

## Axon Guidance: Stretching Gradients to the Limit

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Neuronal growth cones, the sensory-motile structures at the tips of developing axons, navigate to their targets over distances that can be many times greater than their diameter. They may accomplish this impressive task by following spatial gradients of axon guidance molecules in their environment (Bonhoeffer & Gierer, 1984; Tessier-Lavigne & Placzek, 1991; Baier & Bonhoeffer, 1994). We calculate the optimal shape of a gradient and the distance over which it can be detected by a growth cone for two competing mechanistic models of axon guidance. The results are surprisingly simple: Regardless of the mechanism, the maximum distance is about 1 cm. Since gradients and growth cones have coevolved, we suggest that the shape of the gradient *in situ* will predict the mechanism of gradient detection. In addition, we show that the experimentally determined dissociation constants for receptor-ligand complexes implicated in axon guidance are about optimal with respect to maximizing guidance distance. The relevance of these results to the retinotectal system is discussed.

### 1 Introduction

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The mechanisms that guide axons to appropriate targets in the developing brain are largely unknown. A popular notion, first suggested by Cajal, is that spatial gradients of axon guidance molecules are detected by the growth cone and provide directional information. Experimental evidence for the existence of such mechanisms is gradually mounting. However, so far there has been little consideration of the theoretical limits on axon guidance by gradients imposed by physical limits on the detection of a concentration difference across a small sensing device. Here, using a few pieces of experimental data and some simple approximations, we address these limits.

For a growth cone to be guided by a gradient, it must be able to sense a sufficiently large difference in ligand concentration over its length. The ligand may be attractive or repellent, and may be substrate bound, freely

diffusing, or a combination of both. Two possible mechanisms for gradient detection by a growth cone are (1) internal amplification of a small percentage change in external concentration across the width  $w$  of the growth cone (Bonhoeffer & Gierer, 1984; Gierer, 1987), and (2) a shifting internal baseline that reduces the effective concentration at one edge of the growth cone to zero (Walter, Allsop, & Bonhoeffer, 1990). Gradient detection by the former mechanism requires a sufficiently high percentage change  $p$  in concentration over distance  $w$ , while the latter requires a sufficiently high absolute concentration difference  $\Delta C$  over  $w$ . Three additional constraints limit gradient detection. First, the local external concentration must be less than a critical value  $C_{\text{high}}$ , at which most receptors are saturated. Second, it must be greater than a critical value  $C_{\text{low}}$ , at which an insufficient number of receptors are bound to overcome noise.  $C_{\text{low}}$  and  $C_{\text{high}}$  vary relative to the dissociation constant  $k_d$  for the receptor-ligand complex. Third, the local concentration must also be greater than a physical limit  $C_{\text{noise}}$ , which is  $k_d$  independent. At this concentration, the number of ligand molecules in the vicinity of the growth cone is so small that over the time scales of relevance to the growth cone, thermally induced fluctuations wash out the gradient signal (Tranquillo & Lauffenburger, 1987).

## 2 Maximum Guidance Distance

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What is the maximum range  $r_{\text{max}}$  for which guidance is possible for the two mechanisms above? The optimal gradient for case 1 has a constant fractional change across the width of the growth cone  $w$  for all positions: an exponential gradient. Consider  $C(r) = C_0 e^{-ar}$  where  $C$  is concentration,  $r$  is distance, and  $C_0$  and  $a$  are constants. Requiring a percentage change of  $p$  ( $= \Delta C/C$ ) across distance  $w$  yields  $a = p/w$ . The maximum distance for which  $C \geq C_{\text{low}}$  is achieved when  $C_0 = C_{\text{high}}$ . This gives

$$r_{\text{max}} = \frac{w}{p} \log_e \frac{C_{\text{high}}}{C_{\text{low}}}. \quad (2.1)$$

The optimal gradient for case 2 has a constant absolute concentration change across the width of the growth cone: a linear gradient. Consider  $C(r) = C_0 - ar$ . Requiring a concentration change of  $\Delta C$  over distance  $w$  yields  $a = \Delta C/w$ . Again the optimal value of  $C_0$  is  $C_{\text{high}}$ . For the analogous case of leukocyte chemotaxis, it is known that sensitivity to gradients is optimized when the external concentration is equal to the dissociation constant  $k_d$  of the relevant receptor (Devreotes & Zigmond, 1988), which yields  $\Delta C = pk_d$ . This gives

$$r_{\text{max}} = \frac{w}{p} \frac{C_{\text{high}} - C_{\text{low}}}{k_d}. \quad (2.2)$$

What are plausible parameter values? We assume a growth cone diameter  $w$  (including filopodia) of  $20\ \mu\text{m}$ . Direct evidence (Baier & Bonhoeffer, 1992), analogous data for leukocyte chemotaxis (Devreotes & Zigmond, 1988), and theoretical considerations (Tranquillo & Lauffenburger, 1987) suggest that  $p$  is about 2 percent. Data for leukocyte chemotaxis suggest that  $C_{\text{low}} \approx k_d/100$  and  $C_{\text{high}} \approx 10k_d$  (the asymmetry is due to down-regulation of receptors at high external concentrations) (Zigmond, 1981). Assuming  $C_{\text{low}} > C_{\text{noise}}$  yields  $r_{\text{max}} \approx 0.7\ \text{cm}$  for the exponential case (see equation 2.1) and  $r_{\text{max}} \approx 1\ \text{cm}$  for the linear case (see equation 2.2). Note that these values scale linearly with the size of the growth cone and do not depend on  $k_d$ . The calculation assumes that the growth cone can detect  $p = 2$  percent for  $C_{\text{high}} \geq C \geq C_{\text{low}}$ , whereas in fact it is likely that  $p$  needs to be much larger away from  $C = k_d$ . Correcting for this would reduce  $r_{\text{max}}$  in both cases. Similarly if growth cones employ a combination of the two mechanisms,  $r_{\text{max}}$  would again be reduced: 1 cm is thus an upper bound.

Three obvious scenarios for how axons could be guided over larger distances are as follows. First, there could exist a series of spaced gradients of different ligands, each binding to the same or different receptors and guiding the growth cone over only a portion of the full distance. Second, there could exist overlaid gradients of different ligands, each competing for occupancy of the same receptor. Appropriate differences in affinity would allow guidance in multiple regions. Third, there could exist multiple receptors on the growth cone for the same ligand, with different affinities. Each would guide the growth cone over the segment of the gradient lying within its appropriate concentration range. Note that these considerations apply to attractant as well as repellent guidance molecules, or to combinations of both.

### 3 Noise Limits to Receptor-Ligand Affinity

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To maximize guidance distance, it is clearly necessary to choose  $C_{\text{low}} > C_{\text{noise}}$ . An accurate calculation of  $C_{\text{noise}}$  requires knowledge of parameters such as the length of time over which an axon integrates signals from its receptors before assessing a gradient value, which has not been measured. Here instead a conservative order of magnitude estimate for  $C_{\text{noise}}$  is made. We assume, as an extremely rough estimate, that 100 molecules in the vicinity of the growth cone are sufficient for a 2 percent gradient to be detected. This means that the growth cone can distinguish 50 molecules on one side from 51 on the other. Imagine that the growth cone plus filopodia occupies a cube of side length  $20\ \mu\text{m}$ ; this has a volume of approximately  $10^{-11}$  liters. One hundred ligand molecules in this volume correspond to a ligand concentration  $C_L \approx 0.01\ \text{nM}$  (note that the proportion of the cube occupied by the body of the growth cone, and thus unavailable to the ligand molecules, is small). Equating this with the lower limit due to the dynamics of receptor binding,  $C_{\text{low}} = k_d/100$ , yields  $k_d \approx 1\ \text{nM}$ . We suggest that a  $k_d$  of very

roughly 1 nM represents a lower limit for axon guidance receptor-ligand complexes.<sup>1</sup> A receptor-ligand affinity significantly higher than this (i.e.,  $k_d \ll 1$  nM) would not improve the accuracy of gradient reading. A significantly lower affinity would require comparatively large amounts of factor to be produced. An alternative reverse-engineering argument based on the same principle is that the  $k_d$  of the receptor-ligand complex could predict the actual signal-to-noise requirements of gradient reading.

#### 4 Applications to the Retinotectal System

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Two recently identified repellent axon guidance molecules are believed to be involved in the formation of the retinotectal projection: ephrin-A5 (Drescher et al., 1995) and ephrin-A2 (Cheng, Nakamoto, Bergemann, & Flanagan, 1995; Nakamoto et al., 1996). Both are expressed as gradients in the chick optic tectum, and both bind to one family of receptors, some members of which are expressed on retinal growth cones (for review, see Friedman & O'Leary, 1996a). The ephrin-A2 gradient spans the entire tectum, while the ephrin-A5 gradient is shifted posteriorly in the tectum, being absent from the anterior tectum (where retinal axons enter) (Cheng et al., 1995; Drescher et al., 1995; Nakamoto et al., 1996).  $k_d$  values have recently been measured in vitro for ephrin-A5 and ephrin-A2 for three growth cone receptors: EphA3, EphA5, and EphA4. These values are as follows, for ephrin-A5 and ephrin-A2, respectively: EphA3: 0.144 nM/0.86 nM; EphA5: 0.616 nM/8.62 nM; EphA4: 0.622 nM/12.7 nM (Monschau et al., 1997). In each case, the value for ephrin-A2 is roughly an order of magnitude higher than that for ephrin-A5.

The chick optic tectum extends over 6–9 mm during formation of the retinotectal map. The distance that the farthest projecting retinal growth cones have to travel across its (bent) surface is well over 1 cm. Our calculations predict that if retinal axons are guided within the tectum solely by gradient mechanisms, then some method for extending guidance must be operating.<sup>2</sup> We suggest that retinal growth cones could use the same receptor(s) for both ephrin-A2 and ephrin-A5, with the low-affinity ephrin-A2 gradient providing guidance in the anterior tectum, the high-affinity ephrin-A5 gradient providing guidance in the posterior tectum, and a combination of both gradients providing guidance in the middle. In addition,

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<sup>1</sup> This calculation applies to both substrate-bound and freely diffusing ligands and also analogously to the sensing of a gradient on a two-dimensional surface.

<sup>2</sup> The situation is apparently more involved: only the nasal-most retinal axons traverse the entire tectum. The more temporal the axons' site of origin in the retina, the farther anteriorly they terminate in the tectum. This graded response to tectal cues, such as ephrin-A5, is possibly reflected by a gradient of receptor level, such as EphA3 (Drescher et al., 1995), in the retina. However, temporal axons are able to navigate to their appropriate tectal target if misrouted or surgically displaced, suggesting that they can utilize gradient information in tectal regions that they normally do not encounter.

the affinity values for ephrin-A5 and ephrin-A2 given above are all within an order of magnitude of our theoretical lower limit of 1 nM, which is reasonable agreement given the crudeness of our calculation. (However, these are *in vitro* measurements, which may differ from values *in vivo*.)

## 5 Regulation of Gradient Shape

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An unresolved issue of both biological and theoretical interest is how gradient shape can be regulated in an embryonic field (Crick, 1970). Some axon guidance molecules, like netrin-1 (Kennedy et al., 1994; Serafini et al., 1994), are diffusible factors that are secreted by target cells (Tessier-Lavigne & Placzek, 1991; Kennedy et al., 1994). Simple diffusion yields gradients that are inefficient when growth cones have to traverse distances greater than 1 mm (Tessier-Lavigne & Placzek, 1991; Goodhill, 1997). Binding of the factor to the substrate (e.g., the extracellular matrix) could modify the shape of the gradient to maximize the distance and optimize the accuracy of guidance. The positional information conferred by the gradients of ephrin-A2 and ephrin-A5 in the tectum is initially set up by gradients of morphogens (Crick, 1970) and by transcription factors such as *en-1* or *en-2* (Itasaki & Nakamura, 1996; Logan et al., 1996; Friedman & O'Leary, 1996b). The local concentrations of these have to be translated into local concentrations of guidance molecules. The translation mode is unknown, but we expect, given the size constraints discussed here, that nature has made some effort to optimize it.

## 6 Conclusions

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For the two possible mechanisms of gradient detection across the width of the growth cone (measuring a fractional change versus a difference from an adjustable baseline), the maximum guidance distance is surprisingly similar (0.7–1.0 cm). However, the shape of the optimal gradient is different in the two cases (exponential versus linear). Therefore, it should be possible to predict the actual gradient-reading mechanism by accurately measuring the shape of gradients of axon guidance protein *in situ*. Our result also has important implications for the scalability of axon guidance mechanisms to animals substantially larger than the rats and chickens that are most commonly studied.

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