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Multiple EphB receptors mediate dorsal–ventral retinotopic mapping via similar bi-functional responses to ephrin-B1

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ABSTRACT

The projection from the retina to the superior colliculus in mice is organized in a retinotopic map that develops through the formation and guidance of interstitial branches extended by retinal ganglion cell axons. Bidirectional branch guidance along the lateral–medial collicular axis is critical to mapping the dorsal–ventral retinal axis. EphB receptor tyrosine kinases expressed in an overall low to high dorsal–ventral retinal gradient have been implicated in this mapping in response to the graded low to high lateral–medial expression of a ligand, ephrin-B1, in the superior colliculus. However, the relative contributions of EphBs and ephrin-B1 are not well understood. We examined EphB1, EphB2, and EphB3 mutant mice and find that each has ectopic arborizations of retinal axon branches lateral to their appropriate termination zone, with no qualitative differences in aberrant mapping, suggesting a similar role for each EphB. However, the frequency of cases with map defects progressively rises in compound EphB mutants coincident with the number of EphB null alleles from one to five of the six total alleles indicating that EphB level is critical. We analyzed branch extension in vitro and find that dorsal branches, with low EphB levels, exhibit a negative response to ephrin-B1, whereas ventral branches, with high EphB levels, exhibit a positive response to ephrin-B1. Using EphB mutant retina, we show that both of these differential branch extension responses are dependent on EphB level. Our findings show a bifunctional action of ephrin-B1 regulated by EphB levels that can account for the bidirectional extension of interstitial branches required to establish a retinotopic map.

1. Introduction

The projection of retinal ganglion cells (RGCs) to the superior colliculus (SC) is the predominant system for studying mechanisms of topographic map development. The dorsal–ventral (DV) axis of the retina maps along the lateral–medial (LM) SC axis. However, the initial projection of RGC axons is diffuse and has only a coarse topographic order within the SC (Simon and O'Leary, 1992a). RGC axons extend far posterior to the location of their future termination zone (TZ), and axons originating from neighboring RGCs are dispersed across the entire LM axis of the SC, although biased for the LM position of their future TZ (Fig. 1A). However, most axons are located either medial or lateral to their future TZ and connect to it through branches that form interstitially along the axon shaft (Simon and O'Leary, 1992b, 1992c). Interstitial branches are directed either medially or laterally along the LM axis to their correct TZ depending upon the initial LM position of their parent RGC axons in the SC. Thus, the DV retinotopic map is established by the bidirectional guidance of interstitial branches along the LM axis to the topographically appropriate SC region of their TZ.

Several molecules have been implicated in DV retinotopic mapping, most prominently the EphBs and ephrin-Bs (Feldheim and O'Leary, 2010). Ephrin-B1 is expressed in a low-to-high LM gradient across the SC (Hindges et al., 2002), whereas EphB1, EphB2, and EphB3 are expressed in an overall low-to-high DV gradient by RGCs (Hindges et al., 2002; Thakar et al., 2011). Two studies (Hindges et al., 2002; Thakar et al., 2011) have reported that EphB1/EphB2 and EphB2/EphB3 double mutants, as well as each individual mutant of EphB1, EphB2, and EphB3, have DV mapping defects, demonstrating a role for EphB forward signaling for each receptor (Fig. 1B). Significantly, each of these EphB mutants has lateral, ectopic TZs for central and ventral RGCs, attributed to a defect in LM branch guidance (Hindges et al., 2002).

Here we first examined EphB null allelic combinations more in depth and find that every allelic combination has similar DV mapping defects, but the frequency of cases with defects rises with the number of null EphB alleles. Thus, the overall level of EphBs is a critical factor in DV mapping rather than distinct functional contributions from each EphB type. To test the hypothesis that the differential guidance of interstitial branches is due to their bifunctional responses to ephrin-B1 mediated

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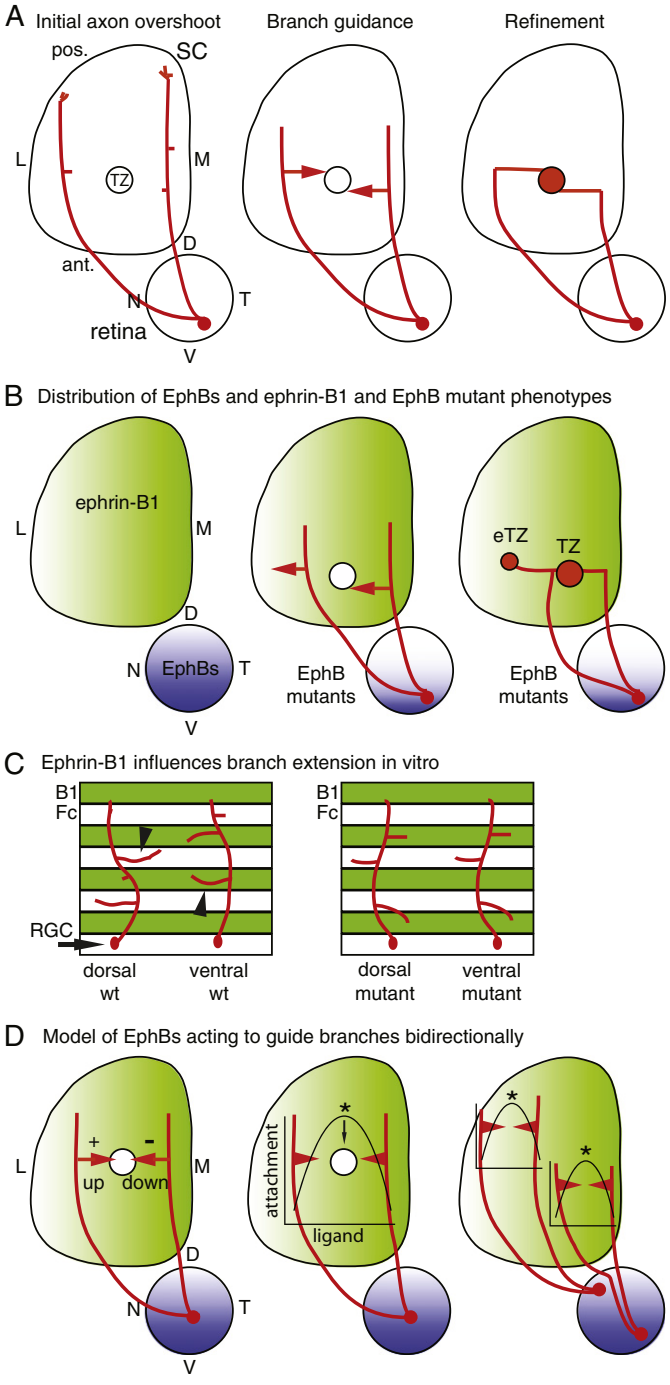

Fig. 1. Retinocollicular map development and the roles of EphBs. (A) Retinal ganglion cell (RGC) axons enter the superior colliculus (SC) at its anterior (ant.) border and overshoot the future termination zone (TZ) into posterior (pos.) SC (left). Axons then form branches which extend along the medial (M)–lateral (L) SC axis in a guided manner towards the TZ (middle). Branches that reach the nascent TZ arborize and contribute to a dense array of connections (right). D, dorsal; N, nasal; T, temporal; V, ventral. (B) EphB1, EphB2, EphB3, and EphB4 are distributed in RGCs in an overall high ventral–low dorsal gradient (blue) and ephrin-B1 is distributed in a high-M-to-low-L gradient (green) in the SC during retinocollicular map development. In EphB mutant mice, RGC axons from VT retina form a TZ in the appropriate location, but in addition form an ectopic TZ (eTZ) laterally (center). (C) In vitro, dorsal retinal axons prefer to extend branches on control lanes (Fc), avoiding ephrin-B1. Ventral retinal axons prefer to extend branches on ephrin-B1 lanes (B1). Both preferences are lost in EphB mutants. (D) A potential model for the roles of EphBs is informed by in vitro studies (Huynh-do et al., 1999). RGCs have a different point on the ephrin-B1 gradient at which adherence is maximal, dependent on their EphB level. Thus, distinct branch responses to the ephrin-B1 gradient are based on not only the EphB level of the parent RGC, but also the position of the branch on the gradient.

by EphB level, we have used the protein stripe assay to examine branch response to ephrin-B1. We show in this assay that dorsal and ventral RGCs exhibit differential branch extension and, by using retina from EphB mutants, that the differential response to ephrin-B1 by branches extended from retinal axons is dependent on EphB level. Further, these responses are EphB-dependent and, importantly, recapitulate the responses observed in vivo for RGC axons in wild type and EphB mutant mice. Our findings provide a mechanism consistent with a bifunctional action of ephrin-B1 regulated by EphB levels that can account for the bidirectional extension of interstitial branches from RGC axons required to establish a DV retinotopic map.

2. Results

2.1. EphB1 is required for DV retinocollicular mapping

We first analyzed EphB1 mutant mice to determine the characteristics and frequency of retinocollicular map defects in mice homozygous or heterozygous for an EphB1 null allele, providing baseline data for our analysis of the contributions of EphB null alleles to DV mapping. Focal injections of the axon tracer, DiI, were made near the middle of the DV axis of the peripheral temporal retina to label RGC axonal projections in the contralateral SC. Mice were injected at P7 and analyzed at P8, when in wild type mice (WT) the projection is topographically mature. Such a focal injection densely labels a single TZ in anterior SC at the appropriate LM location and never labels arborizations outside the immediate area of the TZ (Fig. 2). In EphB1$^{+/-}$ mice, a comparable DiI injection results in a normal appearing TZ in the appropriate location in the SC, but in addition ectopic TZs (eTZs) are labeled lateral to the appropriate TZ in 39% of EphB1$^{+/-}$ mice ($n = 39$ mice, $p < 0.03$ Fisher's exact test; Fig. 2B). EphB1$^{-/-}$ mice have qualitatively similar defects, with all eTZs found lateral to the appropriate TZ, but at a higher frequency than EphB1$^{+/-}$ mice (48% with eTZs, $n = 23$ mice, $p < 0.02$; Fig. 2C). Injections of DiI into the dorsal retina in EphB1$^{-/-}$ mice reveal no aberrancies in retinocollicular mapping in a limited number of cases (data not shown; $n = 4$).

2.2. EphB1, EphB2, and EphB3 null alleles produce similar aberrancies in DV retinocollicular maps with frequency of aberrant maps correlated to number of null alleles

To investigate the relative contribution of EphB1, EphB2, and EphB3 to DV retinotopic mapping, we analyzed retinocollicular maps in mice with combinations of null alleles for each receptor. We examined 18 of the 27 possible allelic combinations for triple mutants, with at least 9 cases examined for ten of the combinations, and at least 3 cases for an additional eight combinations (182 total mice; Fig. 3D). We have not been able to generate viable triple homozygous mutants, although Thakar et al. (2011) have reported them.

In mice with one or more mutant alleles of EphB1, EphB2, or EphB3, and in every combination of EphB1, EphB2, and EphB3 null alleles examined in sufficient numbers, including null mutants for each EphB, we find an aberrant topographic map in a subset of cases with one or more eTZs present lateral to a normal appearing TZ at the topographically appropriate site (Fig. 3). Axon branches are found to extend along the LM axis from the appropriate TZ to the eTZs, often ending with clearly identified arborizations forming an eTZ (Fig. 3C). We find no substantial qualitative difference in phenotype and the overall severity of the phenotype and the number of eTZs does not correlate neither with the number of null alleles nor with a null allele for any specific EphB receptor. For example, EphB1$^{+/-}$; EphB2$^{+/+}$; EphB3$^{+/-}$ mice have defects similar to EphB1$^{+/-}$; EphB2$^{+/-}$; EphB3$^{-/-}$ mice (Fig. 3B) and to EphB1$^{+/-}$; EphB2$^{+/-}$; EphB3$^{-+/-}$ mice (Fig. 3C). However, the total number of null alleles for EphB1, EphB2, and EphB3 does correlate strongly with the frequency of cases with eTZs in the SC (Fig. 3D). All 18 allelic combinations examined have mapping