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Appendix A. Growth in Diffusive Chemical Gradients

In this Appendix, we describe how a set of interacting diffusible chemicals μ may control axonal development. These chemicals diffuse in the extracellular space with diffusion constants D_μ and decay with rate constants δ_μ. The diffusion equations with sources and losses thus take the form

$$\partial\rho_\mu/\partial t = D_\mu\nabla^2\rho_\mu - \delta_\mu\rho_\mu + S_\mu(\mathbf{x},t), \qquad (A1)$$

where ρ_μ is the concentration of chemical μ, $\nabla\rho_\mu$ its concentration gradient, and $S_\mu(\mathbf{x},t)$ represents the source flux of chemical μ. If the chemical μ is released from fixed targets that are treated as point sources at positions $\mathbf{x}_i$, then

$$S_\mu(\mathbf{x},t) = \sum_i \sigma_\mu(\{\rho(\mathbf{x}_i,t)\})\delta(\mathbf{x}-\mathbf{x}_i), \qquad (A2)$$

where $\mathbf{x}$ is a point in the extracellular space and $\delta(\mathbf{x})$ is the Dirac delta function. If the migrating growth cones at $\mathbf{r}_\alpha(t)$ are themselves treated as point sources of the chemical μ,

$$S_\mu(\mathbf{x},t) = \sum_\alpha \sigma_\mu(\{\rho(\mathbf{r}_\alpha(t),t)\})\delta(\mathbf{x}-\mathbf{r}_\alpha(t)). \qquad (A3)$$

In equations (A2) and (A3) we have allowed for the possibility that the rate of release $\sigma_\mu(\{\rho\})$ could depend on the concentration $\{\rho\}$ of other chemoattractants and chemorepellants at the release site.

The growth cones will respond to these diffusible chemicals by growing up the gradients of chemoattractants and down the gradients of chemorepellants. Thus, the combined effect of several diffusible cemicals on the growth of axon α will be given by

$$d\mathbf{r}_\alpha/dt = \sum_\mu \lambda_{\mu,attract} \nabla \rho_\mu(\mathbf{r}_\alpha(t), t) - \sum_\mu \lambda_{\mu,repel} \nabla \rho_\mu(\mathbf{r}_\alpha(t), t), \qquad (A4)$$

where $\lambda_{\mu,attract}$ and $\lambda_{\mu,repel}$ are the rates of growth to the gradients of chemoattractants and chemorepellants, respectively.

As axon growth usually occurs on a much longer time-scale than the time needed for the diffusive fields to equilibrate, we can replace equation (A1) by the quasi-steady-state approximation

$$[\nabla^2 - \kappa_\mu^2]\rho_\mu = -S_\mu(\mathbf{x}, t)/D_\mu, \qquad (A5)$$

where $\kappa_\mu = \sqrt{\delta_\mu/D_\mu}$ is the inverse diffusive length for chemical μ. Note that diffusive gradients can therefore control development on a range of scales, depending on the biochemistry of the molecules involved. Quasi-steady-state equations such as these we actually integrate in our simulations.

Because equation (A5) is linear, it can be solved using Green's functions such as

$$\rho_\mu(\mathbf{x}, t) = \int G_\mu(\mathbf{x} - \mathbf{x}') S_\mu(\mathbf{x}', t)/D_\mu d\mathbf{x}'. \qquad (A6)$$

The nature of the Green's functions to be used depends on the dimensionality of the extracellular space and on the boundary conditions imposed experimentally. For the two-dimensional simulations, the Green's function can be expressed in terms of the modified Bessel function $K_0(x)$ as $G_\mu(\mathbf{x} - \mathbf{x}') = K_0(\kappa_\mu|\mathbf{x} - \mathbf{x}'|)/2\pi$ (*e.g.* Bronstein & Semendyayev 1997).

Thus, using equations (A2) and (A3), we can write down expressions for the diffusive fields:

$$\rho_\mu(\mathbf{x},t) = \begin{cases} (1/2\pi)\sum_i[\sigma_\mu(\{\rho(\mathbf{x}_i,t)\})/D_\mu]K_0(\kappa_\mu|\mathbf{x}-\mathbf{x}_i|) & \text{(target)} \\ (1/2\pi)\sum_\alpha[\sigma_\mu(\{\rho(\mathbf{r}_\alpha(t),t)\})/D_\mu]K_0(\kappa_\mu|\mathbf{x}-\mathbf{r}_\alpha(t)|) & \text{(gr. cone)} \end{cases} \quad \text{(A7)}$$

or, provided we are only interested in the concentration far from the source, we can replace the modified Bessel function by its asymptotic form $K_0(x) \approx \sqrt{\pi/2x}\exp -x$, and

$$\rho_\mu(\mathbf{x},t) = \begin{cases} \sqrt{1/8\pi}\sum_i[\sigma_\mu(\{\rho(\mathbf{x}_i,t)\})/D_\mu]\exp -\kappa_\mu|\mathbf{x}-\mathbf{x}_i|/\sqrt{\kappa_\mu|\mathbf{x}-\mathbf{x}_i|} & \text{(target)} \\ \sqrt{1/8\pi}\sum_\alpha[\sigma_\mu(\{\rho(\mathbf{r}_\alpha(t),t)\})/D_\mu]\exp -\kappa_\mu|\mathbf{x}-\mathbf{r}_\alpha(t)|/\sqrt{\kappa_\mu|\mathbf{x}-\mathbf{r}_\alpha(t)|} & \text{(gr. cone)} \end{cases} \quad \text{(A8)}$$

Similarly, the instantaneous concentration gradient of chemical μ at growth cone α, a quantity required for calculating the response of the growth cone to chemical μ (see equation (A4)), is given by

$$\nabla\rho_\mu(\mathbf{r}_\alpha(t),t) = \begin{cases} \sqrt{1/8\pi\kappa_\mu}\sum_i[\sigma_\mu(\{\rho(\mathbf{x}_i,t)\})/D_\mu]\exp -\kappa_\mu|\mathbf{r}_\alpha(t)-\mathbf{x}_i| \\ |\mathbf{r}_\alpha(t)-\mathbf{x}_i|^{-3/2}[\kappa_\mu|\mathbf{r}_\alpha(t)-\mathbf{x}_i|+1/2](\hat{\mathbf{r}}_\alpha(t)-\hat{\mathbf{x}}_i) & \text{(target)} \\ \sqrt{1/8\pi\kappa_\mu}\sum_{\beta\neq\alpha}[\sigma_\mu(\{\rho(\mathbf{r}_\beta(t),t)\})/D_\mu]\exp -\kappa_\mu|\mathbf{r}_\alpha(t)-\mathbf{r}_\beta(t)| \\ |\mathbf{r}_\alpha(t)-\mathbf{r}_\beta(t)|^{-3/2}[\kappa_\mu|\mathbf{r}_\alpha(t)-\mathbf{r}_\beta(t)|+1/2](\hat{\mathbf{r}}_\alpha(t)-\hat{\mathbf{r}}_\beta(t)) & \text{(gr. cone)} \end{cases} \quad \text{(A9)}$$

References

Bronstein, I. N. & Semendyayev, K. A. 1997 *Handbook of Mathematics*, Springer: Berlin.

Appendix B. Scaling The Equations of Motion

Converting variables into non-dimensional ones usually reduces the number of parameters. In equations (1), (2), and (3) (in main text) there appear to be nine relevant parameters (excluding those of the Michaelis-Menten functions), but by scaling the length and time in terms of the growth cone parameters (*i.e.* measure lengths in terms of $L = \kappa_{cone}^{-1}$, and time in terms of $T = [\sqrt{8\pi} D_{cone}/\lambda_{cone}\kappa_{cone}^2\sigma_{cone}]$) and defining dimensionless concentrations

$$\begin{aligned} \rho_{cone} &= [\sigma_{cone}/\sqrt{8\pi} D_{cone}]\rho_{cone,dim} \\ \rho_{target} &= [\sigma_{target}/\sqrt{8\pi} D_{target}]\rho_{target,dim} \\ \rho_{rep} &= [\sigma_{max}/\sqrt{8\pi} D_{rep}]\rho_{rep,dim}, \end{aligned} \tag{B1}$$

we can reduce the number of parameters to only four dimensionless parameters:

$$\begin{aligned} \chi_1 &= \kappa_{target}/\kappa_{cone} \\ \chi_2 &= \kappa_{rep}/\kappa_{cone} \\ \chi_3 &= (\lambda_{target}/\lambda_{cone})[\sigma_{target}/D_{target}]/[\sigma_{cone}/D_{cone}]\sqrt{\kappa_{cone}/\kappa_{target}} \\ \chi_4 &= (\lambda_{rep}/\lambda_{cone})[\sigma_{max}/D_{rep}]/[\sigma_{cone}/D_{cone}]\sqrt{\kappa_{cone}/\kappa_{rep}}. \end{aligned} \tag{B2}$$

Two parameters control the geometry by setting the relative length scales: χ_1 is the ratio of the diffusive length scales of the axon-derived chemoattractant and the 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.