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Effects of *trkB* knockout on topography and ocular segregation of uncrossed retinal projections

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Abstract

TrkB is an important receptor for brain-derived neurotrophic factor and NT4, members of the neurotrophin family. TrkB signaling is crucial in many activity-dependent and activity-independent processes of neural development. Here, we investigate the role of *trkB* signaling in the development of two distinct, organizational features of retinal projections—the segregation of crossed and uncrossed retinal inputs along the “lines of projection” that represent a single point in the visual field and the “retinotopic” mapping of retinofugal axons within their cerebral targets. Using anterograde tracing, we obtained quantitative measures of the distribution of retinal projections in the dorsal nucleus of the lateral geniculate body (LGd) and superior colliculus (SC) of wild-type mice and mice homozygous for constitutive null mutation (knock-out) of the full-length *trkB* receptor ($trkB_{FL}^{-/-}$). In $trkB_{FL}^{-/-}$ mice, uncrossed retinal projections cluster normally but there is a topographic expansion in the distribution of these clusters across the SC. By contrast, the absence of *trkB* signaling has no significant effect on the segregation of crossed and uncrossed retinal projections along the lines of projection in LGd or SC. We conclude that the normal topographic organization of uncrossed retinal projections depends upon *trkB* signaling, whereas the segregation of crossed and uncrossed retinal projections is *trkB*-independent. We also found that in $trkB_{FL}^{-/-}$ mice, neuronal number was reduced in the LGd and SC and in the caudate-putamen. Previous studies by ourselves and others have shown that the number of retinal ganglion cells (RGCs) is unchanged in $trkB_{FL}^{-/-}$ mice. Together, these results demonstrate that there is no matching of the numbers of RGCs with neuronal numbers in the LGd or SC.

Keywords

Lateral geniculate nucleus; Superior colliculus; Neurotrophin; Visual system

Introduction

Elucidation of the cellular mechanisms that contribute to the ontogeny of orderly neural connections is a major theme of developmental neurobiology. Retinal projections have been a

widely used experimental system for studies of this issue. In mammals, the dorsal nucleus of the lateral geniculate body (LGd) and the superior colliculus (SC) are, respectively, the principal thalamic and midbrain targets of the retina. The representation of the two-dimensional visual field is described in three dimensions of both these structures by a “point-to-line” transformation (Kaas et al. 1972; Frost et al. 1979; Frost and Caviness 1980): a point in the visual field is represented along a cylinder of tissue [termed a “line of projection” by neurophysiologists (Bishop et al. 1962)] that extends into the LGd or SC from its surface. Neighboring points in the visual field are represented within adjacent cylinders of tissue. This arrangement produces retinotopic maps of the visual field on the surface of each structure. In mature animals, within the representation of the binocular region of the visual field, inputs from the two eyes are segregated in non-overlapping zones along the lines of projection (Rakic 1977; Frost et al. 1979; Godement et al. 1984; Shatz and Kirkwood 1984; Reese 1986).

Ontogenetically, the mature mammalian pattern of retinal projections arises from a less distinct one. In both the LGd (Hubel et al. 1977; Rakic 1977; Frost et al. 1979; Bunt et al. 1983; Shatz 1983; Godement et al. 1984) and the SC (Frost et al. 1979; Land and Lund 1979; Godement et al. 1984), axons originating in the contralateral and the ipsilateral eyes, though biased toward their appropriate termination zones at the one or the other end of the lines of projection, initially overlap and progressively segregate. The retinotopic organization of both crossed (O’Leary et al. 1986; Simon and O’Leary 1990; Jhaveri et al. 1991; Hindges et al. 2002) and uncrossed (Frost et al. 1979; Land and Lund 1979; Cowan et al. 1984; Frost 1984; Godement et al. 1984; Insausti et al. 1985; Woo et al. 1985) retinocollicular axons is also progressively refined from an initially less focused pattern. The progressive topographic focusing of retinal projections occurs by a combination of axonal pruning and directed axonal growth (Sretavan and Shatz 1986; Simon and O’Leary 1990; Bhide and Frost 1991; Jhaveri et al. 1991). Elimination of inappropriately projecting retinal ganglion cells (RGCs) can also contribute to the topographic focusing of crossed (O’Leary et al. 1986) and uncrossed (Cowan et al. 1984; Insausti et al. 1984) retinal projections.

The emergence of eye-specific segregation is activity-dependent in both the LGd and SC (Shatz and Stryker 1988; Sretavan et al. 1988; Rossi et al. 2001; Muir-Robinson et al. 2002). At least in the SC, the topographic focusing of crossed (Simon et al. 1992; McLaughlin et al. 2003) and uncrossed (Cowan et al. 1984; Thompson and Holt 1989) retinal projections is also activity-dependent. The sets of cellular mechanisms by which electrical activity contributes to ocular segregation and retinotopic organization could be different but overlapping. For example, NMDA receptor signaling appears to contribute to the emergence of retinotopic order (Simon et al. 1992) but not to ocular segregation (Smetters et al. 1994). Brain-derived neurotrophic factor (BDNF)/trkB signaling is an important mediator of many activity-dependent processes (Poo 2001; Ernfors and Bramham 2003). In order to assess the contribution of trkB signaling to retinotopic order and ocular segregation, we examined these two features in mice that are homozygous for null mutation (knockout) of the full-length trkB receptor ($\text{trkB}_{\text{FL}}^{-/-}$). We found that in the absence of trkB signaling, the topographic distribution of uncrossed retino-SC projections is altered but ocular segregation is normal in LGd and SC. Thus, the topographic organization of uncrossed retinal projections appears to be determined by mechanisms that depend on trkB receptor signaling, whereas the segregation of crossed and uncrossed retinal projections is trkB independent.

Methods

Experimental subjects

The constitutive null mutation of trkB used in this study affects full length, but not truncated, trkB (Klein et al. 1993). WT mice and mice heterozygous for trkB_{FL} null mutation ($\text{trkB}_{\text{FL}}^{-/-}$),

both on a hybrid C57Bl6/129 background, were purchased for use as breeders from Jackson Laboratories (catalog numbers JR101045 and 002544, respectively). The $\text{trkB}_{\text{FL}}^{-/-}$ and WT mice used in this study of retinal projections were obtained by mating of $\text{trkB}_{\text{FL}}^{-/-}$ breeding pairs. Although most homozygous mice of the original trkB null mutant line, on a C57Bl/6 background, died by the end of the first postnatal week (Klein et al. 1993), a significant fraction of the hybrid line that we used survive to postnatal days 12–13 (see the Jackson Laboratories specification sheet at <http://jaxmice.jax.org/strain/002544.html>), allowing us to study their retinal projections at this relatively advanced stage of development (see “Discussion”). All procedures were performed blind to genotype.

Tracing of retinal projections

On postnatal day 10 or 11 (P10 or P11; P0 = day of birth), the mice were anesthetized by inhalation of Fluothane (ca. 2% in oxygen) and given bilateral intraocular injections of fluorescent-labeled cholera toxin B (CTB; Molecular Probes; 1% in a solution of 2% DMSO in distilled water; 1 μl /eye). Green fluorescent (Alexa 488 labeled) CTB was injected into one eye and red fluorescent (Alexa 546 labeled) CTB was injected into the opposite eye, using a transcorneal approach. Two days later, the mice were deeply anesthetized with Nembutal (60 mg/kg) and perfused transcardially with 0.9% NaCl in 0.1 M phosphate buffer (pH 7.2) followed by 4% paraformaldehyde in the same buffer. The brains were then cryoprotected by immersion in 30% sucrose buffer at 4°C.

Histology

The brains were frozen in dry ice and cut frozen in the coronal plane on a cryostat at 40 μm . Sections were mounted on Superfrost Plus slides (ThermoFisher). A one in four series of sections was cover slipped using Fluoromount-G (Southern Biotech) and reserved for confocal analyses, providing a 25% sample at a spacing of 160 μm . A second one in four series was stained with cresyl violet and used for the analysis of neuronal numbers and neuronal size.

Confocal microscopy

For each section, using a 10X (NA = 0.3) objective, Z-stacks of 5 μm optical slices were obtained using a Biorad confocal microscope on slow scan with kalman filtering.

Quantitative analysis

Retinal projections—Only cases in which the retinal projections of both eyes were completely labeled were used for quantitative analysis. The completeness of labeling was confirmed by examining the crossed retinal projections to the stratum griseum superficiale (SGS) of the SC, which normally contains a topographic representation of the entire contralateral retina. Any gap in the labeling of the crossed retinal input indicates damage to the contralateral retina or failure of tracer to spread into the corresponding region of the contralateral retina. 6 WT and 6 $\text{trkB}_{\text{FL}}^{-/-}$ mice were analyzed quantitatively. Quantitative analysis of the collapsed Z-stack from each section was performed using Image J software (National Institutes of Health).

We performed our quantitative analysis of the segregation of crossed and uncrossed retinal inputs along the lines of projection only in the LGd because the distribution of uncrossed retino-SC projections makes them unsuitable: Uncrossed retino-SC axons project densely to the deep ends of lines of projection in the stratum opticum (SO), but diffusely to the superficial regions in the SGS where crossed retino-SC projections are most dense. Thus, at the resolution of our analysis, even in normal animals, the crossed and uncrossed retino-SC projections largely overlap in their distribution along the lines of projection. At higher levels of resolution the

projections do not overlap (Lyckman et al. 2005)—a trivial consequence of the fact that two different axons cannot occupy the same space exactly.

We determined the volume of crossed (V_C) and uncrossed (V_U) retinogeniculate projections (C and U, respectively, in Fig. 1a) and the total volume of the LGd (V_{LGd}) by measuring the cross-sectional area of the projections and of the LGd as seen in the collapsed Z-stack of each section, adding the measures obtained from all the sections through the LGd and multiplying by the section spacing. We quantified the segregation of crossed and uncrossed retinogeniculate projections by dividing the sum of V_C and V_U by $V_{LGd}[(V_C + V_U)/V_{LGd}]$ to derive a segregation index (SI). SI = 1 indicates perfect segregation; SI > 1 indicates overlap; SI < 1 indicates the existence of LGd regions with no retinal projection.

We performed our quantitative analysis of retinotopic mapping only in the SC because the regionally varying orientation of lines of projection in the LGd and the distance of the uncrossed projections from the surface of the LGd render such an analysis impractical in that structure (Frost and Schneider 1979). Because the rodent SC contains a representation of the entire contralateral retina on its dorsal surface (Drager and Hubel 1976; Frost and Schneider 1979; Godement et al. 1984), the crossed retinal projection was used to define the surface extent of the SC. In the rodent SC, unlike in the LGd, the lines of projection representing a single point in the visual field are in a plane close to the plane of sectioning (Frost and Schneider 1979). This permitted us to perform one-, two- and three-dimensional analyses of the distribution of uncrossed retinal projections within the SC. Our analysis includes the uncrossed retinal projections to the SGS and stratum opticum (SO) that are biased toward the rostrolateral quadrant of the SC [and that lie within the representation of the binocular visual field in normal animals (U in Fig. 1b–e)], but excludes a longitudinally oriented cylinder of projections at the medial edge of the SO that lies outside the representation of the binocular visual field [(U_X in Fig. 1b–e); see (Drager 1974; Drager and Hubel 1976; Frost and Schneider 1979; Godement et al. 1984)].

For the one-dimensional analysis, we calculated the anterior–posterior span of the SC (S_{SC} , Fig. 1b) as the number of sections containing the SC multiplied by the section spacing. We calculated the anterior–posterior span of the uncrossed retinal projection (S_U , Fig. 1b) as the number of sections containing the uncrossed retino-SC projection multiplied by the section spacing. Similarly, we calculated the span from the anterior tip of the SC to the posterior limit of the uncrossed retinal projection (S_{UPOST} , Fig. 1b).

For the two-dimensional analysis, we measured the surface area of the SC (A_{SC}) by summing, for all sections through the SC, the length of the surface of the SGS as seen in each section (L_{SC} in Fig. 1c) and multiplying by the section spacing. The area of the SC surface that represents the ipsilateral eye (A_U) was determined by projecting the borders of the regions containing uncrossed retino-SC projections onto the surface of the SGS, adding, for all sections, the lengths of the segments of the SGS surface onto which the uncrossed projection was projected (sum of the segments labeled L_U in Fig. 1c) and multiplying by section spacing.

For the three-dimensional analysis, we measured the volume of the SGS (V_{SGS}) by summing the cross sectional area of the SGS as seen in all sections through the SC and multiplying by section spacing. Similarly, we determined the volume of the uncrossed retino-SC projection (V_U) by adding, for all sections, the areas of regions containing uncrossed retino-SC projections (sum of the areas labeled U in Fig. 1c) and multiplying by section spacing. For analysis of SGS thickness, in each section, we measured the thickness of the SGS at the mid point of the medial–lateral extent of the SC and, using ImageJ, averaged across all the sections from each animal to obtain a measure for that animal.

The primary quantitative measurements for each case were average measures for the right and left sides of the brain. We confirmed that data were normally distributed using an equality of variance *F*-test (Statview) and for each of the measures in LGd and SC, the statistical significance of differences between WT and $\text{trkB}^{-/-}$ mice was determined using a two-tailed *t*-test.

Neuronal number—Using the optical disector method (Coggeshall and Lekan 1996) we estimated the number of neurons in the LGd ($n = 4/\text{genotype}$), SC ($n = 5/\text{genotype}$) and (as a non-visual control structure that also expresses trkB (Merlio et al. 1992; Altar et al. 1994) the caudate-putamen ($n = 5/\text{genotype}$). For every section through each of these structures, we determined neuronal density in three $100 \times 100 \mu\text{m}$ sampling regions and then multiplied the average density within each structure by the total volume of that structure to estimate neuronal number. The samples spanned the dorso-ventral extent of the LGd and caudate-putamen, and the medio-lateral extent of the SC. Neurons were identified using morphological criteria: Nissl stained neurons have round, densely stained profiles that are distinct from astrocytes and oligodendroglia (which have little cytoplasm and lighter nissl staining) and blood vessels (Oorschot 1996).

To ensure that the optical disector method could be accurately employed, we confirmed that neuronal soma size was not significantly different between genotypes by measuring the cross sectional area of neuronal cell bodies in each brain region (LGd, SC and caudate-putamen). Four sections that were randomly distributed through the rostro-caudal extent of each region were photographed at $1000\times$ magnification. The cell body area of 10–20 randomly selected cells was measured from each photograph, using ImageJ image analysis. Areas were averaged for each animal and then for each genotype and values compared using a two-tailed *t*-test.

Results

Qualitative analysis

The overall pattern of retinal projections is similar in WT and $\text{trkB}_{\text{FL}}^{-/-}$ mice (Fig. 1d, e). In the LGd of both genotypes, crossed retinal input fills all of the LGd except for a small region adjoining the medial surface of the LGd (at the deep ends of the lines of projection), which receives uncrossed retinal input. In the SC of both genotypes, the crossed retinal projection terminates densely within all of the SGS except for sectors in the deep 1/3 of the SGS rostromedially, where the projection is sparser (Fig. 1d, e). There are also terminating crossed retinal inputs within the SO. As seen in coronal sections, the uncrossed retino-SC projection consists principally of clusters of label that span the interface of the SO and SGS. Occasional, generally sparse patches of label may extend more superficially into the SGS. At most levels of the SC, there is also a dense patch of uncrossed retinal input at the medial extremity of the SO that is not included in our quantitative analysis because it is not included within the representation of the binocular part of the visual field (Drager and Hubel 1976). These findings are consistent with previous reports in rodents (Drager and Hubel 1976; Frost et al. 1979; Land and Lund 1979; Cowan et al. 1984; Frost 1984; Godement et al. 1984; Insausti et al. 1985; Woo et al. 1985).

Quantitative analysis

Topographic analysis in the SC—In $\text{trkB}_{\text{FL}}^{-/-}$ mice, the anterior–posterior span of the SC (S_{SC} , Fig. 1b) is reduced to 77% of that in WT mice (Fig. 2). Despite this shrinkage, the anterior–posterior span of the uncrossed retino-SC projection (S_{U} , Fig. 1b) increases absolutely by about 13% (Fig. 2). As a consequence of the shrinkage of the SC and the expansion of the uncrossed retino-SC projection, the anterior–posterior span of the uncrossed retino-SC projection as a

fraction of the anterior–posterior span of the SC (S_U/S_{SC} , Fig. 2) increases from about 49% in WT mice to about 67% in $\text{trkB}_{\text{FL}}^{-/-}$ mice, i.e., about 1.4 times the normal value. Similarly, the posterior limit of the uncrossed retinal projection (S_{UPOST} , Fig. 1b) shifts from about 52% of the distance from the anterior to the posterior limit of the SC (S_{UPOST}/S_{SC} , Fig. 2) in WT mice to about 78% of that distance in $\text{trkB}_{\text{FL}}^{-/-}$ mice, i.e., 1.5 times the normal value.

The surface area of the SC (A_{SC}) in $\text{trkB}_{\text{FL}}^{-/-}$ mice is reduced to 73% of that in WT mice (Fig. 2). Despite this, in $\text{trkB}_{\text{FL}}^{-/-}$ mice the absolute area of the representation of the ipsilateral eye on the surface of the SC (A_U) is about 2.5-fold that in WT mice (Fig. 2). As a result of these two changes, in $\text{trkB}_{\text{FL}}^{-/-}$ mice the percentage of the surface of the SC that receives uncrossed (and crossed) retinal input (A_U/A_{SC} , Fig. 2) is about 3.5-fold the percentage in WT mice (44.3 and 12.6%, respectively, Fig. 2). Thus, the percentage of the visual field representation that receives binocular retinal input is enlarged in $\text{trkB}_{\text{FL}}^{-/-}$ mice. Because, in $\text{trkB}_{\text{FL}}^{-/-}$ mice, the relative surface area of the uncrossed retino-SC projection increases 3.5-fold and the anterior–posterior extent of the projection increases only 1.5-fold, one can calculate that the relative medial–lateral extent of the uncrossed projection is increased (on average, by about 2.3-fold). Taken together, the increased anterior–posterior span and increased surface area of the uncrossed retinal projection in $\text{trkB}_{\text{FL}}^{-/-}$ mice suggest a spread of uncrossed retinal inputs into parts of the contralateral visual field representation that normally receive only crossed retinal input.

In $\text{trkB}_{\text{FL}}^{-/-}$ mice, the volume of the SGS (V_{SGS}) is reduced to about 64% of the WT value, due to reductions in both its area and the thickness (Fig. 2). Despite this, the volume of the uncrossed retino-SC projection (V_U) expands to about 2.2 times the volume in WT mice and the volume of the uncrossed retinal projection as a fraction of the volume of the SGS (V_U/V_{SG}) increases from about 2 to 8% (Fig. 2).

Ocular segregation analysis in the LGd—In $\text{trkB}_{\text{FL}}^{-/-}$ mice, the volumes of the LGd and of the crossed and uncrossed retinal projections (V_{LGd} , V_C and V_U , respectively, Fig. 3) are significantly reduced to approximately 40% of the corresponding measures in WT mice. Despite this reduction, there is no significant difference between the two genotypes in the relative sizes of the crossed and uncrossed projections (93 and 11% of total LGd volume, respectively, Fig. 3) or in the mean “segregation index”. Thus, although the absolute sizes of the LGd and the crossed and uncrossed retinal projections are significantly reduced in $\text{trkB}_{\text{FL}}^{-/-}$ mice, the proportion of the nucleus in which retinal projections overlap (about 4%), is not significantly different across genotypes ($\text{SI} = 1.041 \pm 0.036$ for WT and 1.038 ± 0.017 for $\text{trkB}_{\text{FL}}^{-/-}$ mice, respectively, Fig. 3).

Neuronal numbers—In $\text{trkB}_{\text{FL}}^{-/-}$ mice, the total number of neurons (Fig. 4) is reduced to 41, 68 and 66% of WT values in LGd ($P = 0.0002$), SC ($P = 0.003$) and caudate-putamen ($P = 0.005$), respectively. This loss of neurons is accompanied by shrinkage of all three structures (LGd: Fig. 3; SC: Fig. 2; caudate putamen: WT: $3.64 \pm 0.38 \text{ mm}^3$; $\text{trkB}_{\text{FL}}^{-/-}$: $2.60 \pm 0.35 \text{ mm}^3$). However, there is no significant difference between WT and $\text{trkB}_{\text{FL}}^{-/-}$ mice with respect to the density of neurons in the LGd (WT: $135,829 \pm 11,403 \text{ cells/mm}^3$; $\text{trkB}_{\text{FL}}^{-/-}$: $138,461 \pm 12,493 \text{ cells/mm}^3$; $P = 0.76$), SC (WT: $26,819 \pm 4,393 \text{ cells/mm}^3$; $\text{trkB}_{\text{FL}}^{-/-}$: $28,527 \pm 4452 \text{ cells/mm}^3$; $P = 0.56$) or caudate-putamen (WT: $130,427 \pm 12,354 \text{ cells/mm}^3$; $\text{trkB}_{\text{FL}}^{-/-}$: $121,096 \pm 13,311 \text{ cells/mm}^3$; $P = 0.37$). The cross sectional area of neuronal somata was not significantly different in $\text{trkB}_{\text{FL}}^{-/-}$ mice (LGd: $106.10 \pm 32.76 \mu\text{m}^2$; SC: $85.20 \pm 27.70 \mu\text{m}^2$;

caudate putamen: $81.65 \pm 23.97 \mu\text{m}^2$) compared to WT mice (LGd: $128.64 \pm 40.96 \mu\text{m}^2$; SC: $80.85 \pm 19.81 \mu\text{m}^2$; caudate putamen: $87.99 \pm 27.54 \mu\text{m}^2$). Because there are no significant changes in neuronal density or soma size, the shrinkage of the LGd, SC and caudate putamen in $\text{trkB}_{\text{FL}}^{-/-}$ mice must be due principally to the loss of neurons in those structures.

Discussion

The normal, mature ocular segregation and topographic organization of retinal projections are both activity-dependent. This raises the question of whether activity exerts its effects on these two organizational features using similar cellular mechanisms. A vast literature has demonstrated the role of BDNF/trkB signaling in mediating the effects of electrical activity on the development and plasticity of neural connections (Poo 2001; Ernfors and Bramham 2003), although the specific role of activity is incompletely understood (Huberman et al. 2003). Thus, we investigated the role of trkB signaling on the topography and ocular segregation of retinal projections. Our principal findings are that in $\text{trkB}_{\text{FL}}^{-/-}$ mice, (1) the number of neurons in the LGd and SC is reduced and contributes to a reduction in the size of these structures; (2) uncrossed retinal projections to the SC cluster normally; (3) the uncrossed retino-SC projection expands absolutely across the surface of the SC despite an absolute shrinkage in SC surface area (Fig. 5a); (4) segregation of uncrossed and crossed projections along lines of projection is qualitatively normal in both the SC and LGd, an observation that is confirmed by quantitative analysis in LGd (Fig. 5b). These data indicate that the normal topographic organization of uncrossed retinal projections depends upon trkB signaling, whereas eye-specific segregation of retinal projections is trkB-independent. The respective roles of the two trkB ligands, BDNF and NT4, in topographic organization are incompletely understood, although cross-phylum comparison suggests that NT-4 may have become dispensable in some vertebrate classes (Hallböök et al. 2006).

Our data show a significant loss of neurons in both the LGd and SC of $\text{trkB}_{\text{FL}}^{-/-}$ mice. Although the number of neurons in the LGd, SC and caudate putamen is reduced compared to WT, the density of neurons is not significantly altered. The data from WT mice are in line with those of previous studies in juvenile or adult rats and mice when differences in brain size due to age, species and/or inbred mouse lines are taken into account LGd: (Seecharan et al. 2003; Ito et al. 2008; SC: Smith and Bedi 1997; caudate putamen: Oorschot 1996; Rosen and Williams 2001; McCarthy et al. 2007). Our data are also consistent with the reduction in neuronal number in the visual cortex of mice with forebrain-specific conditional null mutation of trkB (Xu et al. 2000) and the number of neurons in the corpus striatum of mice with a Dlx5/6 conditional null mutation of trkB (Baydyuk et al. 2008). The $\text{trkB}_{\text{FL}}^{-/-}$ induced loss of neurons in the caudate-putamen demonstrates that similar alterations can occur in central neurons that express trkB both within and outside the visual system. TrkB knockout does not alter the survival of retinal ganglion cells (Rohrer et al. 2001; Pollock et al. 2003), thus illustrating that neuronal survival is not similarly affected by the mutation in all visual (or central nervous system) structures. In $\text{trkB}_{\text{FL}}^{-/-}$ mice, the lack of effect on RGC number, in combination with the reduced number of LGd neurons, demonstrates that there is no numerical matching between these two neuronal populations. This conclusion is supported by comparisons across mouse lines of the numbers of RGCs and LGd neurons (Seecharan et al. 2003). Thus, during normal development, anterograde transport of endogenous BDNF and/or NT4 may not have a major role in determining the survival of immature LGd neurons. However, higher levels of *exogenous* BDNF transported anterogradely from the eye or *exogenous* NT4 transported retrogradely from the cortex can protect LGd neurons from dysfunction or degeneration due to visual cortex lesions (Caleo et al. 2003) or monocular deprivation (Riddle et al. 1995), respectively.

Our finding that $\text{trkB}_{\text{FL}}^{-/-}$ null mutation does not affect ocular segregation is consistent with previous data demonstrating that null mutation of the BDNF gene does not alter the segregation of crossed and uncrossed retinal projections (Lyckman et al. 2005). Serotonin (Upton et al. 1999), nitric oxide (Vercelli et al. 2000; Wu et al. 2000), calcium influx (Cork et al. 2001) and adenylate cyclase (Ravary et al. 2003) have all been implicated in ocular segregation and may mediate the effects of electrical activity on this organizational feature of retinal projections.

The combination of absolute increases in the anterior–posterior span, posterior limit and surface area of the uncrossed retino-SC projection, despite absolute decreases in all three dimensions of the SC, assures that there is a real increase in the topographic extent of the projection, not simply a relative increase due to the shrinkage of the SC. Previous studies show that following neonatal partial retinal lesions, the projections of intact retinal regions can spread into zones normally innervated by the ablated parts of the retina; the increased spread appears to be due to reduced competition among retino-SC axons (Frost and Schneider 1979). In the present study, the trkB knockout-induced expansion of the uncrossed retinal projection may also be due to reduced competition among retino-SC axons in the plane of the retinotopic map. TrkB-dependent axonal competition occurs in the visual cortex, where trkB signaling regulates the focusing of thalamocortical projections into ocular dominance columns (Cabelli et al. 1995; Cabelli et al. 1997).

Our data demonstrate that trkB signaling is required in order to attain the normal topographic distribution of uncrossed retino-SC projections. This result is consistent with observations that focal over-expression of BDNF in the SC, prior to and during the period when the topography of uncrossed retinal projections is normally refined, results in corresponding, topographically inappropriate foci of uncrossed retinal projections (Isenmann et al. 1999). TrkB signaling could be part of a phylogenetically conserved mechanism to focus uncrossed retinal input to the representation of the binocular visual field, despite variations across species in the laterality of the eyes and, consequently, in the size of the binocular field. Although this process may be activity-dependent, it is not vision-dependent, as it is virtually complete before the photoreceptor layer becomes synaptically connected to the rest of the retina (Weidman and Kuwabara 1968; Cowan et al. 1984). As we have found for trkB signaling, NMDA receptor signaling is not required for ocular segregation (Smetters et al. 1994), although it is necessary for the normal topographic organization of retinal projections (Simon et al. 1992). By contrast, serotonin (Upton et al. 1999) contributes to both processes. Thus, different but overlapping sets of cellular mechanisms mediate the ocular segregation and topographic organization of uncrossed retinal projections.

An interesting feature of our data is that although null mutation of trkB disrupts the overall topographic distribution of uncrossed retino-SC projections, it does not disrupt their clustering in discrete regions within the overall zone of termination, even though clustering and retinotopia are both represented in the plane parallel to the surface of the SC. This may be due to the fact that crossed retinal projections extend into the SO, where uncrossed retinal projections are concentrated (Frost 1984; Godement et al. 1984). Clustering in the SO, although it occurs in the same plane as retinotopia, is likely to reflect ocular segregation, as it does in the visual cortex of carnivores and primates (Hubel and Wiesel 1962; Hubel and Wiesel 1977), where both features are also represented in the same plane. Thus, trkB signaling does not appear to contribute to ocular segregation along lines of projection *or* in the plane of topographic representation, even though it does regulate the topographic distribution of the uncrossed retinal projection.

It might be argued that the abnormally widespread topographic distribution of uncrossed retino-SC projections in $\text{trkB}_{\text{FL}}^{-/-}$ mice is simply due to a retardation of the normal refinement process.

This cannot be studied directly in mice with constitutive null mutation of trkB_{FL} , owing to their limited life span. However, several observations argue against this possibility: (1) The rate of developmental elimination of RGCs in $\text{trkB}_{\text{FL}}^{-/-}$ mice is accelerated compared to WT mice, rather than retarded (Pollock et al. 2003). Indeed, by P14 the total number of RGCs in $\text{trkB}_{\text{FL}}^{-/-}$ mice is normal (Rohrer et al. 2001). (2) The clusters of uncrossed projections in the SC (“U” in Fig. 1b, c) are initially continuous and develop into discrete patches (Frost et al. 1979; Godement et al. 1984). Normal clustering of the uncrossed retino-SC projection in $\text{trkB}_{\text{FL}}^{-/-}$ mice (Fig. 1d, e), despite a topographically expanded distribution, also argues that the topographic effects of trkB null mutation are not due to developmental retardation.

Conclusions

In $\text{trkB}_{\text{FL}}^{-/-}$ mice, (1) uncrossed retinal projections to the SC cluster normally but have an enlarged topographic distribution; (2) segregation of uncrossed and crossed projections is qualitatively normal in both the SC and LGd, an observation that is confirmed by quantitative analysis in LGd. These data indicate that the normal topographic organization of uncrossed retinal projections depends upon trkB signaling, whereas eye-specific segregation of retinal projections is trkB -independent.

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Fig. 1.

Crossed and uncrossed projections in the LGd and SC. **a** Schematic outline of the LGd as seen in a coronal section, showing the termination regions of the crossed (C: unshaded) and uncrossed (U: shaded) retinal inputs. OT is the optic tract. **b** Schematic dorsal view of the surface of the entire SC. Terminating crossed retinal inputs (C) are distributed across the entire extent of the SC, principally in the SGS, but also in the stratum opticum (SO). Individually variable, uncrossed retinal inputs (U) terminate in longitudinally oriented patches, principally in the SO but also in the SGS. The medial-most patch (U_X) is excluded from our quantitative analysis (see text). S_{SC} , S_U and S_{UPOST} indicate, respectively, the anterior–posterior span of the SC, the anterior–posterior span of the quantitatively analyzed, uncrossed retino-SC projections and the span from the anterior tip of the SC to the posterior tip of the uncrossed retino-SC projections. The horizontal line represents the level of a coronal cut through the SC, which is illustrated in frame **c**. **c** Schematic outline of the SC as seen in coronal section (perpendicular to the surface view in **b**). L_C and L_U are as explained in “Methods”. U and U_X are as in **b**. **d** Montages of coronal sections (as schematized in **a** and **c**) spanning the rostro-caudal extent of the LGd and SC in WT mice. For each panel, crossed projections are labeled in green and uncrossed projections in red. For the LGd, sections are separated by 240 μm , and for the SC, by 360 μm . Arrows in LGd micrographs indicate the border of the LGd with the ventral nucleus of the lateral geniculate body, which also receives retinal input. PT pretectum; other abbreviations are as defined above and in the text. Scale as in **e**. **e** Montages of coronal

sections spanning the rostro-caudal extent of the LGd and SC in $\text{trkB}_{\text{FL}}^{-/-}$ mice. All conventions and abbreviations are as in **d**. *Scale bars* LGd 200 μm ; SC 100 μm

Fig. 2.

Anterior–posterior (A–P) spans, surface area and volume of the SC and its crossed and uncrossed retinal inputs. Histograms are group means, error bars are standard deviations. Note that the numerical scale on the Y axes applies both to raw measurements and to ratios (shown on the far right of each histogram), but the dimension does not apply to the ratios. Asterisks indicate that for all measures, the differences between WT and *trkB*^{-/-} mice are statistically significant ($P < 0.0001$ for all measures except for S_U , for which $P = 0.0002$). The techniques used to determine all measures are described in the “Methods”. S_{SC} , S_U and S_{UPOST} are, respectively, the A–P spans of the SC and the uncrossed retino-SC projection and the distance from the anterior limit of the SC to the posterior limit of the uncrossed retino-SC projection.

A_{SC} and A_U are, respectively, the surface area of the SC and the area of the uncrossed retinal input as projected onto the surface of the SC. V_{SGS} and V_U are, respectively, the volumes of the SGS and the uncrossed retino-SC projection. The *inset* shows the average thickness of the SGS

Fig. 3.

Volume of the LGd, its crossed and uncrossed retinal inputs (V_{LGd} , V_C and V_U , respectively) in WT and $trkB_{FL}^{-/-}$ mice. The techniques used to determine all measures are described in “Methods”. The segregation index (SI ; inset) is a measure of the extent of segregation of the crossed and uncrossed retino-SC projections. $SI = 1$ indicates perfect segregation; $SI > 1$ indicates overlap; $SI < 1$ indicates the existence of LGd regions with no retinal projection.

Asterisks indicate significant differences between WT and $trkB_{FL}^{-/-}$ mice for the volume measures but not for the segregation index

Fig. 4. Number of neurons in the SC, LGd and caudate putamen of TrkB^{-/-} mice compared to WT. Asterisks indicate that in all three regions, the differences between WT and TrkB^{-/-} mice are statistically significant ($P < 0.05$). Error bars are standard deviations

Fig. 5.

Summary of our findings of the effects of *trkB* null mutation on the distribution of crossed (C) and uncrossed (U) retinal projections. **a** Surface view of the SC. The crossed retinal input (not labeled) is distributed across the entire surface of the SC and forms a retinotopic map of the visual field (see text). In *trkB*^{-/-} mice, there is an absolute increase in the areal spread of the uncrossed retinal input across the surface of the SC and the surface area of the SC is diminished, so that the topographic organization of the uncrossed projection is altered. Thus, the topographic organization of the uncrossed retino-SC projection depends upon *trkB* signaling. **b** Coronal sections through the LGd show that the segregation of retinal inputs from

the two eyes is unaffected by *trkB* null mutation, although the LGd is reduced in size. Thus, the segregation of crossed and uncrossed retinal projections is *trkB*-independent. *D* dorsal, *V* ventral, *M* medial, *L* lateral, *A* anterior, *P* posterior