Molecular Gradients and Development of Retinotopic Maps

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Annu. Rev. Neurosci.
2005. 28:327–55

doi: 10.1146/
annurev.neuro.28.061604.135714

0147-006X/05/0721-
0327$20.00

Key Words

axon branching, axon guidance, bidirectional signaling, Ephs, ephrins, visual system development

Abstract

Gradients of axon guidance molecules have long been postulated t control the development of the organization of neural connectior into topographic maps. We review progress in identifying molecul required for mapping and the mechanisms by which they act, fc cusing on the visual system, the predominant model for map dc velopment. The Eph family of receptor tyrosine kinases and the ligands, the ephrins, remain the only molecules that meet all cr teria for graded topographic guidance molecules, although othe fulfill some criteria. Recent reports further define their modes c action and new roles for them, including EphB/ephrin-B contr of dorsal-ventral mapping, bidirectional signaling of EphAs/ephrir As, bifunctional action of ephrins as attractants or repellents in context-dependent manner, and complex interactions between mu tiple guidance molecules. In addition, spontaneous patterned neur activity has recently been shown to be required for map refineme during a brief critical period. We speculate on additional activiti required for map development and suggest a synthesis of molecul and cellular mechanisms within the context of the complexities o map development.

Contents

INTRODUCTION

A critical function of the nervous system is to interpret the environment through the connections of various sensory organs to the brain. To accomplish this task, incoming information must be organized in an efficient manner. Perhaps the most efficient organization is achieved through the use of topographic maps, which are present throughout the brain, to process sensory information (Kaas 1997). In general a topographic map is a projection from one set of neurons to another wherein the receiving set of cells reflects the neighbor relationships of the projecting set. In the nervous system of higher vertebrates topographic maps are common and include sensory maps of the body, tonotopic maps for auditory stimuli, and maps of the visual field. Furthermore, topographic maps persist in some form throughout the circuitry from first-order to higher-order connections.

Map development has been studied in several vertebrate projection systems, including thalamocortical (Dufour et al. 2003, Vanderhaeghen & Polleux 2004), hippocamposeptal (Gao et al. 1996, Yue et al. 2002), olfactory/vomeronasal (Sidebar 1), motor axons to muscles (Feng et al. 2000, Nguyen et al. 2002), and retina to its targets in the brain (see below). However, this latter system, the primary visual projection formed by the axons of retinal ganglion cells (RGCs) to their most prominent midbrain target—the optic tectum (OT) of fish, amphibians, and chick, or the superior colliculus (SC) of mammals—has been far and away the predominant model for studying the development of topographic maps and the gradients of guidance molecules that control their formation. Therefore, we focus on the visual system and primarily on the mechanisms of mapping in the target, with the goal of providing a detailed account of the development of a vertebrate neural map and the molecular mechanisms that control it, though we recognize the importance of growth cone guidance to the target and the intricacies of multiple interacting signaling pathways

(see recent reviews Huber et al. 2003, van Horck et al. 2004). We devote most attention to mammals and chickens in which axonal mechanisms of map development require unique actions of topographic guidance molecules in a specific temporal sequence, but we do provide examples of mechanisms and molecules involved in developing maps in lower vertebrates such as frogs and fish.

The representation of the retina onto the OT or SC can be simplified to the mapping of two sets of orthogonally oriented axes: the temporal-nasal (TN) axis of the retina along the anterior-posterior (AP) axis of the OT/SC, and the dorsal-ventral (DV) axis of the retina along the lateral-medial (LM) axis of the OT/SC (corresponding to the ventral-dorsal OT axis in nonmammalian vertebrates). Criteria for a topographic guidance molecule in the retinotectal projection are that it is expressed in a graded or restricted manner in the retina or OT/SC, that RGC axons from different parts of the retina exhibit distinct responses to it, and that it affects RGC mapping in vivo; an additional criterion to provide a stricter definition states that the molecule is required for the development of a proper topographic map, although some molecules can be involved in map development but their role is masked by functional redundancy.

Being well into the molecular era with entire genomes becoming available, one might presume that most major players in topographic guidance are known, although their precise roles and interactions are not. Surprisingly, though, to date, the Eph/ephrin families of receptors and ligands are the only molecules described to meet all criteria for topographic guidance molecules, not only in the retinotectal system but also in other systems in which topographic map development has been studied. A small number of additional molecules, for example, RGM (repulsive guidance molecule) and semaphorins, meet a subset of the criteria.

The diversity and complexity of the expression patterns of Ephs and ephrins, spatially and temporally, and features of their function provide for a vast array of signaling possibilities and therefore guidance activities. The regulation of mRNA and protein localization and the intracellular integration of guidance cues, as well as the influence of the growth substrate, have become major issues (Bassell & Kelic 2004, Huber et al. 2003, van Horck et al. 2004). Many guidance molecules, including Ephs and ephrins, have multiple, often opposite, activities, and the choice of which activity dominates in a given context is critical to the development of topographic maps. Signaling pathways connecting guidance receptors to the cytoskeleton and cell-attachment molecules such as integrins are being defined and provide explanations for the observed functional activities (Davy & Robbins 2000, Gallo & Letourneau 2004, Nakamoto et al. 2004).

THE ACCESSORY OLFACTORY SYSTEM

The vomeronasal organ (VNO), located in the ventral nasal cavity, projects to the accessory olfactory bulb (AOB), found posterior-dorsal to the main olfactory bulb using zonal topography. Neurons in apical VNO project to anterior AOB, and neurons in basal VNO project to posterior AOB (**Figure 5**). VNO neurons expressing a given pheromone receptor form multiple glomeruli within each AOB half (Belluscio et al. 1999, Rodriguez et al. 1999). The distributions and known activities of several families of guidance molecules are described in **Figure 5**. As in the visual system, distinct combinations of attractive and repellent molecular activities guide VNO axons to their topographically appropriate zones (Knoll & Drescher 2002). The possibility that neuropilin-2 shapes the gradient of secreted semaphorins is an important concept to be considered in all systems. Furthermore, the apparent use of the GPI-linked ephrin-As as guidance receptors (or part of a receptor complex) has important consequences for models of topographic mapping. The accessory olfactory system, owing to its expression of several molecular guidance families in relatively simple patterns and its zonal topography, is an excellent model system to examine the combinatorial interactions between, and within, families of guidance molecules.

Recent system-level progress in the development of retinotopic maps includes the first direct evidence of guidance molecules involved in mapping the DV retinal axis along the LM axis of the OT/SC. In addition, more precise roles for the molecules involved in mapping the TN axis of the retina along the AP axis of the OT/SC have been identified. Also, the refinement of the retinotopic map has been examined through computational models and genetics, lending support to a combinatorial hypothesis involving molecular activities and correlated spontaneous waves of retinal activity.

Toward Discovering Graded Topographic Guidance Molecules

The mechanisms that control the establishment of topographic maps have been intensively studied for many decades, but only in recent years has the molecular control of this process begun to be defined. The chemoaffinity hypothesis, formally proposed by Roger Sperry nearly a half century ago, presaged the dawning of the era of molecular mechanisms of map development (1963). Sperry proposed that molecular tags on projecting axons and their target cells determine the specificity of axonal connections within a neural map. Further, he suggested that these molecular tags might be distributed in complementary gradients that mark corresponding points in both sensory and target structures. Although Sperry based this hypothesis on his studies of regeneration of the retinotectal projection in newts and frogs, it gave direction to the burgeoning field of map development. The basic tenet of the chemoaffinity hypothesis has largely been borne out (Benson et al. 2001), but subsequent mathematical models have substantially refined it to add countergradients of attractants (e.g., Gierer 1983) and graded repellents (e.g., Gierer 1987) and to account more accurately for map development, in particular the sequential phases of complex behaviors exhibited by RGC axons during map development in the OT/SC of higher vertebrates (Yates et al. 2004).

On the basis of the chemoaffinity hypothesis, each point in the OT/SC would have a unique molecular address determined by the graded distribution of topographic guidance molecules along the two tectal axes, and similarly each RGC would have a unique profile of receptors for those molecules that would result in a position-dependent, differential response to them by RGC axons. Over the next several decades, the specificity of the projections of RGC axons to tectal cells was investigated further by analyzing axonal projections in normal animals and following experimental manipulations, first in the regenerating retinotectal system of fish and amphibians, and later in the developing retinotectal/retinocollicular projections when new high-resolution axon-tracing techniques became available. This body of evidence supported the basic tenet of the chemoaffinity hypothesis that the establishment of topographic projections involves RGC axons responding to positional information in the OT/SC.

Searches for molecules with features suggestive of roles in mapping have been carried out by many labs using numerous approaches. Several cell-surface molecules, such as TRAP (McLoon 1991) and TOP_{AP} (Savitt et al. 1995), with graded or restricted patterns in the retina and/or OT/SC consistent with a role in mapping were identified prior to the mid-1990s, but functional studies have yet to show such a role (for review see Roskies et al. 1995). The first description of graded molecules that proved to have properties of topographic guidance molecules came only a decade ago with the cloning of two related genes, ephrin-A2 (originally called Eph ligand family-1, or ELF-1) by Flanagan and colleagues (Cheng et al. 1995, Cheng & Flanagan 1994) and ephrin-A5 by Bonhoeffer, Drescher, and colleagues (originally called repulsive axon guidance signal, or RAGS) (Drescher et al. 1995), both of which are ligands of the receptor tyrosine kinase EphA3 (originally named MEK4), expressed in a

graded pattern by RGCs (Cheng et al. 1995). Both ephrin-A2 and ephrin-A5 were thereafter shown to meet functional criteria for graded topographic guidance molecules, including differential responses of RGC axons to them (ephrin-A2: Nakamoto et al. 1996; ephrin-A5: Drescher et al. 1995, Monschau et al. 1997) and their influence on retinotopic mapping in vivo, as well as their requirement for proper map development (ephrin-A2: Nakamoto et al. 1996, Feldheim et al. 2000; ephrin-A5: Frisen et al. 1998).

Prior to the discovery of the ephrins, the most compelling evidence for topographic guidance molecules came from the work of Bonhoeffer's group using in vitro assays, including the membrane stripe and growth cone collapse assays. Using the membrane stripe assay, they showed that chick temporal RGC axons, given a choice between growing on alternating lanes of anterior and posterior tectal membranes, show a strong preference to grow on their topographically appropriate anterior membranes, whereas nasal RGC axons exhibit no preference. One critical finding showed that the growth preference of temporal axons is not due to an attractant or growth-promoting activity associated with anterior tectal membranes but instead to a repellent activity associated with posterior tectal membranes (Walter et al. 1987a,b).

Posterior tectal membranes also preferentially collapse the growth cones of temporal axons, a feature that facilitated biochemically isolating the repellent activity to a 33-kDa, GPI-anchored protein referred to as RGM (Cox et al. 1990, Stahl et al. 1990). RGM is expressed in a graded pattern in the OT similar to ephrin-As, and inactivation of RGM using the CALI (chromophore assisted laser inactivation) technique resulted in a loss of the selective repellent effect of posterior OT membranes on temporal RGC axons (Muller et al. 1996). Chick RGM was recently cloned, and recombinant RGM expressed in 293T cells has a repellent effect on chick RGC axons (Monnier et al. 2002). RGC axons transfected to express neogenin, an RGM receptor, are also repelled by RGM (Rajagopalan et al. 2004). Therefore, it was reasonable to assume that RGM has a required role in retinotopic mapping. Suprisingly, though, mice with targeted deletions of RGMa reportedly have no mapping defects in the retinocollicular projection (Niederkofler et al. 2004), possibly because of a functional redundancy, for example with other RGM family members or with ephrin-As.

MECHANISMS OF MAP FORMATION

Determining the process by which RGCs establish topographic connections is critical for defining the roles of graded guidance molecules in map development and for creating conceptual or computational models of the process. Investigators have used predominantly frogs, fish, chicks, and rodents as vertebrate models for development of retinotopic maps. These species exhibit important differences in the development of the visual system and retinotopic maps, as well as substantial differences in the absolute size of the OT/SC; for example, the AP axis of the chick OT is about 50 times greater than that in frog and fish (**Figure 1**). Although each species has unique features that can be exploited, they also have substantial differences in mechanisms employed by RGC axons to target their correct termination zone (TZ) and therefore the actual roles of topographic guidance molecules in controlling the topographic targeting of RGC axons.

Development of retinotectal topography in chicks (Nakamura & O'Leary 1989, Yates et al. 2001) and rodents (Hindges et al. 2002; Simon & O'Leary 1992a,b,c) is a multistep process that involves axon overshoot and interstitial branching. Detailed quantitative analyses have indicated that this is the exclusive mechanism for map development and have begun to define the relative importance of directed axon extension and branching and the roles of guidance molecules in controlling them (Hindges et al. 2002, McLaughlin et al. 2003b, Yates et al. 2001). Initially, the primary growth cones of RGC axons enter the OT/SC and extend posteriorly past the location of their future TZ (Simon & O'Leary 1992a,c; Yates et al. 2001) (**Figure 1**). RGC axons from a given DV location have a broad distribution along the LM tectal axis, with a peak centered on the location of the future TZ, mirroring their coarse ordering within the optic tract (Simon & O'Leary 1991, 1992b; Yates et al. 2001). In rodents, these two features result in RGC axons originating from a focal source in the retina covering virtually the entire SC at perinatal ages and covering a sizeable fraction of the chick OT at E10 to E13.

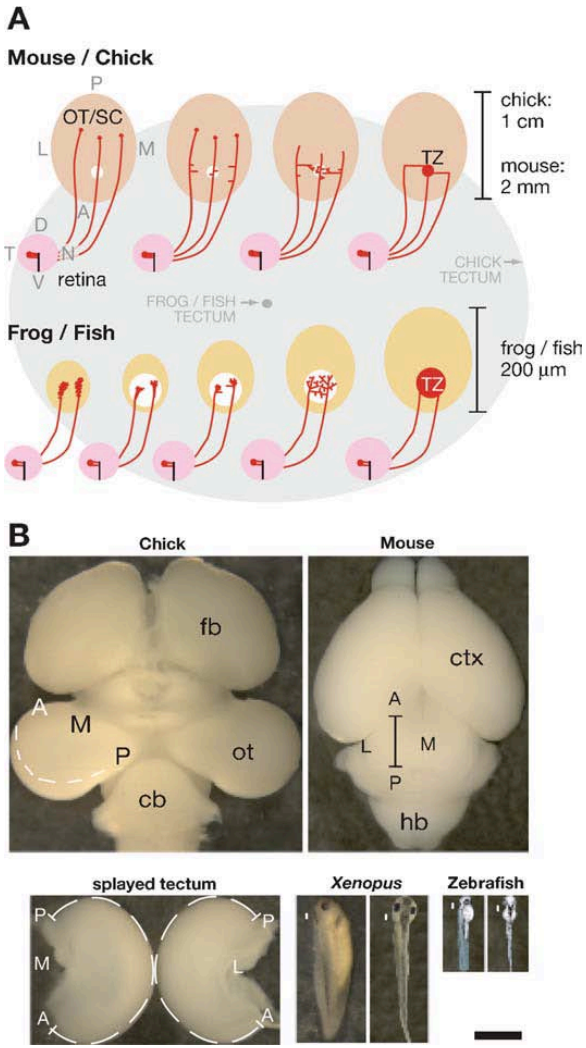


Figure 1

Development of the retinotopic projection and relative scale of the tectum in primary model species. (*A*, *Top*) In mouse and chick, RGC axons enter the OT/SC and initially extend well posterior to the location of their future termination zone (TZ) (*circle*). Interstitial branches form along the axon shaft in a distribution biased for the AP location of the TZ and subsequently exhibit bidirectional growth along the LM axis toward their correct TZ. Upon reaching their TZ, branches elaborate complex arbors and the initial axon overshoot is eliminated. All arbors are formed by interstitial branches. (*A*, *Bottom*) In frog (*Xenopus laevis*) and zebrafish, the tectum and retina expand throughout the development of the retinal projection. During retinotopic map development the tectum is much smaller in relation to a typical growth cone in frog and fish than in chick and mouse. RGC axons extend into the tectum and elaborate many small branches from the base of the growth cone. Arbors elaborate from these backbranches and the thinned growth cone. The TZ becomes dense and refines as the tectum enlarges. (*A*, *Background*) The two ovals in the background represent the relative sizes of the chick tectum (*large oval*) and frog or zebrafish tectum (*small oval*). (*B*) The photographs are at the same scale. The chick OT rotates during development such that the posterior pole (P) is near the midline. The OT is cut along the AP axis at the LM midline (*dashed line*) and splayed. The distance from the anterior to posterior pole along the cut edge is 1 cm (*dashed lines in the splayed tectum*). The mouse SC is about 2 mm along the AP pole at the LM midline (*bar*). For frog and zebrafish the entire animal is shown in lateral and dorsal views. The white bar on the left of each panel represents the approximate AP position and size of the tectum. The tecta for these organisms are approximately 200 μm along the AP axis. cb, cerebellum; ctx, cortex; fb, forebrain; hb, hindbrain. Scale bar = 2 mm. Adapted from McLaughlin et al. 2003a.

A period of interstitial branch formation begins and is the first indication of appropriate topography. Branches form de novo from the axon shaft hundreds of microns or even millimeters behind the growth cone. Interstitial branching exhibits a significant degree of topographic specificity along the AP axis; the highest percentage of branches are found at the AP location of the future TZ (Yates et al. 2001). Interstitial branches form roughly perpendicular to the primary axon and preferentially extend along the LM axis toward their future TZ (Hindges et al. 2002, McLaughlin et al. 2003b, Nakamura et al. 1989). The branches arborize at the appropriate LM and AP location of their TZ and are the exclusive means by which RGCs form permanent, topographically ordered synaptic connections (Yates et al. 2001). Although RGC axons originating from the same site along the DV retinal axis are broadly distributed across the LM extent of the OT/SC, with most being well outside the LM position of their appropriate TZ, their distribution does not change even though their number declines as the map undergoes considerable refinement coincident with the death of a substantial proportion of RGCs (Hindges et al. 2002, Simon & O'Leary 1992b). Therefore, the position of an RGC axon along the LM axis relative to its TZ does not bias its ability to make a connection to the TZ and to be maintained.

In frogs and fish, initial DV mapping along the LM axis is much more accurate than in chicks and rodents. In addition, RGC axons extend along the AP axis directly to the correct location of their TZ (**Figure 1**). As the growth cone of the primary RGC axon reaches the location of its future TZ, it stops and exhibits a phenomenon termed backbranching. Backbranching is characterized by the formation of short terminal branches at or near the base of the growth cone, which itself often acquires a branch-like morphology, and together they locally elaborate a terminal arborization of the distal part of the primary axon (Harris et al. 1987, Kaethner & Stuermer 1992, O'Rourke et al. 1994). Thus, backbranching, as originally defined in frogs and fish, is a phenomenon distinct in scale, location, and purpose from interstitial branching in chicks and rodents.

The size of an individual arbor in frog and fish is much larger in relation to the OT than in chick and mouse, in part owing to the fact that RGC axons reach the OT and arborize at relatively early stages of tectal neurogenesis when the OT is very small. Therefore, though RGC axons are not described to overshoot their TZ in frogs or fish, early on their RGC axonal arbors are disproportionately large compared with the OT, particularly along its AP axis, and cover a greater percentage of its surface area than at later stages. In frogs and fish, arbors cover progressively less of the AP axis over the period of map development because the OT expands substantially more than the arbors, and some arbor refinement occurs (Debski & Cline 2002). In contrast, the surface area of the OT/SC of chick and rodents expands relatively little over the period of map development.

Axon Extension and Target Overshoot During AP Mapping

RGC axons enter their midbrain target at its anterior edge and extend posteriorly parallel to the AP axis of the OT/SC. In amphibians and fish the primary RGC axonal growth cone halts its posterior extension at, or just past, its TZ (Harris et al. 1987). However, in higher vertebrates, essentially all RGC axons extend well posterior to the topographically appropriate location of their future TZ (Nakamura & O'Leary 1989; Simon & O'Leary 1992a,c; Yates et al. 2001). Thus, the growth cones of RGC axons in birds and mammals do not target their future TZ but instead extend a millimeter or more posterior to it.

The extent of the posterior overshoot in higher vertebrates and targeted posterior extension in lower vertebrates is controlled, in large part, by repellent EphA/ephrin-A interactions (**Figure 2**) (Ciossek et al. 1998; Drescher et al. 1995; Feldheim et al. 2000,

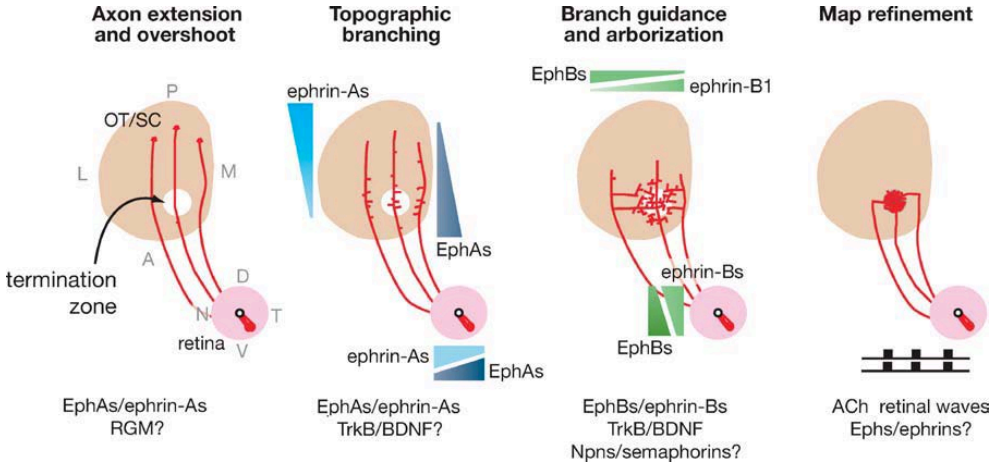


Figure 2

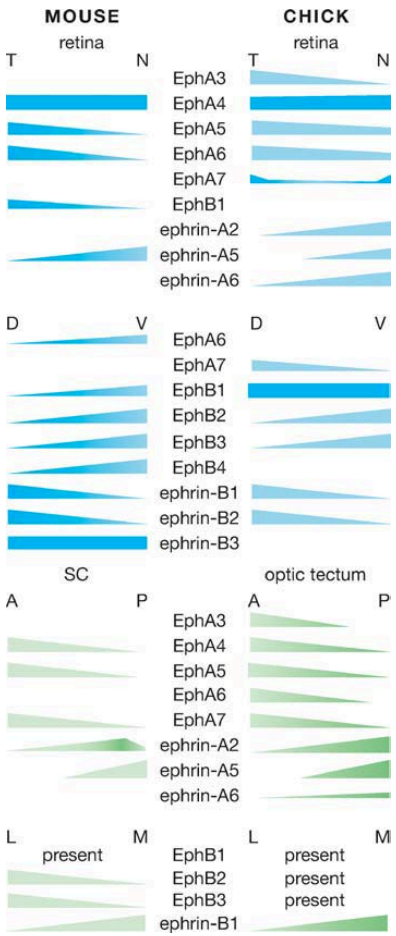
Mechanisms and molecules controlling retinotopic mapping in chicks and rodents. The names and/or distributions of molecules known, or potentially able, to control the dominant mechanisms at each stage are listed. The gradients represent the consensus distribution for a combination of related molecules (i.e., ephrin-A's), which are not listed individually owing to distinctions in the individual members expressed and the precise distributions between species. Molecules other than those listed are likely to participate.

2004; Nakamoto et al. 1996). EphAs are expressed in an overall low-to-high NT gradient in RGCs, and ephrin-As are expressed in an overall low-to-high AP gradient in the OT/SC, though the individual receptors and ligands and their exact distributions vary between species (**Figure 3**). Thus, in lower vertebrates, which lack a posterior overshoot of the TZ, this single repellent gradient can guide RGC axons to the appropriate location of their TZ (**Figure 4*A***). Furthermore, in species with an extended posterior overshoot, the shape of the ephrin-A gradient in the OT/SC predicts the extent of the overshoot. For example, in chick, temporal RGC axons extend a greater distance past their future TZ than do nasal RGC axons, a feature predicted by the relatively shallow slope of ephrin-As in anterior and central OT and the steep slope of ephrin-As in posterior OT (Yates et al. 2001). These correlations are consistent with in vitro (Rosentreter et al. 1998) and in vivo (Brown et al. 2000) data indicating that the incremental change in ligand concentration is a critical factor and that absolute concentration (at least, apparently, in physiological ranges) is not.

Considerable evidence indicates that ephrin-As are repellents acting through EphAs to control the advance of the primary axon's growth cone, dependent on the amount of EphA present on the growth cone and the shape of the ephrin-A gradient it encounters. Reducing signaling through EphAs expressed by RGCs results in a decrease in the repellent response of temporal RGC axons to ephrin-As in vitro (Ciossek et al. 1998; Feldheim et al. 2000, 2004; Frisen et al. 1998) and an increase in the extent of the posterior overshoot in vivo (Frisen et al. 1998, Sakurai et al. 2002). Blocking EphA/ephrin-A interactions in vitro results in a decreased repellent response (Ciossek et al. 1998; Feldheim et al. 2000, 2004). Complementing these findings, increasing signaling through EphAs by overexpression (Brown et al. 2000), or ectopic expression of EphAs (Feldheim et al. 2004) or ephrin-As

(Nakamoto et al. 1996), results in an increase in the repellent response of EphA expressing axons to ephrin-As in vitro (Feldheim et al. 2004, Nakamoto et al. 1996) and a decreased extension of RGC axons along the AP axis in vivo (Brown et al. 2000, Nakamoto et al. 1996).

In zebrafish and frog, the growth cones of RGC axons directly target their appropriate AP location in the OT. The in vitro action of two ephrin-A homologs in zebrafish and their expression patterns, one in a low-to-high AP gradient and another in a dense band posterior to the OT, suggest that ephrin-As act as a molecular barrier to prevent RGC axons from exiting the posterior end of the OT (Brennan et al. 1997). Furthermore, the in vitro action and in vivo distribution of sema3A in posterior OT and neuropilin-1 in RGCs in frog suggest that it may be involved in controlling the posterior extension (and terminal branching, described below) of RGC axons (Campbell et al. 2001). In mice ephrin-As also act as a molecular barrier to prevent RGC axons from extending posteriorly from the SC into the inferior colliculus (Feldheim et al. 2004, Frisen et al. 1998).



TN Retinotopic Mapping Achieved Through AP-Specific Interstitial Branching

In frogs and fish RGC axon extension along the AP axis is determined in part by EphAs and ephrin-As; RGC axonal growth cones stop at or very near the appropriate topographic location and undergo terminal arborization, in part via backbranching (Harris et al. 1987, Kaethner & Stuermer 1992). In vitro studies suggest that backbranching may be causally linked to growth cone collapse or to the halting of axonal extension: For example, a neuropilin-1-mediated collapse of the growth cones of frog RGC axons in response to Sema3A leads to an increase in backbranching around the collapsed growth cone (Campbell et al. 2001). RGC axon arborization via terminal branching is also likely controlled, at least in part, by TrkB/BDNF interactions (Alsina et al. 2001, Cohen-Cory 2002, Cohen-Cory & Fraser 1995).

In mammals and birds, critical events in retinotopic mapping are the topographically selective addition and stabilization of interstitial branches, which subsequently form all RGC axonal arbors after they directionally extend to their topographically correct site (Simon & O'Leary 1992a,b,c; Yates et al. 2001). In these species, growth cones of RGC axons typically grow well over a millimeter posterior to the location of their future TZ before they halt their extension. The majority of branches extending from the axon shaft do so at or near the AP location of the nascent TZ, with a paucity of branches anterior and posterior to it (Simon & O'Leary 1992a,b,c; Yates et al. 2001). At least two distinct activities

Figure 3

Expression of Ephs and ephrins in the retinocollicular/retinotectal systems of mouse and chick. The table represents our view of the consensus expression patterns for individual Ephs and ephrins in the retina (and likely RGCs) and OT/SC (in positions likely to affect mapping) during the primary molecular-dependent events in topographic map formation (about E15-P7 in mouse and E6-E14 in chick). The list may not be complete, and exclusion from this chart does not necessarily signify absence of expression in vivo. The sizes and shapes of the gradients are generalized, and relative expression levels should not be inferred (for an example of relative expression levels of Eph receptors see Reber et al. 2004). We have included only Eph family members with published expression domains determined by in situ hybridization with antisense riboprobes or specific antibodies in preparations where the listed pattern is evident at an appropriate age. A, anterior; D, dorsal; L, lateral; M, medial; N, nasal; P, posterior; present, receptor is expressed but the pattern is unclear; T, temporal; V, ventral.

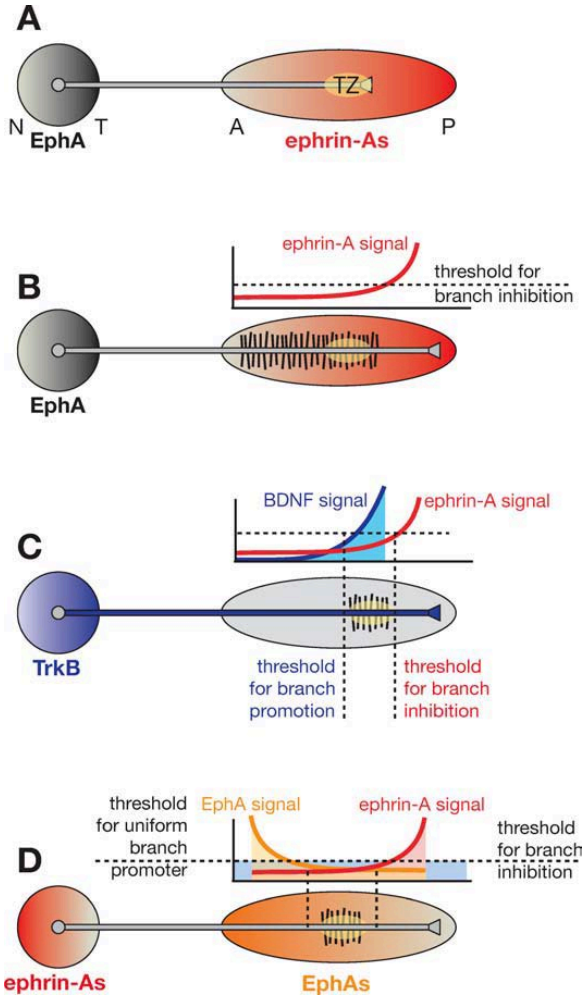


Figure 4

Requirement for two molecular activities to control branch distribution along the AP axis. (*A*) A gradient of repulsive guidance molecules, such as ephrin-As, is in principle sufficient to guide RGC axonal growth cones topographically to their appropriate TZ. This is the mapping mechanism in lower vertebrates. (*B*) However, a single repulsive gradient cannot result in the topographic branching observed in chicks and rodents. If the inhibitory signal allows for branching at the TZ, it must also allow for branching anterior to the TZ at the same, or higher, levels. This is not observed in vivo. (*C*) Gradients of molecules with branch-promoting activities could act in concert with inhibitory activities to result in branching restricted to the topographically appropriate domain. TrkB, in a similar distribution to EphAs in the retina (and/or if TrkB were graded along each RGC axon), and brain-derived neurotrophic factor (BDNF), in the OT/SC, have the appropriate activities to act with EphAs/ephrin-As in mapping. (*D*) Gradients of branch-inhibiting molecules in gradients opposite the EphA/ephrin-A gradients depicted in (*A*) could also result in topographic branching. Dual inhibitory gradients would require either a branching mechanism intrinsic to RGC axons or a branch-promoting molecule (*blue shading*) distributed to allow branching at the low point of the combined inhibitory gradients. The distributions of ephrin-As in the retina and EphAs in the OT/SC fulfill these requirements.

are required to center the distribution of branches around the future TZ: one activity to limit branching posterior to the TZ and one activity to limit branching anterior to the TZ (O'Leary et al. 1999; Yates et al. 2001, 2004). A single graded activity, whether negative or positive, cannot do both, though a single molecule with two activities could, in theory.

In addition to controlling the posterior overshoot, in vitro branching assays show that ephrin-As also inhibit branch formation along RGC axons (Roskies & O'Leary 1994, Yates et al. 2001). This conclusion is supported by in vivo studies showing enhanced RGC axon branching in the OT coincident with a local inactivation of ephrin-As using CALI (Sakurai et al. 2002). Consistent with these data, temporal axons form ectopic TZs in aberrantly posterior locations in ephrin-A-deficient mice (Feldheim et al. 2000, Frisen et al. 1998). These findings indicate that the low-to-high AP gradient of ephrin-As in the OT/SC exposes RGC axons posterior to their correct TZ to levels of ephrin-As that inhibits their branching and thereby helps generate the topographic bias in branching along the AP axis of the OT/SC observed in vivo (**Figure 2**) (Simon & O'Leary 1992a, Yates et al. 2001). This mechanism of branch inhibition is a primary role for ephrin-As in retinotopic map development (Yates et al. 2001).

Mechanisms for AP Branch Specificity

Though an activity that limits branching along the portion of RGC axons anterior to their TZ remains to be identified experimentally, one must exist, and investigators have suggested some candidates (O'Leary et al. 1999; Yates et al. 2001, 2004). The "activity" could be an intrinsic bias for RGC axons to branch distally due, for example, to a proximal-to-distal gradient in the maturity of the axonal cytoskeleton and the polymerization dynamics of actin and tubulin into neurofilaments and microtubules, possibly coupled with a trigger, for example, collapse of the growth cone of the primary axon in response to ephrin-As as suggested by in vitro work (Davenport et al. 1999). Alternatively, the activity may be due to activation of branch-promoting and/or branch-inhibiting signaling pathways by exogenous ligands binding to their receptors along the axon shaft. Straightforward examples would include a low-to-high AP gradient of a signal that promotes branching along each RGC axon or a high-to-low proximal AP gradient of a signal that inhibits branching along each RGC axon (**Figure 4**). These alternative models are not mutually exclusive and could cooperate to develop AP-specific branching.

Parallel AP Gradients of Promoters and Inhibitors of Branching

In this model, the graded branch-promoting activity is at a level sufficient to overcome the graded ephrin-A branch inhibitory activity and thereby to promote branching only near the TZ; posterior to the TZ its level is insufficient to overcome the ephrin-A branch-inhibitory activity, and anterior to the TZ its level is below a threshold required to promote branching (**Figure 4**) (O'Leary et al. 1999, Yates et al. 2001). In chick, BDNF/TrkB signaling is a good candidate for this activity on the basis of expression patterns and the finding that focal application of BDNF to the shaft of RGC axons selectively induces, via activation of TrkB receptors, the formation of primary branches in vitro (Choi et al. 1998, Choi & O'Leary 2000). Other potential candidates include the ephrin-As, -A2 and -A5, if they acted bifunctionally as branch promoters in addition to their demonstrated role as branch inhibitors (Yates et al. 2001).

Recent findings are consistent with this proposed bifunctional action for ephrin-As. Several guidance molecules, including semaphorins, netrin, and ephrin-B1 (see below), can have both attractive and repulsive functions, depending on developmental context, substrate, and intracellular concentrations of second messengers. In vitro, soluble ephrin-A5 can act as either an attractant or a repellent for frog RGC axons, dependent on the substrate (Weinl et al. 2003), and in vivo ephrin-A5 can have positive or inhibitory effects on distinct subsets of EphA4-expressing motor neurons (Eberhart et al. 2004). More directly relevant is a recent in vitro study concluding that ephrin-A2 can have an adhesive, attractive, or growth-promoting effect on RGC axons at concentrations below those that result in its previously defined repellent effect (Hansen et al. 2004).

Opposing AP Gradients of Branch Inhibitors

An alternative to parallel gradients of branch promoters and inhibitors is a set of opposing gradients along the AP axis, each of which inhibits branching (Yates et al. 2001, 2004). In this scenario, one gradient is the low-to-high AP gradient of ephrin-A2 and -A5, which inhibits branching along RGC axons posterior to their TZ; opposing it is a high-to-low AP signaling gradient that inhibits branching along RGC axons anterior to their TZ (**Figure 4**).

Several ephrin-As and EphAs are expressed by RGCs and the OT/SC in opposing expression gradients that complement the high-to-low graded TN expression of EphAs by RGCs and low-to-high graded AP expression of ephrin-As in the OT/SC (Brennan et al. 1997, Connor et al. 1998, Hornberger et al. 1999, Marcus et al. 1996); in addition to EphAs, ephrin-As are present along RGC axons and exhibit a low-to-high graded TN distribution (Hornberger et al. 1999). The opposing gradients of ephrin-As in the retina sharpen the graded distribution of functional EphA receptors (Hornberger et al. 1999). Others investigators have proposed that the opposing gradients in the retina and OT/SC act as opposing gradients of branch inhibition through EphA-ephrin-A reverse signaling (Yates et al. 2001, 2004). Some ephrin-As and EphAs are expressed in ways that suggest that they act in map development predominantly via reverse signaling. For example, in chick, ephrin-A6 is expressed in a high-to-low NT gradient by RGCs but is sparsely expressed in the OT (Menzel et al. 2001).

EphB-ephrin-B binding is well established to initiate both forward and reverse signaling (Bruckner et al. 1997, Henkemeyer et al. 1996, Holland et al. 1996). Reverse signaling into ephrin-A-expressing cells upon binding EphAs has been implicated in topographic mapping in the accessory olfactory system, although in this system, axonal ephrin-As act as attractant receptors for EphAs in the target (Davy et al. 1999, Knoll et al. 2001) (See sidebar on the Accessory Olfactory System and **Figure 5**). Computational modeling of retinotopic mapping shows that opposing gradients of EphAs and ephrin-As can act as branch inhibitors through bidirectional signaling and generate the major phases of map development in chick and mouse, including progressive increases in the topographic specificity of AP branching exhibited by RGC axons originating from all TN positions, and can recapitulate the phenotypes reported for ephrin-A knockout and EphA knock-in (KI) mice (Yates et al. 2004).

Lateral-Medial Mapping Accomplished by Directed Growth of Interstitial Branches

In zebrafish and frog, the growth cones of RGC axons target directly to the appropriate LM location of their future TZ. However, in rodents and chicks, RGC axons from the same retinal location enter and grow across the OT/SC with a broad distribution over its LM axis, though biased for the LM location of their future TZ (Hindges et al. 2002; Simon & O'Leary 1991, 1992a,b,c). Topographic specificity along the LM axis emerges through the bidirectional guidance of branches that form along RGC axons (Hindges et al. 2002, McLaughlin et al. 2003b, Nakamura & O'Leary 1989) with an AP bias as described above. Branches that extend

from RGC axons located lateral to their future TZ grow medially, branches that extend from RGC axons located medial to their future TZ grow laterally, and branches extending from RGC axons located within the LM extent of the future TZ exhibit no directional bias (Hindges et al. 2002, McLaughlin et al. 2003b, Nakamura & O'Leary 1989). Branches that reach the area of the nascent TZ selectively form complex arbors. In summary, in mammals and chicks, the bidirectional guidance of interstitial branches is the critical feature in retinotopic mapping along the LM axis of the OT/SC, analogous to the importance of topographic specificity in branch formation in AP mapping.

EphBs and Ephrin-Bs Control DV Retinotopic Mapping

Characterization of the molecular control of DV mapping along the LM axis of the OT/SC has lagged behind that of TN mapping along the AP axis of the OT/SC, in part because in vitro assays that reveal strong TN responses to endogenous AP target tissues fail to reveal differential DV responses from RGC axons. Only in the past few years have defined molecules been shown to control DV mapping; the reports are few and demonstrate roles for EphBs and ephrin-Bs but implicate both bidirectional signaling and bifunctional action.

In retina, EphB receptors are expressed by RGCs during map development in an overall low-to-high DV gradient, complemented by an overall high-to-low DV gradient of ephrin-Bs (McLaughlin et al. 2003a). In both chick OT and mouse SC, ephrin-B1 is expressed in a low-to-high LM gradient (Braisted et al. 1997, Hindges et al. 2002), complemented by an overall high-to-low LM EphB gradient (Hindges et al. 2002). Analyses of EphB2 and EphB3 mutant mice, with and without reverse signaling intact, show aberrant LM mapping due to defects in the guidance of interstitial branches; these findings show that ephrin-B1 acts as a branch attractant via EphB2/B3 forward signaling; modeling of these data indicates that bidirectional branch extension requires a branch repellent in a distribution paralleling ephrin-B1 (Hindges et al. 2002). This model is supported by the demonstration that high levels of ephrin-B1 repel interstitial branches in a selective manner (McLaughlin et al. 2003b). Taken together, these studies show that in mice and chicks ephrin-B1 acts through EphB forward signaling as both an attractant and repellent: A branch located lateral to its nascent TZ is attracted up the gradient of ephrin-B1 toward its future TZ, whereas a branch located medial to its nascent TZ is repelled down the ephrin-B1 gradient toward its future TZ (**Figure 6**) (Hindges et al. 2002, McLaughlin et al. 2003b). Importantly, the trajectories of primary RGC axons are not changed in wild-type OT/SC nor in the SC of EphB mutant mice or when encountering domains of ectopic ephrin-B1 expression in chick OT (Hindges et al. 2002, McLaughlin et al. 2003b). Therefore, the response of RGC axons to ephrin-B1 is specific to their interstitial branches (and later their arborizations; McLaughlin et al. 2003b) and is context dependent—the location of the branch on the ephrin-B1 gradient in relation to the location of its future TZ and its EphB level determine its response.

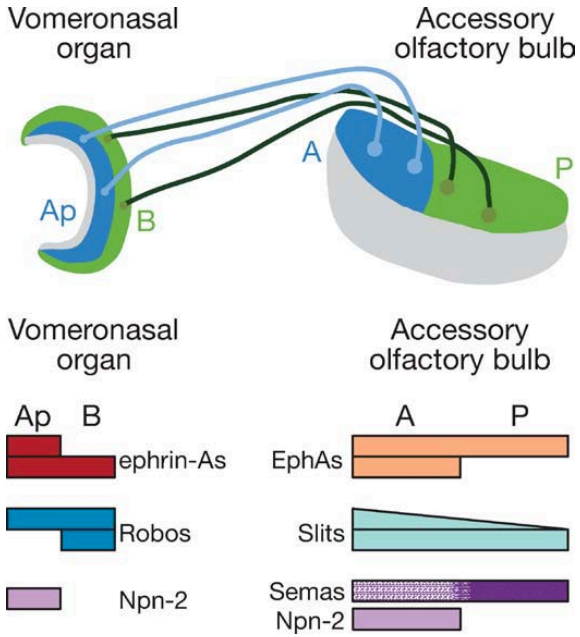


Figure 5

Representation of the accessory olfactory system in cross section. Apical (Ap, *blue*) vomeronasal (VNO) neurons (*light blue*) extend axons into the anterior (A, *blue*) accessory olfactory bulb (AOB) and form glomeruli. Basal (B, *green*) VNO neurons (*dark green*) extend axons into posterior (P, *green*) AOB and form glomeruli. This zonal topography is controlled by the guidance molecules charted below the illustration in representative form. Neuropilin-2 (Npn-2) in the AOB is present primarily in the anterior external plexiform layer of the AOB (*gray*) and may act to sequester secreted semaphorins (Semas), thus converting the uniform expression of secreted semaphorins in the AOB into a functional distribution of semaphorin protein (*purple stippled area*) highest in posterior AOB by reducing the availability of ligand in anterior AOB. Apical VNO neurons are guided to anterior AOB by attractive ephrin-A/EphA interactions and repellent Npn-2/sema interactions, whereas basal VNO neurons are guided to posterior AOB by repellent Robo/Slit interactions (Cloutier et al. 2002, 2004; Knoll et al. 2001, 2003; Walz et al. 2002). Other activities may play a role as well, and the described activities likely act in concert with pheromone receptors and other guidance cues (such as MHC class molecules; Loconto et al. 2003) to control the formation of glomeruli (Belluscio et al. 1999, Rodriguez et al. 1999). The gray area in the VNO represents the lumen.

In frog, ephrin-B reverse signaling has been implicated in retinotopic mapping (Mann et al. 2002). Increasing ephrin-B expression in retina results in defects in RGC axon targeting, consistent with an attractant response for retinally expressed ephrin-Bs being activated on RGC axons by EphBs expressed in the OT (Mann et al. 2003). Expression of a dominant-negative construct in retina to interfere with this interaction results in a response also consistent with an attractant effect of ephrin-Bs acting by reverse signaling (Mann et al. 2002). It remains to be determined if reverse signaling has a role in mapping in mice and chicks and that forward signaling has a role in mapping in frog. In zebrafish, ephrin-B2a expressed in the OT has a repellent effect on RGC axons via forward signaling through EphB receptors (Wagle et al. 2004). In zebrafish, DV retinotopic mapping is also likely controlled, in part, by Sema3D, which is expressed primarily in ventral (lateral) OT and repels ventral RGC axons that map to dorsal (medial) OT (Liu et al. 2004).

Distinctions in Guidance of Primary Axons and Interstitial Branches Require Unique Mechanisms

In frog and zebrafish, EphBs/ephrin-Bs affect the primary axon growth cone in vivo and in vitro (Mann et al. 2002, 2003; Wagle et al. 2004). Conversely, in mice and chicks, EphBs/ephrin-B1 do not influence the trajectories of primary RGC axons in the OT/SC but do affect the guidance of interstitial branches (Hindges et al. 2002, McLaughlin et al. 2003b). A priori, a potential explanation is that RGC axons extend parallel to the ephrin-B1 gradient and thus do not encounter a gradient along their primary direction of extension, whereas their interstitial branches extend perpendicular to the gradient and therefore extend either directly up or directly down the ephrin-B1 gradient. However, primary RGC axons do not respond even to very high levels of graded ephrin-B1 achieved by electroporation of ephrin-B1 retroviral expression vectors (McLaughlin et al. 2003b), whereas they do stop their posterior extension across the OT when they confront an ectopic domain of ephrin-A2 created by retroviral infection (Nakamoto et al. 1996). A potential explanation is that growth cones of primary RGC axons lack sufficient EphB receptors and signaling to respond to ephrin-B1 in the OT/SC. An intriguing possibility is that the transport of EphB mRNA and its subsequent translation, or the export of EphB receptors, may be preferentially targeted to interstitial branches as they form rather than to the primary RGC axon growth cones; such differential mRNA transport, local translation, and protein export to selected parts of the axon have been described for other proteins and RNAs (Brittis at al. 2002, Campbell & Holt 2001).

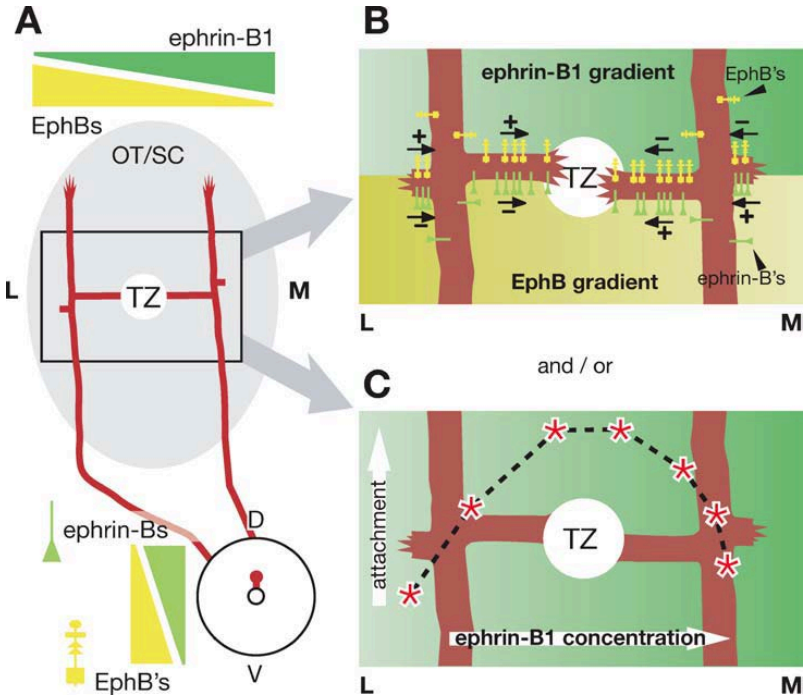


Figure 6

Potential bidirectional and bifunctional interactions resulting in topographic branch guidance along the LM axis of the OT/SC. (*A*) Representation of the projection from two RGCs in the same retinal location. One RGC axon has extended lateral (L) to the TZ and preferentially extends branches medially, toward the TZ. The other RGC axon has extended medial (M) to the TZ and preferentially extends branches laterally, toward the TZ. (*B*) EphBs and ephrin-Bs can cooperate via bifunctional and bidirectional signaling to guide branches appropriately both medially and laterally. The top half of this panel summarizes in vivo data demonstrating branch guidance by EphBs (*yellow icons*) on RGC axons. Lateral to the TZ (*left axon*), EphB receptors encounter an ephrin-B1 level (*green gradient*) lower than that at their TZ (*circle*) and, at that relatively low level of ephrin-B1, branches are attracted up the ephrin-B1 gradient. Branches initially probing down the ephrin-B1 gradient from this axon have no incentive to do so and do not extend. Medial to the TZ (*right axon*), EphB receptors encounter a relatively high level of ephrin-B1 that repels branches down the ephrin-B1 gradient. The bottom half of this panel illustrates potential reverse signaling events. Lateral to the TZ, ephrin-Bs (*green icons*) on RGC axons encounter a higher level of EphBs (*yellow gradient*) than that at the TZ and thus are repulsed down the gradient. Medial to the TZ, ephrin-Bs along RGC axons encounter a relatively low level of EphBs, and branches are attracted up the EphB gradient laterally toward the TZ. (*C*) Branches may also be guided to the TZ by forward signaling if EphBs act as ligand density sensors. Overlaid on the schematic are data from Huynh-do et al. (1999) showing EphB-expressing cell attachment (*y-axis*) on substrates containing different concentrations of ephrin-B1 (*x-axis*). At low concentrations (i.e., lateral to the TZ), attachment is favored at a higher concentration (i.e., medial positions). At high concentrations (i.e., medial to the TZ) increased attachment is favored at a lower concentration (i.e., lateral positions). RGC axonal branches may be guided by a similar principle. The point of maximal attachment for each RGC axon is centered on the TZ and is dependent on its EphB concentration.

Multiple Actions and Models of EphBs and Ephrin-Bs in DV Map Development

EphBs and ephrin-Bs control map development along the LM axis of the OT/SC likely by acting both bifunctionally (i.e., one molecule acting as an attractant and repellent) and bidirectionally (i.e., both EphBs and ephrin-Bs acting as receptors or ligands). Although several guidance molecules have been shown to be bifunctional (van Horck et al. 2004), and EphB/ephrin-Bs have long been known to signal bidirectionally (Bruckner et al. 1997, Henkemeyer et al. 1996, Holland et al. 1996), an individual RGC axon has the unique ability to exhibit a response to all of these signaling possibilities simultaneously (**Figure 6**). For example, two neighboring RGCs may extend axons with multiple EphBs and ephrin-Bs on their membranes and may encounter multiple ephrin-Bs and EphBs in the OT/SC. The responses to these cues, being transmitted by forward signaling through EphBs and reverse signaling through ephrin-Bs, are dependent on the location of each RGC axon in relation to its future TZ. One RGC axon may be located medial to its future TZ and extend branches laterally toward its future TZ through a combination of a repellent response of EphBs binding ephrin-B1 in the OT/SC and an attractant response of ephrin-Bs binding EphBs in the OT/SC (**Figure 6**). Its neighboring RGC, which may have extended lateral to their future TZ, will respond in the exact opposite manner, despite expressing an identical complement of EphBs/ephrin-Bs and responding to identical guidance cues, though at different concentrations, reflecting its different location on the LM axis and therefore on the gradients of EphBs and ephrin-B1.

The bifunctional action of ephrin-B1 through EphBs present along RGC axons may be due to the balance of distinct responses through each receptor type (i.e., EphB2 signaling results in attraction and EphB1 signaling in repulsion) or, alternatively, to a combinatorial thresholding mechanism in which the combined signaling through all EphBs results in either attraction or repulsion, controlled by a transition of EphB signaling between attraction and repulsion to ephrin-B1 that is balanced at the TZ, with lower signaling levels occurring lateral to the TZ and resulting in branch attraction and higher levels occurring medial to the TZ and resulting in branch repulsion (Hindges et al. 2002, McLaughlin et al. 2003b). Trans-endocytosis of EphBs and ephrin-Bs may be responsible for the switch from attraction to repulsion (Marston et al. 2003, Zimmer et al. 2003). At signaling levels above threshold, endocytosis, which initiates repulsion, is favored, whereas at low signaling levels attraction is favored (Marston et al. 2003, Zimmer et al. 2003).

A third model, and in particular an alternative mechanism to a receptor threshold model, is that EphB receptor signaling may act as a ligand-density sensor to control DV retinotopic mapping (**Figure 6**) (McLaughlin et al. 2003b). This model is based on studies showing that EphB1-induced attachment of cell lines to a substrate of extracellular matrix molecules is dependent on the concentration of ephrin-B1 in the substrate (Huynh-Do et al. 1999). Within a critical concentration range, cells attach to their substrate in an integrin-dependent manner at a much higher density; if ephrin-B1 concentration is either above or below this optimal level, cell attachment is decreased. Furthermore, as described above, the point of maximal attachment may depend on the point at which trans-endocytosis of EphB/ephrin-Bs is favored (Marston et al. 2003, Zimmer et al. 2003).

Such a ligand-density sensor model could account for the bidirectional targeted extension of interstitial branches observed in LM mapping. An interstitial branch senses the ephrin-B1 concentration (i.e., ligand density) through EphB receptors and is directed toward its TZ. The amount of EphB receptors expressed by the parent RGC is determined by its DV location and sets the most favorable concentration of ephrin-B1 for its

interstitial branches that by definition is found at the appropriate LM position of the TZ. A branch located either medial or lateral to the TZ would encounter a gradient of increasingly favored attachment centered on its TZ (**Figure 6**). EphB-ephrin-B1 signaling on the branch would control the density of other molecules (e.g., integrins, cell adhesion molecules, etc.) that mediate its attachment to ECM components and cells in the OT/SC as well as cytoskeletal changes required for axon growth to promote branch extension toward the optimal ephrin-B1 concentration found at the future TZ.

Accounting for Species Differences in Development of Topographic Maps

Analyses of topographic map development in frogs (O'Rourke & Fraser 1990), fish (Kaethner & Stuermer 1992, Yoda et al. 2004), chick (Nakamura & O'Leary 1989, Yates et al. 2001), rat (Simon & O'Leary 1992a,b), mouse (Hindges et al. 2002), ferret (Chalupa et al. 1996, Chalupa & Snider 1998), and wallaby (Ding & Marrote 1997) show that the initial topographic targeting of RGC axons differs substantially across species. Differences in the initial DV ordering of RGC axons along the LM axis of the OT/SC are a direct reflection of their ordering in the optic tract as they enter the OT/SC from its anterior margin. The broad LM distribution of axons arising from neighboring RGCs in chicks and mammals relative to the tight, topographically ordered distribution in frog and fish requires an additional mechanism to achieve DV retinotopic mapping, such as the directed growth of interstitial branches. Differences in the initial targeting of RGC axons over the AP axis of the OT/SC are likely due to differences in the slope of gradients of guidance molecules, the length of the axis over which the gradient is distributed, and the sensitivity of RGC axons to these molecules. Clear differences do exist between species in the expression of Ephs and ephrins (**Figure 3**, see sidebar on EPH Family of Receptor Tyrosine Kinases).

EPH FAMILY OF RECEPTOR TYROSINE KINASES

The Eph family of receptor tyrosine kinases (RTKs) is the largest known family of RTKs, composed of 14 Ephs and 8 ephrins in mouse and 15 Ephs and 9 ephrins in chick. Signaling through Ephs and ephrins has been implicated in a wide variety of processes including axon guidance, cell migration, vascular development, synapse development, structure and plasticity, and midline fusion events (Himanen & Nikolov 2003, Murai & Pasquale 2003, Palmer & Klein 2003, Poliakov et al. 2004, Rossant & Hirashima 2003, Surawska et al. 2004). Ephs and ephrins are separated into two subclasses on the basis of homology, the EphA/ephrin-As and EphB/ephrin-Bs, within which receptor-ligand binding and activation are promiscuous. In addition, some cross talk occurs between subclasses and may be functionally relevant in some systems. Ephs and ephrins are membrane bound, Ephs and ephrin-Bs are transmembrane proteins, and ephrin-As are GPI-linked to the plasma membrane, allowing for complex and precise patterns of expression within a single tissue or organ and between a projecting set of neurons and their targets (e.g., the retinocollicular/retinotectal projection; **Figure 3**). Eph-ephrin binding initiates signal transduction cascades by both Ephs and ephrins, resulting in bidirectional signaling. Additionally, Eph/ephrin signaling can be bifunctional, resulting in opposing responses (i.e., attraction or repulsion) to the same cues, depending on context (e.g., level of signaling). These features combine to make the Ephs and ephrins major players in many intricate problems of development, including the development of topographic maps described here.

The shape of topographic guidance molecule gradients is an integral part in forming theories of topographic mapping and axon guidance. Early studies indicated that a ~1% difference in concentration of a repellent guidance molecule across the growth cone is required to halt the forward extension of a growth cone (Baier & Bonhoeffer 1992). Calculations based on this estimate and other parameters including growth cone size and effective concentration range of a guidance molecule indicate that a single, graded guidance activity could function over a maximum distance of 5–10 mm (Goodhill & Baier 1998), which intriguingly approximates the 10-mm length of the AP axis of the chick OT during map development (**Figure 1**). However, recent technological advances suggest a more sensitive mechanism that allows for directed growth along much more shallow gradients, implying that relatively shallow gradients of a single class of molecule can be effective for axon guidance, theoretically allowing for guidance over a longer distance than the 5–10 mm previously calculated (Rosoff et al. 2004). Nonetheless, intuitively, target size should be a variable in determining the slope of the gradient of a topographic guidance molecule across a target axis and therefore the distance over which the molecule is effective. For example, if the same concentration range of ephrin-As is distributed along the AP tectal axis in zebrafish as in chick, because the AP axis of the zebrafish OT is only ~2% of the length of the chick OT axis during map development, the slope of the gradient of the full range of effective concentration could be much steeper in the smaller zebrafish OT. If the threshold of growth cone response to the ephrin-A repellent is conserved, a steeper gradient should result in a greater degree of topographic precision in growth cone targeting, as is observed in zebrafish compared with chick. This proposal is supported by the correlation between the position-dependent differential growth cone overshoot and the gradients of ephrin-As in the chick OT (Yates et al. 2001): The greater overshoot exhibited by temporal axons within anterior OT than by nasal axons in posterior OT correlates with the shallow slope of the graded distribution of ephrin-As in anterior OT (solely due to ephrin-A2) and the steep ephrin-A slope in posterior OT (due to ephrin-A2 and -A5 combined).

Refinement of the Retinotopic Map: Patterned Activity and Axon Repellents

As described above, in mice and chicks, all arbors are formed by interstitial branches that preferentially arborize at or in the vicinity of the topographically appropriate TZ. In frog, refinement of individual arbors is a dynamic process involving the addition and subtraction of higher-order branches (Alsina et al. 2001, Ruthazer et al. 2003); further, the continued but disparate growth of the retina and OT requires a continuous small-scale remodeling throughout life (Cline 1998). These processes shape and refine developing arbors and are dependent on TrkB/BNDF interactions and neural activity (Alsina et al. 2001, Ruthazer et al. 2003). Map refinement in fish and frogs is a precise shaping of arbors rather than the large-scale remodeling of a topographically diffuse projection that occurs in rodents and chicks.

In chicks and mice, the initial collection of arbors is loosely organized around the topographically appropriate position of the future TZ and requires a substantial degree of remodeling to develop the precise connections evident in the mature retinotopic map. This large-scale remodeling requires the elimination of overshooting portions of RGC axons as well as the removal of inappropriately located branches and even entire ectopic arbors. In mice, the retinotopic map resembles its mature form by P8, days before the onset of visually evoked activity and the opening of the eyes (Tian & Copenhagen 2003). However, the large-scale map remodeling is coincident with a period of correlated waves of spontaneous neural activity that propagate across the retina (Galli & Maffei 1998, Meister et al. 1991, Wong et al. 1993). These waves are mediated by a network of cholinergic amacrine cells and correlate the activity of neighboring RGCs, thereby relating an RGC's position to its pattern of activity (Wong 1999). Correlated activity has long been postulated to refine topographic connections by strengthening coordinated inputs and/or weakening uncorrelated inputs (Butts 2002, Debski & Cline 2002, Hebb 1949, Stent 1973). Pharmacological activity blockade in mice and chicks suggested a small but evident role for neural activity in map

remodeling (Kobayahsi et al. 1990, Simon et al. 1992).

However, a recent analysis of mice lacking cholinergic-mediated retinal waves indicates that correlated patterns of RGC activity are required for the large-scale remodeling of the retinocollicular projection into a refined map (McLaughlin et al. 2003c). Mice lacking the $\beta 2$ subunit of the nicotinic acetylcholine receptor maintain spontaneous activity, but the correlation evident in nearby RGCs in wild-type retina is lost (McLaughlin et al. 2003c). The topographic projection in $\beta 2$ mutant mice is aberrant in that RGC axons from a given retinal location do not form a dense TZ but rather maintain a loose collection of diffuse arborizations around the appropriate location of their TZ (McLaughlin et al. 2003c). In $\beta 2$ mutant mice, correlated activity does resume during the second postnatal week through a glutamatergic process, and visually evoked activity begins soon thereafter, but neither process leads to proper map refinement, indicating a brief early critical period for retinotopic map remodeling in mice (McLaughlin et al. 2003c).

In addition to the required role for correlated RGC activity in retinotopic map refinement, the graded expression of guidance molecules may play a role as well (Yates et al. 2004). Indeed, in the $\beta 2$ mutant mice some remodeling does occur; for example, the portions of the primary RGC axons that overshoot the aberrantly diffuse TZ are eliminated (McLaughlin et al. 2003c). Although the mechanisms responsible for the elimination of these overshooting axon segments are unknown, this finding and other evidence suggest that is due to graded repellents that are also involved in generating the initial retinotopic map (Yates et al. 2004). The opposing gradients of EphAs/ephrin-As in the retina result in EphAs predominantly along temporal RGC axons and ephrin-As predominantly along nasal RGC axons. These distributions create imposed countergradients across the OT/SC, which presumably add to the EphA/ephrin-A countergradients expressed by OT/SC cells, but which progressively increase over time as axons branch and arborize (Yates et al. 2001, 2004). Thus, as development proceeds, the level of ephrin-As in posterior OT/SC increases as nasal RGC axons elaborate branches, and the level of EphAs in anterior OT/SC increases as temporal RGCs branch. These changes in EphA/ephrin-A over time amplify the endogenous OT/SC gradients and could be responsible, in part, for the restriction in branching patterns around the TZ, observed over the course of map development, as well as the elimination of the initial RGC axon overshoot (Yates et al. 2001, 2004).

This dynamic type of axon-axon interaction could also provide a partial explanation for aspects of phenotypes observed in mutant mice. One example comes from the analysis of the Isl2-IRES-EphA3 knock-in mouse (EphA3 KI), in which EphA3 is misexpressed by approximately half of the RGCs (RGCs expressing the LIM homeodomain transcription factor Isl2) (Brown et al. 2000). RGCs in homozygous EphA3 KI mice form two topographic maps in the SC: One map comprised essentially entirely from Isl2-positive cells (which therefore misexpress EphA3) forms in anterior SC, and the second map is composed of Isl2-negative RGCs (which have wild-type levels of EphAs) and forms in posterior SC (Brown et al. 2000). Thus, temporal RGCs with wild-type levels of EphAs project to mid-SC, rather than their topographically appropriate TZ in anterior SC, indicating that absolute signaling of EphAs is not the dominant determinant of mapping (Brown et al. 2000, Reber et al. 2004). Crossing EphA3 KI mice with an EphA knockout allows for many combinations and levels of EphA signaling. Results from such crosses confirm that absolute EphA levels are not the absolute determinant of mapping but rather that secondary interactions must take place to form a continuous map (Reber et al. 2004). These studies (Brown et al. 2000, Reber et al. 2004) suggest that competition-exclusion rules (Prestige & Willshaw 1975) may be acting in retinotopic mapping. Thus, another potential way gradients shape topographic maps is by being dynamic over the period of map development.

Additional Activities and Interactions Potentially Required for Map Development

Some mutant phenotypes are not easily explained by the reported actions and distributions of known guidance molecules. For example, an appropriately located TZ (in addition to ectopic TZs) is found in all mice deficient for the Ephs or ephrins required for retinotopic mapping in the SC (Feldheim et al 2000, 2004; Frisen et al. 1998; Hindges et al. 2002), which indicates the action of additional guidance activities along both the AP and LM axes. The dramatic LM mapping defects observed in mice deficient for the homeodomain protein Vax2 also suggest the action of DV guidance molecules other than EphBs and ephrin-Bs. Vax2 is expressed in a tilted gradient in the developing retina, being highest in nasal-ventral RGCs and lowest in temporal-dorsal RGCs (Mui et al. 2002). Targeted deletion of Vax2 in mice results in flattened or diminished gradients of retinal EphBs and ephrin-Bs and a complete shift in the TZs of temporal-ventral RGC axons from antero-medial SC to antero-lateral SC (Mui et al. 2002; also see Schulte et al. 1999), a phenotype much more dramatic than seen in EphB2/B3 double mutants (Hindges et al. 2002). Finally, the functional interactions of Ephs and ephrins are still being detailed and new ones uncovered. For example, EphB2 and ephrin-A5, guidance molecules critical for LM and AP mapping, respectively (Frisen et al. 1998, Hindges et al. 2002), reportedly bind one another, leading to activation of EphB2 signaling pathways (Himanen et al. 2004). Such interactions could potentially influence retinotopic mapping along both axes of the OT/SC.

Though guidance molecules play critical roles in map formation, other interactions, e.g., axon-axon interactions, are likely critical for mapping. Mice deficient for the cell adhesion molecule, L1, which is transiently expressed on RGC axons during pathfinding and mapping, reportedly have defects in both AP and LM mapping in the SC (Demyanenko & Maness 2003). Why L1 is required for proper retinotopic mapping is not known; but considering its roles in other systems, the investigators suggest that it modulates RGC axon-axon interactions required for mapping or the appropriate function of Ephs and ephrins (Itoh et al. 2004, Suh et al. 2004).

Other interactions suggested to influence mapping include competitive interactions for limiting diffusible factors (such as BDNF) or synaptic sites, as well as the interplay between neural activity, response to guidance molecules, and branch dynamics (Alsina et al. 2001, Ruthazer et al. 2003). One example of evidence for this type of secondary interaction comes from analysis of EphA3 KI mice described above. In these mice, Isl2-negative temporal RGCs form TZs in aberrant locations, despite having wild-type levels of topographic guidance molecules, e.g., Ephs and ephrins (Brown et al. 2000). One explanation for this result is that the TZs of Isl2-positive, EphA3 KI RGCs are limited to anterior SC, owing to their enhanced sensitivity to the low-to-high AP gradient of ephrin-A repellents, and exclude Isl2-negative RGC TZs through axon-axon interactions and/or competitive interactions resulting in their orderly, ectopic mapping in posterior SC (Brown et al. 2000).

Genetic Screens: Mapping the Future?

To date, only certain members of the Eph and ephrin families fulfill all criteria for topographic guidance molecules, with a handful of other molecules being studied. Several groups, though, have carried out forward- and reverse-genetic screens to identify additional candidate genes involved in retinotopic mapping or screens that could produce candidate genes as a by-product. The most prominent such undertaking has

been the large-scale Tubingen genetic screen in mutagenized zebrafish designed in part to identify genes involved in RGC axon pathfinding and mapping (Baier et al. 1996, Karlstrom et al. 1996, Trowe et al. 1996). This near-saturation screen resulted in the identification of about a hundred mutants, representing scores of genes; a subset of these mutants have defects in DV (LM) or AP mapping in the retinotectal projection, some of which appear to be primary defects (Hutson & Chien 2002, Karlstrom et al. 1997). A subsequent behavioral screen of mutants previously obtained from broad-based screens of mutagenized zebrafish has identified a subset as defective in visual behavior and therefore may provide additional means to identify genes required for retinotopic mapping (Neuhauss 2003, Neuhauss et al. 1999).

A recent microarray screen has identified many known and unknown genes expressed in gradients or restricted patterns along the TN or DV axes of the developing mouse retina; a common pattern is differential gene expression along the DV axis (Diaz et al. 2003). On the basis of their expression pattern and functional class, a subset of these genes will likely prove to be involved in retinotopic mapping.

FUTURE DIRECTIONS

Future work will be focused most likely on small-scale events within the growth cone and axon shaft as well as on population-wide effects, such as the dynamics in gradients of topographic guidance moelcules and the effects of the progressive elaboration of the projection itself on its own development. Furthermore, given the complexity and array of guidance molecules, along with their complementary expression both in the projecting neurons and in the target, conditional mutants in which a gene can be deleted from a defined structure or neuronal population at a defined time point will be fruitful. Additionally, systems in which reciprocal expression of ligands and receptors is asymmetric (i.e., a receptor present in the projecting population but not in the target population) will prove useful in sorting through the actions of multiple related genes expressed in complex expression patterns.

Among the topics most amenable to examination are the roles of ephrin-As in the retina and their potential as axon guidance receptors in the visual system. Identification of conditional mutants in which Ephs can be selectively removed from the SC, or in which ephrin-As can be removed from the retina or from the SC, would be informative. Furthermore, determining the molecular cascade involved with ephrin-A reverse signaling is of fundamental importance (Davy et al. 1999). In addition to sorting out the potential bifunctional and bidirectional actions of Ephs and ephrins, interactions between various families of guidance molecules may provide evidence of new molecular mechanisms to control topographic map formation and are likely to indicate a level of control not yet fully appreciated. For example, the Eph/ephrins have been linked to multiple guidance molecules including slits, Trk receptors, L1, and laminin (Kong et al. 2001, Suh et al. 2004, Wong et al. 2004). In addition, phosphatases clearly have a critical role in axon guidance, and their roles require further examination (Ensslen-Craig & Brady-Kalnay 2004, Johnson et al. 2001, Palmer et al. 2002).

Lastly, we find that the role a system plays in its own development is an intriguing facet of mapping. In many systems the projecting axons themselves likely affect the development of the projection. These potential interactions are not well defined but may include competition for synaptic space or diffusible factors, direct axon-axon interactions, or the addition of guidance molecules present along projecting axons to the target, to name a few. For example, RGC axons may alter the landscape of guidance molecules as they elaborate arbors in the target, owing in part to the presence of guidance molecules on RGC axons, such as ephrin-As on nasal RGC axons (Hornberger et al. 1999). As nasal RGC axons branch in posterior OT/SC, the level of ephrin-As should increase in that domain, adding to the expression of ephrin-As by posterior tectal or collicular cells. This alteration of the ephrin-A gradient will likely affect topographic mapping (Yates et al. 2004). Thus, guidance information is unlikely to be static, and therefore the sum total of analyses of small numbers of axons may not yield data that seem able to account fully for topographic mapping. However, in the dynamic developing topographic map the same information may, in fact, be sufficient.

AOB: accessory olfactory bulb

AP: anterior-posterior

DV: dorsal-ventral

IRES: internal ribosome entry site

KI: knock-in

LM: lateral-medial

NT: nasal-temporal

OB: olfactory bulb

OT: optic tectum

RGC: retinal ganglion cell

SC: superior colliculus

TZ: termination zone

VNO: vomeronasal organ

ACKNOWLEDGMENTS

Work in the authors' lab on the topic of this review is supported by National Eye Institute grant R01 EY 07025 (DO'L). We thank current and former members of the O'Leary lab (especially Carol Armstrong, Octavio Choi, Glenn Friedman, Robert Hindges, Winnie Pak, Adina Roskies, Alicia Santiago, David Simon, and Paul Yates) who have worked on this topic and have made valued contributions to discussions of concepts of mapping. We also thank our collaborators, in particular Mariano Barbacid, Jonas Frisen, Mark Henkemeyer, and Greg Lemke, for sharing mutant mice for our analyses of molecular mechanisms of retinotopic mapping.

LITERATURE CITED

Alsina B, Vu T, Cohen-Cory S. 2001. Visualizing synapse formation in arborizing optic axons in vivo: dynamics and modulation by BDNF. *Nat. Neurosci.* 4:1093–101

Baier H, Bonhoeffer F. 1992. Axon guidance by gradients of a target-derived component. *Science* 255:472–75

Baier H, Klostermann S, Trowe T, Karlstrom RO, Nusslein-Volhard C, Bonhoeffer F. 1996. Genetic dissection of the retinotectal projection. *Development* 123:415–25

Bassell GJ, Kelic S. 2004. Binding proteins for mRNA localization and local translation, and their dysfunction in genetic neurological disease. *Curr. Opin. Neurobiol.* 14:574–81

Belluscio L, Koentges G, Axel R, Dulac C. 1999. A map of pheromone receptor activation in the mammalian brain. *Cell* 97:209–20

Benson DL, Colman DR, Huntley GW. 2001. Molecules, maps and synapse specificity. *Nat. Rev. Neurosci.* 2:899–909

Braisted JE, McLaughlin T, Wang HU, Friedman GC, Anderson DJ, O'Leary DDM. 1997. Graded and lamina-specific distributions of ligands of EphB receptor tyrosine kinases in the developing retinotectal system. *Dev. Biol.* 191:14–28

Brennan C, Monschau B, Lindberg R, Guthrie B, Drescher U, et al. 1997. Two Eph receptor tyrosine kinase ligands control axon growth and may be involved in the creation of the retinotectal map in the zebrafish. *Development* 124:655–64

Brittis PA, Lu Q, Flanagan JG. 2002. Axonal protein synthesis provides a mechanism for localized regulation at an intermediate target. *Cell* 110:223–35

Brown A, Yates PA, Burrola P, Ortuño D, Vaidya A, et al. 2000. Topographic mapping from the retina to the midbrain is controlled by relative but not absolute levels of EphA receptor signaling. *Cell* 102:77–88

Bruckner K, Pasquale EB, Klein R. 1997. Tyrosine phosphorylation of transmembrane ligands for Eph receptors. *Science* 275:1640–43

Butts DA. 2002. Retinal waves: implications for synaptic learning rules during development. *Neuroscientist* 8:243–53

Campbell DS, Holt CE. 2001. Chemotropic responses of retinal growth cones mediated by rapid local protein synthesis and degradation. *Neuron* 32:1013–26
Campbell DS, Regan AG, Lopez JS, Tannahill D, Harris WA, Holt CE. 2001. Semaphorin 3A elicits stage-dependent collapse, turning, and branching in *Xenopus* retinal growth cones. *J. Neurosci.* 21:8538–47
Chalupa LM, Snider CJ. 1998. Topographic specificity in the retinocollicular projection of the developing ferret: an anterograde tracing study. *J. Comp. Neurol.* 392:35–47
Chalupa LM, Snider CJ, Kirby MA. 1996. Topographic organization in the retinocollicular pathway of the fetal cat demonstrated by retrograde labeling of ganglion cells. *J. Comp. Neurol.* 368:295–303
Cheng HJ, Flanagan JG. 1994. Identification and cloning of ELF-1, a developmentally expressed ligand for the Mek4 and Sek receptor tyrosine kinases. *Cell* 79:157–68
Cheng HJ, Nakamoto M, Bergemann AD, Flanagan JG. 1995. Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographic retinotectal projection map. *Cell* 82:371–81
Choi O, O'Leary DDM. 2000. Potential roles for BDNF and TrkB in developing topographic retinotectal projections. *Soc. Neurosci. Abstr.* 26 (Abstr.)
Choi SB, Yates PA, O'Leary DDM. 1998. Localized BDNF application induces branch-like structures along retinal axons. *Soc. Neurosci. Abstr.* 24 (Abstr.)
Ciossek T, Monschau B, Kremoser C, Loschinger J, Lang S, et al. 1998. Eph receptor-ligand interactions are necessary for guidance of retinal ganglion cell axons in vitro. *Eur. J. Neurosci.* 10:1574–80
Cline HT. 1998. Topographic maps: developing roles of synaptic plasticity. *Curr. Biol.* 8:R836–39
Cloutier JF, Giger RJ, Koentges G, Dulac C, Kolodkin AL, Ginty DD. 2002. Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. *Neuron* 33:877–92
Cloutier JF, Sahay A, Chang EC, Tessier-Lavigne M, Dulac C, et al. 2004. Differential requirements for semaphorin 3F and Slit-1 in axonal targeting, fasciculation, and segregation of olfactory sensory neuron projections. *J. Neurosci.* 24:9087–96
Cohen-Cory S. 2002. The developing synapse: construction and modulation of synaptic structures and circuits. *Science* 298:770–76
Cohen-Cory S, Fraser SE. 1995. Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. *Nature* 378:192–96
Connor RJ, Menzel P, Pasquale EB. 1998. Expression and tyrosine phosphorylation of Eph receptors suggest multiple mechanisms in patterning of the visual system. *Dev. Biol.* 193:21–35
Cox EC, Muller B, Bonhoeffer F. 1990. Axonal guidance in the chick visual system: posterior tectal membranes induce collapse of growth cones from the temporal retina. *Neuron* 4:31–37
Davenport RW, Thies E, Cohen ML. 1999. Neuronal growth cone collapse triggers lateral extensions along trailing axons. *Nat. Neurosci.* 2:254–59
Davy A, Gale NW, Murray EW, Klinghoffer RA, Soriano P, et al. 1999. Compartmentalized signaling by GPI-anchored ephrin-A5 requires the Fyn tyrosine kinase to regulate cellular adhesion. *Genes Dev.* 13:3125–35
Davy A, Robbins SM. 2000. Ephrin-A5 modulates cell adhesion and morphology in an integrin-dependent manner. *EMBO J.* 19:5396–405
Debski EA, Cline HT. 2002. Activity-dependent mapping in the retinotectal projection. *Curr. Opin. Neurobiol.* 12:93–99

Demyanenko GP, Maness PF. 2003. The L1 cell adhesion molecule is essential for topographic mapping of retinal axons. *J. Neurosci.* 23:530–38
Diaz E, Yang YH, Ferreira T, Loh KC, Okazaki Y, et al. 2003. Analysis of gene expression in the developing mouse retina. *Proc. Natl. Acad. Sci. USA* 100:5491–96
Ding Y, Marotte LR. 1997. The initial stages of development of the retinocollicular projection in the wallaby (Macropus eugenii): distribution of ganglion cells in the retina and their axons in the superior colliculus. *Anat. Embryol.* 194:301–17
Drescher U, Kremoser C, Handwerker C, Loschinger J, Noda M, Bonhoeffer F. 1995. In vitro guidance of retinal ganglion cell axons by RAGS, a 25 kDa tectal protein related to ligands for Eph receptor tyrosine kinases. *Cell* 82:359–70
Dufour A, Seibt J, Passante L, Depaepe V, Ciossek T, et al. 2003. Area specificity and topography of thalamocortical projections are controlled by ephrin/Eph genes. *Neuron* 39:453–65
Eberhart J, Barr J, O'Connell S, Flagg A, Swartz ME, et al. 2004. Ephrin-A5 exerts positive or inhibitory effects on distinct subsets of EphA4-positive motor neurons. *J. Neurosci.* 24:1070–78
Ensslen-Craig SE, Brady-Kalnay SM. 2004. Receptor protein tyrosine phosphatases regulate neural development and axon guidance. *Dev. Biol.* 275:12–22
Feldheim DA, Kim YI, Bergemann AD, Frisen J, Barbacid M, Flanagan JG. 2000. Genetic analysis of ephrin-A2 and ephrin-A5 shows their requirement in multiple aspects of retinocollicular mapping. *Neuron* 25:563–74
Feldheim DA, Nakamoto M, Osterfield M, Gale NW, DeChiara TM, et al. 2004. Loss-of-function analysis of EphA receptors in retinotectal mapping. *J. Neurosci.* 24:2542–50
Feng G, Laskowski MB, Feldheim DA, Wang H, Lewis R, et al. 2000. Roles for ephrins in positionally selective synaptogenesis between motor neurons and muscle fibers. *Neuron* 25:295–306
Frisen J, Yates PA, McLaughlin T, Friedman GC, O'Leary DDM, Barbacid M. 1998. Ephrin-A5 (AL-1/RAGS) is essential for proper retinal axon guidance and topographic mapping in the mammalian visual system. *Neuron* 20:235–43
Galli L, Maffei L. 1988. Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. *Science* 242:90–91
Gallo G, Letourneau PC. 2004. Regulation of growth cone actin filaments by guidance cues. *J. Neurobiol.* 58:92–102
Gao PP, Zhang JH, Yokoyama M, Racey B, Dreyfus CF, et al. 1996. Regulation of topographic projection in the brain: Elf-1 in the hippocamposeptal system. *Proc. Natl. Acad. Sci. USA* 93:11161–66
Gierer A. 1983. Model for the retino-tectal projection. *Proc. R. Soc. London B. Biol. Sci.* 218:77–93
Gierer A. 1987. Directional cues for growing axons forming the retinotectal projection. *Development* 101:479–89
Goodhill GJ, Baier H. 1998. Axon guidance: stretching gradients to the limit. *Neural Comput.* 10:521–27
Hansen MJ, Dallal GE, Flanagan JG. 2004. Retinal axon response to ephrin-As shows a graded, concentration-dependent transition from growth promotion to inhibition. *Neuron* 42:717–30
Harris WA, Holt CE, Bonhoeffer F. 1987. Retinal axons with and without their somata, growing to and arborizing in the tectum of *Xenopus* embryos: a time-lapse video study of single fibres in vivo. *Development* 101:123–33
Hebb DO. 1949. *The Organization of Behavior: A Neuropsychological Theory*. New York: Wiley

Henkemeyer M, Orioli D, Henderson JT, Saxton TM, Roder J, et al. 1996. Nuk controls pathfinding of commissural axons in the mammalian central nervous system. *Cell* 86:35–46

Himanen J-P, Chumley MJ, Lackmann M, Li C, Barton WA, et al. 2004. Repelling class discrimination: ephrin-A5 binds to and activates EphB2 receptor signaling. *Nat. Neurosci.* 7:501–9

Himanen JP, Nikolov DB. 2003. Eph signaling: a structural view. *Trends Neurosci.* 26:46–51

Hindges R, McLaughlin T, Genoud N, Henkemeyer M, O'Leary DDM. 2002. EphB forward signaling controls directional branch extension and arborization required for dorsal ventral retinotopic mapping. *Neuron* 35:475–87

Holland SJ, Gale NW, Mbamalu G, Yancopoulos GD, Henkemeyer M, Pawson T. 1996. Bidirectional signalling through the EPH-family receptor Nuk and its transmembrane ligands. *Nature* 383:722–25

Hornberger MR, Dutting D, Ciossek T, Yamada T, Handwerker C, et al. 1999. Modulation of EphA receptor function by coexpressed ephrinA ligands on retinal ganglion cell axons. *Neuron* 22:731–42

Huber AB, Kolodkin AL, Ginty DD, Cloutier JF. 2003. Signaling at the growth cone: ligand-receptor complexes and the control of axon growth and guidance. *Annu. Rev. Neurosci.* 26: 509–63

Hutson LD, Chien CB. 2002. Wiring the zebrafish: axon guidance and synaptogenesis. *Curr. Opin. Neurobiol.* 12:87–92

Huynh-Do U, Stein E, Lane AA, Liu H, Cerretti DP, Daniel TO. 1999. Surface densities of ephrin-B1 determine EphB1-coupled activation of cell attachment through alphavbeta3 and alpha5beta1 integrins. *EMBO J.* 18:2165–73

Itoh K, Cheng L, Kamei Y, Fushiki S, Kamiguchi H, et al. 2004. Brain development in mice lacking L1-L1 homophilic adhesion. *J. Cell Biol.* 165:145–54

Johnson KG, McKinnell IW, Stoker AW, Holt CE. 2001. Receptor protein tyrosine phosphatases regulate retinal ganglion cell axon outgrowth in the developing Xenopus visual system. *J. Neurobiol.* 49:99–117

Kaas JH. 1997. Topographic maps are fundamental to sensory processing. *Brain Res. Bull.* 44: 107–12

Kaethner RJ, Stuermer CA. 1992. Dynamics of terminal arbor formation and target approach of retinotectal axons in living zebrafish embryos: a time-lapse study of single axons. *J. Neurosci.* 12:3257–71

Karlstrom RO, Trowe T, Bonhoeffer F. 1997. Genetic analysis of axon guidance and mapping in the zebrafish. *Trends Neurosci.* 20:3–8

Karlstrom RO, Trowe T, Klostermann S, Baier H, Brand M, et al. 1996. Zebrafish mutations affecting retinotectal axon pathfinding. *Development* 123:427–38

Knoll B, Drescher U. 2002. Ephrin-As as receptors in topographic projections. *Trends Neurosci.* 25:145–49

Knoll B, Schmidt H, Andrews W, Guthrie S, Pini A, et al. 2003. On the topographic targeting of basal vomeronasal axons through Slit-mediated chemorepulsion. *Development* 130:5073–82

Knoll B, Zarbalis K, Wurst W, Drescher U. 2001. A role for the EphA family in the topographic targeting of vomeronasal axons. *Development* 128:895–906

Kobayashi T, Nakamura H, Yasuda M. 1990. Disturbance of refinement of retinotectal projection in chick embryos by TTX and grayanotoxin. *Dev. Brain Res.* 57:29–35

Kong H, Boulter J, Weber JL, Lai C, Chao MV. 2001. An evolutionarily conserved transmembrane protein that is a novel downstream target of neurotrophin and ephrin receptors. *J. Neurosci.* 21:176–85

Liu Y, Berndt J, Su F, Tawarayama H, Shoji W, et al. 2004. Semaphorin3D guides retinal axons along the dorsoventral axis of the tectum. *J. Neurosci.* 24:310–18

Loconto J, Papes F, Chang E, Stowers L, Jones EP, et al. 2003. Functional expression of murine V2R pheromone receptors involves selective association with the M10 and M1 families of MHC class Ib molecules. *Cell* 112:607–18

Mann F, Miranda E, Weinl C, Harmer E, Holt CE. 2003. B-type Eph receptors and ephrins induce growth cone collapse through distinct intracellular pathways. *J. Neurobiol.* 57:323–36

Mann F, Ray S, Harris WA, Holt CE. 2002. Topographic mapping in dorsoventral axis of the *Xenopus* retinotectal system depends on signaling through ephrin-B ligands. *Neuron* 35:461–73

Marcus RC, Gale NW, Morrison ME, Mason CA, Yancopoulos GD. 1996. Eph family receptors and their ligands distribute in opposing gradients in the developing mouse retina. *Dev. Biol.* 180:786–89

Marston DJ, Dickinson S, Nobes CD. 2003. Rac-dependent trans-endocytosis of ephrinBs regulates Eph-ephrin contact repulsion. *Nat. Cell Biol.* 5:879–88

McLaughlin T, Hindges R, O'Leary DDM. 2003a. Regulation of axial patterning of the retina and its topographic mapping in the brain. *Curr. Opin. Neurobiol.* 13:57–69

McLaughlin T, Hindges R, Yates PA, O'Leary DDM. 2003b. Bifunctional action of ephrin-B1 as a repellent and attractant to control bidirectional branch extension in dorsal-ventral retinotopic mapping. *Development* 130:2407–18

McLaughlin T, Torborg CL, Feller MB, O'Leary DDM. 2003c. Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. *Neuron* 40:1147–60

McLoon SC. 1991. A monoclonal antibody that distinguishes between temporal and nasal retinal axons. *J. Neurosci.* 11:1470–77

Meister M, Wong RO, Baylor DA, Shatz CJ. 1991. Synchronous bursts of action potentials in ganglion cells of the developing mammalian retina. *Science* 252:939–43

Menzel P, Valencia F, Godement P, Dodelet VC, Pasquale EB. 2001. Ephrin-A6, a new ligand for EphA receptors in the developing visual system. *Dev. Biol.* 230:74–88

Monnier PP, Sierra A, Macchi P, Deitinghoff L, Andersen JS, et al. 2002. RGM is a repulsive guidance molecule for retinal axons. *Nature* 419:392–95

Monschau B, Kremoser C, Ohta K, Tanaka H, Kaneko T, et al. 1997. Shared and distinct functions of RAGS and ELF-1 in guiding retinal axons. *EMBO J.* 16:1258–67

Mui SH, Hindges R, O'Leary DD, Lemke G, Bertuzzi S. 2002. The homeodomain protein Vax2 patterns the dorsoventral and nasotemporal axes of the eye. *Development* 129:797–804

Muller BK, Jay DG, Bonhoeffer F. 1996. Chromophore-assisted laser inactivation of a repulsive axonal guidance molecule. *Curr. Biol.* 6:1497–502

Murai KK, Pasquale EB. 2003. 'Eph'ective signaling: forward, reverse and crosstalk. *J. Cell Sci.* 116:2823–32

Nakamoto M, Cheng HJ, Friedman GC, McLaughlin T, Hansen MJ, et al. 1996. Topographically specific effects of ELF-1 on retinal axon guidance in vitro and retinal axon mapping in vivo. *Cell* 86:755–66

Nakamoto T, Kain KH, Ginsberg MH. 2004. Neurobiology: new connections between integrins and axon guidance. *Curr. Biol.* 14:121–23

Nakamura H, O'Leary DDM. 1989. Inaccuracies in initial growth and arborization of chick retinotectal axons followed by course corrections and axon remodeling to develop topographic order. *J. Neurosci.* 9:3776–95
Neuhauss SC. 2003. Behavioral genetic approaches to visual system development and function in zebrafish. *J. Neurobiol.* 54:148–60
Neuhauss SC, Biehlmaier O, Seeliger MW, Das T, Kohler K, et al. 1999. Genetic disorders of vision revealed by a behavioral screen of 400 essential loci in zebrafish. *J. Neurosci.* 19:8603–15
Nguyen QT, Sanes JR, Lichtman JW. 2002. Pre-existing pathways promote precise projection patterns. *Nat. Neurosci.* 5:861–67
Niederkofler V, Salie R, Sigrist M, Arber S. 2004. Repulsive guidance molecule (RGM) gene function is required for neural tube closure but not retinal topography in the mouse visual system. *J. Neurosci.* 24:808–18
O'Leary DDM, Yates P, McLaughlin T. 1999. Mapping sights and smells in the brain: distinct mechanisms to achieve a common goal. *Cell* 96:255–69
O'Rourke NA, Cline HT, Fraser SE. 1994. Rapid remodeling of retinal arbors in the tectum with and without blockade of synaptic transmission. *Neuron* 12:921–34
O'Rourke NA, Fraser SE. 1990. Dynamic changes in optic fiber terminal arbors lead to retinotopic map formation: an in vivo confocal microscopic study. *Neuron* 5:159–71
Palmer A, Klein R. 2003. Multiple roles of ephrins in morphogenesis, neuronal networking, and brain function. *Genes Dev.* 17:1429–50
Palmer A, Zimmer M, Erdmann KS, Eulenburg V, Porthin A, et al. 2002. EphrinB phosphorylation and reverse signaling: regulation by Src kinases and PTP-BL phosphatase. *Mol. Cell* 9:725–37
Poliakov A, Cotrina M, Wilkinson DG. 2004. Diverse roles of eph receptors and ephrins in the regulation of cell migration and tissue assembly. *Dev. Cell* 7:465–80
Prestige MC, Willshaw DJ. 1975. On a role for competition in the formation of patterned neural connexions. *Proc. R. Soc. London B Biol. Sci.* 190:77–98
Rajagopalan S, Deitinghoff L, Davis D, Conrad S, Skutella T, et al. 2004. Neogenin mediates the action of repulsive guidance molecule. *Nat. Cell Biol.* 6:756–62
Reber M, Burrola P, Lemke G. 2004. A relative signalling model for the formation of a topographic neural map. *Nature* 431:847–53
Rodriguez I, Feinstein P, Mombaerts P. 1999. Variable patterns of axonal projections of sensory neurons in the mouse vomeronasal system. *Cell* 97:199–208
Rosentreter SM, Davenport RW, Loschinger J, Huf J, Jung J, Bonhoeffer F. 1998. Response of retinal ganglion cell axons to striped linear gradients of repellent guidance molecules. *J. Neurobiol.* 37:541–62
Roskies A, Friedman G, O'Leary DDM. 1995. Molecules and mechanisms in the development of retinotopic maps. *Perspect. Dev. Neurobiol.* 3:63–75
Roskies AL, O'Leary DDM. 1994. Control of topographic retinal axon branching by inhibitory membrane-bound molecules. *Science* 265:799–803
Rosoff WJ, Urbach JS, Esrick MA, McAllister RG, Richards LJ, Goodhill GJ. 2004. A new chemotaxis assay shows the extreme sensitivity of axons to molecular gradients. *Nat. Neurosci.* 7:678–82
Rossant J, Hirashima M. 2003. Vascular development and patterning: making the right choices. *Curr. Opin. Genet. Dev.* 13:408–12
Ruthazer ES, Akerman CJ, Cline HT. 2003. Control of axon branch dynamics by correlated activity in vivo. *Science* 301:66–70

Sakurai T, Wong E, Drescher U, Tanaka H, Jay DG. 2002. Ephrin-A5 restricts topographically specific arborization in the chick retinotectal projection in vivo. *Proc. Natl. Acad. Sci. USA* 99:10795–800
Savitt JM, Trisler D, Hilt DC. 1995. Molecular cloning of TOP_{AP}: a topographically graded protein in the developing chick visual system. *Neuron* 14:253–61
Schulte D, Furukawa T, Peters MA, Kozak CA, Cepko CL. 1999. Misexpression of the Emx-related homeobox genes cVax and mVax2 ventralizes the retina and perturbs the retinotectal map. *Neuron* 24:541–53
Simon DK, O'Leary DDM. 1991. Relationship of retinotopic ordering of axons in the optic pathway to the formation of visual maps in central targets. *J. Comp. Neurol.* 307:393–404
Simon DK, O'Leary DDM. 1992a. Development of topographic order in the mammalian retinocollicular projection. *J. Neurosci.* 12:1212–32
Simon DK, O'Leary DDM. 1992b. Influence of position along the medial-lateral axis of the superior colliculus on the topographic targeting and survival of retinal axons. *Dev. Brain Res.* 69:167–72
Simon DK, O'Leary DDM. 1992c. Responses of retinal axons in vivo and in vitro to position-encoding molecules in the embryonic superior colliculus. *Neuron* 9:977–89
Simon DK, Prusky GT, O' Leary DDM, Constantine-Paton M. 1992. N-methyl-D-aspartate receptor antagonists disrupt the formation of a mammalian neural map. *Proc. Natl. Acad. Sci. USA* 89:10593–97
Sperry R. 1963. Chemoaffinity in the orderly growth of nerve fiber patterns and connections. *Proc. Natl. Acad. Sci. USA* 50:703–10
Stahl B, Muller B, von Boxberg Y, Cox EC, Bonhoeffer F. 1990. Biochemical characterization of a putative axonal guidance molecule of the chick visual system. *Neuron* 5:735–43
Stent GS. 1973. A physiological mechanism for Hebb's postulate of learning. *Proc. Natl. Acad. Sci. USA* 70:997–1001
Suh LH, Oster SF, Soehrman SS, Grenningloh G, Sretavan DW. 2004. L1/Laminin modulation of growth cone response to EphB triggers growth pauses and regulates the microtubule destabilizing protein SCG10. *J. Neurosci.* 24:1976–86
Surawska H, Ma PC, Salgia R. 2004. The role of ephrins and Eph receptors in cancer. *Cytokine Growth Factor Rev.* 15:419–33
Tian N, Copenhagen DR. 2003. Visual stimulation is required for refinement of ON and OFF pathways in postnatal retina. *Neuron* 39:85–96
Trowe T, Klostermann S, Baier H, Granato M, Crawford AD, et al. 1996. Mutations disrupting the ordering and topographic mapping of axons in the retinotectal projection of the zebrafish, Danio rerio. *Development* 123:439–50
van Horck FP, Weinl C, Holt CE. 2004. Retinal axon guidance: novel mechanisms for steering. *Curr. Opin. Neurobiol.* 14:61–66
Vanderhaeghen P, Polleux F. 2004. Developmental mechanisms patterning thalamocortical projections: intrinsic, extrinsic and in between. *Trends Neurosci.* 27:384–91
Wagle M, Grunewald B, Subburaju S, Barzaghi C, Le Guyader S, et al. 2004. EphrinB2a in the zebrafish retinotectal system. *J. Neurobiol.* 59:57–65
Walter J, Henke-Fahle S, Bonhoeffer F. 1987b. Avoidance of posterior tectal membranes by temporal retinal axons. *Development* 101:909–13
Walter J, Kern-Veits B, Huf J, Stolze B, Bonhoeffer F. 1987a. Recognition of position-specific properties of tectal cell membranes by retinal axons in vitro. *Development* 101:685–96
Walz A, Rodriguez I, Mombaerts P. 2002. Aberrant sensory innervation of the olfactory bulb in neuropilin-2 mutant mice. *J. Neurosci.* 22:4025–35

Weinl C, Drescher U, Lang S, Bonhoeffer F, Loschinger J. 2003. On the turning of *Xenopus* retinal axons induced by ephrin-A5. *Development* 130:1635–43

Wong EV, Kerner JA, Jay DG. 2004. Convergent and divergent signaling mechanisms of growth cone collapse by ephrinA5 and slit2. *J. Neurobiol.* 59:66–81

Wong RO, Meister M, Shatz CJ. 1993. Transient period of correlated bursting activity during development of the mammalian retina. *Neuron* 11:923–38

Wong RO. 1999. Retinal waves and visual system development. *Annu. Rev. Neurosci.* 22:29–47

Yates PA, Holub AD, McLaughlin T, Sejnowski TJ, O' Leary DD. 2004. Computational modeling of retinotopic map development to define contributions of EphA-ephrinA gradients, axon-axon interactions, and patterned activity. *J. Neurobiol.* 59:95–113

Yates PA, Roskies AR, McLaughlin T, O' Leary DDM. 2001. Topographic specific axon branching controlled by ephrin-As is the critical event in retinotectal map development. *J. Neurosci.* 21:8548–63

Yoda H, Hirose Y, Yasuoka A, Sasado T, Morinaga C, et al. 2004. Mutations affecting retinotectal axonal pathfinding in Medaka, Oryzias latipes. *Mech. Dev.* 121:715–28

Yue Y, Chen ZY, Gale NW, Blair-Flynn J, Hu TJ, et al. 2002. Mistargeting hippocampal axons by expression of a truncated Eph receptor. *Proc. Natl. Acad. Sci. USA* 99:10777–82

Zimmer M, Palmer A, Kohler J, Klein R. 2003. EphB-ephrinB bi-directional endocytosis terminates adhesion allowing contact mediated repulsion. *Nat. Cell Biol.* 5:869–78

Annual Review of Neuroscience

Volume 28, 2005

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