and (2) a chemoattractant, which is released by the axonal growth cones at rate J_{cone}, has diffusion constant D_{cone}, and concentration $\rho_{cone}(\mathbf{x})$. More complex models can be created involving chemorepellants to control debundling [14].

For the two diffusive fields described above, the equations for the concentration gradients are

$$[\nabla^2 - \kappa_{cone}^2]\rho_{cone} = -[J_{cone}/D_{cone}] \sum_{\alpha} \delta(\mathbf{x} - \mathbf{r}_\alpha(t)) ,$$
$$[\nabla^2 - \kappa_{target}^2]\rho_{target} = -[J_{target}/D_{target}] \sum_{i} \delta(\mathbf{x} - \mathbf{x}_i) , \quad (1)$$

where $\mathbf{r}_\alpha(t)$ is the position of growth cone α at time t, and the target cells and growth cones are treated as point sources. Both chemoattractants are degraded in the extracellular space, resulting in finite diffusion lengths $1/\kappa_{target}$, and $1/\kappa_{cone}$.

Axons will move up the gradients of the chemoattractants at rates proportional to the gradients [15], but are also subject to random movements due to random variations in the substrate over which the axons move and due to random exploration of extracellular space by the growth cone. Thus, the total response of the growth cone is

$$d\mathbf{r}_\alpha/dt = \lambda_{cone}\nabla\rho_{cone}(\mathbf{r}_\alpha(t), t) + \lambda_{target}\nabla\rho_{target}(\mathbf{r}_\alpha(t), t) + \xi_\alpha(t) , \quad (2)$$

where λ_{cone}, and λ_{target} are the rate constants of growth of the axon to the cone and target chemoattractant gradients; and $\xi_\alpha(t)$ are the random growth cone movements assumed to have only short-range correlations i.e., $\langle \xi_{\alpha,i}(t)\xi_{\beta,j}(t')\rangle = \Gamma\delta_{\alpha,\beta}\delta_{i,j}\delta(t-t')$. Eqs. (1) and (2) are an adiabatic approximations valid on length scales $l \ll D/V_{growth}$, where $V_{growth} = d\mathbf{r}_\alpha/dt$. This includes the present case as we are considering development on scales $10\ \mu m < l < 1000\ \mu m$, while $D/V_{growth} \approx 1$ cm (using $D \approx 10^{-6}$ cm^2/s [16,17], and in the presence of typical gradients $V_{growth} \approx 10^{-6}$ cm/s [15]), which is about the size of the whole embryo.

In Fig. 1, a complete simulation of growth in the presence of the diffusive fields [14] can be seen. We shall now describe the results of our simulations and their implications for the self-organization of biological neural networks.

2.1. Fasciculation

In our simulations, the target cells and the initial positions of the growth cones were taken to lie in two thin layers separated by a distance of three diffusive length scales of the target-derived chemoattractant. With this choice, the initial concentrations of the target-derived chemoattractant are just perceptible at the layer of the growth cones. The initial configuration of the growth cones is dense compared to the length scale of the axon-derived chemoattractant, $1/\kappa_{cone}$. As a result fasciculation occurs readily (see Fig. 2).

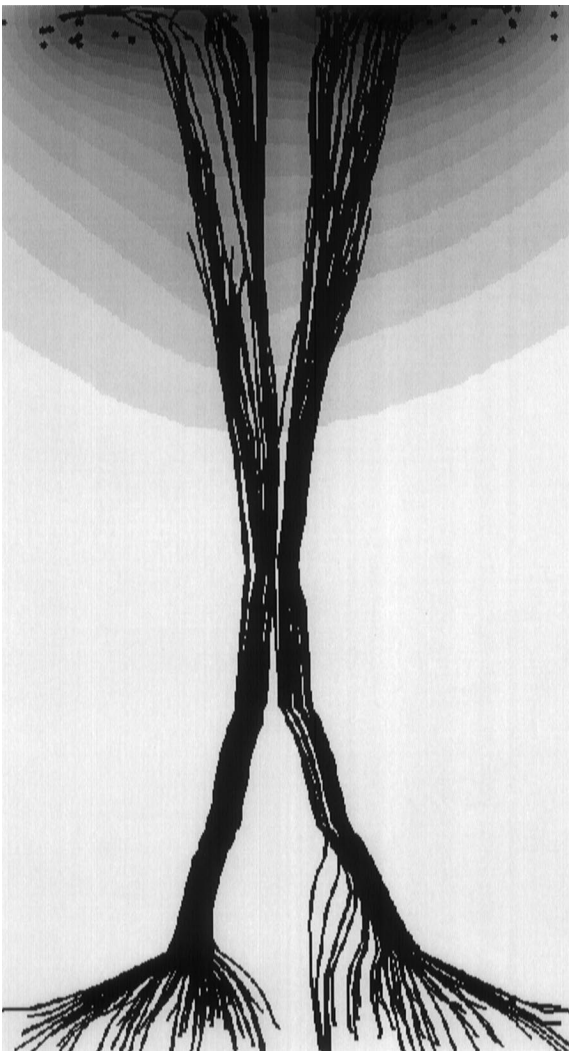

Fig. 1. A complete simulation of axonal migration from a source to a target layer of cells in the presence of a chemoattractant released by the axonal growth cone and a chemoattractant released by the target. Bundling of the individual axons can be observed. The target chemoattractant is visualized as higher in the darker regions of the figure.

It is interesting to note that the axons organize themselves into two bundles each containing axons from a portion of the sites. By appropriate choices of the strength and diffusive length scale of the axon-derived chemoattractant, the beginning of a spatial segregation of sites occurs early in development.

Growth in the absence of cone-released chemoattractant can still lead to local bundling, provided random motion and CAMs are present, but there is no tendency for the global fasciculation observed in the presence of chemoattractants. More complex growth

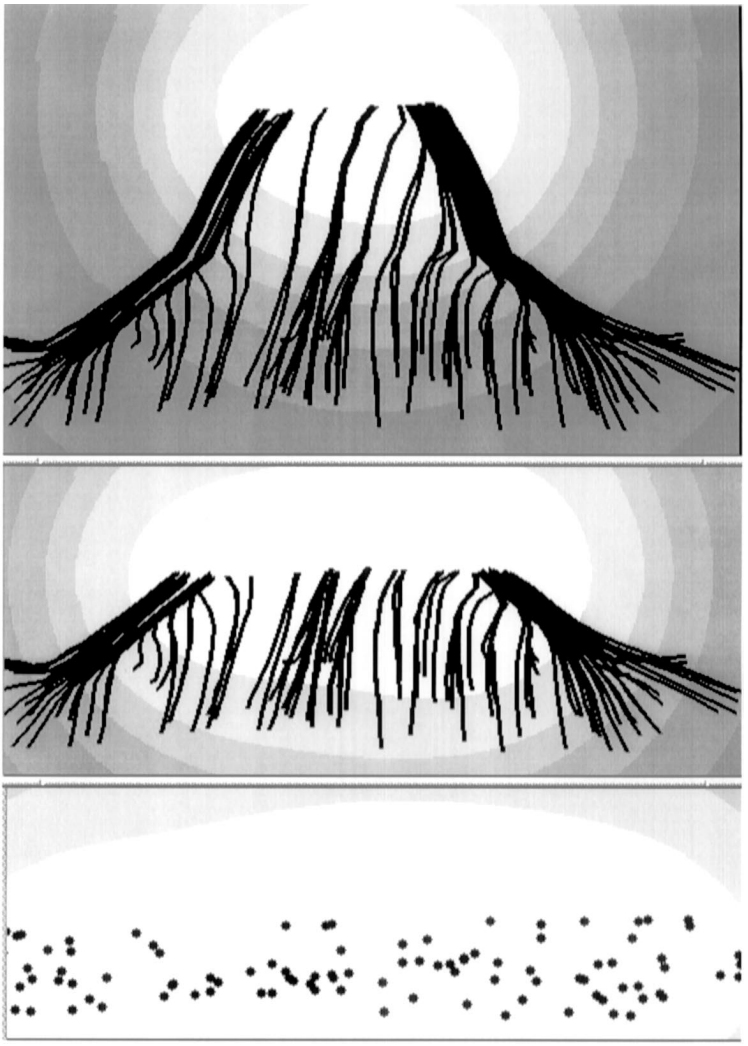

Fig. 2. Axon fasciculation in the presence of a chemoattractant released by the growth cone. The growth cone chemoattractants are higher in the lighter regions of the image. Starting with the bottom image consisting of neural cells before axonal outgrowth and proceeding upwards the sequence of events in which nerve fibres grow from these cells and bundle can be seen.

conditions may partially remedy this situation. For example, diffusible chemorepellants released by surrounding tissue as well as contact repellants on the surface of surrounding cells or in the extracellular matrix may serve to push the axons together after which contact attraction can come into play [2,14]. The transition from chemoattractant-dominated bundling to diffusion-dominated bundling can be estimated as follows. If the typical initial distance between fibers is l, then the time for fibers to start bundling due to the random motion of the growth cones (see Eq. (2)) is

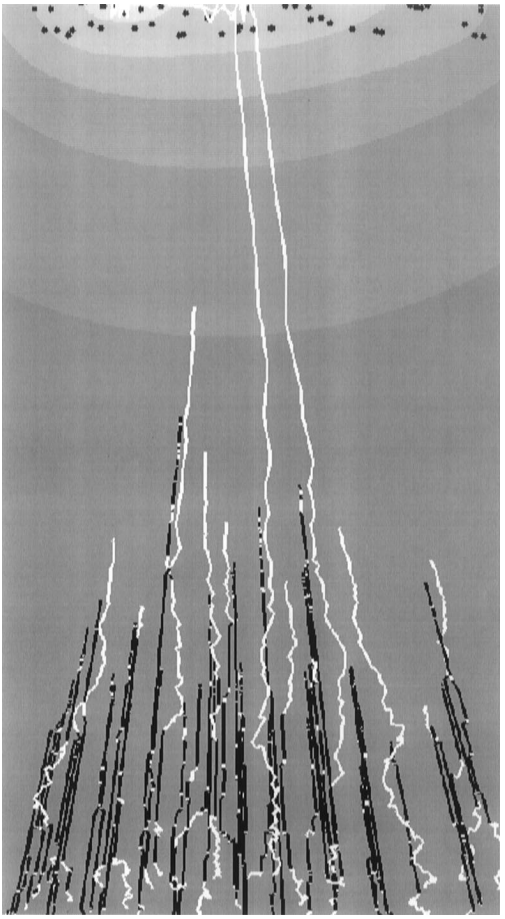

Fig. 3. The emergence of pathfinding neurons in the presence of noise and a chemoattractant released by the target. In this figure, the black fibers represent bundled axons due to CAMs while the white fibers represent free axons.

$t_{random} \sim l^2/\Gamma$, while the time for fibers to start bundling due to a long-range cone attractant is $t_{cone} \sim l/v_{cone} \sim D_{cone} l^3/(\lambda_{cone} J_{cone})$. When the noise levels become greater than $\Gamma \sim \lambda_{cone} J_{cone}/(D_{cone} l)$ the bundling will be noise-dominated. When this happens, proper target innervation may no longer be possible due to the loss of topographic ordering in any bundling.

As fasciculation by axon fibers may be due either to growth cone-released chemoattractants (see Fig. 2), or due to the presence of CAMs on the membrane surface causing axons, that are close enough, to have a probability of sticking to each other (see Fig. 3), this bundling probability $P_{bundling}$ needs to be incorporated into simulations.

This probability is not a constant but decreases close to the target layer of cells in order to allow defasciculation to occur. The factors triggering defasciculation are at present unknown, but for example in vertebrate limbs, the branched innervation may

depend on mesodermal cues such as the connective tissue that organizes muscle pattern [18]. The detailed mechanism involved seems less clear. A reasonable possibility is that the axons sense the target-released chemoattractant $\rho_{target}(\mathbf{x})$ that increases close to the target and switches on a genetic mechanism reducing the production of CAMs. For example, the beat gene has been implicated in encoding such proteins [19].

In our simulations we assumed that sites exist on the growth cone that can bind to CAM molecules responsible for contact attraction on another axon membrane. The probability for CAMs to bind to these receptor sites was assumed to be reduced in the presence of target-derived chemoattractant. Ligand–receptor kinetics then lead to a Michaelis–Menten kinetic form for the probability

$$P_{bundling}(\rho_{target}) = 1 - \rho_{target}^m/[\rho_c^m + \rho_{target}^m]\,, \tag{3}$$

where ρ_c is the value of ρ_{target} where the bundling probability is 50%, and m is the Hill coefficient of the receptor kinetics involved.

2.2. Axon guidance

The target-derived chemoattractant is necessary for axon guidance to the target region. The interplay between the tendency to bundle (as a result of the axon-derived chemoattractant) and the tendency to grow up the gradient of the target-derived chemoattractant affects the topographic ordering within the bundle. If the concentration gradient of the target-derived chemoattractant, $\nabla\rho_{target}$, is relatively small and/or λ_{target} is relatively small, the axons form a random clustering and show no tendency to develop into organized bundles. In contrast, if $\nabla\rho_{target}$ and/or λ_{target} are very large, growth is ordered and directed to the targets but no bundling occurs. At optimal conditions both bundling and axon guidance occur (see Fig. 1).

2.3. Pathfinding

Migrating axons are guided to their targets through the concentration gradients of target-derived chemoattractant. New in these simulations is the emergence of what might be called ‘pathfinding axons’.

This interesting emergent property (see Fig. 3) is a noise-enhanced process. The mechanism for their emergence appears to be the following. As the steepness of the gradient of the target-derived chemoattractant increases toward the target region, the rate at which the axons grow increases exponentially, as do their fluctuations. For example, if the typical distance from the source to target is L, then expanding $v_{target}(z) = v_{target}(0) + \beta z$, where z is the distance from the source of the axons and $\beta \sim \lambda_{target} J_{target}/(D_{target} L^3)$ leads to an initial average growth of the axon toward the target

$$\langle z(t)\rangle = v_{target}(0)(\mathrm{e}^{\beta t} - 1)/\beta\,, \tag{4}$$

while the fluctuations grow as

$$\sigma^2(t) = (\langle z(t)^2 \rangle - \langle z(t) \rangle^2) = \Gamma(e^{2\beta t} - 1)/2\beta \,. \tag{5}$$

This is because the existence of random axon movements, which are stronger for unbundled than for bundled axons, allows unbundled axons to move into steep chemoattractant gradients. As a result, the axons will grow faster toward the targets and so come into even steeper gradients – a form of positive feedback exists. Once the unbundled axons have reached the targets, they form 'paths': as a result of contact attraction, the slower bundles will become attached to these paths (provided they find them), and they will subsequently follow them. Pathfinding will occur if $\sigma(t_{cone}) \gg l$ for then individual fibers will escape the cone chemoattractant gradients encouraging bundling and these fibers may enter an exponential growth phase in the target chemoattractant gradient.

2.4. Defasciculation and target innervation

Once the target-derived chemoattractant has guided the axon bundle to the target region, debundling does not occur automatically. It may be thought that the chemoattractant concentration gradients from different targets might be sufficient to pull the axons apart. Simulations [14] show, however, that the gradients of the target-derived chemoattractant are often not large enough across an axon bundle, and the axon bundle will consequently behave as a single entity responding to the target chemoattractants.

Experimental observations indicate, however, that this is not a problem, because defasciculation depends on both a reduction of the expression of fasciculation-enhancing adhesion molecules, such as L1 and the neural cell adhesion molecule (NCAM), and also on recognition molecules that actively induce defasciculation by triggering second-messenger systems [20] inside the cell. For example, the beat gene has been implicated in encoding a protein with an antiadhesive function [19].

A trigger is required for this to occur, possibly some variation in the properties of the extracellular space in which the nerve fiber moves or some diffusant reaching a threshold concentration. We shall model this process here assuming that the presence of chemorepellants released by the growth cones in response to the target chemoattractant is sufficient to cause defasciculation [14] in agreement with previous theoretical arguments [21] on the requirements for topographic map formation in neural network formation.

To estimate the likelihood of debundling as the target is approached, we note that the average velocity of an individual fiber in a bundle is proportional to the difference in bound receptors B over the growth cone $v_{fiber} \sim \delta B \sim |\langle \partial B/\partial \rho_{target} \rangle||\nabla \rho_{target}|d$ where d is the diameter of a growth cone, while the deviation in this value Δv across a bundle of $\mathcal{N}$ fibers of thickness $W \sim \mathcal{N}^{1/2} d$ can be expected to scale as

$$\Delta v \sim |\delta B(\mathbf{x}) - \delta B(\mathbf{x} + \mathbf{W})| \sim |\langle \partial B/\partial \rho_{target} \rangle||\nabla^2 \rho_{target}|dW \,. \tag{6}$$

Clearly when $\Delta v/v_{fiber} \sim W/R \approx 1$ debundling may occur. Whether it actually does occur depends on the extent to which CAM molecules can efficiently bind the fibers together. From Eq. (3) we see that debundling is likely to occur when $\rho_{target} \ll \rho_c$, or for $J_{target}/(4\pi D_{target} W) \ll \rho_c$. The final innervation of the individual targets occurs in a biologically plausible manner in simulations [14] (see Fig. 1). For example, as the target region is approached the axons curve and grow transversely into the slice of target cells innervating them in the process.

3. Concluding remarks

We have investigated the strengths and weaknesses of various mechanisms that have been proposed for axon guidance, bundling, and debundling. These mechanisms involve diffusible chemoattractants and chemorepellants as well as contact attraction and repulsion.

Our simulations show that the diffusion of a target-derived chemoattractant is an effective mechanism by which axons and axon bundles can be guided to the target region. We estimated the range over which such diffusive mechanisms should be effective and find that if the chemoattractant concentration is either too large or too small a 'real' growth cone will not be able to sense a concentration gradient in agreement with the work of Goodhill [16,17]. If the chemoattractant concentration is too large, the receptors become saturated; if it is too small, noise effects become dominant. In both cases, there is no detectable difference in receptor binding across the growth cone for it to sense a gradient. This effect can be accommodated in our model by making the rate of outgrowth, λ_{target}, dependent on the chemoattractant concentration in such a way that for either too high or too low concentrations, it becomes zero. This imposes a maximum length range over which growth cone guidance by a diffusible factor is possible.

A kind of 'pathfinding axons' emerge in the model if random axon movements are included. Pathfinding axons could certainly exist in the absence of chemoattractants and random movements: owing to a genetic programme, some axons may simply grow out first, creating a path to the targets that can then be followed by the other axons. Nevertheless, we find it intriguing that such genetic mechanisms do not need to be employed and that pathfinding appears as an emergent property of the dynamics.

Axons within a bundle can be kept together by contact attraction, but for this to work axons need to come together in the first place. An important outcome of our simulations is that although random axon movements and repulsive signals from the surrounding environment may be helpful in this, a long-range signal derived from the axonal growth cones themselves could provide a much more effective mechanism for bringing axons together. Our model allows for two interpretations of the nature of this long-range signal: (i) a diffusible chemoattractants released by the axonal growth cones, and (ii) the filopodia of the growth cones, which extend over some radius (although their effective range will be typically smaller than that of a diffusible signal).

Another important result of our study is that proper debundling and target innervation does not occur solely as the result of a breakdown of contact attraction (or indeed active contact repulsion) and the presence of concentration gradients of target-derived chemoattractants. The reason for this is that the concentration gradients of target-derived chemoattractant are simply not large enough across the relatively small axon bundle (whether it is broken up or not) to further separate the axons. A diffusible, long-range, axon-derived chemorepellant appears, therefore, necessary for debundling and proper target innervation; this is a testable prediction of the model, and experimental studies should be carried out to find out whether such chemorepellants exist. Our findings are in agreement with those of Fraser [21] who argues that chemorepellants appear to be necessary for proper target innervation.

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