Visual Neuroscience (2005), **22**, 27–36. Printed in the USA.

DOI: 10.1017/S095252380522103X

Normal eye-specific patterning of retinal inputs to murine subcortical visual nuclei in the absence of brain-derived neurotrophic factor

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(Received March 9, 2004; Accepted October 18, 2004)

Abstract

Brain-derived neurotrophic factor (BDNF) is a preferred ligand for a member of the tropomyosin-related receptor family, trkB. Activation of trkB is implicated in various activity-independent as well as activity-dependent growth processes in many developing and mature neural systems. In the subcortical visual system, where electrical activity has been implicated in normal development, both differential survival, as well as remodeling of axonal arbors, have been suggested to contribute to eye-specific segregation of retinal ganglion cell inputs. Here, we tested whether BDNF is required for eye-specific segregation of visual inputs to the lateral geniculate nucleus and the superior colliculus, and two other major subcortical target fields in mice. We report that eye-specific patterning is normal in two mutants that lack BDNF expression during the segregation period: a germ-line knockout for BDNF, and a conditional mutant in which BDNF expression is absent or greatly reduced in the central nervous system. We conclude that the availability of BDNF is not necessary for eye-specific segregation in subcortical visual nuclei.

Keywords: Optic tract, Neurotrophic factor, Synaptic specificity, Plasticity, Competition

Introduction

The segregation of the retinal inputs from the two eyes is an established model system for understanding the developmental mechanisms that pattern neural circuits. Neuronal activity appears to be an essential patterning determinant in this system. A large body of work has shown that normal eye-specific segregation of retinal inputs to the dorsal nucleus of the lateral geniculate (LGd) is dependent on activity (Shatz & Stryker, 1988; Penn et al., 1998; Cook et al., 1999; Sengpiel & Kind, 2002; Huberman et al., 2003). Segregation and refinement of ipsilateral and/or contralateral retinal inputs to the superior colliculus (SC) also requires activity (Meyer, 1982; Reh & Constantine-Paton, 1985; Schmidt & Tieman, 1985; Thompson & Holt, 1989). Although there is a propensity for the two eyes to establish innervation within their appropriate eye-specific layers even in the absence of retinal activity in one or both eyes, retinogeniculate projections show incomplete segregation in the absence of activity (Cook et al., 1999; Huberman et al., 2003). Moreover, blockade of retinal activity after normal segregation is established subsequently leads to desegregation (Chapman, 2000).

Activation of trkB by BDNF profoundly influences synaptic transmission (Tyler et al., 2002; Zakharenko et al., 2003) and synaptic plasticity (Bartoletti et al., 2002; Messaoudi et al., 2002; Minichiello et al., 2002; Ernfors & Bramham, 2003; Jiang et al., 2003), and is widely held as a central molecular link through which patterned synaptic activity controls the differentiation of neurons and their synaptic connections (Bonhoeffer, 1996; Berardi & Maffei, 1999; Klintsova and Greenough, 1999; Thoenen, 2000). During development of the primary visual cortex, perturbing the activation of trkB during the critical period for ocular dominance plasticity inhibits the consolidation of ocular dominance columns (Cabelli et al., 1995; Galuske et al., 1996; Cabelli et al., 1997; Hata et al., 2000). Conversely, early enhancement of BDNF expression in the forebrain accelerates the critical period for ocular dominance plasticity and the development of visual acuity (Hanover et al., 1999; Huang et al., 1999). Both trkB and BDNF are expressed by retinal ganglion cells during the development of retinofugal pathways (Frost et al., 2001; Vecino et al., 2002) as well as by cells in the retinorecipient target zones (Allendoerfer et al., 1994; Cabelli et al., 1996; von Bartheld, 1998; Lein et al., 2000; Lein & Shatz, 2000; Silver & Stryker, 2001). Activation of trkB by BDNF

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promotes the growth (Atkinson et al., 1999; Bosco & Linden, 1999; Lom & Cohen-Cory, 1999; Tropea et al., 2003) and maintenance (Isenmann et al., 1999) of retinal terminal arbors. Thus, either through effects on activity-dependent synaptic rearrangement or on axonal terminal arborization, trkB activation by BDNF may also mediate activity-dependent patterning in the subcortical visual nuclei. Here, we tested whether such signaling was required for segregation of eye-specific inputs in subcortical target zones, a process that is highly dependent on retinal ganglion cell activity. Rather unexpectedly, our results show that eye-specific patterning of retinal inputs to subcortical targets is normal in mouse mutants which lack BDNF expression.

Materials and methods

Control and BDNF transgenic mouse strains

All procedures on live animals were approved by the Committee on Animal Care at MIT. We have analyzed the following two strains of BDNF mutant mice and their littermate controls.

1. BDNF knockout mice (BDNF-KO). The genetic background of these mice is pure129/Svjae. The generation of this mutation has been described (Ernfors et al., 1994). The homozygous mutant mice, which do not survive past the age of 3 weeks postnatally, were produced by intercrossing BDNF+/− mice with normal longevity. The lethality of BDNF−/− (BDNF-KO) is likely caused by severe sensory deficits in the peripheral nervous system (Ernfors et al., 1994). Wild-type or heterozygous littermates served as controls.
2. BDNF conditional mutant mice (BDNF-CM). These mice were derived by intercrossing the following two strains of mice: (1) Conditional $BDNF^{2lox}$ allele mice in 129/Svjae-Balb/c genetic background (Rios et al., 2001); (2) nestin-cre transgenic mice with backcrossing into 129/Svjae background (Bates et al., 1999; Fan et al., 2001). The littermate control $BDNF^{2lox/+}$ and conditional mutant mice Nestin-cre; $BDNF^{2lox/2lox}$ (BDNF-CM) were used in the experiments. Nestin-cre mediated BDNF gene deletion in the brain starts at E9–10 and is completed by E12.5. In contrast to the early postnatal lethality, BDNF-CM mice survive well into adulthood over the age of one year and older (G. Fan et al., unpublished data).

Anterograde tracing of retinal projections

Eye-specific projection patterns in the various subcortical, retinorecipient targets were examined after intraocular injections of cholera toxin B (CTB) or fluorescent CTB-conjugates (List Biological Labs., Campbell, CA). We first examined the ipsilateral projections in BDNF-KO and wild-type mice by monocular injections of CTB. Prior to eye injections, animals were anesthetized with Avertin (Aldrich, Milwaukee, WI; 5 μl/g for BDNF-knockouts; 16μl/g for conditional mutants and controls). The sclera was pierced at the limbus with a 26-gauge needle and the posterior chamber of the eye was injected with 1 μl of a 1% CTB solution in normal saline using a 30-gauge Hamilton microsyringe. Animals were revived under a heat lamp and returned to home cages. Two days later, animals were terminally anesthetized with sodium pentobarbital (100 mg/kg) and transcardially perfused, sequentially, with phosphate-buffered saline (PBS, pH 7.4) and 4% paraformaldehyde in PBS. Brains were dissected out, postfixed for 2 h, equilibrated in 30% sucrose, and then sectioned in the coronal plane at 40 μm on a freezing-sliding microtome. Sections were processed for CTB immunohistochemistry as previously described (Lyckman et al., 2001). Digital images were acquired under bright-field optics (Zeiss Axiophot).

Secondly, we directly examined the disposition of the both ipsilateral and contralateral retinal projections simultaneously in BDNF-KO, BDNF-CM, and control mice by injecting (as described above for CTB) the right eye with CTB-rhodamine and the left eye with CTB-fluorescein (List Biological Labs.). In addition, we also examined retinal projections prior to eye-specific segregation at P2 in the wild type. Sections were obtained as described above, omitting the immunohistochemistry, and were mounted in Vectashield (Vector Labs., Burlingame, CA) under cover glass and examined with a laser scanning confocal microscope (Biorad MRC 1024 ES, Mercury, CA).

Image analysis

To examine effects of BDNF on the size of the ipsilateral projection, we examined digitized, bright-field images of the LGd, taken at five locations spanning its rostrocaudal extent, in wild-type and BDNF-KO animals that had received monocular injections of CTB. Images were set a to 50% threshold and binarized, and pixels representing retinal innervation of the LGd were counted (ImageJ software, RSB/NIH).

To examine the effects of BDNF on local patterning of ipsilateral and contralateral retinal terminals in LGd, we obtained digitized, confocal images of the dorsal half of the LGd in animals that had received intraocular injection of red (right) and green (left) fluorescent CTBs. Each channel (red or green) of these images was set to a 50% threshold and binarized. We then asked, for each nonzero pixel in the ipsilateral channel, what percentage of the nonzero pixels (whether ipsilateral or contralateral) in concentric cells (in 5-pixel steps) surrounding that pixel were also ipsilateral pixels. These results are presented as:

$$\frac{\text{Number of ipsilateral pixels}}{\text{Number of ipsilateral + Contralateral pixels}} \times 100\%.$$

Unlabeled gaps, that is, pixels that failed to reach the binarization threshold, were not included in the analysis. If ipsilateral pixels were locally clustered, then this percentage would be highest for the innermost shells, and would drop off with increasing shell diameter. Measurements were averaged for each shell diameter and each ipsilateral pixel across the entire image, and then all measurements from all images for each mouse strain were averaged and plotted (MatLab, The Mathworks, Natick, MA). We then statistically compared these curves to determine if this index of local ipsilateral clustering differed between mutants and controls (Statview). As a further control, we performed the same analysis using shuffled binarized images of the ipsilateral channel, demonstrating that the technique reveals considerable local order in the unshuffled images (see Results).

Results

To determine if BDNF expression is required for eye-specific segregation of retinal projections, we examined the ipsilateral and

contralateral retinal projections in mature control and BDNF transgenic mice, focussing on four retinorecipient subcortical nuclei: the lateral geniculate nucleus, the superior colliculus, the suprachiasmatic nucleus, and the medial terminal nucleus. Systemic absence of BDNF expression is well documented for the BDNF-KO mice (Ernfors et al., 1994; Fan et al., 2000; Kernie et al., 2000). For the BDNF-CM mice, the BDNF-flox strain is also well documented using crosses with the Cre-CamKII strain that expresses late in development (Rios et al., 2001). Here, we show that the Cre-nestin strain (Fan et al., 2001) we have used in the present study shows virtually complete neural recombination in the thalamus and midbrain (Fig. 1A) (encompassing the target zones we study here), and substantial recombination in the ganglion cell layer of the retina (Fig. 1B). Thus, the targets of the retina are deprived of locally synthesized BDNF in both mutants. Retinal ganglion cells are deprived completely in the BDNF-KO and significantly in the BDNF-CM mice, though a small number of retinal cells may have persistent BDNF expression in the BDNF-CM mice during the developmental periods examined.

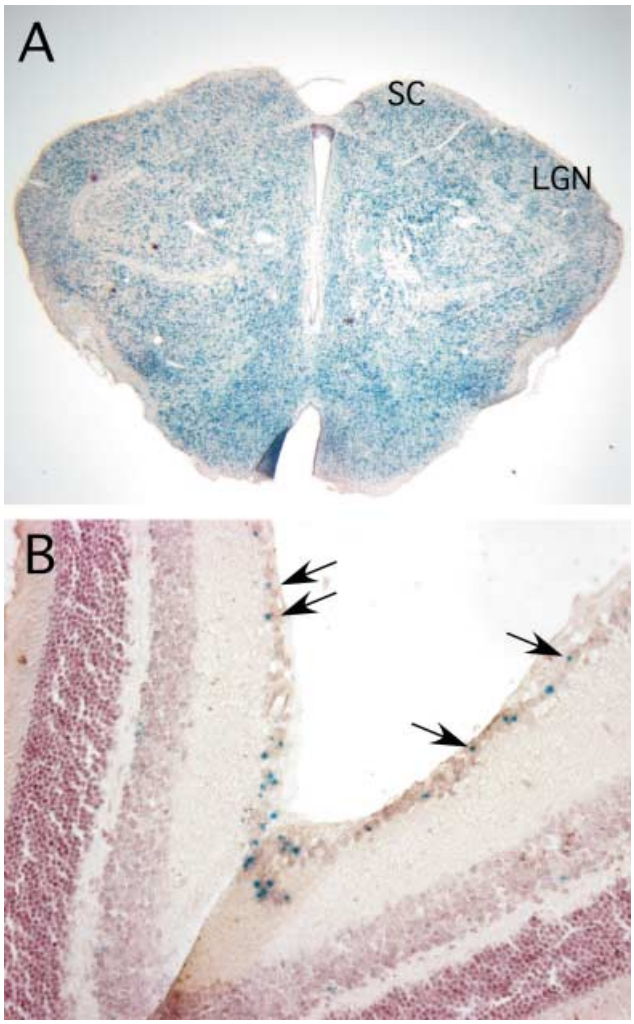

Fig. 1. Recombination in the nestin-Cre strain. Beta-galactosidase staining (blue nuclei) marks cells with Cre-recombinase activity in sections from adult BDNF-CM mice. A: Recombination was virtually complete throughout the posterior thalamus and anterior midbrain, encompassing the caudal LGd and rostral SC. B: Recombination was extensive throughout the ganglion cell layer of the retina. Black arrows point to some examples of nuclear beta-galactosidase staining in retinal ganglion cells.

The retino-LGd projection

In mice, retinal ganglion cell axons reach the LGd by approximately E15 through P2 (Godement et al., 1984; Edwards et al., 1986). The anatomical segregation of ipsilateral and contralateral of retinal inputs is complete by P8 in the LGd (Godement et al., 1984). We first compared the ipsilateral retinal projections to LGd in the wild-type (Fig. 2A) and in the BDNF-KO (Fig. 2B) mice at P17 that received monocular injections of CTB on P15. In the wild type (Fig. 2A), the ipsilateral projection (see red arrowheads) is seen as a dense clustering of retinal axon terminals in the most dorsal region of the rostral LGd (Fig. 2A, left panel). Progressing caudalward through the LGd (left to right in Fig. 2A), the ipsilateral projection enlarges, becomes less compact, and moves to the medial border of LGd. In the most caudal regions of LGd, the area of the ipsilateral projection is reduced in size compared to the immediately rostral regions (Fig. 2A, right panel). In any given section, the ipsilateral projection is typically densest at its centroid, but its outer contour can be very irregular. This is in stark contrast to the stereotyped delineation of the lateral geniculate nucleus as a whole, which shows a remarkably stable configuration across individuals (Godement et al., 1980, 1984; Upton et al., 2002).

In the BDNF-KO mice (Fig. 2B), the ipsilateral projection showed the same general pattern of innervation of LGd as occurs in the wild type (Fig. 2A). Since the profile of the ipsilateral projection appeared slightly larger in some sections (compare, for instance, the middle panels of Figs. 2A and 2B), we quantified the area occupied by the ipsilateral projections at the five rostrocaudal levels shown in Figs. 2A and 2B (Fig. 2C). This analysis shows that there is no significant difference in the area occupied by the ipsilateral projection for wild-type versus BDNF-KO mice at any of the five levels analyzed ($P > 0.50$, *t*-test, for each level; $n = 3$ animals each group). We also note that the disposition of the ipsilateral projection to two other parts of the lateral geniculate nucleus, the ventral nucleus of the lateral geniculate (LGv, Figs. 2A & 2B), and the intergeniculate leaflet (IGL, Figs. 2A & 2B), are similarly comparable between the wild type and the BDNF-KO. BDNF-CM mice and their controls examined by confocal microscopy after injection of fluorescent tracers showed no obvious differences in the sizes of ipsilateral and contralateral target zones (see Fig. 3).

At higher resolution (Fig. 3A) and magnification (Fig. 3B), confocal imaging of the LGd in control mice at P2 shows few yellow pixels in the neuropil of the LGd, indicating that there is little superpositioning (or coincidence) of retinal axon terminals from the two eyes within the nascent LGd. However, there is a considerably higher density of yellow pixels in images of the superficial optic tract (Fig. 3A, arrows), indicative of a very dense intermingling of retinal axons from the two eyes in the optic tract. Where the optic tract is not yellow, it is red (and not green), reflecting the greater contribution of the contralateral versus ipsilateral retinal axons to the retinogeniculate projection. Despite the close proximity of ipsilateral and contralateral axons in the superficial optic tract, retinal axon terminals from the two eyes nonetheless clearly occupy different target zones within the neuropil of the nascent LGd (Fig. 3A), in a pattern that presages the formation of the mature and segregated pattern of innervation (Figs. 3C–3F; in these panels, red is contralateral, green is ipsilateral). Red and green terminals are generally distinct yet intermixed within the

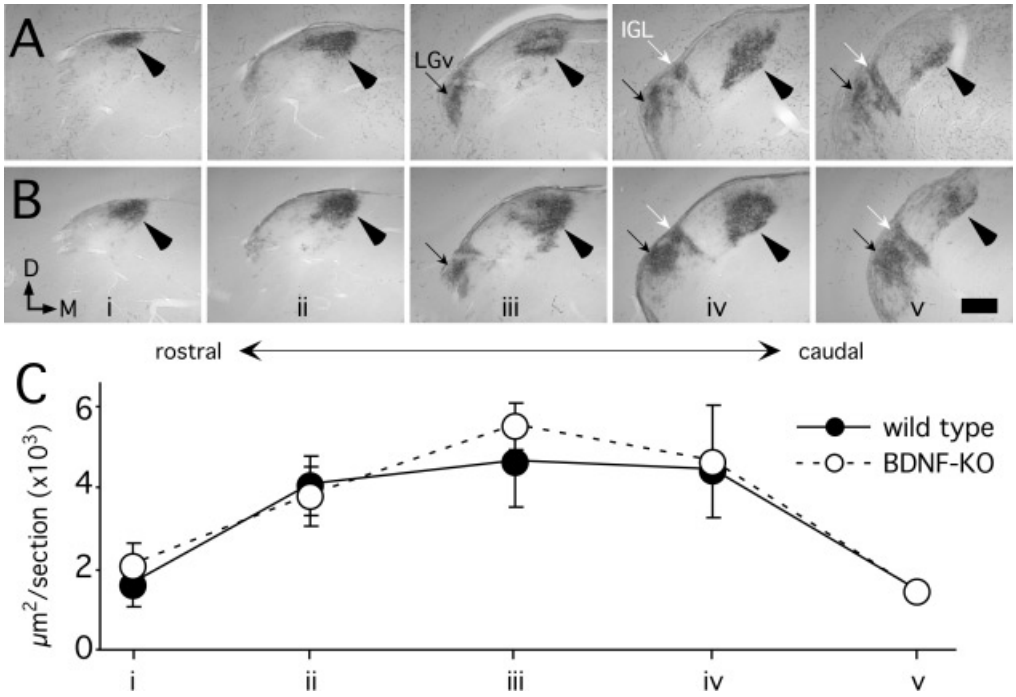

Fig. 2. Eye-specific segregation of retinal inputs in the dorsal nucleus of the lateral geniculate (LGd). A&B: The ipsilateral retinal projections to the LGd (black arrowheads) as seen in light micrographs of coronal sections taken at five levels (Roman numerals i–v, rostral to caudal, left to right) from wild-type (A) or BDNF-KO (B) mice brains processed for CTB immunohistochemistry on P17 after monocular injection of CTB on P15. Black arrows indicate the ventral nucleus of the lateral geniculate, LGv. White arrows indicate the intergeniculate leaflet, IGL. Anatomical axes (D, dorsal; M, medial) and scale bar (200 μm) apply to all panels. C: Quantitation of the area of the ipsilateral projections to LGd in the wild type and in the BDNF-KO at the same five levels (i–v) in A and B.

neuropil of the LGd at P2 (Figs. 3A & 3B), which is prior to the completion of eye-specific segregation at P8.

The relatively condensed central zone of the ipsilateral projections, as well as the irregularity of the interface between the ipsilateral and contralateral projection zones, are evident in confocal images of the dorsal half of the LGd from control and mutant strains (Figs. 3C–3F). Comparisons of the BDNF-KO and its control (Figs. 3C & 3D) at P15, and of the BDNF-CM and its control (Figs. 3E & 3F) at P13, show equivalent degrees of segregation of ipsilateral and contralateral projection zones. Note that in these confocal images, there is almost a complete absence of yellow pixels (which would indicate superposition of retinal axons terminals from both the ipsilateral and contralateral eye). However, at the borders between the contralateral and ipsilateral projections zones, retinal axon terminals from the two eyes clearly intermingle and fail to maintain nearest-neighbor relations with terminals from the same eye. Such is rarely the case in the densest regions of any of these ipsilateral target zones, all of which show virtually total exclusion of retinal axon terminals from the contralateral eye. Thus, in all cases examined, the degree of eye-specific segregation is qualitatively equivalent and independent of genetic strain, whether BDNF expression is normal or deficient.

To examine whether there were more subtle differences in the local clustering of retinal terminals, we examined the neighborhood relationships of ipsilateral terminals. We postulated that, on average, an ipsilateral terminal's nearest neighbors would be other ipsilateral terminals, and that the probability would decrease with distance. We further expected that, if BDNF deficiency disrupted segregation, then the proportions of terminals at a given distance from those of the same eye would be lower overall in mutants versus controls. To examine this, we binarized the digital images (Figs. 4A–4B) in order to count pixels representing retinal terminals (whether ipsilateral or contralateral). As a negative control, the binarized ipsilateral images were shuffled (Fig. 4C). The percentage of ipsilateral innervation was measured for each ipsilateral pixel in six shells that extended away from the ipsilateral pixel in 5-pixel intervals.

For the BDNF-KO and its control (Fig. 4D), the percentages for each shell for the knockout strain were marginally, though not significantly, greater than those of the control strain ($P > 0.19$, t-test, for each shell; $n = 6$ images from 2–3 animals each group). For the BDNF-CM and its control strain (Fig. 4E), the percentages of ipsilateral innervation also did not differ significantly ($P > 0.54$, t-test, for each shell, $n = 6$ images from two animals from group). Shuffling the ipsilateral image reduced the percentage of ipsilateral innervation within a given shell to about 4% in each shell for both the BDNF-KO and BDNF-CM and their controls. We conclude that absence of BDNF during eye-specific segregation does not reduce this index of local clustering of ipsilateral terminals.

The retinocollicular projection

The retinocollicular projection achieves mature eye-specific segregation at least by P14–15 (Godement et al., 1984; Colonnese & Constantine-Paton, 2001). In this pathway, the contralateral and ipsilateral projections are considerably intermixed before P4, but by P8, terminals from the contralateral projection occupy the

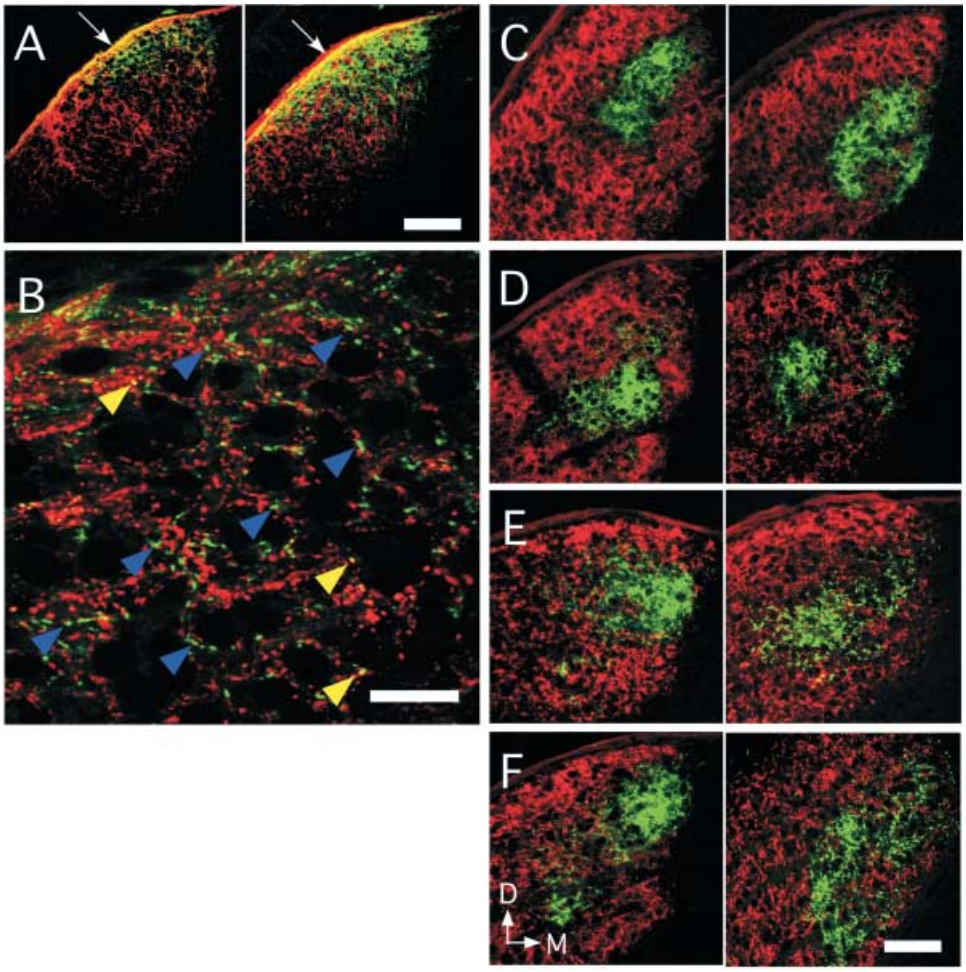

Fig. 3. Confocal images of coronal sections from the binocular region of LGd (level ii as per Figs. 2A & 2B) after intraocular injection of fluorescently labeled CTB into the two eyes. To aid comparisons in this figure, pseudocoloring was reversed for some panels so that the green channel always represents the ipsilateral projection, and red represents the contralateral projection. [*Actual* side of origin is *left* for A (both), C (1st), D (both), E (first); and *right* for B (both), C (2nd), and E (2nd)]. A: Wild type, P2. Arrows indicate areas of high retinal axon density in the superficial optic tract. Scale bar = 100 μm. B: Wild type, P2. Higher magnification of the neuropil. Yellow arrowheads indicate examples of occasional yellow pixels within the neuropil. Blue arrowheads indicate examples of numerous distinct ipsilateral terminals. Scale bar = 20 μm. C: BDNF-KO, P15. D: BDNF-KO Control strain, P15. E: BDNF-CM, P13. F: CM Control strain, P13. Scale bar (100 μm) in F applies to panels C–F. Anatomical axes (D, dorsal; M, medial) apply to all panels.

stratum griseum superficiale (SGS), while those from the ipsilateral projections coalesce into longitudinally oriented columns within stratum opticum (SO). In coronal sections, these columns of ipsilateral terminals appear as dense, irregular clusters. In sections from wild-type mice at P17, we found that rostral coronal sections of the SC typically show 4–6 such clusters (Fig. 5A, arrowheads). Progressing caudalward, the lateral clusters disappear, so that in the caudalmost sections of the SC only one medial cluster is present (Fig. 5B, arrowhead). The contralateral projection (Figs. 5A & 5B, asterisks), by contrast, makes a continuous, dense projection throughout the SGS. Tangentially projecting retinal axons in the SO from the contralateral projection (Fig. 5A, arrow) are also detected because the contralateral projection is relatively heavy and immunohistochemical reaction for CTB is sufficiently sensitive to detect small fibers.

In analogous sections taken from the BDNF-KO at P17, the ipsilateral projection follows the same pattern: the rostral sections show 4–6 clusters of ipsilateral retinal terminals (Fig. 5C, arrowheads), while caudal sections have a single, medial cluster (Fig. 5D, arrowheads). The contralateral projection occupies the SGS, and staining of tangential fibers in the SO is also apparent (Fig. 5C, arrow). The segregation pattern in the BDNF-CM mice also appeared normal (data not shown).

Confocal images of rostral sections of the SC from the wild type at P15 (Fig. 5E) and the BDNF-KO at P15 (Fig. 5F) show, in both cases, that the lamina bearing the clustered ipsilateral projection (arrowheads) excludes terminals from the contralateral projection. Similarly, no ipsilateral terminals are seen in the SGS, where the contralateral retina makes a heavy, continuous projection. The demarcation between the ipsilateral and contralateral projection zones in the SC (and the lack of overlap) is clear in the confocal sections because the fluorescent signal derives largely from retinal axon terminals and not from the fibers themselves. This is an important observation because it shows directly that the

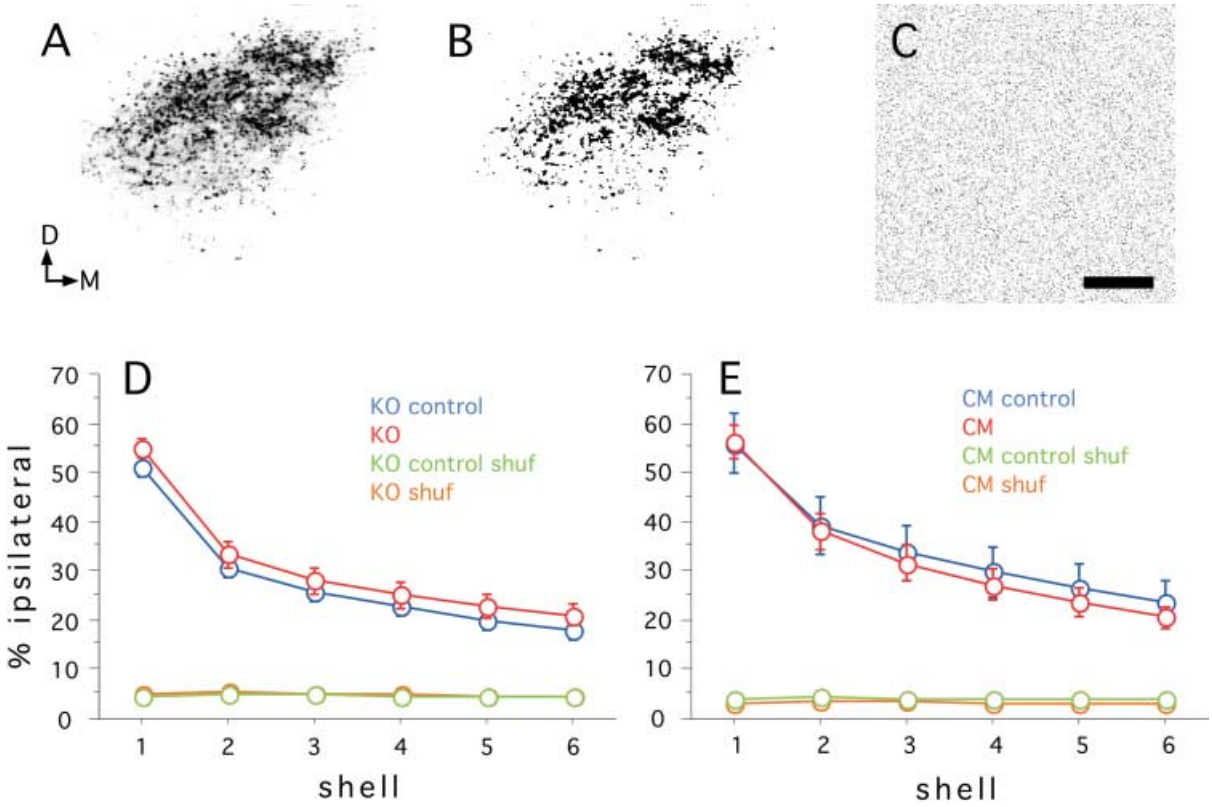

Fig. 4. Analysis of local clustering of retinal terminals in binarized images. A–C: An example of a gray-scale fluorescent image of the ipsilateral channel before (A) and after (B) binarization, and after shuffling of the binarized image (C). Contralateral images were also binarized (see Methods). Scale bar in C is (100 μm) and applies to A–C. Anatomical axes (in A) apply to A–C. D: Shell analysis for the BDNF-KO and its control. Curves indicate the average percentage ($\pm$ standard error) of ipsilateral pixels within six successive shells around each ipsilateral pixel. Lower curves indicate the result of the same analysis using shuffled ipsilateral images. E: Shell analysis for the BDNF-CM and its control as described for D.

ipsilateral and contralateral retinal terminals differ completely in laminar preference within the SC, and that this occurs regardless of the level of BNDF expression.

The suprachiasmatic nucleus (SCN) and the medial terminal nucleus (MTN)

We examined two other prominent retinal targets in diencephalon, the SCN and the MTN. Immunohistochemical detection of anterogradely transported CTB after monocular injection in the wild type at P17 (Fig. 6A) and in the BDNF-KO at P17 (Fig. 6B) shows that, in both the wild type and in the BDNF-KO, the SCN is virtually completely bilaterally innervated, although the contralateral projection appears slightly larger than the ipsilateral projection in both strains. These sections (Figs. 5A & 5B) are posterior to the decussation of optic fibers in the optic chiasm, and as a result the contralateral optic tract is heavily labeled. By contrast, the MTN, in the wild type, is a retinorecipient target that is almost exclusively innervated by the contralateral eye (Fig. 6C)—the ipsilateral projection to the MTN (Fig. 6D) being wispy or faint at best even as detected by immunohistochemistry. In the BDNF-KO, the contralateral (Fig. 6E) and ipsilateral (Fig. 6F) projections show the same intensity of staining as in the wild type. Comparing the patterning of ipsilateral and contralateral retinal inputs to the MTN versus the SCN thus reveals another important finding, namely that different retinal targets can differ greatly in the ratio of ipsilateral to contralateral inputs. The SCN represents a target which is almost uniformly bilaterally innervated by the retina, while the MTN has extremely sparse ipsilateral inputs.

Discussion

The present study has examined whether BDNF, a strong molecular candidate in many neural systems for mediating the effects of neuronal activity on synaptic plasticity in the visual system (Bonhoeffer, 1996; von Bartheld, 1998; Berardi & Maffei, 1999; Elliott & Shadbolt, 1999; Caleo & Maffei, 2002; Maffei, 2002), is required for normal eye-specific segregation in the major subcortical centers to which the two retinas project. In the two strains of BDNF transgenics with deficient expression of BDNF examined in the present study (a germ-line knockout and a conditional mutant), we saw normal eye-specific patterning in four principal retinorecipient targets, the LGd, the SC, the SCN, and the MTN. We also show that early ipsilateral retinogeniculate projections appear to be predisposed to innervate the dorsal region of LGd, that is, the stereotypical location of the ipsilateral target zone. Taken together, these data show that segregation occurs normally in the absence of BDNF, and suggest that eye-specific patterning involves a strong (activity-independent) targeting component.

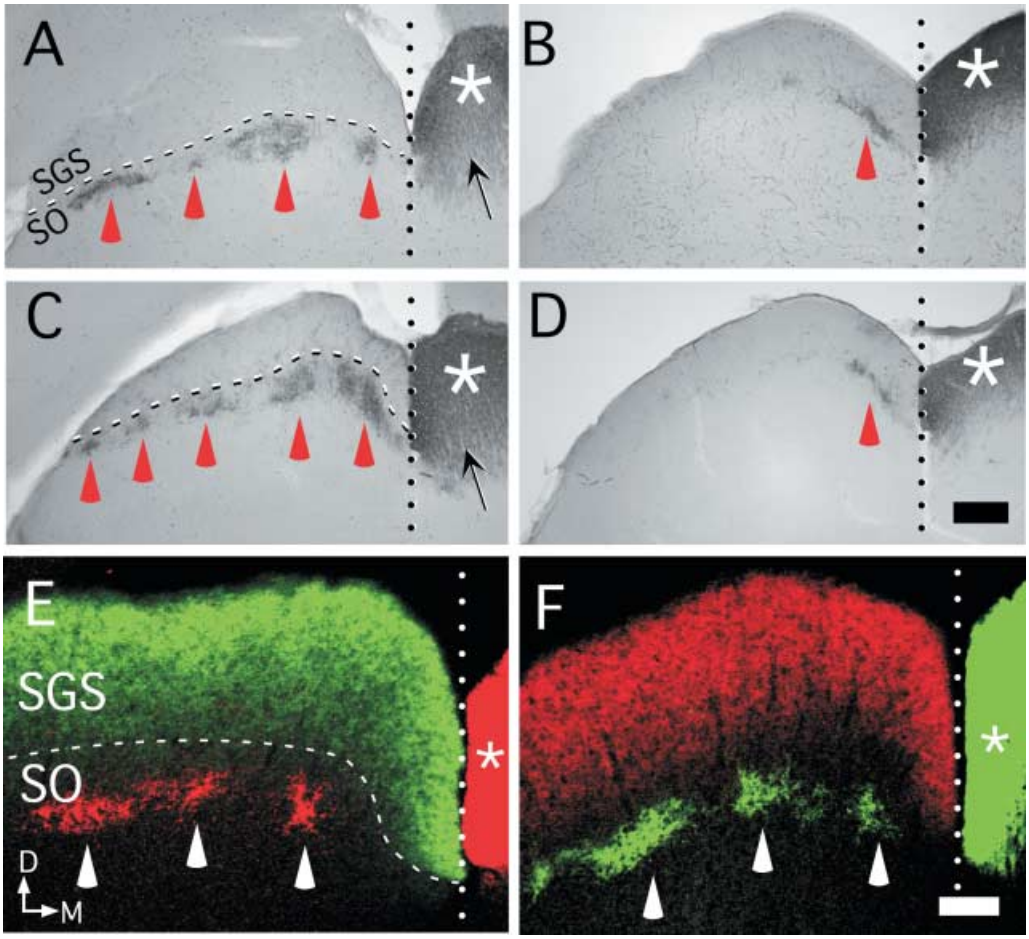

Fig. 5. Eye-specific segregation of retinal inputs in the superior colliculus (SC). A–D: Bright-field images of coronal sections through the rostral (A & C) or caudal (B & D) SC from P17 wild-type (A & B) or P17 BDNF-KO (C & D) mice. Brains were processed for CTB immunohistochemistry 2 days after monocular injection of CTB. Red arrow heads indicate clusters of ipsilateral projections. The dashed line separates the SGS and the SO. The dotted vertical line indicates the vertical midline of the midbrain. The asterisks indicate the contralateral projection to the SGS. The arrows indicate tangential fibers in the contralateral SO. Scale bar (200 μm) applies to A–D. E–F: Confocal images of the rostral SC from sections processed for imaging 2 days after injection of fluorescent CTB conjugates (red channel, right eye's projection; green channel, left eye's projection). E: BDNF-KO control, P15. Red channel is the ipsilateral projection. Anatomical axes (D, dorsal; M, medial) apply to all panels. F: BDNF-KO, P15. Green channel is the ipsilateral projection. Symbols as per A–D above. SGS, stratum griseum superficiale; SO, stratum opticum. Scale bar (100 μm) applies to E and F.

In the normal animals, BDNF could play either an instructive or permissive role in determining whether individual nascent synapses are stabilized or removed based on activity-dependent mechanisms (Zhang & Poo, 2002; Lu, 2003). In the present study, we find normal eye-specific patterning whether BDNF expression is eliminated or reduced throughout the body or only in the central nervous system (CNS). We do note, however, that elimination of expression might be compensated for by other trkB ligands such as NT-4 (Riddle et al., 1995; Bosco & Linden, 1999; Fan et al., 2000), although NT-4 may be cortically derived (Wahle et al., 2003).

In the LGd and the SC, there is evidence that the development of eye-specific segregation is affected by retinal activity in rodents and in carnivores (Shatz & Stryker, 1988; Penn et al., 1998; Cook et al., 1999; Chapman, 2000; Huberman et al., 2002;), as well as by disruption of specific molecular systems which have been shown to, or can plausibly be expected to, mediate the effects of neuronal activity (Upton et al., 1999; Vercelli et al., 2000; Wu et al., 2000; Cork et al., 2001; Ravary et al., 2003). Yet, consensus in this field is lacking concerning a specific role for activity. For instance, while retinal activity has been reported to be critical to the development of eye-specific segregation, doubt has been cast on the role of *correlated* retinal activity (Huberman et al., 2003). This may explain the finding that blockade of N-methyl-D-aspartate (NMDA) receptors fails to block eye-specific segregation (Smetters et al., 1994), since correlated activity would be a major target of this blockade. Moreover, an overt emphasis on activity-dependent mechanisms has overshadowed evidence for activity-independent mechanisms of eye-specific patterning. In particular, both axon guidance cues present at the optic chiasm (Erskine et al., 2000; Herrera et al., 2003; Williams et al., 2003) and targeting molecules present on retinal axons and in the LGd can significantly influence eye-specific patterning (Feldheim et al., 1998; Ellsworth et al., 2005). In the present study, we show that some retinal targets zones either show virtually complete integration of eye-specific projections, as in the suprachiasmatic nucleus, or virtually completely unilateral projections, such as the medial

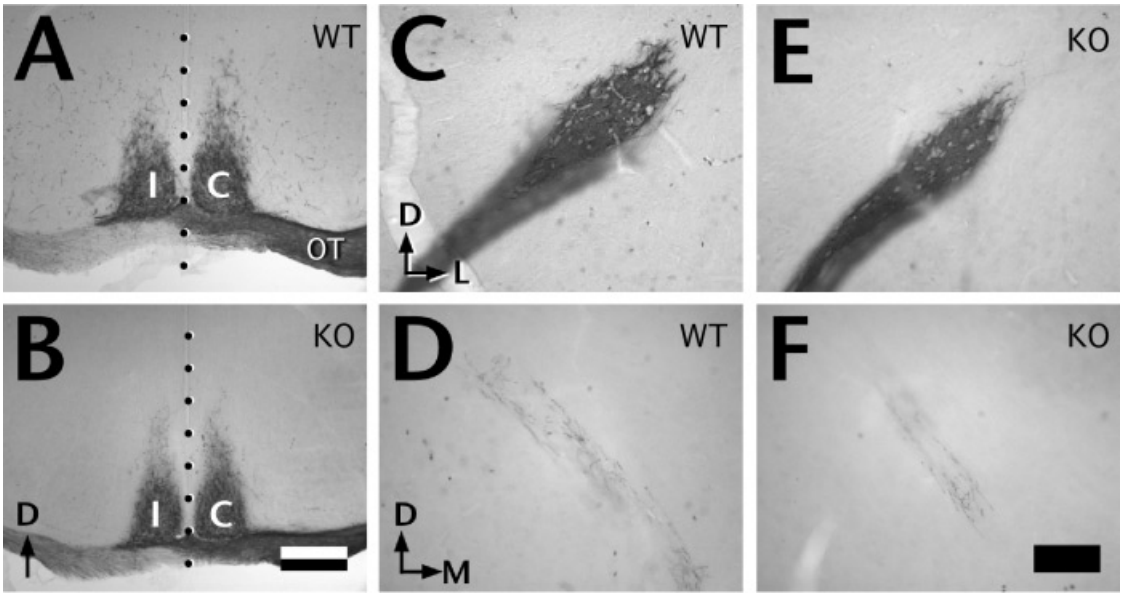

Fig. 6. Eye-specific projections to the suprachiasmatic nucleus (SCN) and to the medial terminal nucleus (MTN) at P17. Bright-field images showing CTB immunohistochemistry after monocular injection of CTB on P15. A: SCN in the wild type. B: SCN in the BDNF-KO. Dotted vertical line indicates the midline. I, ipsilateral; and C, contralateral to injected eye. Scale bar (200 μm) applies to A and B. Dorsal is upward. C & D: Contralateral and ipsilateral, respectively, retinal projections to the MTN in the wild type. E & F: Contralateral and ipsilateral, respectively, retinal projections to the MTN in the BDNF-KO. Scale bar (100 μm) applies to C–F. Anatomical axes in C (D, dorsal; L, lateral) apply to C and E. Anatomical axes in D (D, dorsal; M, medial) apply to D and F.

terminal nucleus. We suggest that unique patterns of molecular expression intrinsic to these target zones probably attract specific subsets of retinal axons (Sherman & Spear, 1982), substantially determining the diverse patterns of eye-specific segregation.

The innervation of LGd by the two retinas remains a powerful model to investigate the interplay of multiple developmental patterning mechanisms. We are struck by the appearance of an early targeting preference for retinal axon terminals from the two eyes to the LGd in a pattern that will result in the mature pattern without the need for extensive remodeling of the projections. Out data show, at very early stages in development, that the ipsilateral projection preferentially targets the dorsal region of LGd, while the contralateral projection preferentially targets the ventral region. This observation is highly suggestive of targeting mechanisms that are more related to axonal guidance than to activity. Two possible candidate mechanisms are temporal ordering and molecular chemoaffinity. The ipsilateral axons may be delayed relative to the contralateral axons (Godement et al., 1984), although this is not universally accepted (Drager, 1985; Chalupa et al., 1996; Jhaveri et al., 1996). In this case, contralateral axons might have earlier access to more ventral regions of LGd, but it would also be necessary to further posit competition for target space in this model. Even still, there will be a considerable period over which nascent axons from the two projections will arrive contemporaneously, and thus this model alone cannot explain the lack of integration of the projections.

The present findings suggest that activity-independent mechanisms, such as the expression of a set of matching labels between retinal ganglion cells and their appropriate target zones, represent important patterning determinants in this system. Developmental labels for left eye versus right eye are unknown. Yet because the ipsilateral projection arises from the ventrotemporal region of retina (Sretavan, 1990; Sretavan & Kruger, 1998), a retinal region that selectively expresses a variety of markers (Feldheim et al., 1998; Pak et al., 2002; Herrera et al., 2003; Ellsworth et al., 2005), specific targeting of the ipsilateral and contralateral projections may be largely determined by molecular cues related to retinal position. Retinal activity may provide some permissive modulation (Cook et al., 1999; Huberman et al., 2003) of some aspects of this patterning. However, whatever the effects of activity in this system, BDNF, acting as either a signal or effector of activity, or as an activity-independent trophic factor, appears to be dispensable to normal eye-specific patterning in subcortical visual pathways.

Acknowledgments

This work was supported by NIH grants EY07023 and EY14134 (M.S.).

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