Retinotectal maps: molecules, models and misplaced data

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The mechanisms underlying the formation of topographic maps in the retinotectal system have long been debated. Recently, members of the Eph and ephrin receptor–ligand family have been found to provide a molecular substrate for one type of mechanism, that of chemospecific gradient matching, as proposed by Sperry. However, experiments over several decades have demonstrated that there is more to map formation than gradient matching. This article briefly reviews the old and new findings, argues that these two types of data must be properly integrated in order to understand map formation fully, and suggests some experimental and theoretical ways to begin this process.

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In spite of a great deal of debate over the years, the idea that cell surface molecules mediate recognition remains an attractive explanation for many aspects of patterned connections. It is clear, however, that surface recognition molecules provide a relatively weak force that modulates the development of connectivity rather than dictating it. Attempts to establish the biochemical basis of such recognition are still at an early stage, but there seems no reason why they should not ultimately succeed. One should hasten to emphasize, however, that surface recognition is only one of a number of mechanisms that lead to patterned innervation…Thus, although important, the biochemical identity of surface recognition molecules is not really the Holy Grail.

Purves and Lichtman (1985)[1].

ONE of the most-common characteristics of axonal connection patterns in the brain is their organization into topographic maps, whereby neighboring points in one structure project to neighboring points in a target structure. For many decades, neuroscientists have attempted to understand the cues that axons use during development in order to achieve such precise targeting. There is no shortage of candidate mechanisms; the challenge is to determine the relative importance of each in both normal and perturbed situations. The most extensively studied model system in this regard is the projection from the retina to the tectum. From the 1960s to the 1980s elegant experimental and theoretical research looked at the effects of numerous types of experimental manipulations on this system, and concluded that multiple mechanisms are involved. More recently, the exciting discovery of gradients of erythropoietin-producing hepatocellular (Eph) receptors in the retina, and their ligands, the ephrins, in the tectum, has sharply focussed attention on a single mechanism, that of gradient matching, as proposed by Sperry[2]. However, while the molecular biology is impressive, the renewed discussion of mechanisms is overly simplistic: current debate largely ignores previous work showing that gradient matching alone is an incomplete explanation. The purpose of this article is to draw attention to the limitations of current gradient-matching ideas, and to suggest ways of integrating the old and new data in a more satisfactory way. Only the mapping of retinal axons along the rostral–caudal axis of the tectum is discussed, and how axons reach the tectum itself will not be considered.

Gradient matching and the Eph and ephrin family

In order to account for the experimental finding that retinal axons could find their normal sites of termination in the tectum, even when optic nerve fibers were artificially scrambled, Sperry postulated a scheme of chemical matching between retina and tectum[2].

The establishment and maintenance of synaptic associations [is] conceived to be regulated by highly specific cytochemical affinities that arise systematically among the different types of neurons involved via self-differentiation, induction through terminal contacts, and embryonic gradient effects…[I propose] an orderly cytochemical mapping in terms of two or more gradients of embryonic differentiation that spread across and through each other with their axes roughly perpendicular. These separate gradients successively superimposed on the retinal and tectal fields and surroundings would stamp each cell with its appropriate latitude and longitude expressed in a kind of chemical code with matching values between the retinal and tectal maps. The inversion of the retinal map on the tectum suggests complementary relations in the affinity forces involved in linking the corresponding points in the two fields.

This idea of 'chemospecificity' has provided a powerful organizing hypothesis for subsequent studies of retinotectal development. Although some molecules were identified that exist in gradients in the retina [9-*O*-acetyl GD3 (Ref. 3), TOP (toponymic molecule[4,5]) and TRAP (temporal retinal axon protein[6])] and in the tectum [RGM (repulsive guidance molecule[7])], a clear role for these in the formation of retinotectal topography was not established. Meanwhile, Sperry's hypothesis was tested in many creative ways by comparing qualitative (and sometimes also quantitative) predictions with the outcome of a variety of experimental manipulations. However, over the past five years a new family of molecules has been found to exist in gradients in the retina and in the tectum, and has been shown clearly to have an important role in retinotectal map formation. Some of these results are summarized briefly in this article and in Fig. 1, and are reviewed in Refs 8–12.

Retinal gradients

In chick, the Eph family of receptor tyrosine kinases are found in the retina and on the growth cones of retinal ganglion cells[13,14]. *EPHA3* is expressed in an

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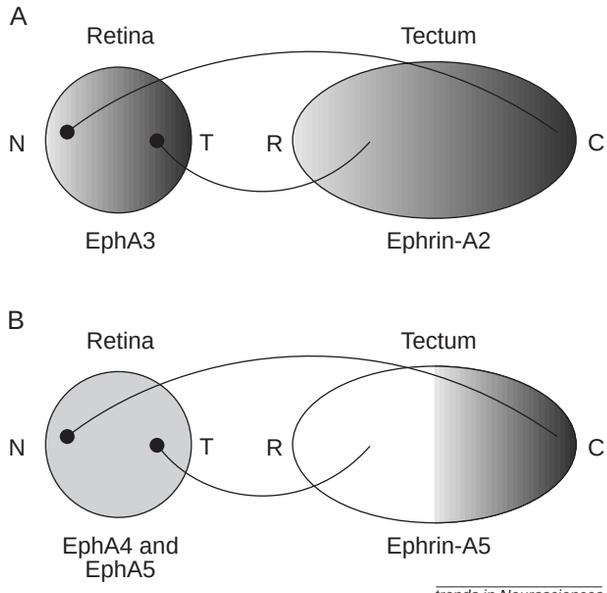

Fig. 1. ***Eph and ephrin distributions in retina and tectum.*** *The normal retinotectal mapping in the chick. The nasal (N) retina maps to the caudal (C) tectum and the temporal (T) retina maps to the rostral (R) tectum.* **(A)** *The shading indicates the levels of EphA3 (retina) and the levels of ephrin-A2 (tectum) (darker shading represents higher levels). Note that gradients run in opposite directions: regions of high EphA3 levels map to regions of low ephrin-A2 and ephrin-A5 [see (B)] and vice versa.* **(B)** *The shading indicates the levels of EphA4 and EphA5 (retina) and the levels of ephrin-A5 (tectum) (darker shading represents higher levels). EphA4 and EphA5 are uniformly distributed across the retina.*

increasing nasal-to-temporal gradient while *EPHA4* and *EPHA5* are expressed uniformly (see Fig. 1). In mouse, *Epha4* and *Epha5* are expressed in the retina with *Epha4* being expressed uniformly and *Epha5* expressed in an increasing nasal-to-temporal gradient[15].

Tectal gradients

Ephrin-A2 and ephrin-A5, which are ligands for the EphA3, EphA4 and EphA5 receptors, are both produced in an increasing anterior to posterior gradient in the tectum[13,16]. In chick, the levels of mRNA for both ephrin-A2 and ephrin-A5 rise from anterior to posterior tectum, with the latter being restricted to more-posterior locations and rising more steeply. In mouse, levels of ephrin-A5 rise from anterior to posterior while levels of ephrin-A2 decrease at both ends of the superior colliculus[17]. The *engrailed* gene has been strongly implicated in the establishment of tectal polarity and in the control of tectal ephrin gradients (Refs 18–22). (Ephrin-A2 and ephrin-A5 are also found in high nasal to low temporal gradients in the retina, and very recently it has been shown that these gradients might also be important for map formation[23].)

Several lines of evidence suggest that these receptor–ligand interactions are repulsive. *In vitro*, axons of the temporal retina prefer to grow on lanes of cell membranes from rostral tectum in the membrane-stripe assay, while axons of the nasal retina grow on both rostral and caudal membranes[24]. *In vivo*, high levels of receptor map to low levels of ligand, and vice versa; temporal axons avoid regions of tectum where *engrailed* and, thus, the genes for ephrin-A2 and ephrin-A5 are overexpressed[18–22]; and in ephrin-A5-knockout mice, retinal axons overshoot the superior colliculus[17]. These findings have been interpreted in terms of simple qualitative models for how gradient matching could generate topographic maps[8,11,12,25]. These models postulate that each axon seeks out the concentration of ligand(s) in the tectum that are appropriate for the concentration of receptor(s) present on the growth cone. The interaction of receptor(s) and ligand(s) produces a 'topographic signal' (referred to as *S*) in the axon that causes either the growth cone to stop or the axon to branch. This occurs either when *S* reaches a certain threshold or critical value, or when it reaches a local optimum with respect to tectal position. It is postulated that the position along the tectum at which this occurs varies smoothly with retinal origin, thus establishing a map. Nakamoto *et al.*[25] further propose that the level of this topographic signal is simply the product of the receptor and ligand concentrations, a 'law of mass action'. However, such models are problematic for at least two reasons. First, their precise requirements to produce a topographic map under normal conditions in terms of mechanisms and gradient shapes have not been investigated quantitatively. Second, there has so far been no analysis of how these models might function or need to be modified in light of previous data regarding map formation in the retinotectal system. In Box 1, the assumptions underlying some of the recently proposed gradient models are formalized and their quantitative requirements for establishing a map are investigated. Some of the older data are reviewed below, and it is argued that, unless receptor and ligand gradients can change shape under particular circumstances, gradient matching alone is an insufficient explanation for map formation.

Earlier data regarding retinotectal map formation

Many experiments between the 1960s and the present have probed the mechanisms of retinotectal map formation using a variety of ingenious methods for disturbing the normal development or regeneration of the map. These results have been reviewed many times (see, for example, Refs 26–34), though are not much discussed in more-recent publications about the role of Eph and ephrins in retinotectal map formation.

Shifting connections and ectopic targeting

In fish and frogs the retina grows by addition of new neurons radially during development, while the tectum grows mostly along one dimension. The retinotectal map remains ordered throughout this time, indicating that the retinotectal projection is shifting continually[35,36]. Retinal axons entering the tectum via abnormal trajectories can still find their appropriate termination sites[37–39].

Rotation

If a presumptive tectum in *Xenopus* is rotated early enough during development, a map is formed that is normal relative to the whole animal (Fig. 2B), whereas later rotations lead to a rotated map[40] (Fig. 2C). Initially it was thought that eye rotation could also lead to both a normal outcome, if performed early enough, and a rotated outcome, if performed later[41,42]. However, more-recent experiments have always found rotated maps (Fig. 2D) (reviewed in Refs 33,34).

Retinal ablation and tectal ablation ('expansion' and 'compression')

The map formed after removal of half the retina covers half the tectum initially[43], but then expands gradually to fill the whole tectum[44] (Fig. 2E). If the optic nerve is then made to regenerate again, an expanded map is formed immediately[45]. If half the tectum is ablated, the regenerated map is compressed into the remaining

Box 1. Mathematical analysis of a gradient-based model

Although the qualitative reasoning about gradient matching used by Sperry and others has been useful, claims about whether or not any particular set of rules is sufficient to produce a map can only be definitively answered by quantitative reasoning. We shall analyze quantitatively some specific versions of the 'mass action' model of Nakamoto *et al.*[a] (see also Ref. b).

Let R be the concentration of a receptor present on a growth cone or axon, and L be the concentration of a ligand present in the tectum. Refer to position along the nasal–temporal axis of the retina as x and position along the rostral–caudal axis of the tectum as y, so that $R = R(x)$ and $L = L(y)$. Gierer[c,d] discussed how topographic information could be signaled by interactions between ligands and receptors. A particular type of interaction, which has been proposed by Nakamoto *et al.*[a], is that the concentration of a 'topographic signal', the signal that tells axons where to stop, is related to the concentrations of receptor and ligand by the law of mass action:

$$S(x,y) = kR(x)L(y) \quad (1)$$

where $S(x,y)$ is the concentration of topographic signal produced within an axon that originates from position x in the retina when it is at position y in the tectum, and k is a constant. In the general case of multiple receptors and ligands, with promiscuous interactions between them, this equation becomes:

$$S(x,y) = \sum_{i,j} k_{ij} R_i(x) L_j(y) \quad (2)$$

Whether each receptor–ligand interaction is attractive or repulsive is indicated by the sign of the relevant k_{ij}. Two possibilities for how $S(x,y)$ might produce a stop (or branch) signal in the growth cone (or axon) are that this occurs when:

- a set point is reached (discussed in, for example, Refs a,e), that is $S(x,y) = P$, where P is a constant; or
- when attraction (or repulsion) reaches a local maximum (or minimum), that is

$$\frac{\delta S(x,y)}{\delta y} = 0 \quad (3; \text{Refs c,d})$$

For a smooth, uniform mapping, one of these conditions must hold along a line $y = cx$. The constant of proportionality c can be used to control for whether this is a normal map, a compressed map from a whole retina to a half tectum, or an expanded map from a half retina to a whole tectum.

Set-point rule

For one gradient in the retina and one gradient in the tectum (Eqn 1), this rule requires that the ligand gradient be inversely proportional to the receptor gradient:

$$L(cx) = \frac{P}{kR(x)} \quad (4)$$

This implies that the gradients run in opposite directions. If $R(x)$ is linear (cf. the gradient of EphA3 in the retina), the ligand concentration is required to go to infinity at one end of the tectum. One way of circumventing this is to assume $R(x)$ does not reach zero at $x = 0$: the experimental data are not precise enough to decide on this point. However, the addition of a second receptor gradient gives:

$$L(cx) = \frac{P}{k_1 R_1(x) + k_2 R_2(x)} \quad (5)$$

If $R_1(x)$ is linear and $R_2(x)$ is flat (cf. the gradient of EphA4 in the retina), then it is no longer necessary for $L(y)$ to reach infinity. For two receptor and two ligand gradients, many combinations of gradient shapes are possible. Note that the receptor or ligand distribution must have a different form to reproduce a normal, compressed or expanded map, that is, one or both gradients must be respecified under these circumstances.

Local optimum rule

For one retinal and one tectal gradient we have the requirement:

$$R(x)\frac{\delta L(y)}{\delta y} = 0 \quad (6)$$

If this is true for any value of x it is true for all values of x, and thus no map is generated. The same problem arises with two receptor gradients, whatever their shapes. For two receptor and two ligand gradients, many combinations of gradient shapes are possible. (Gierer investigated this case, but for a more-complicated reaction law for generating the topographic signal than mass action[c,d].) However, note that changing the value of c again requires the gradient shapes to be respecified for an appropriate map to form.

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tectal space[46–49] (Fig. 2F). If 'mismatched' halves of the retina and tectum are ablated a topographic map still forms[50] (Fig. 2G).

Compound-eye experiments

When a whole eye is created by fusing together two half-eye rudiments before connections are made, the two halves being from opposite eyes but of the same type (for example, nasal, ventral or temporal), they each map across the whole tectum in the mirror image of each other[51,52] (Fig. 2H). When smaller fragments than half a retina are substituted early in development ('pie-slice' eyes), the retinal fragments map appropriately for their original position[53], though they can also show some degree of reprogramming[54].

Translocation

If two parts of the tectum are translocated reciprocally, regenerating retinal axons innervate their normal piece of tectum[55,56] and also appropriately reverse their order if the tectal fragment is rotated[57] (Fig. 2I). However, in some cases, a map can be formed that ignores the translocation, that is, the fibers tend to align with fibers in the surrounding tectum, regardless of the orientation of the transplant[58] (Fig. 2J).

The outcome of the experiments that concern retinal rotation and ectopic targeting, and some of the outcomes of the tectal translocation, tectal rotation and compound-eye experiments, are consistent with a purely chemospecific matching mechanism between stable gradients in retina and tectum. However, as has been discussed previously (see, for example, Refs 26–34), the other results imply that either the gradients are not stable and are modified in response to experimental manipulation, or that additional mechanisms are also involved. The other principal mechanisms invoked previously are competition of retinal axons for tectal space, which explains the expansion and compression results (also known as 'systems matching'[26]), and fiber–fiber interactions, which explain how, for example, the abnormal polarity of rotated tectal grafts can be ignored when they are small (see Ref. 29 for further discussion). (There is also extensive evidence of a role for activity-dependent refinement in the later stages of map development, but

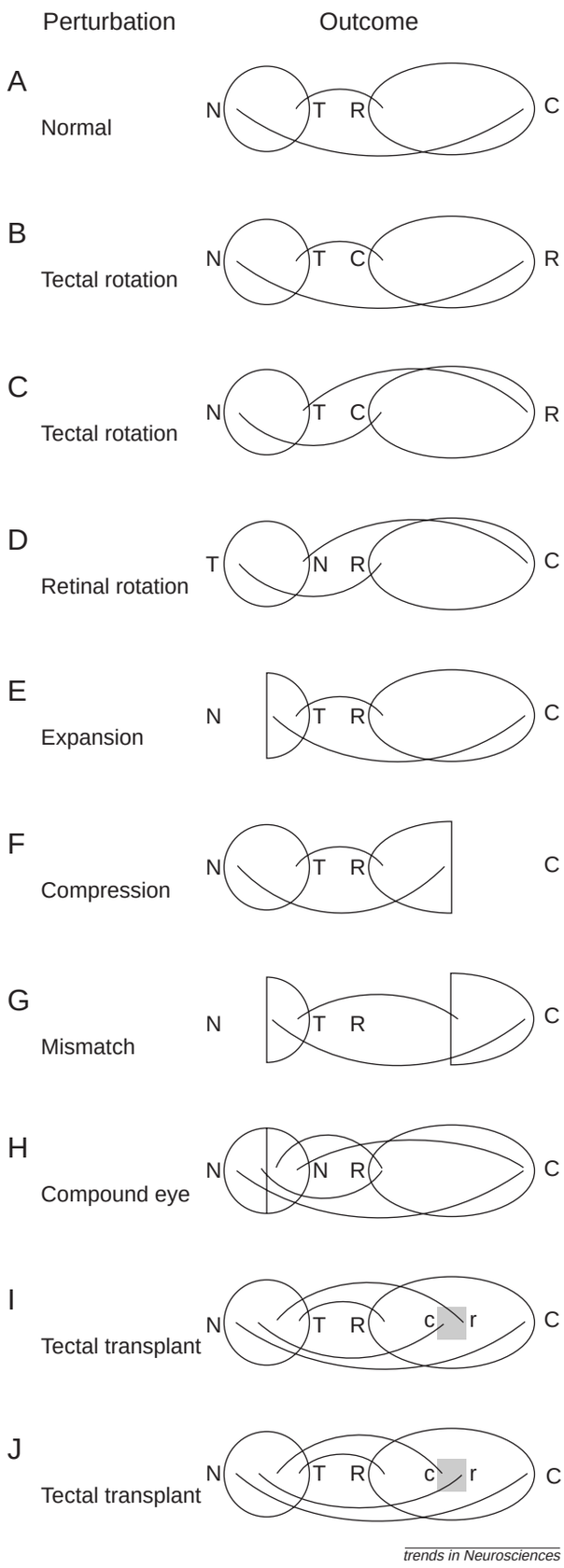

Fig. 2. ***Schematic representation of some of the experimental perturbations to the retinotectal system that have been performed, and their outcomes.*** *The first row* **(A)** *shows the normal retinotectal mapping as in Fig. 1. The nasal (N) retina maps to the caudal (C) tectum and the temporal (T) retina maps to the rostral (R) tectum.* **(B)**–**(J)** *summarize how this map is altered when parts of the retina or tectum are rotated or transplanted. The shaded areas in* (I) *and* (J) *repesent a tectal transplant that has been rotated by 180°.*

this article is concerned only with activity-independent axon targeting. In addition, strong distinctions have not been made between developmental and regeneration experiments, nor between experiments in different species. Although these caveats are important, they do not detract from our main point: that mechanisms in addition to the matching of stable gradients must be invoked in order to provide a complete account of retinotectal map formation.)

Understanding how the outcomes observed under different experimental circumstances could be produced by the interaction between gradient matching, competition and fiber–fiber interactions is a challenging task. Computational models have had an important role in clarifying intuitions and establishing the precise capabilities of different mechanisms acting alone or in concert (Refs 58–69; reviewed in Ref. 70). Each of these models suggests quantitative, testable hypotheses, and can be judged by how well they reproduce the data described above (some examples are given later). However, with some exceptions (see, for example, Refs 14,71), most of the recent Eph and ephrin literature mentions only the model of Gierer[72,73]. This relies purely on the matching of stable gradients, and is thus limited in its ability to explain the full range of experimental data described above.

Discussion

By combining the previous experimental and theoretical data with current molecular findings, an exciting opportunity now exists to address the mechanisms regulating topographic targeting in the retinotectal system more precisely. How might the current thinking on retinotectal map formation be enhanced by including evidence from the previous experimental paradigms? Clearly, some of the previous results cannot be accounted for directly by the type of models currently being proposed if gradients are stable. In the half-retinal and half-tectal ablation studies, the observed result of systems matching suggests that either the Eph and ephrin gradients are not stable in response to these experimental manipulations ('respecification'[46]; see also Box 1), or that mechanisms in addition to pure gradient matching are operating. While respecification has been criticized as a *post hoc* explanation[26], the molecular tools are now available to investigate this hypothesis directly. Do the gradients become twice as steep in a hemi-retina or hemi-tectum? Does their steepness shift over time in line with the gradual expansion or compression of the map? What do regenerating nasal and temporal retinal axons do in the membrane-stripe assay when confronted with stripes made from the two rostral or two caudal quarters of a half tectum after a compressed map has formed? What happens to the retinal gradients in compound or pie-slice eyes?

The hypothesis of competition for tectal space to account for systems matching without invoking respecification was first investigated computationally by Prestige and Willshaw[59]. For retinal and tectal gradients of 'labels' (receptors and ligands) that run in the same direction, they suggested that all retinal axons seek to connect to tectal regions with the highest level of tectal label. Competition for tectal space ensures that only those retinal axons with the highest available level of retinal label can connect to any particular region. A map forms because the 'best' retinal axons occupy the 'best' regions of tectum, and so on. Such a model can also be

applied when axons compete for low levels of ligand. In the more-abstract multiple-constraint model of Fraser[32,63,64], systems matching arises as the optimal balance is reached between the opposing forces of competition for tectal space and matching by stable gradients. A similar result, based on more biologically motivated rules, occurs in the model of Cowan and colleagues[65,67–69]. However, a simple rule that 'retinal axons expand to fill the available space' does not explain, for example, why temporal axons do not merely expand over all lanes in the membrane-stripe assay: clearly there is a delicate balance between expansion and specificity.

The idea of competition for tectal space was recently rekindled by Feldheim *et al*.[15] to explain their findings in the retinogeniculate projection in the ephrin-A5-knockout mouse. In this mutant, at least initially, there is a greatly expanded territory of arborization for nasal retinal axons in 'caudal' lateral geniculate nucleus (LGN) and a slight expansion of temporal retinal axons in 'rostral' LGN. This is surprising given that nasal axons show no response to ephrin-A5 in the membrane-stripe assay: one would expect the temporal axons to be affected more strongly by the absence of ephrin-A5. Given the often seen result from the previous literature, that the initial map can be different from the final map, it would be interesting to examine the longer-term development of maps in these knockout animals. In addition, it would be useful to try to tease apart the different roles of ephrin-A2 and ephrin-A5 by including the known properties of these gradients more directly in computational models with multiple constraints, such as those of Cowan and Fraser. Simulated gene manipulations could then be compared with reality, and predictions could be made about future biological experiments. Another recent finding that has not yet been included in computational models is that retinal axons seem to grow to a specific concentration of ligand relative to a starting point concentration[74], rather than responding to an absolute concentration of ligand or a particular steepness of ligand gradient. It is possible that nasal retinal axons require an encounter with a specific ligand concentration in rostral tectum to be able to respond to higher ligand concentrations in caudal tectum, and this is why there is no apparent effect of caudal tectum on nasal retinal axons *in vitro*. Theories for how growth cones actually detect concentration differences across their spatial extent caused by ligand gradients[75] have so far not been included in models of retinotectal map formation.

It is now possible to enquire directly about the molecular substrate of the tectal translocation results. Are levels of ephrin ligand preserved in tectal grafts that are rotated or moved to ectopic locations? What about the case where the graft is ignored and a normal map forms: is the gradient still abnormal in this case and do other mechanisms dominate, or does the gradient itself become respecified in the graft? In the aforementioned models of Fraser and Cowan, the gradient remains the same but fiber–fiber interactions can override this in appropriate cases.

Although we have not yet focused on species differences in the development of topographic maps in the retinotectal system (retinocollicular system in mammals), a significant difference exists in the formation of these projections in frogs and fish[76–81], and chicks and rodents[82–85]. In frogs and fish, retinal axons grow to their final termination zones directly (although some remodeling occurs in *Xenopus*[86]), a developmental phenomenon that lends itself more easily to the chemoaffinity mechanism and the 'law of mass action'. In these species, retinal axons might use molecular gradients in the tectum to determine where to stop growing. In chicks and rodents, however, retinal axons grow past their final termination zone initially, form axon collaterals along the whole axon shaft (but preferentially in their topographically correct region) and then select their specific topographic termination zone by stabilizing an axon collateral while the distal part of the axon is pruned back[84,85]. Therefore, three distinct steps occur during the development of the retinotectal (retinocollicular) projection in chicks and rodents: (1) retinal-axon invasion of the tectum and growth to the most caudal region; (2) the formation of axon collaterals; and (3) the stabilization and arborization of topographically correct collaterals and the elimination of the distal part of the axon. The Eph and ephrins have been implicated in regulating multiple steps in the development of topographic targeting in chicks and rodents. In particular in the ephrin-A5-knockout mouse, retinal axons overshoot the superior colliculus and invade the inferior colliculus[17], thus implicating this molecule in regulating axon 'stopping', which is similar to the mechanism suggested for how molecules might regulate topographic targeting in frogs and fish. As axons grow to the most-caudal part of the tectum, this mechanism was not initially as obvious in chicks and rodents prior to this experiment. Membrane-associated molecules have also been implicated in regulating axon collateral (branch) formation. In a modification of the membrane-stripe assay, Roskies and O'Leary[87] showed that a GPI-linked molecule in rostral tectum could preferentially induce branch formation of temporal retinal axons in chicks; this molecule might be a member of the ephrin-A family[12,88]. Castellani *et al*.[89] have shown that ephrin ligands can regulate layer-specific branching in the cortex. In addition, the ephrin-A5-knockout mouse displays an increase in ectopic arbors of temporal axons in the tectum as well as maintaining topographically correct temporal axon arbors[17]. In order to explain these results, it was proposed that ephrin-A5 normally inhibited the stabilization of axon collaterals in topographically incorrect regions; in the case of temporal axons, in the caudal colliculus[17]. However, as previously described, this does not account for the preferential formation of axon collaterals in topographically appropriate regions during normal development. An additional chemoattractive gradient has been hypothesized to account for this[12]. The computational models referred to above have not so far considered the role of topographic branching in map formation; including this would allow further insight into the differences in mapping mechanisms between species.

Concluding remarks

In this article, we have drawn together data from a wide variety of model systems and experimental paradigms. It is not yet clear to what extent one can generalize across the mechanisms and molecules at work in different cases: in development versus regeneration; in the retinotectal projection in animals such as frogs and fish, where connections continually shift throughout life; in the retinotectal projection in chicks; and in the retinocollicular and retinogeniculate projections in mice, which develop somewhat differently. There are

Box 2. Some outstanding questions

- Does the matching of Eph and ephrin gradients constitute a general mechanism for topographic map formation in the developing nervous system, or is it specific to only a few systems?
- How do Eph and ephrin gradients evolve during normal development and how are the gradients regulated?
- Why are Eph and ephrin gradients different shapes in different species?
- Why do ephrins sometimes cause axon stopping and sometimes axon branching?
- Are Eph and ephrin gradients modified in response to surgical manipulations? For example, do the gradients become twice as steep in a hemi-retina or hemi-tectum? What happens to the retinal gradients in compound or pie-slice eyes? Are levels of ephrin ligand preserved in tectal grafts that are rotated or moved to ectopic locations?
- Are gradients of molecules other than the Eph or ephrins also involved in retinotectal map formation?
- Why do the results of the membrane-stripe assay sometimes not match *in vivo* results?
- Can the fiber–fiber interactions required by many computational models be interpreted in terms of interactions between Eph receptors and ephrin ligands?
- What is the effect of including known details of Eph and ephrin gradients in existing computational models: do they still produce sensible results?

still several outstanding questions (Box 2), and, at the very least, the appropriate homologs of the Eph and ephrins will need to be identified in *Xenopus* for some of the direct experimental comparisons suggested above to be performed. However, the conclusion remains that the long and rich history of the retinotectal system, both experimental and theoretical, might be able to provide novel insight into the newer molecular findings, and vice versa.

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