European Journal of Neuroscience, Vol. 9, pp. 1414–1421, 1997

Diffusion in Axon Guidance

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Keywords: chemotaxis, chemotropism, diffusible factors, gradients, growth cone

Abstract

Axon guidance by target-derived diffusible factors plays an important role in the development of the nervous system. This paper considers the constraints imposed on this process by the mathematics of diffusion. A point source continuously producing a factor into an infinite three-dimensional volume is considered as a model for both the *in vivo* and *in vitro* situation. Basic constraints for effective guidance are assumed to be that the concentration falls between certain maximum and minimum limits, and that the percentage change in concentration across the width of the growth cone exceeds a certain minimum value. The evolution of the shape of the gradient over time is analysed. Using biologically reasonable parameter values, it is shown that the maximum range over which growth cone guidance by a diffusible factor is possible for large times (several days) after the start of the production of the factor is 500–1000 μm. This maximum distance is independent of the diffusion constant of the diffusing molecule, applies to both chemoattractants and chemorepellents, and agrees with experimental data. At earlier times, however, the constraints may be satisfied for distances up to several millimetres. The time it takes for this maximum guidance distance to fall to the asymptotic value depends on the diffusion constant. This time is a few hours for a small molecule but as much as a few days for a large molecule. The model therefore predicts that guidance over distances larger than 1000 μm is possible if the start of production of the factor is carefully matched to the time when guidance is required.

Introduction

In the developing nervous system, growing axons are guided to targets that may be some distance away. Several mechanisms contribute to this (Tessier-Lavigne and Goodman, 1996). One such mechanism is the diffusion of a factor from the target through the extracellular space, creating a gradient of increasing concentration that axons can sense and follow (Tessier-Lavigne and Placzek, 1991; Kennedy and Tessier-Lavigne, 1995; Keynes and Cook, 1995). In the central nervous system, such a process seems to occur in at least three cases: the guidance of axons from the trigeminal ganglion to the maxillary process in the mouse (Lumsden and Davies, 1983, 1986), of commissural axons in the spinal cord to the floor plate (Tessier-Lavigne *et al.*, 1988), and of axons and axonal branches from the corticospinal tract to the basilar pons (Heffner *et al.*, 1990). The evidence for this comes from both *in vivo* and *in vitro* experiments. *In vivo*, target cells can be ectopically positioned by irradiation of the embryo when the cells are migrating (O'Leary *et al.*, 1991), or a normally present piece of the target can be deleted by mutation (Bovolenta and Dodd, 1991). In both these cases growth towards the new or remaining target is seen, implicating a target-derived diffusible signal. For the *in vitro* studies, a piece of target tissue is generally embedded in a three-dimensional collagen gel near a piece of tissue containing the appropriate population of neurons. Axon growth is then observed directed towards the target, again implicating a target-derived signal (Lumsden and Davies, 1983, 1986; Tessier-Lavigne *et al.*, 1988; Heffner *et al.*, 1990). *In vivo*, for the systems described, the target is always <500 μm from the population of axons. *In vitro*, where the distance between axons and target can readily be varied, guidance is generally not seen for distances >500 μm. Can such a limit be explained in terms of the mathematics of diffusion?

There are two related constraints that the distribution of a diffusible factor must satisfy to provide an effective guidance cue at a point. Firstly, the *absolute concentration* of factor must not be too small or too large. Secondly, the *fractional change in concentration* of factor across the width of the gradient-sensing apparatus, generally assumed to be the growth cone, must be sufficiently large. These constraints are related because in both cases the problem is to overcome statistical noise. At very low concentrations, noise exists due to thermal fluctuations in the number of molecules of the factor in the vicinity of the growth cone. At higher concentrations, the limiting source of noise is stochastic variation in the amount of binding of the factor to receptors distributed over the growth cone. At very high concentrations, all receptors will be saturated and no gradient will be apparent.

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Received 30 October 1996, revised 31 January 1997, accepted 6 February 1997

TABLE 1. Summary of symbols used

Symbol	Description
r	distance between source and growth cone (cm)
t	time since source started producing factor (s)
$C(r,t)$	concentration of factor at distance r from source at time t (mol)
q	rate at which factor is produced (mol/s)
D	diffusion constant of factor in substrate (cm^2/s)
Δr	width of growth cone (cm)
$p(\Delta r, r, t)$	percent concentration change across Δr at distance r from source at time t

The closer the concentration is to the upper or lower limits, the higher the gradient that is needed to ensure detection (Devreotes and Zigmond, 1988; Tessier-Lavigne and Placzek, 1991).

The purpose of this paper is to investigate how the region over which effective guidance can occur changes as a function of space and time, given a set of simple assumptions about the diffusion and sensing processes and some rough estimates for the relevant parameters. It is shown that these limits impose biologically significant constraints on axon guidance, and that for plausible parameters 500–1000 μm emerges as a limit on the spatial range over which guidance can occur once the gradient has stabilized. This limit is linearly related to the width of the growth cone, but is independent of the diffusion constant of the diffusing molecule. However, it is also shown that before the gradient has stabilized the region of effective guidance is larger, up to several millimetres from the source. The model therefore predicts that guidance over much greater distances than previously observed could occur under certain conditions.

Mathematical model

The mathematics of how gradients can be set up by diffusion has been extensively studied in the embryological literature. In some embryos, regional specification may be driven by the existence of different concentration levels of a diffusible factor at different distances from a source (e.g. Wolpert, 1969; Eichele and Thaller, 1987; Driever and Nüsslein-Volhard, 1988; Slack, 1991). A now standard mathematical approach, pioneered by Crick (1970), is to consider a one-dimensional system containing a point source at which the concentration is held constant. Crick assumed a point sink, which for large times yields a linearly decreasing concentration from source to sink. A more complicated stable form is given by assuming a distributed sink, i.e. uniform decay everywhere (Eichele and Thaller, 1987; Slack, 1991). In such studies a key concern has been whether there is sufficient time for the gradient to be set up during the appropriate stage of embryogenesis, i.e. for the concentration to reach suitable threshold levels at appropriate distances from the source. A measure of this has been taken to be the time required for the gradient to everywhere reach within 1% of its stable value (Crick, 1970). Such studies have led to the general conclusion that diffusion gradients suitable for morphogenesis cannot be set up over distances greater than ~1 mm during developmental time periods (Crick, 1970; Slack, 1991).

The mathematical requirements for axon guidance are somewhat different from those for morphogenesis. Firstly, for axon guidance the concentration at a point merely needs to fall within a broad range, whereas for appropriate differentiation it may need to achieve a rather more narrowly constrained value (e.g. Eichele, 1987). Secondly, the fractional change across the width of the growth cone must also exceed a minimum value at appropriate distances, a constraint of little relevance in morphogenesis. The regions of space over which both of these constraints are satisfied will vary with time. For systems in which axon guidance by diffusible factors has been studied *in vivo*, and also for three-dimensional collagen gel assays, it is more appropriate to consider diffusion in three dimensions rather than one dimension. In addition, to assume that the concentration at the source is held constant requires the existence of a regulatory mechanism. A simpler model is that the source is just producing factor at a constant rate. Consider such a source releasing a factor with diffusion constant D cm^2/s at rate q mol/s into an infinite, spatially uniform three-dimensional volume. Initially, zero decay of the factor is assumed. The notation used is summarized in Table 1, and a schematic representation of the main conclusions of the analysis is shown in Figure 1.

For radially symmetrical Fickian diffusion in three dimensions, the equation satisfied by the concentration $C(r,t)$ at distance r from the source at time t is

$$\frac{\partial C(r,t)}{\partial t} = D\left[\frac{\partial^2 C(r,t)}{\partial r^2} + \frac{2}{r}\frac{\partial C(r,t)}{\partial r}\right] \tag{1}$$

For the above assumptions, this has the solution

$$C(r,t) = \frac{q}{4\pi Dr}\,\mathrm{erfc}\,\frac{r}{\sqrt{4Dt}} \tag{2}$$

(e.g. Crank, 1975) where $\mathrm{erfc}(x) = 1 - \mathrm{erf}(x)$, and $\mathrm{erf}(x)$ is the error function:

$$\mathrm{erf}(x) = \frac{2}{\sqrt{\pi}}\int_0^x e^{-\zeta^2} d\zeta$$

As $t \to \infty$, $C(r,t) \to q/4\pi Dr$. Note that the concentration at the origin is always infinite. Intuitively this is because the derivation assumes that each new molecule of the factor is initially released into an infinitely small volume, from which it diffuses. The discrepancy between this and a real physical system quickly becomes negligible as one moves away from the source.

Two forms of chemical reaction are now considered. If the factor is reversibly bound to the substrate so that the amount S bound at any time equals a constant R times the local free concentration C, then it is straightforward to show that the relevant solution of the diffusion equation is the same as for the case without binding but with an effective diffusion constant of $D/(R + 1)$: the speed of diffusion is simply reduced (Crank, 1975). If the factor is irreversibly removed everywhere at a rate equal to k times the concentration, then equation 1 becomes

$$\frac{\partial C(r,t)}{\partial t} = D\left[\frac{\partial^2 C(r,t)}{\partial r^2} + \frac{2}{r}\frac{\partial C(r,t)}{\partial r}\right] - kC \tag{3}$$

By inspection it can be verified that the solution to this equation is simply the solution to equation 1 multiplied by e^{-kt}. The case of irreversible binding to the substrate to create a bound gradient is discussed later.

What about the gradient? The percentage change in concentration p across a small distance Δr (the width of the growth cone) is given by

$$p(r,t) = \frac{\partial C}{\partial r}\,\frac{\Delta r}{C}$$

For equation 2, this can straightforwardly be calculated:

$$p = -\frac{\Delta r}{r}\left[1 + \frac{r}{\sqrt{\pi D t}}\,\frac{e^{-r^2/4Dt}}{\mathrm{erfc}(r/\sqrt{4Dt})}\right]$$

The form of p is identical for the two cases considered above of reversible binding and irreversible decay. This function has two perhaps surprising characteristics. Firstly, for fixed r $|p|$ *decreases* with t. That is, the largest percentage change at any distance occurs immediately after the source starts releasing the factor. For large t, $|p|$ asymptotes at $\Delta r/r$. Secondly, for fixed $t < \infty$ numerical results show that p is *non-monotonic* with r. In particular it decreases with distance, reaches a minimum, then increases again. The position of this minimum moves to larger distances as t increases.

The general characteristics of the above constraints can be summarized as follows. (i) At small times after the start of production the factor is very unevenly distributed. The concentration C falls quickly to almost zero moving away from the source, the gradient is steep, and the percentage change p across the growth cone is everywhere large. (ii) As time proceeds the factor becomes more evenly distributed; C everywhere increases, but p everywhere decreases. (iii) For large times, C tends towards inverse variation with the distance from the source r, while $|p|$ tends to $\Delta r/r$ independent of all other parameters. This means that for large times the maximum distance over which guidance by diffusible factors is possible scales linearly with growth cone diameter Δr. (iv) If there is a dispersed sink for the diffusible factor the gradient limit is unchanged. The concentration is everywhere divided by an amount increasing exponentially with time; the exponent depends on the rate at which the factor is absorbed by the sink.

Figure 1 summarizes these results in qualitative form, while quantitative graphs for particular parameter values are shown in Figure 2.

Parameter values

In order to explore the quantitative consequences of these equations, it is necessary to estimate appropriate values for the parameters D, q, Δr, the minimum and maximum C for which gradient detection is possible, and the minimum percentage concentration change p detectable by the growth cone.

Diffusion constant, D

Crick (1970) estimated the diffusion constant in cytoplasm for a molecule of mass 0.3–0.5 kDa to be ~10^{-6} cm^2/s. Subsequently, a direct determination of the diffusion constant for a molecule of mass 0.17 kDa in the aqueous cytoplasm of mammalian cells yielded a value of ~3.3×10^{-6} cm^2/s (Mastro *et al.*, 1984). By fitting a particular solution of the diffusion equation to their data on limb bud determination by gradients of a morphogenetically active retinoid, Eichele and Thaller (1987) calculated a value of 10^{-7} cm^2/s for this molecule (mass 348.5 kDa) in embryonic limb tissue. One chemically identified diffusible factor known to be involved in axon guidance is the protein netrin-1, which has a molecular mass of ~75 kDa (Kennedy *et al.*, 1994; Serafini *et al.*, 1994). D should scale roughly inversely with the radius of a molecule, i.e. with the cube root of its mass. Taking the value of 3.3×10^{-6} cm^2/s and scaling it by yields 4.0×10^{-7} cm^2/s.s[1]

However, these estimates of D for cytoplasm may not be applicable either to the *in vivo* situation or to collagen gels. *In vivo* the axon guidance factor is diffusing not through cells but through the extracellular space. This presumably has a very complex geometry, slowing diffusion, but a much smaller volume than the whole three-dimensional space, speeding diffusion. It is thus hard to estimate the net effect on D. For collagen gels, Ogston *et al.* (1973) predicted $D_{collagen}/D_{H_2O}$ on theoretical grounds in terms of the length of collagen chains, their radius, and the Stokes' radius of the diffusing molecule. However, direct measurements of $D_{collagen}$ by Shaw and Schy (1981) yielded consistently lower values than those predicted by Ogston *et al.* (1973), about one quarter to one half as large, for molecules with a range of molecular masses. For instance, for ovalbumin (mass 45 kDa) they found $D_{collagen} \approx 6 \times 10^{-8}$ cm^2/s. This was for a collagen gel of concentration 5% prepared from bovine Achilles tendon. The collagen gel used in axon guidance assays is generally derived from bovine dermis (Lumsden and Davies, 1983) or, most commonly, from rat tails (Lumsden and Davies, 1986; Heffner *et al.*, 1990), and is of variable concentration. It is possible that D in these cases may be different from the value given by Shaw and Schy (1981), and may vary between laboratories using different collagen preparation protocols. It is difficult to know how big these variations might be. Given these uncertainties, some rough guesses are that D both *in vivo* and *in vitro* is definitely unlikely to be any bigger than 10^{-6} cm^2/s, and probably unlikely to be an order of magnitude smaller than 10^{-7} cm^2/s.

Rate of production of factor q

This is very hard to estimate *in vivo*: some insight can be gained by considering *in vitro* experiments. Gundersen and Barrett (1979, 1980) found a turning response in chick spinal sensory axons towards a nearby pipette filled with a solution of nerve growth factor. They estimated the rate of outflow from their pipette to be 1 μl/h, and found an effect when the concentration in the pipette was as low as 0.1 nM nerve growth factor (Tessier-Lavigne and Placzek, 1991). This corresponds to a value for q of 3×10^{-11} nM/s. Lohof *et al.* (1992) studied growth cone turning induced by a gradient of cell membrane-permeant cAMP from a pipette containing a 20 mM solution and a release rate of the order of 0.5 pl/s: $q = 10^{-5}$ nM/s. Below a further calculation for q is performed, which suggests that an appropriate value may be $q = 10^{-7}$ nM/s.

Growth cone diameter, Δr

For the three systems mentioned above, the diameter of the main body of the growth cone is <10 μm. However, this ignores filopodia, which can increase the effective width for gradient sensing purposes. The values of 10 and 20 μm are considered below.

Minimum concentration for gradient detection

Studies of cell chemotaxis suggest that when gradient detection is limited by the dynamics of receptor binding rather than physical limits due to a lack of molecules of factor, optimal detection occurs when the concentration at the growth cone is equal to the dissociation constant for the receptor (Zigmond, 1981; Devreotes and Zigmond, 1988). Such studies also suggest that the low concentration limit is ~1% of the dissociation constant (Zigmond, 1981). The transmembrane protein 'Deleted in Colorectal Cancer' has recently been shown to possess netrin-1 binding activity, with an order of magnitude estimate for the dissociation constant of 10 nM (Keino-Masu *et al.*, 1996). For comparison, the dissociation constant of the low-affinity nerve growth factor receptor P75 is ~1 nM (Meakin and Shooter,

[1]A much faster drop has been measured for the diffusion constant of large biological molecules in cytoplasm than would be implied by the cube root law: Mastro et al. (1984) found a ratio of $D_{H_2O}/_{cytoplasm}$ of 2.6 for sucrose (mass 0.324 kDa), but 71.0 for bovine serum albumin (mass 68 kDa; $D_{cytoplasm} = 10^{-8}$ cm^2/second). However, this could be due to binding in the cytoplasm.

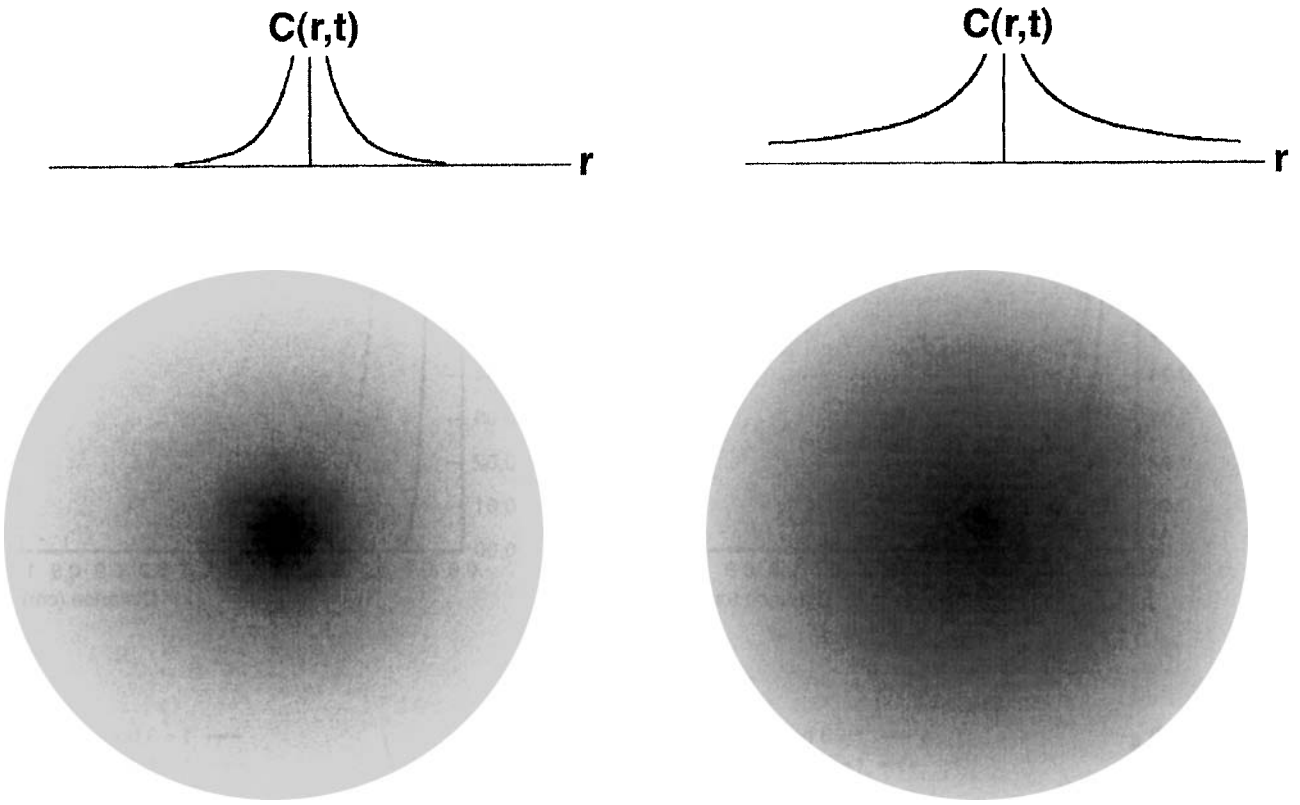

FIG. 1. This picture shows qualitatively the distribution of factor at a short (left) and long (right) time after factor production starts. The source, in the centre of each picture, is producing factor at a rate q mol/s. At early times (left) the concentration is relatively small everywhere, but the gradient is steep. At late times (right) the gradient has stabilized and the concentration is higher everywhere, but the gradient is shallower. The rate at which the gradient evolves to its stable state depends on the diffusion constant D. When D is large this happens rapidly (a few hours for the parameters used in this paper), and when D is small this happens slowly (a few days).

1992). Therefore, low concentration limits of both 10^{-1} and 10^{-2} nM will be considered.

Maximum concentration for gradient detection

Theoretical considerations suggest that, for leukocyte chemotaxis, sensitivity to a fixed gradient should fall off symmetrically in a plot against the log of background concentration, with the peak at the dissociation constant for the receptor (Zigmond, 1981). Raising the concentration to several hundred times the dissociation constant appears to prevent axon guidance (discussed in Tessier-Lavigne and Placzek, 1991). At concentrations very much greater than the dissociation constant, the number of receptors may be down-regulated, reducing sensitivity (Zigmond, 1981). Given the dissociation constants above, 100 nM thus constitutes a reasonable upper bound on concentration.

Minimum percentage change detectable by a growth cone, p

By establishing gradients of a repellent, membrane-bound factor directly on a substrate and measuring the response of chick retinal axons, Baier and Bonhoeffer (1992) estimated p to be ~1%. Studies of cell chemotaxis in various systems have suggested optimal values of 2%; for concentrations far from the dissociation constant for the receptor, p is expected to be larger (Devreotes and Zigmond, 1988). Both $p = 1\%$ and $p = 2\%$ are considered below.

Results

In order to estimate bounds for the rate of production of factor q for biological tissue, the empirical observation is used that, for collagen gel assays lasting of the order of 1 day, guidance is generally seen over distances of at most 500 μm (Lumsden and Davies, 1983, 1986; Tessier-Lavigne *et al.*, 1988). Assume first that this is constrained by the low concentration limit. Substituting the above parameters (with $D = 10^{-7}$ cm^2/s) into equation 2 and specifying that C (500 μm, 1 day) = 0.01 nM gives $q \approx 10^{-9}$ nM/s. On the other hand, assuming constraint by the high concentration limit, i.e. C (500 μm, 1 day) = 100 nM, gives $q \approx 10^{-5}$ nM/s. Thus it is reasonable to assume that, roughly, 10^{-9} nM/s $< q < 10^{-5}$ nM/s. The results discussed below use a value in between, namely $q = 10^{-7}$ nM/s. For this and the above parameters, graphs of C and p at various times are shown in Figure 2.

The constraints arising from equations 2 and 4 are plotted in Figure 3. The cases of $D = 10^{-6}$ cm^2/s and $D = 10^{-7}$ cm^2/s are shown in panels A, C and B, D respectively. In all four pictures the constraints $C = 0.01$ nM and $C = 0.1$ nM are plotted. In panels A and B the gradient constraint $p = 1\%$ is shown, whereas in C and D $p = 2\%$ is shown. These are for a growth cone diameter of 10 μm. The graph for a 2% change and a growth cone diameter of 20 μm is identical to that for a 1% change and a diameter of 10 μm. Each constraint is satisfied for regions to the left of the relevant line. The line $C = 100$ nM is approximately coincident with the vertical axis in all cases. For these parameters, the high concentration limit does not therefore prevent gradient detection until the axons are within a few microns of the source, and it is thus assumed that it is not an important constraint.

As expected, for large t the gradient constraint aymptotes at $\Delta r/r = p$, i.e. r = 100 μm for $p = 1\%$ and $r = 500$ μm for $p = 2\%$ and

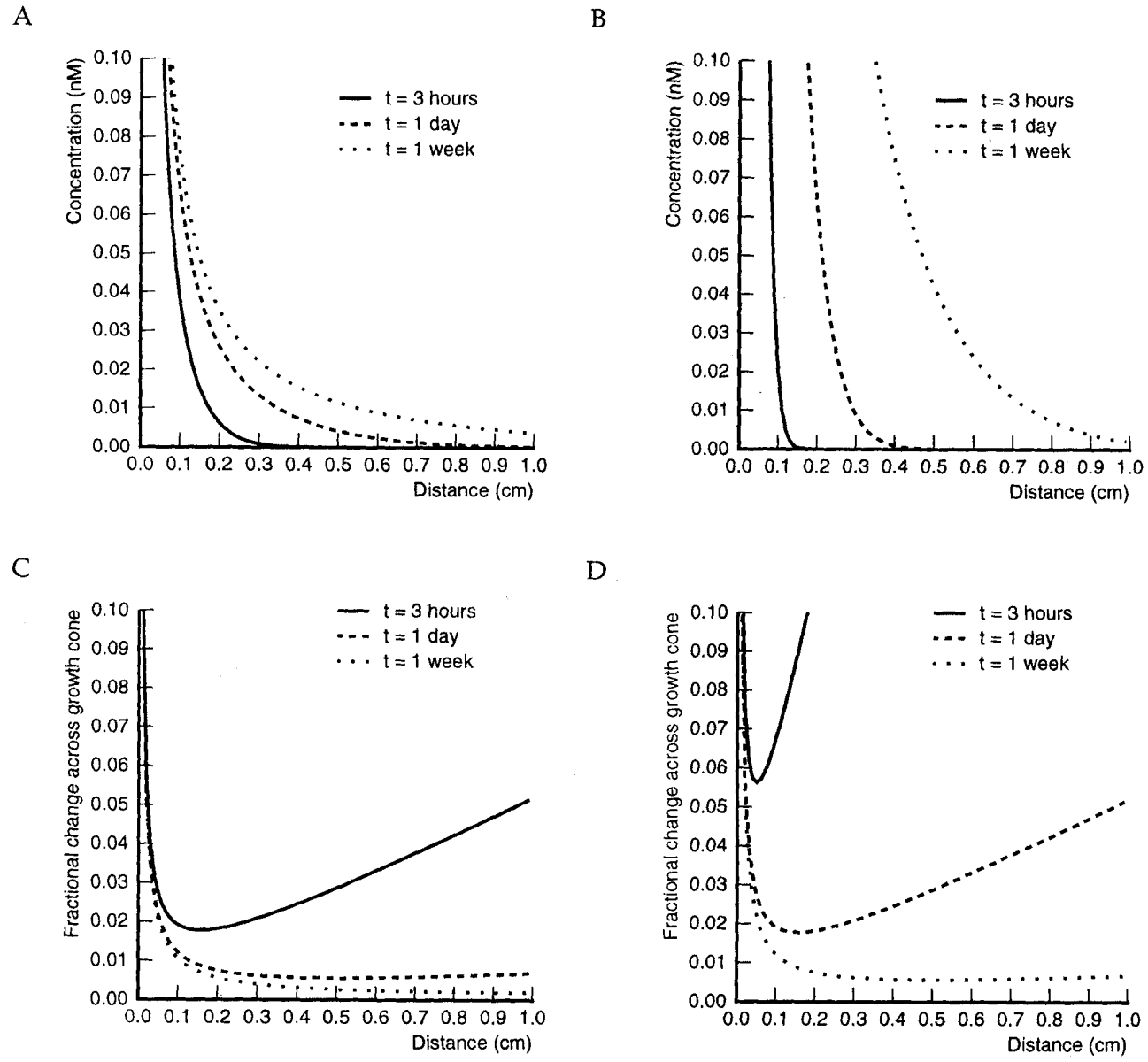

FIG. 2. Graphs showing the distribution of factor as a function of distance from the source for three times (3 h, solid line; 1 day, dashed line; 1 week, dotted line). The top row (A, B) shows the concentration while the bottom row (C, D) shows the percentage change in concentration across the growth cone. The left column (A, C) is calculated for a relatively quickly diffusing molecule ($D = 10^{-6}$ cm^2/s), while the right column (B, D) is calculated for a relatively slowly diffusing molecule ($D = 10^{-7}$ cm^2/s). Note that at early times the gradient is steeper for $D = 10^{-7}$ cm^2/s than $D = 10^{-6}$ cm^2/s. As time increases the gradients for the two cases become more similar.

a 10 μm growth cone. That is, the gradient constraint is satisfied at all times when the distance from the source is <500 μm for $p = 2\%$ and $\Delta r = 10$ μm. The gradient constraint lines end to the right because at earlier times p exceeds the critical value over all distances[2]. As t increases from zero, guidance is initially limited only by the concentration constraint. The maximum distance over which guidance can occur increases smoothly with t, reaching for instance 1500 μm (assuming a concentration limit of 0.01 nM) after ~2 h for $D = 10^{-6}$ cm^2/s and ~6 h for $D = 10^{-7}$ cm^2/s. However, at a particular time the gradient constraint starts to take effect and rapidly reduces the maximum range of guidance towards the asymptotic value as t increases. This time (for $p = 2\%$) is ~2 h for $D = 10^{-6}$ cm^2/s, and ~1 day for $D = 10^{-7}$ cm^2/s. It is clear from these pictures that although the exact size of the diffusion constant does not affect the position of the asymptote for the gradient constraint, it does play an important role in the interplay of constraints while the gradient is evolving. The effect is, however, subtle: reducing D from 10^{-6} to 10^{-7} cm^2/s increases the time for the $C = 0.01$ nM limit to reach 2000 μm, but *decreases* the time for the $C = 0.1$ nM limit to reach 2000 μm.

[2]Since the formula for p is non-monotonic with r, there is sometimes another branch of each p curve (not shown) off the graph to the right.

Discussion

Taking the gradient constraint to be a fractional change of at least 2% across a growth cone of width 10 or 20 μm yields asymptotic

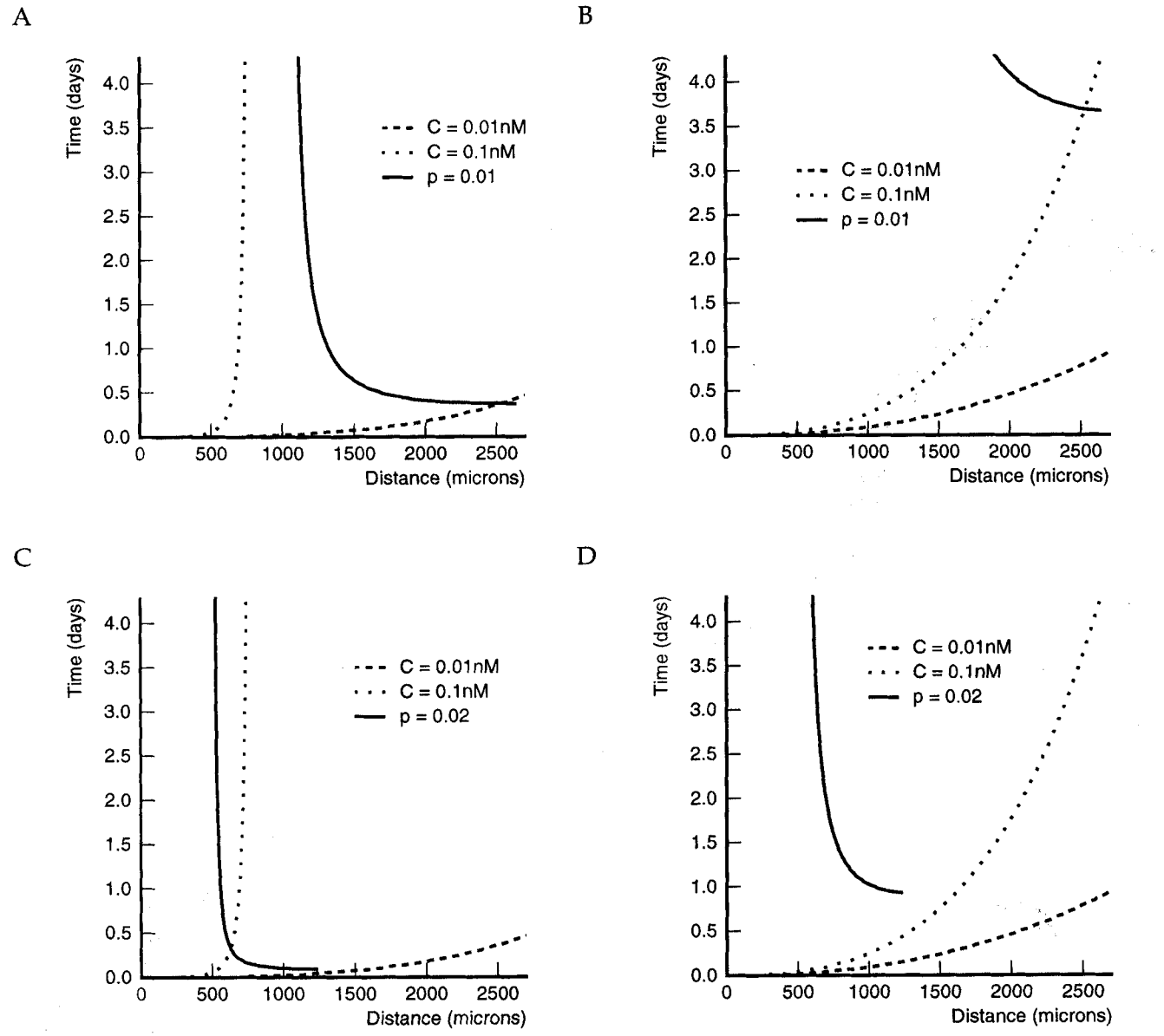

FIG. 3. Graphs showing how the gradient constraint (solid line) interacts with the minimum concentration constraint (dashed/dotted lines) to limit guidance range, and how these constraints evolve over time. The top row (A, B) is for $p = 1\%$, the bottom row (C, D) for $p = 2\%$. The left column (A, C) is for $D = 10^{-6}$ cm^2/s, the right column (B, D) for $D = 10^{-7}$ cm^2/s. Each constraint is satisfied to the left of the appropriate curve. It can be seen that for $D = 10^{-6}$ cm^2/s the gradient limit quickly becomes the dominant constraint on maximum guidance range. In contrast, for $D = 10^{-7}$ cm^2/s the concentration limit is the dominant constraint at times up to several days. However, after this the gradient constraint starts to take effect and rapidly reduces the maximum guidance range.

values for the maximum distance over which guidance can occur once the gradient has stabilized of 500 and 1000 μm respectively. This fits well with both *in vitro* data and the fact that for the systems mentioned in the introduction the growing axons are always ~500 μm from the target *in vivo*. The concentration limits seem to provide a weaker constraint than the gradient limit on the maximum distances possible. However, this is very dependent on the value of q, which has been estimated only very roughly: if q is significantly less than 10^{-7} nM/s, the low concentration limits will provide more restrictive constraints (q may well have different values in different target tissues). The gradient constraint curves are independent of q. The gradient constraint therefore provides the most robust explanation for the observed guidance limit. These conclusions apply identically to diffusible chemorepellents and chemoattractants. The model considers only a point source and so does not address what happens when the axons get very close to and enter a more realistic target of finite size.

The model makes the prediction that guidance over longer distances than have hitherto been observed may be possible before the gradient has stabilized. In the early stages following the start of factor production the concentration falls off more steeply, providing more effective guidance. The time at which guidance range is at a maximum depends on the diffusion constant D. For a rapidly diffusing molecule ($D \approx 10^{-6}$cm^2/s) this occurs after only a few hours. For a more slowly diffusing molecule ($D \approx 10^{-7}$ cm^2/s), however, this occurs after a few days, which would be easier to investigate *in vitro*. *In vivo*, molecules such as netrin-1 may thus be large because, during

times immediately following the start of production by the source, there could be a definite benefit (i.e. steep gradient) to a slowly diffusing molecule. Also, it is conceivable that nature has optimized the start of production of factor relative to the time that guidance is required in order to exploit an evolving gradient for extended range. This could be especially important in larger animals, where axons may need to be guided over longer distances in the developing embryo.

In the guidance of axonal branches to the basilar pons, it is believed that the gradient is detected across the axon shaft rather than the growth cone (Sato *et al.*, 1994). This implies a Δr of at most 2 μm, and therefore a maximum possible distance for guidance of 100–200 μm, depending on p. This constraint takes effect very rapidly with time. The distance between the basilar pons and axons in the corticospinal tract is generally <100 μm at the time branches form. However, *in vitro*, directed branch formation has been observed at distances up to 300 μm (Sato *et al.*, 1994). One possible explanation is that the axon is capable of detecting a lower fractional concentration change. Another explanation could be that branches initially extend for a few microns before deciding whether to extend further or retract, and are capable of comparing binding of factor at their base with that at their tip.

Some guidance factors, most notably netrin-1, appear to bind strongly to the substrate. It is not clear whether it is the free distribution, substrate-bound distribution, or some combination of the two, which guides axons. It would be interesting to calculate the substrate-bound distribution for a particular diffusion process. However, this requires assumptions about the process of binding of free factor to the substrate. The substrate presumably has a finite capacity for binding the factor: thus at large enough times the substrate will be everywhere saturated and there will be no gradient. The approach to this state at smaller times is beyond the scope of the present paper. Two further possible extensions of the model are as follows. Firstly, it is unlikely that factor is produced at a constant rate. A more reasonable, though mathematically substantially more complicated, model would assume a value of q increasing from zero to a maximum and then decreasing back to zero, over a time scale of a small number of days. Secondly, and most significantly, it is desirable to model in more detail the gradient-sensing process itself (Berg and Purcell, 1977; Gierer, 1987). A simple model is that of two groups of receptors, one on each side of the growth cone, that communicate a difference-in-binding signal between them. Such a model has been investigated in detail for leukocyte chemotaxis (Tranquillo and Lauffenburger. 1987; Tranquillo *et al.*, 1988). However, growth cones show some importantly different behaviours from leukocytes. For instance, in the absence of a gradient they tend to grow in straight lines rather than taking a random walk. It would also be interesting to explore theoretically the way that the minimum fractional change in concentration across the growth cone required for guidance changes as a function of the absolute concentration.

Clearly the mathematical framework developed in this paper is a crude model, particularly for the *in vivo* situation. In reality there will be geometrically complicated boundary conditions for both the source and the volume, the volume will consist of heterogeneous tissue types in different regions that yield different diffusion constants, and the diffusing molecule will interact chemically in complicated ways with, for instance, cell surface receptors and the extracellular matrix. Diffusion through the complicated geometry of the extracellular space may have rather different properties from that for a uniform substrate, and may not even be Fickian. However, the present model still gives good quantitative agreement with the observed maximum guidance distance. It provides a starting point for thinking quantitatively about what may be possible both *in vivo* and *in vitro*, how different parameters contribute to the limits on guidance, and which may be the most important constraints in different situations.

Acknowledgements

I am very grateful to Linda Richards, Herwig Baier, Dennis O'Leary, Marc Tessier-Lavigne, David Price and David Litwack for stimulating discussions, and to Linda Richards, Herwig Baier and David Price for helpful comments on the manuscript. I thank the anonymous reviewers for useful suggestions regarding presentation, and Eric Klineberg for help preparing Figure 1. This work was supported by the Alfred P. Sloan Foundation.

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