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Models of axon guidance and bundling during development

H. G. E. Hentschel[1] **and A. van Ooyen**[2*]

[1]*Department of Physics, Emory University, Atlanta, GA 30322, USA*
[2]*Netherlands Institute for Brain Research, Graduate School of Neurosciences Amsterdam, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands*

Diffusible chemoattractants and chemorepellants, together with contact attraction and repulsion, have been implicated in the establishment of connections between neurons and their targets. Here we study how such diffusible and contact signals can be involved in the whole sequence of events from bundling of axons, guidance of axon bundles towards their targets, to debundling and the final innervation of individual targets. By means of computer simulations, we investigate the strengths and weaknesses of a number of particular mechanisms that have been proposed for these processes.

Keywords: axon guidance; fasciculation; chemoattraction; contact attraction; diffusion; neurite outgrowth

1. INTRODUCTION

During the development of the nervous system, neurons send out axons, which migrate to their targets. One of the mechanisms by which migrating axons are guided to their targets is the diffusion of chemoattractant molecules from the target through the extracellular space. Such target-derived chemoattractants, e.g. netrins (Kennedy *et al.* 1994; Shirasaki *et al.* 1995; Mitchell *et al.* 1996) and neurotrophins (Tessier-Lavigne & Goodman 1996; ElShamy *et al.* 1996), create a gradient of increasing concentration, which the growth cone at the tip of a developing axon can sense and follow (Goodhill 1997). Axons are also repelled by diffusible molecules, e.g. netrins and semaphorins (chemorepellants) (Tessier-Lavigne & Goodman 1996), which are secreted by tissues the axons need to grow away from (Tamada *et al.* 1995; Colamarino & Tessier-Lavigne 1995). Not only diffusible molecules but also molecules in the extracellular matrix and molecules on the surface of cells, such as cell adhesion molecules (CAMs, e.g. cadherins), can attract and repel axons (Van Ooyen 1994), so-called contact attraction and contact repulsion, respectively.

Growing axons often form bundles or fascicles, a process called fasciculation or bundling (Nornes & Das 1972; Goodman *et al.* 1984; Jessell 1991). Contact attraction mediated by CAMs on the surface of axons has been implicated in this (Harrelson & Goodman 1988; Jessell 1991; Stoeckli & Landmesser 1995). Contact attraction, however, will only serve to keep axons together, and there remains the question of how axons come together in the first place. Various mechanisms have been suggested for bringing axons together. (i) Axons come together as a result of their random movements. Once the axons are near each other, but still separated, contact interactions between the filopodia of the growth cones may further draw the axons together. (ii) Contact repulsive signals (e.g. semaphorins) from surrounding cells push axons together (Tessier-Lavigne & Goodman 1996). (iii) Axons are attracted by diffusible molecules that they themselves secrete. Migrating axons are capable of secreting neurotransmitters (Hume *et al.* 1983; Young & Poo 1983), which have been implicated as chemoattractants (Tessier-Lavigne 1994; Zheng *et al.* 1994).

Upon reaching the target region, the axons in the bundle must steer away from each other (a process called defasciculation or debundling) in order to innervate their specific targets. Defasciculation could involve (i) a breakdown of contact attraction, (ii) active contact repulsion, and (iii) diffusible chemorepellants secreted by the axons themselves. Because defasciculation must occur close to the target region, it may be triggered by target-derived molecules. Indeed, the adhesiveness of CAMs can be modulated by factors encountered in the environment through which the axons grow (Tessier-Lavigne & Goodman 1996), and also by factors expressed by the axons themselves, e.g. polysialic acid (Tang *et al.* 1994).

In summary, both diffusible and contact signals have been implicated in the whole sequence of events from fasciculation, guidance of axon bundles towards their targets, to defasciculation and the final innervation of individual targets. The aim of this study is to uncover by means of computer simulations the strengths and weaknesses of a number of particular mechanisms that have been implicated in these processes.

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

*Author for correspondence (a.van.ooyen@nih.knaw.nl).

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paper, we consider some mechanisms of the latter category, namely diffusion and contact interaction. In simulations of two specific models, we study the consequences of these 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. In the first model, there are only long-range signals controlling growth, whereas in the second model there are also contact interactions.

(a) *Model I: only diffusible signals*

In this model, we consider the possibility that not only axon guidance to the target region but also axon bundling and debundling are controlled by diffusible molecules, i.e. by signals that have a long-range effect. We consider the interaction of three types of diffusible molecules: (i) a chemoattractant that is released by the target cells at rate σ_{target}, has diffusion constant D_{target} and a spatially varying concentration ρ_{target}; (ii) a chemoattractant that is released by the axonal growth cones at rate σ_{cone}, has diffusion constant D_{cone} and a spatially varying concentration ρ_{cone}; and (iii) a chemorepellant that is released by the axonal growth cones at rate σ_{rep}, has diffusion constant D_{rep} and a spatially varying concentration ρ_{rep}. In addition, the chemoattractants and chemorepellant are degraded or taken up by the extracellular space with rate constants δ_μ (where μ is target, cone, or rep), which results in concentration gradients that exist over length scales $1/\kappa_\mu$, where $\kappa_\mu = \sqrt{(\delta_\mu/D_\mu)}$ (see Appendix A on the Royal Society Web site).

Biologically, (i) is now fairly well established (e.g. Kennedy & Tessier-Lavigne 1994; Keynes & Cook 1995). For (ii) and (iii), there is less direct evidence, although they are certainly plausible because growth cones secrete various chemicals that may operate as chemoattractants and chemorepellants, e.g. neurotransmitters (see §1). Our simulations results show that (ii) and (iii) are necessary for bundling and debundling if we restrict ourselves to diffusive mechanisms. What is more, however, we find that they are more effective than the non-diffusive mechanisms that we have investigated (see model II). Furthermore, it should be noted that there are two quite different interpretations of (ii). The essential aspect of (ii) is that the signal has a long-range effect. This can be achieved either through a real chemoattractant or through the growth cones' filopodia. Filopodia extend over some radius and can bind to, and pull, filopodia of other growth cones through contact attraction. In this case, ρ_{cone} represents the actual density of filopodial contacts in space (which decreases with distance away from the growth cone), while $1/\kappa_{\text{cone}}$ represents the spatial extent of these filopodia. Similarly, the chemorepellant in (iii) could also describe contact repulsion through filopodial interactions. Usually, we will use the language of chemoattractants and chemorepellants, but this second interpretation should be kept in mind. Although diffusion and filopodial interactions are both long-range signals, they differ in that the spatial extent of filopodia is typically smaller than the effective range of a diffusible signal.

The target-derived chemoattractant controls axon guidance, while the axon-derived chemoattractant and chemorepellant control bundling and debundling, respectively. The axon-derived chemorepellant is needed because the concentration gradients of the target-derived chemoattractant are not large enough across the relatively small axon bundle to pull the axons apart (see §3(a)). For debundling to occur near the target region, the rate of release of axon-derived chemorepellant, σ_{rep}, must depend on the local concentration of the target-derived chemoattractant, ρ_{target} (see equation (2)).

The general equations for axonal growth in diffusive fields are described in Appendix A on the Royal Society Web site. For the three diffusive fields described above, the concentration gradients at equilibrium for given positions of the growth cones and target cells obey

$$[\nabla^2 - \kappa^2_{\text{cone}}]\rho_{\text{cone}} = -[\sigma_{\text{cone}}/D_{\text{cone}}]\sum_\alpha \delta(\boldsymbol{x} - \boldsymbol{r}_\alpha(t)),$$

$$[\nabla^2 - \kappa^2_{\text{target}}]\rho_{\text{target}} = -[\sigma_{\text{target}}/D_{\text{target}}]\sum_i \delta(\boldsymbol{x} - \boldsymbol{x}_i),$$

$$[\nabla^2 - \kappa^2_{\text{rep}}]\rho_{\text{rep}}$$

$$= -\sum_\alpha [\sigma_{\text{rep}}(\rho_{\text{target}}(\boldsymbol{r}_\alpha(t),t))/D_{\text{rep}}]\delta(\boldsymbol{x} - \boldsymbol{r}_\alpha(t)), \qquad (1)$$

where $\nabla\rho$ is the concentration gradient, $\boldsymbol{x}$ is a point in the extracellular space, $\boldsymbol{x}_i$ is the fixed position of target cell i, $\boldsymbol{r}_\alpha(t)$ is the position of growth cone α at time t, and $\delta(\boldsymbol{x})$ is the Dirac delta function, which means that the target cells and growth cones are treated as point sources. Equation (1) is a quasi-steady-state approximation for the diffusive fields. This approximation is valid if the time-scale for growth, t_{growth}, for a typical growth rate V_{growth} across a length scale l, is much longer than the time-scale for setting up the concentration gradient, t_{diffuse}. Because $t_{\text{growth}} \sim l/V_{\text{growth}}$ and $t_{\text{diffuse}} \sim l^2/D$, if $t_{\text{growth}} \gg t_{\text{diffuse}}$, the approximation is valid for length scales $l \ll D/V_{\text{growth}}$. Because $D \approx 10^{-6}\text{cm}^2\,\text{s}^{-1}$ (e.g. Goodhill 1997) and $V_{\text{growth}} \approx 10^{-6}\text{cm}\,\text{s}^{-1}$ (e.g. Rosentreter *et al.* 1998), it follows that $D/V_{\text{growth}} \approx 1\,\text{cm}$. During development, the distances among growth cones and between growth cones and target cells are typically much smaller than D/V_{growth}, so that the quasi-steady-state approximation is valid. Furthermore, axonal growth might commence only after the concentration gradient of the target-derived chemoattractant has been set up (see also Goodhill 1997). Under this condition, the only length scale that matters is that involved in the setting up of the concentration gradients of the axon-derived chemoattractant and chemorepellant; this length scale is the initial distance between the growth cones, which is of the order of 10 μm and much smaller than D/V_{growth}.

For the dependence of the rate of release of the axon-derived chemorepellant, σ_{rep}, on the local concentration of the target-derived chemoattractant, ρ_{target}, in equation (1), we assume a Michaelis–Menten type relationship:

$$\sigma_{\text{rep}}(\rho_{\text{target}}) = \sigma_{\text{rep,max}}\rho^m_{\text{target}}/[\rho^m_{\text{a}} + \rho^m_{\text{target}}], \qquad (2)$$

where $\sigma_{\text{rep,max}}$ is the maximum rate of release, ρ_{a} is the value of ρ_{target} where $\sigma_{\text{rep}}(\rho_{\text{target}})$ is half its maximum, and m is the Hill coefficient. We chose Michaelis–Menten relationships throughout this study because they are the most generic forms that arise from ligand-receptor kinetics in which the concentration of bound receptor is

at equilibrium for a given ligand concentration. In most of our simulations, the Hill coefficients are 2, so that relatively high ligand concentrations (which means in this case close to the target region) are needed to exert effects. The essential results of our simulations, however, do not change if other values are chosen.

The growth cones will respond to the concentration gradients by growing up the gradients of chemoattractants and down the gradients of chemorepellants. Thus, the total response of the growth cone is the result of two attractive and one repellant concentration gradient:

$$\mathrm{d}\boldsymbol{r}_\alpha/\mathrm{d}t = \lambda_{\mathrm{cone}}\nabla\rho_{\mathrm{cone}}(\boldsymbol{r}_\alpha(t),t) + \lambda_{\mathrm{target}}\nabla\rho_{\mathrm{target}}(\boldsymbol{r}_\alpha(t),t) - \lambda_{\mathrm{rep}}\nabla\rho_{\mathrm{rep}}(\boldsymbol{r}_\alpha(t),t), \quad (3)$$

where $\nabla\rho$ is the concentration gradient and λ is the rate constant of growth to the gradient in question.

(b) *Model II: contact interactions and diffusible signals*

In this model, we consider the possibility that the only diffusible molecules involved are those secreted by the target cells. Axons do not secrete diffusible molecules, and bundling is controlled by contact attraction between the axons, e.g. mediated by CAMs, which comes into play when the axons are within a very short distance of each other. The essential difference from model I is that in model II bundling is controlled by a short-range signal. In model I, both diffusion and filopodial interactions would give rise to effective long-range interactions between axons.

Clearly, for short-range contact attraction to become operative, some mechanism must bring the axons together in the first place. We investigate whether random axon movements may be able do this. Such random movements may be due to, for example, fluctuations in the environment. When axons come close enough to each other, contact attraction can come into play to keep them together. The strength of these contact interactions can be modulated by factors encountered in the environment (Tessier-Lavigne & Goodman 1996), and we investigate whether a reduction in strength triggered by the target region is sufficient for debundling and the subsequent innervation of the individual targets.

To include random axon movements, we have added a random component $\boldsymbol{\xi}_\alpha(t)$ to the growth of the axons in the gradient of the target-derived chemoattractant:

$$\mathrm{d}\boldsymbol{r}_\alpha/\mathrm{d}t = \lambda_{\mathrm{target}}\nabla\rho_{\mathrm{target}}(\boldsymbol{r}_\alpha(t),t) + \boldsymbol{\xi}_\alpha(t). \quad (4)$$

When two axons come within a short distance of each other, they have a certain probability of bundling, P_{b}, which we assume to be a decreasing function of the local concentration of the target-derived chemoattractant:

$$P_{\mathrm{b}} = P_{\mathrm{b,max}}\rho_{\mathrm{b}}^k/[\rho_{\mathrm{b}}^k + \rho_{\mathrm{target}}^k], \quad (5)$$

where $P_{\mathrm{b,max}}$ is the maximum probability, ρ_{b} is the value of ρ_{target} where P_{b} is half its maximum, and k is the Hill coefficient. A bundle of two or more axons may incorporate further axons to form even larger bundles. If axons come close together but do not bundle, they are allowed to cross each other. An axon bundle behaves as a single entity and is subject to random movements, which are assumed to be smaller than those for unbundled axons. For example, if we assume that random axon movements are due to variations in the substrate they grow over, the effective random force on a bundle will be inversely proportional to the square root of the number of axons in the bundle.

The axon debundles with a probability that depends on the local concentration of the target-derived chemoattractant:

$$P_{\mathrm{d}} = P_{\mathrm{d,max}}\rho_{\mathrm{target}}^n/[\rho_{\mathrm{c}}^n + \rho_{\mathrm{target}}^n], \quad (6)$$

which is a Michaelis–Menten function, where $P_{\mathrm{d,max}}$ is the maximum probability, ρ_{c} is the value of ρ_{target} where P_{d} is half its maximum, and n is the Hill coefficient.

3. RESULTS OF SIMULATIONS

(a) *Model I: only diffusible signals*

For the simulations, we need to specify the parameters in our equations, the fixed positions of the target cells, and the initial positions of the axonal growth cones. Using dimensionless variables (see Appendix B on the Royal Society Web site), we can show that several parameter variations are equivalent to either spatial or temporal scale changes. For the equations describing growth in the three diffusive fields (equations (1), (2), and (3)), there are four dimensionless parameters:

$$\chi_1 = \kappa_{\mathrm{target}}/\kappa_{\mathrm{cone}},$$
$$\chi_2 = \kappa_{\mathrm{rep}}/\kappa_{\mathrm{cone}},$$
$$\chi_3 = (\lambda_{\mathrm{target}}/\lambda_{\mathrm{cone}})[\sigma_{\mathrm{target}}/D_{\mathrm{target}}]/[\sigma_{\mathrm{cone}}/D_{\mathrm{cone}}]\sqrt{(\kappa_{\mathrm{cone}}/\kappa_{\mathrm{target}})},$$
$$\chi_4 = (\lambda_{\mathrm{rep}}/\lambda_{\mathrm{cone}})[\sigma_{\mathrm{max}}/D_{\mathrm{rep}}]/[\sigma_{\mathrm{cone}}/D_{\mathrm{cone}}]\sqrt{(\kappa_{\mathrm{cone}}/\kappa_{\mathrm{rep}})}. \quad (7)$$

Two parameters control the geometry by setting the relative length scales: χ_1 is the ratio of the diffusive length scales of the the axon-derived chemoattractant and target-derived chemoattractant, while χ_2 is the ratio of the diffusive length scales of the axon-derived chemoattractant and the axon-derived chemorepellant. The other two parameters control the growth rates in response to the diffusive fields: χ_3 controls the growth rate to the target-derived chemoattractant relative to the growth rate to the axon-derived chemoattractant, while χ_4 controls the growth rate to the axon-derived chemorepellant relative to the growth rate to the axon-derived chemoattractant.

Figure 1 shows a complete simulation of growth in the presence of the three diffusive fields. The outcome of the growth process depends on the values of the parameters given by equation (7). For example, by reducing the rate of release of the axon-derived chemoattractant, σ_{cone}, the parameters χ_3 and χ_4 will increase, and instead of a single bundle as in figure 1, two separate bundles will appear.

(i) *Fasciculation*

The target cells and the initial positions of the growth cones are 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

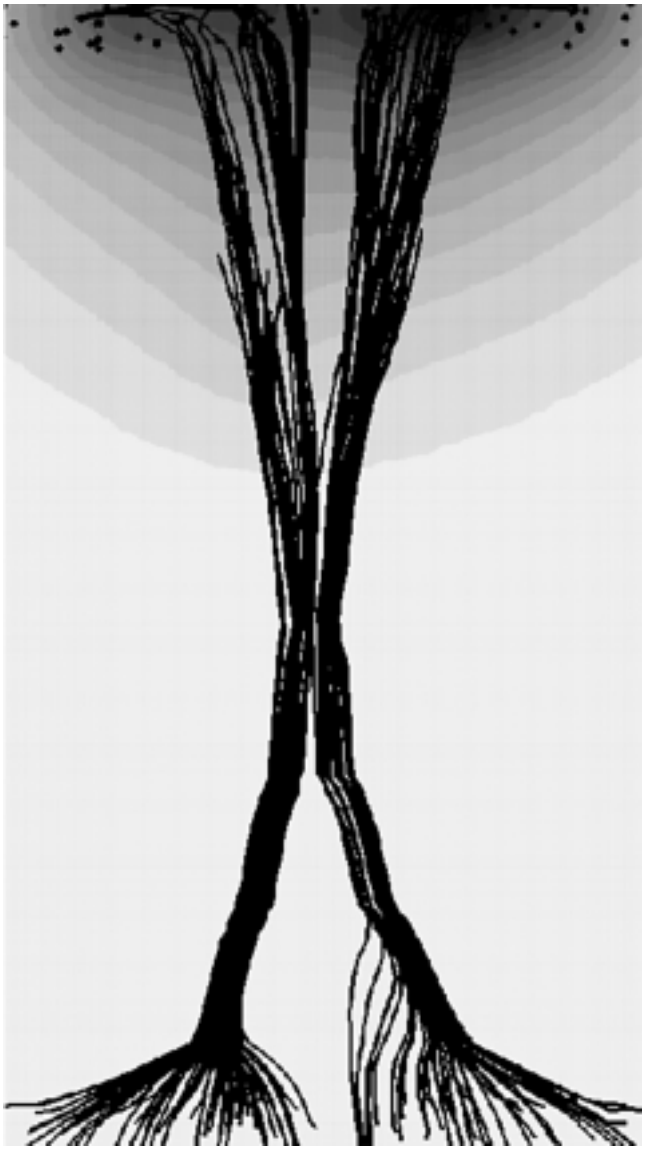

Figure 1. A complete simulation of axon development in the presence of three diffusible fields: (i) a chemoattractant released by the axonal growth cones, causing bundling; (ii) a chemoattractant released by the targets, guiding the axons to their targets; and (iii) a chemorepellant released by the growth cones, causing debundling near the target region. The concentration of the target-derived chemoattractant is higher in the darker regions of the figure.

just perceptible at the layer of the growth cones. The initial configuration of the growth cones is dense compared with the length scale of the axon-derived chemoattractant, $1/\kappa_{\text{cone}}$. As a result, fasciculation occurs readily (figure 2).

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

(ii) *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 chemoatractant, $\nabla\rho_{\text{target}}$, 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 $\nabla\rho_{\text{target}}$ 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 occurs (figure 1).

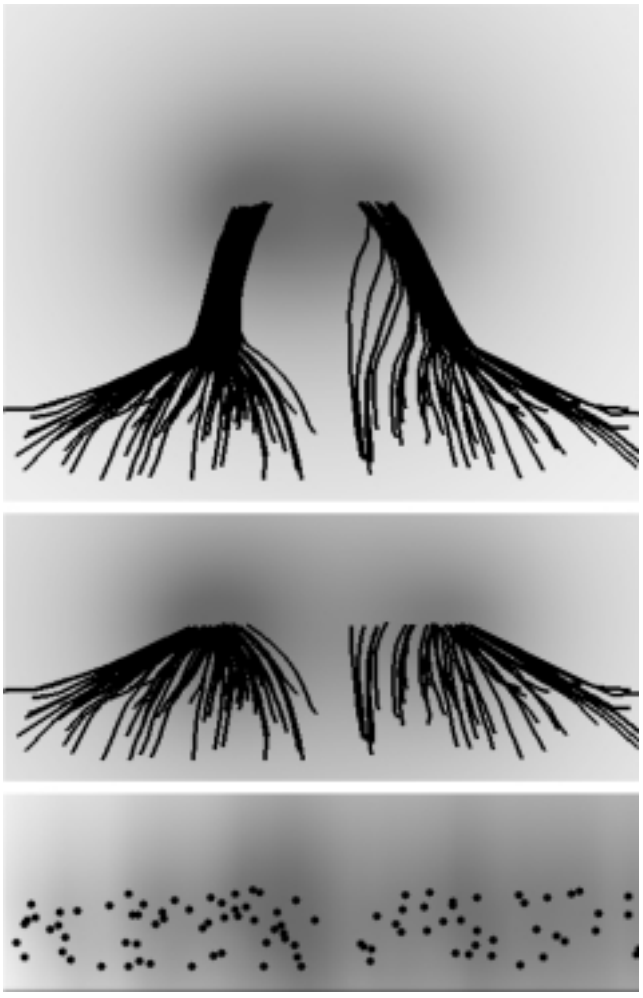

Figure 2. Initial axon development in response to a chemoattractant released by the growth cones. The concentration of this chemoattractant is higher in the darker regions of the figure. The axons are observed to self-organize into bundles in response to the diffusible signal.

(iii) *Defasciculation and target innervation*

Once the target-derived chemoattractant has guided the axon bundle to the target region, the final innervation of the individual target cells requires debundling. This does not occur automatically, especially in the presence of axon-derived chemoattractants, which tend to keep the axons together. It may be thought that the chemoattractant concentration gradients from the different targets are sufficient to pull the axons apart. Simulations show, however, that the gradients of the target-derived chemoattractant are simply not large enough across the relatively small axon bundle to pull

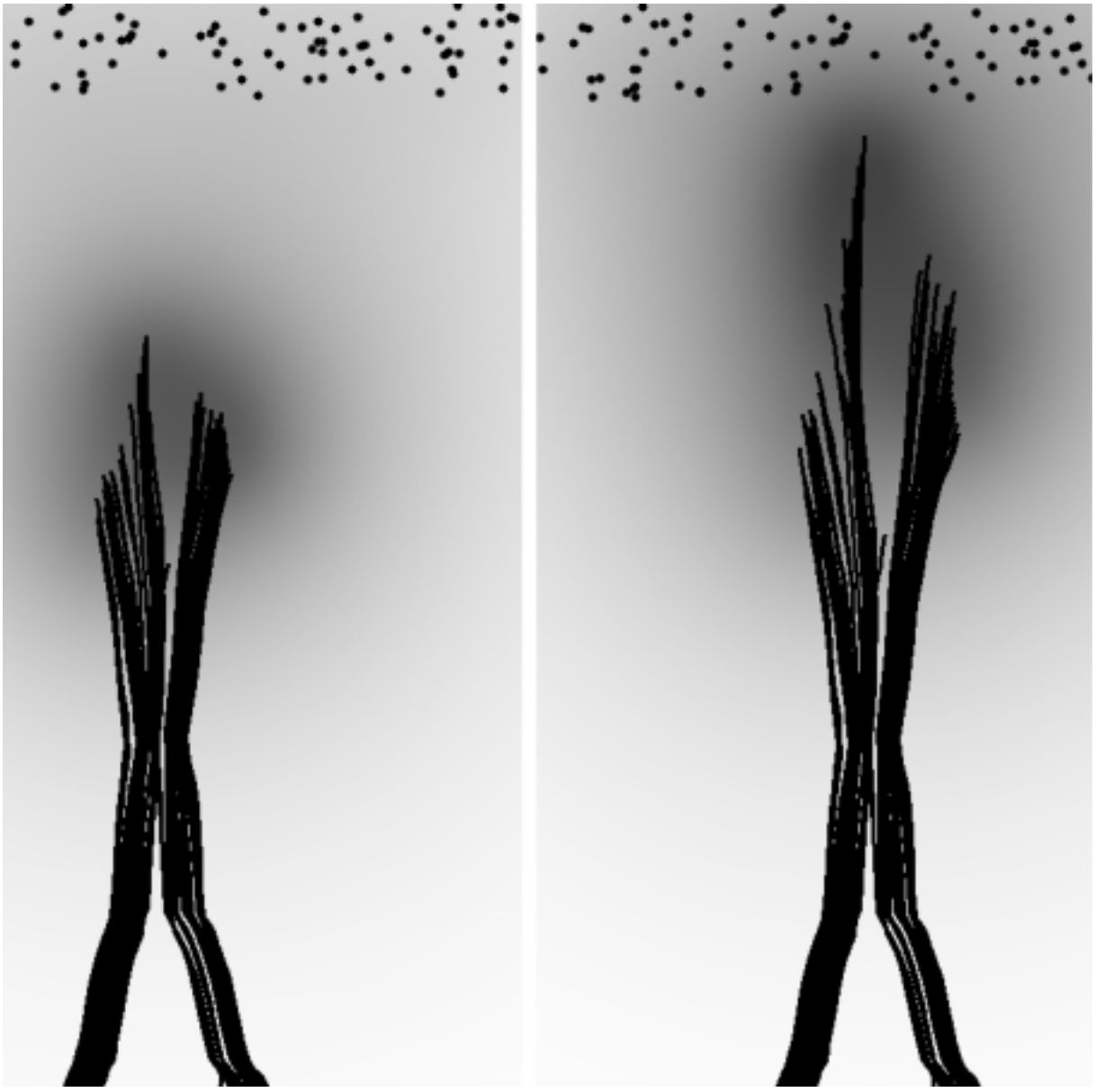

Figure 3. Debundling in the presence of a chemorepellant released by the growth cones at a rate depending on the local concentration of the target-derived chemoattractant. The concentration of the chemorepellant is higher in the darker regions around the tips of the axon bundles.

the axons apart, and the axon bundle will consequently behave as a single entity. It appears that the release of a chemorepellant by the growth cones when the axon bundle approaches the target region is necessary for efficient debundling. In our simulations, we found that release of the chemorepellant triggered by the target-derived chemoattractant (equation (2)) leads to debundling when the axon bundle approaches the target region (figure 3). The final innervation of the individual targets is not random but controlled by the bundling that occurred earlier in development (figure 1).

Bundling and the observed global topographic ordering are robust to adding a small random component to the growth dynamics (equation (3)). Large random axon movements, however, tend to disturb the topographic ordering. In model II, we consider the effects of random axon movements in greater detail.

(b) *Model II: contact interactions and diffusible signals*

The same distribution of target cells and initial position of growth cones are used as in model I. The only diffusible molecules involved are those secreted by the targets; the axons do not secrete diffusible molecules but are, in contrast to model I, subject to short-range contact interactions and random movements.

(i) *Fasciculation*

Although local bundling certainly occurs, there is no tendency for global bundling (figure 4). Any bundling that appears is a direct consequence of the initial positions of the growth cones. More complex growth conditions may partially remedy this situation. For example, diffusible chemorepellants released by surrounding tissue, and also contact repellants on the surface of surrounding cells or in

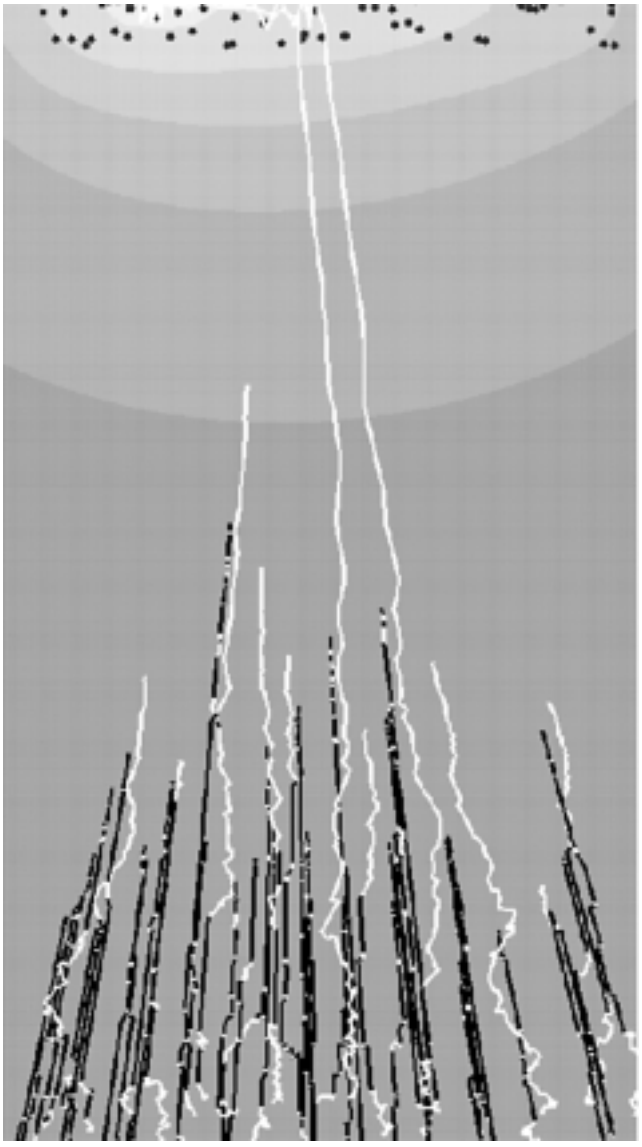

Figure 4. Initial axon development in the presence of contact attraction. The axons are responding to the chemoattractant gradient set up by the layer of target cells and are subject to random movements. The concentration of the target-derived chemoattractant is higher in the lighter regions of the figure. The unbundled axons are drawn in white, the bundled ones in black. Note that the bundled axons move in a less random manner and grow slower than the unbundled ones. Furthermore, note the development of unbundled pathfinding axons.

the extracellular matrix, could serve to push the axons together, after which contact attraction can come into play. Alternatively, the random axon movements may be increased, so that contacts between growing axons will increase. If the random movements become too large, however, they will dominate the effects of the chemoattractant, and proper target innervation will no longer be possible. Furthermore, random axon movements will cause the loss of local topographic ordering in any bundling. So it appears that some form of long-range interaction between axons (model I) is necessary.

(ii) *'Pathfinding'*

As in the previous simulations, migrating axons are guided to their targets through the concentration gradients of the target-derived chemoattractant. New in these simulations is the emergence of what might be called 'pathfinding axons' (figure 4). Although our model was not developed with the intention of modelling pathfinding axons, we find it interesting that they nevertheless arise as an emergent property of the dynamics. The mechanism for their emergence appears to be the following. As the steepness of the gradient of the target-derived chemoattractant increases towards the target region, the rate at which the axons grow increases (see equation (4)). The presence of random axon movements, which are greater for unbundled than for bundled axons, means that unbundled axons have a higher chance to get into steep gradients. As a result, the axons will grow faster towards the targets and so come into even steeper gradients, i.e. a form of positive feedback exists. When the unbundled axons have reached the targets, they have formed '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. Random axon movements can, of course, also move unbundled axons away from steep gradients, but this does not matter provided there exist axons that will stay in the steep gradients; they will form the pathfinding axons.

(iii) *Defasciculation and target innervation*

Debundling and target innervation may seem to be easier than in the presence of diffusible signals. When the contact attraction is broken down close to the target region, there are no axon-derived chemoattractants keeping the axons together. Also in this case, however, the gradients of the target-derived chemoattractant across the relatively small, broken up bundle of axons are not large enough to further separate the axons. Contact repulsion will not be of help either because it becomes ineffective after the axons have separated. So also in this case, a diffusible chemorepellant released by the axonal growth cones upon reaching the target region (model I) would appear to be necessary.

Note that the random axon movements, which are also present after debundling, cause the loss of topographical identity of the individual axons.

4. DISCUSSION

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, together with 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 their target region. It should be noted 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 (Goodhill 1997). 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 taken into account in our model by making the rate of outgrowth, λ, dependent on

the chemoattractant (chemorepellant) concentration in such a way that for concentrations which are either too high or too low, λ becomes zero. This imposes a maximum length range over which growth cone guidance by a diffusible factor is possible.

A kind of 'pathfinding axon' emerges 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 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 bringing axons together, a long-range signal derived from the axonal growth cones themselves could provide a much more effective mechanism. Our model allows for two interpretations of the nature of this long-range signal: (i) 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). Although (i) is certainly plausible (e.g. neurotransmitters, which are released by migrating growth cones (Hume *et al.* 1983; Young & Poo 1983) and which have been implicated as chemoattractants (Tessier-Lavigne 1994; Zheng *et al.* 1994)), direct evidence for it is (as yet) lacking. In view of the results of our simulations, we suggest that experimental studies should be carried out to test whether such chemoattractants exist.

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 chemoattractant. 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 therefore appears to be 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 (1980), who argues that chemorepellants appear to be necessary for proper target innervation. Other types of gradients than the ones studied here may also contribute to debundling and target innervation. Gradients in axonal receptor density in which the density is a function of the topographic origin of the axon, together with complementary gradients in ligand density in the target region, may be involved in both debundling and topographic map formation (Holt & Harris 1993; Cheng *et al.* 1995; O'Leary *et al.* 1999).

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