ELSEVIER

Neurocomputing 26–27 (1999) 39–43

NEUROCOMPUTING

www.elsevier.com/locate/neucom

Limitations on detection of gradients of diffusible chemicals by axons

J.S. Urbach[a,*], Geoffrey J. Goodhill[b]

[a]*Department of Physics, Georgetown University, Washington, DC 20057, USA*
[b]*Georgetown Institute for Cognitive and Computational Sciences, Georgetown University Medical Center, Washington DC 20007, USA*

Abstract

This paper applies a simple analysis of the statistical noise inherent in sensing concentrations of diffusible chemical factors to the problem of guidance of developing axons. We show that growth cones may be able to detect chemical changes of 0.5% across their width, and that guidance by a gradient is limited to distances below about 1 cm, even for a gradient of ideal shape.

Keywords: Axon guidance; Chemotaxis; Growth cone; Gradients

1. Introduction

Axons in the developing brain use a variety of guidance cues to find their targets. In many important cases, guidance is achieved by following gradients in the concentration of a ligand that binds to receptors on the growth cone [10,9]. Ligand gradients can be set by a variety of mechanisms, including diffusion in a three-dimensional volume, diffusion on a two-dimensional substrate, or by binding from a three-dimensional volume onto a two- or three-dimensional substrate, as well as by the graded expression on a substrate. Axons move up or down on these gradients until they reach their targets, or until other guidance cues take over.

In order to move reliably in response to a chemical gradient, the growth cone of the developing axon must perform some relatively sophisticated signal analysis to overcome the noise inherent in a measurement of molecules that move about randomly

* Corresponding author.

0925-2312/99/$ – see front matter
PII: S0925-2312(99)00086-7

through Brownian motion. The fundamental statistical limitations on gradient detection by a small sensing device were originally described by Berg and Purcell [2], in the context of understanding chemotaxis in leukocytes and bacteria. However, very little is known of the biochemical mechanisms of axonal guidance, and no analogous theoretical models of the gradient sensing process have been proposed. In this paper we therefore begin the process of quantitatively analysing axonal gradient detection by applying the approach of Berg and Purcell to several situations important in the developing brain.

2. Statistical limits on gradient sensing

The presence of a ligand gradient will produce a variation in the average occupancy of receptors across the growth cone. There are a number of biochemical pathways by which the difference in receptor occupancy might be converted into directed motion, but those responsible for axon guidance have not been identified. Regardless of the specific mechanism, quantitative limits on the guidance process can be obtained from the fact that, at any instant in time, the actual occupancy of the receptors will differ from the average. If these fluctuations are large compared to the difference in the average that arises from the concentration gradient, the growth cone will not be able to obtain a clean guidance signal from an instantaneous measurement. The random noise can be overcome by making a sufficient number of statistically independent measurements.

A rough estimate for the necessary averaging time can be obtained by considering the kinetics of the diffusion process. A volume V of fluid with an average ligand concentration C will contain, on average, $N = VC$ ligand molecules. The actual number of molecules will differ from the average, and the standard deviation of the fluctuations is simply $\sqrt{N}$. Thus, the root mean square (rms) fluctuation in an instantaneous measure of the concentration, ΔC_{noise}, is given by $\Delta C_{\text{noise}}/C = 1/\sqrt{VC}$. If M statistically independent measurements are averaged, the rms fluctuation is reduced by $1/\sqrt{M}$. For a diffusive process, statistically independent measurements in a single volume can only be made by waiting long enough for molecules to diffuse across the volume. For a spherical volume V, this time is on the order of $V^{2/3}/D$, where D is the diffusion coefficient for the ligand. Thus, averaging over a time T is roughly equivalent to making $TD/V^{2/3}$ independent measurements. Combining the above considerations yields an estimate for the fractional uncertainty of

$$\frac{\Delta C_{\text{noise}}}{C} = \frac{1}{\sqrt{V^{1/3}DTC}}. \tag{1}$$

This calculation neglects all of the details of the sensing process, but turns out to agree quite well with the more sophisticated calculation of Berg and Purcell (BP) in most of the physiologically relevant regimes. BP considered a spherical cell of radius $a = V^{1/3}$, uniformly covered with N receptors, each with an active area s. BP assumed perfectly absorbing receptors (the ligand is internalized), and a diffusion-limited

receptor–ligand interaction (the time required to absorb a ligand is dominated by the time it takes the ligand to diffuse to the receptor). Their result for the fractional uncertainty is

$$\frac{\Delta C_{\text{noise}}}{C} = \sqrt{\frac{1}{2\pi TDaNs/(Ns + \pi a)CC_{1/2}/(C + C_{1/2})}}, \tag{2}$$

where $C_{1/2}$ is the concentration at which half of the receptors are bound (the dissociation constant K_D). In many cases, the term that accounts for the finite area covered by the receptors, $Ns/(Ns + \pi a)$, is close to unity, because even a relatively sparse population of receptors has a high probability of capturing any ligands in the neighborhood. For concentrations on the order of $C_{1/2}$, $CC_{1/2}/(C + C_{1/2})$ is of order C, and Eq. (2) reduces to 1, except for the factor of 2π.

Eq. (2) is more transparent when written in a dimensionless form. Writing the time necessary for diffusion across the volume, as described above, as $T_D = a^2/D$, the number of molecules in volume a^3 at concentration $C_{1/2}$ as $N_{1/2} = C_{1/2}a^3$, and the effective fraction of the surface area covered by the receptors as $f = Ns/(Ns + \pi a)$, Eq. (2) can be written

$$\frac{\overline{\Delta C}_{\text{noise}}}{\overline{C}} = \sqrt{\frac{1}{2\pi(T/T_D)N_{1/2}f\overline{C}/(\overline{C} + 1)}}, \tag{3}$$

where $\overline{C} = C/C_{1/2}$. Combining the concentration-independent factors into a single constant $\alpha = 2\pi(T/T_D)N_{1/2}f$ that measures the effective number of individual receptor measurements going into the average, Eq. (3) becomes simply

$$\frac{\overline{\Delta C}_{\text{noise}}}{\overline{C}} = \sqrt{\frac{\overline{C} + 1}{\alpha\overline{C}}}. \tag{4}$$

An interesting feature of this equation is that the fractional uncertainty tends to a constant for $\overline{C} \gg 1$. As the concentration increases the number of molecules available increases, which increases the effectiveness of the averaging. The number of unbound receptors becomes very small, however, increasing the uncertainty, and the two effects exactly cancel. More sophisticated models, taking into account the dynamics of the receptor–ligand interaction and limitations of the intra-cellular signaling mechanisms, have been developed for certain cases [3,8], and typically produce a more realistic decrease in the fractional sensitivity for $\overline{C} \gg 1$.

Finally note that, in order to detect a concentration gradient between two spatially separated points, the average concentration difference between the two points, $\overline{\Delta C}_{\text{true}}$, must be greater than $\sqrt{2}\,\overline{\Delta C}_{\text{noise}}$. (The factor of $\sqrt{2}$ arises from the fact that the noise at the different points is assumed to be statistically independent.)

3. Results

A typical growth cone has a radius of 10 μm. Ligand concentrations can vary over a very wide range, but a typical value for the dissociation constant for many

receptor–ligand pairs implicated in axon guidance (see references cited in [6]) is 1 nM. Thus $N_{1/2} \approx 2500$, and in this case the instantaneous uncertainty is about 2%. For a freely diffusing molecule the size of the chemoattractant netrin [7] in vivo, $D \approx 10^{-7}\,\mathrm{cm}^2/\mathrm{s}$ (see [4]), so $T_D \approx 10$ s. Growth cones take on the order of 100 s to show a turning response [11], allowing for about 10 independent concentration measurements (assuming the receptor–ligand interaction is diffusion limited). As a result, $1/\sqrt{\alpha} \approx 0.0025$, suggesting a minimum detectable fractional change of about 0.5% across the growth cone. Quantitative experimental data on chemotaxis in axons is quite limited, but a turning response has been seen for gradients as small as 1% across the growth cone [1].

Another interesting number can be derived by considering the optimal gradient shape for guiding the axon over a large distance. The optimal gradient has a percent concentration change across each growth cone diameter equal to the minimum required for detection. Setting $\mathrm{d}(\ln(C))/\mathrm{d}(x/a)$ equal to Eq. (3) and integrating gives

$$\frac{x}{a} = -\sqrt{2\alpha}\log[2(\sqrt{\bar{C}^2 + \bar{C}} + \bar{C} + \tfrac{1}{2})] + B, \tag{5}$$

where B is a constant of integration. The value of the constant can be set by assuming that there exists a maximum allowable concentration, $C_{\max}$, beyond which the concentration cannot be accurately determined. (Thereby, crudely compensating for the inadequacies of the BP model at high concentrations, as discussed above.) Guidance over the maximum possible distance is achieved when B is such that the concentration at $x = 0$ is $C_{\max}$: $B = -a\sqrt{2\alpha}\log[2(\sqrt{\bar{C}_{\max}^2 + \bar{C}_{\max}} + \bar{C}_{\max} + \tfrac{1}{2})]$. The maximum guidance distance is determined by solving Eq. (5) for the value of x where $\bar{C} = 0$, which yields

$$\frac{x_{\max}}{a} = \sqrt{2\alpha}\log[2\sqrt{\bar{C}_{\max}^2 + \bar{C}_{\max}} + 2\bar{C}_{\max} + \tfrac{1}{2}]. \tag{6}$$

Assuming $\bar{C}_{\max} \gg 1$, this reduces to $x_{\max}/a = \sqrt{2\alpha}\log(4\bar{C}_{\max})$. Substituting the value of α estimated above, and setting $\bar{C}_{\max} = 100$ yields $x_{\max} \approx 1$ cm. This is in rough agreement with estimates based on simpler models [6,5]. The natural length scale for this problem is $a\sqrt{\alpha}$, which gives the distance between $\bar{C} = 1$ and $\bar{C} = 0$ for a linear gradient with a slope equal to the minimum detectable gradient at $\bar{C} = 1$. Note that all of the details of the sensing mechanism show up in the logarithm. This presumably accounts for the success of this approach, which so far ignores all details of the biochemistry of developing axons.

Acknowledgements

This work was partly supported by DOD grant DAMD17-93-V-3018 (GJG), and by the Alfred P. Sloan Foundation (JSU).

References

[1] H. Baier, F. Bonhoeffer, Axon guidance by gradients of a target-derived component, Science 255 (1992) 472–475.
[2] H.C. Berg, E.M. Purcell, Physics of chemoreception, Biophys. J. 20 (1977) 193–219.
[3] C. DeLisi, F. Marchetti, G. Del Grosso, A theory of measurement error and its implications for spatial and temporal gradient sensing during chemotaxis, Cell Biophys. 4 (1982) 211–229.
[4] G.J. Goodhill, Diffusion in axon guidance, Eur. J. Neurosci. 9 (1977) 1414–1421.
[5] G.J. Goodhill, Mathematical guidance for axons, Trends in Neurosci. 21 (1998) 226–231.
[6] G.J. Goodhill, H. Baier, Axon guidance: stretching gradients to the limit, Neural Comput. 10 (1998) 521–527.
[7] T.E. Kennedy, T. Serafini, J.R. de al Torre, M. Tessier-Lavigne, Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord, Cell 78 (1994) 425–435.
[8] D.A. Lauffenburger, Influence of external concentration fluctuations on leukocyte chemotactic orientation, Cell Biophys. 4 (1982) 177–209.
[9] M. Tessier-Lavigne, C.S. Goodman, The molecular biology of axon guidance, Science 274 (1996) 1123–1133.
[10] M. Tessier-Lavigne, M. Placzek, Target attraction – are developing axons guided by chemotropism?, Trends Neurosci. 14 (1991) 303–310.
[11] J.G. Zheng, J.-j. Wan, M.-m. Poo, Essential role of filopodia in chemotropic turning of nerve growth cone induced by a glutamate gradient, J. Neurosci. 16 (1996) 1140–1149.

Jeffrey Urbach received a BA in Physics from Amherst College (1985), and a Ph.D. in Physics from Stanford University (1993), where he studied the thermodynamic properties of superconductors. He did postdoctoral work in nonlinear dynamics at the University of Texas at Austin, and became an Assistant Professor at Georgetown University in 1996. He is interested in the statistical physics of non-equilibrium systems, including granular dynamics, ferromagnets, and developing neurons.

Geoffrey Goodhill received a B.Sc. (Joint Honours) in Mathematics and Physics from the University of Bristol (1986), an M.Sc. in Artifical Intelligence from the University of Edinburgh (1988) and a Ph.D. in Cognitive Science from the University of Sussex (1992). Following postdoctoral work at the University of Edinburgh, Baylor College of Medicine and the Salk Institute, he became an Assistant Professor at Georgetown University in 1996. His research aims to understand the biological principles that govern the formation of appropriate neuronal wiring during development.