Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 12564–12569, October 1996
Neurobiology

Suppression of trk_B expression by antisense oligonucleotides alters a neuronal phenotype in the rod pathway of the developing rat retina

(brain-derived neurotrophic factor/neurotrophin/AII amacrine cell)

DENNIS W. RICKMAN AND CATHERINE BOWES RICKMAN

Department of Ophthalmology, Anheuser–Busch Eye Institute, St. Louis University, St. Louis, MO 63104

Communicated by John E. Dowling, Harvard University, Cambridge, MA, August 8, 1996 (received for review February 16, 1996)

ABSTRACT **trk_B is the high-affinity receptor for brain-derived neurotrophic factor (BDNF), a trophic molecule with demonstrated effects on the survival and differentiation of a wide variety of neuronal populations. In the mammalian retina, trk_B is localized to both ganglion cells and numerous cells in the inner nuclear layer. Much information on the role of BDNF in neuronal development has been derived from the study of trk_B- and BDNF-deficient mutant mice. This includes an attenuation of the numbers of cortical neurons immunopositive for the calcium-binding proteins, parvalbumin, and calbindin. Unfortunately, these mutant animals typically fail to survive for >24–48 hr after birth. Since most retinal neuronal differentiation occurs postnatally, we have devised an alternative scheme to suppress the expression of trk_B in the retina to examine the role of BDNF on the postnatal development of neurons of the inner retina. Neonatal rats were treated with intraocular injection of an antisense oligonucleotide (1–2 μl of 10–100 μM solution) targeted to the trk_B mRNA. Immunohistochemistry with a polyclonal antibody to trk_B showed that the expression of trk_B in retinal neurons was suppressed 48–72 hr following a single injection. Northern blot analysis demonstrated that antisense treatment had no effect on the level of trk_B mRNA, even after multiple injections. This suggests an effect of trk_B antisense treatment on protein translation, but not on RNA transcription. No alterations were observed in the thickness of retinal cellular or plexiform layers, suggesting that BDNF is not the sole survival factor for these neurons. There were, however, alterations in the patterns of immunostaining for parvalbumin, a marker for the narrow-field, bistratified AII amacrine cell—a central element of the rod (scotopic) pathway. This was evidenced by a decrease in both the number of immunostained somata (>50%) and in the intensity of immunolabeling. However, the immunostaining pattern of calbindin was not affected. These studies suggest that the ligands for trk_B have specific effects on the neurochemical phenotypic expression of inner retinal neurons and in the development of a well-defined retinal circuit.**

Brain-derived neurotrophic factor (BDNF) is a well-characterized member of the family of trophic molecules, the neurotrophins (NTs), which includes nerve growth factor, NT-3, NT-4/5 and NT-6 (1, 2). The distribution of NT mRNAs to a variety of neuronal structures (3, 4), as well as to neuronally innervated peripheral tissues (5), suggests that these factors have trophic effects on a wide range of neuronal populations, including the visual system. In the rat and *Xenopus* brain, BDNF mRNA has been localized to the retinorecipient layers of the midbrain tectum (6, 7), the primary target of most retinal ganglion cells in these species.

The high-affinity receptors for the NTs are isoforms of the protooncogene, *trk*, a receptor tyrosine kinase (8–13). The presence of the primary high-affinity receptor for BDNF (and NT-4/5), trk_B, has recently been demonstrated in the developing retina (7, 14–17) and is localized primarily to ganglion cells (7, 17). This correlation of NT and receptor expression suggests that trk_B ligands play a role in retinal ganglion cell survival and differentiation. Indeed, BDNF has been shown to support the survival of cultured retinal ganglion cells isolated from the perinatal rat retina (18, 19). The effects of BDNF on adult retinal ganglion cells are suggested by studies in which intraocular injection of BDNF, following optic nerve transection, prolonged the survival of large-diameter ganglion cells (20, 21). Furthermore, in explant cultures of adult rat retina, BDNF and NT-4/5 facilitated dose-dependent neurite outgrowth from retinal ganglion cells (22). Neither NT-4/5 mRNA nor immunoreactivity (IR) has been observed in the retina or central visual nuclei, however, suggesting that BDNF is the primary trk_B ligand affecting retinal development.

Recently, it has been suggested that other retinal neurons may be responsive to BDNF. Indeed, the trk_B receptor also has been localized to numerous cells in the inner nuclear layer (INL) of the rat (17) and *Xenopus* (7) retina. The source of BDNF for these cells is problematic. Only low levels of BDNF mRNA have been reported in the rat (ref. 23; D.W.R., unpublished observations) and *Xenopus* (7) retina, where it is localized primarily to ganglion cells. Thus the precise role of BDNF in the development of retinal interneurons is unknown.

Recently, gene targeting by homologous recombination has allowed investigators to generate transgenic mice lacking specific NTs or their receptors (24, 25). These animals allow *in vivo* analysis of the effects of total deprivation of a specific NT or its receptor on neuronal phenotypic expression (26). Mice in which BDNF or trk_B have been "knocked out" generally display both sensory and motor dysfunctions (24, 25). In mice homozygous for the BDNF deletion, an analysis of cortical neuronal phenotypes has shown a decrease in the number of cells containing the calcium-binding proteins, parvalbumin (PV) and calbindin (CaB; ref. 25). These substances are believed to modulate γ-aminobutyric acid (GABA) neurotransmission and regulate calcium stores, respectively. Interestingly, in the rat, antibodies to these two calcium-binding proteins label specific cell populations in the inner retina (27–31).

Unfortunately, there is a inherent shortcoming to these knockout models, because many of these animals do not survive to maturity. In fact, BDNF- or trk_B-deficient mice

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: BDNF, brain-derived neurotrophic factor; NT, neurotrophin; INL, inner nuclear layer; PV, parvalbumin; CaB, calbindin; IR, immunoreactivity; PND, postnatal day; GCL, ganglion cell layer; IPL, inner plexiform layer.

*To whom reprint requests should be addressed at: Department of Ophthalmology, Anheuser–Busch Eye Institute, 1755 South Grand Boulevard, St. Louis, MO 63014.

typically survive for only 24–48 hr after birth (24, 25). Therefore a neuronal system in which there is considerable postnatal differentiation or remodeling, such as the rodent retina, cannot be thoroughly investigated using even this elegant approach. Therefore we have applied a novel strategy to suppress trk_B expression in the postnatal retina by targeting trk_B mRNA with antisense oligonucleotides. This method allows the introduction of an oligonucleotide whose sequence is complementary to a specific targeted region of mRNA (32, 33). Presumably, the antisense DNA strand hybridizes to the targeted mRNA sequence and prevents translation. Here, these techniques have been used to study the postnatal rat retina to more fully understand the role of BDNF in retinal neuronal differentiation.

METHODS

Antisense oligonucleotide. The nucleotide sequence for full-length trk_B was obtained from the GenBank data base. Oligonucleotides, in the sense and antisense orientations, were designed as follows. For full-length trk_B, we first identified the initiator methionine (AUG) in the rat trk_B nucleotide sequence (nucleotide 665). From that point, we counted 15 nt in both the 3′ and 5′ directions. Within that span we searched for an 18- to 20-mer with the following characteristics: (*i*) containing an equal number of purines and pyrimidines, (*ii*) possessing a terminal purine at both ends, and (*iii*) having a minimum number of nucleotide repeats. For full-length trk_B, this sequence spanned the sequence from nucleotide 651 to 670 (5′-ACTGGCAGCTCGGGATGTCG-3′). The antisense nucleotide, complementary to that sequence, was 5′-CGACATCCCGAGCTGCCAGT-3′. These oligonucleotides were synthesized by a commercial supplier (National Biosciences, Plymouth, MN). To increase resistance to nuclease metabolism, phosphothioated oligonucleotides were used.

Animals. Three litters (n = 12, n = 12, n = 14) of Sprague–Dawley rats were used for these studies. Neonatal and early postnatal animals were anesthetized by inhalation of methoxyflurane. With the aid of a dissecting microscope, a small incision was made in the palpebral fissure to open the eye. For intraocular injection, oligonucleotides were used at a concentration of 10–100 μM in sterile saline. Using a 10-μl Hamilton syringe with a 30-gauge needle, 1–2 μl of sterile antisense oligonucleotide solution was slowly injected into the vitreal chamber of one eye, taking care not to penetrate the lens capsule. The contralateral eye received a similar injection of either sense oligonucleotide in sterile saline or sterile saline alone. The incision was then treated with antibiotic ointment. In some cases, injections were repeated on subsequent days (see Table 1).

Assessment of Antisense Suppression: Immunohistochemical Analysis. To confirm the suppression of trk_B expression following antisense oligonucleotide treatment, retinas were analyzed immunohistochemically, using a rabbit polyclonal antibody to a unique amino acid sequence (794–808) of murine trk_B (Santa Cruz Biotechnology). To assess the specificity of antisense suppression, we also used a rabbit polyclonal antibody directed to a unique amino acid sequence (763–777) of the C terminus of human trk_A. These antibodies are not cross-reactive by immunoprecipitation and Western blotting analysis, as determined by the supplier. To determine specificity of the immunostaining, each antibody was preadsorbed overnight at 4°C, with a 5-fold excess of either the homologous or heterologous peptide. As a further control, the primary antibodies were substituted with normal serum.

On various days following antisense treatment (see Table 1), animals were killed with an overdose of sodium pentobarbital, and the eyes were removed, fixed in 4% paraformaldehyde, cryoprotected in 20% sucrose, and sectioned on a cryostat. Tissue sections were incubated in primary antibody diluted in 0.1 M phosphate buffer (PB) with 0.5% Triton X-100 and 10% normal goat serum for 18 hr. Following a thorough wash in 0.1 M PB, sections were incubated in indocarbocyanine (Cy3)-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) diluted in 0.1 M PB for 2 hr at room temperature. Slides were then washed in PB, coverslipped, and viewed with a Nikon Optiphot microscope equipped with epifluorescence. At least five slides (15 sections) were examined for each experimental group.

Assessment of Antisense Suppression: Northern Blot Analysis. Following trk_B antisense treatment (see Table 1), total RNA was isolated from whole or hemisected retinas as well as brain, and Northern blot analyses were performed to determine the size and relative abundance of trk_B RNA. Total RNA was fractionated on a denaturing (formaldehyde) agarose gel. Before electrophoresis, the samples were denatured by heating to 65°C for 10 min in 6.5% formaldehyde and 50% formamide in Mops running buffer [40 mM morpholinopropanesulfonic acid (pH 7.0) containing 100 mM sodium acetate and 10 mM EDTA]. The samples were then separated in a 2.2 M formaldehyde agarose gel and transferred to a nylon Hybond N^+ membrane (Amersham) by downward capillary transfer using 20× SSC (3 M NaCl and 0.3 M sodium citrate). The membrane was hybridized at 65°C with a PCR-derived fragment of trk_B cDNA (34) radiolabeled with [α-^{32}P] by random priming (35, 36) for detection of the corresponding transcripts. After hybridization the Northern blots were washed to a stringency of 0.2× SSC at 55–60°C and exposed to x-ray film (Hyperfilm MP; Amersham).

Assessment of trk_B Suppression on Calcium-Binding Protein Expression. Retinas were analyzed immunohistochemically using a mouse monoclonal antibody to PV (Sigma) and a rabbit polyclonal antibody to CaB (Chemicon). Tissue sections were processed as described above using Cy3-conjugated secondary antibodies and analyzed for their patterns and intensities of immunolabeling.

RESULTS

Intraocular treatment with either trk_B sense or antisense oligonucleotides did not result in any obvious morphological alterations of the retina. Generally, treatment with as little as 1–2 μl of 10 μM trk_B antisense oligonucleotide resulted in a substantial reduction in trk_B-IR (Fig. 1). This suppression of immunostaining was evidenced as soon as 48 hr after antisense treatment and persisted for at least 72 hr. In retinas examined after 96 hr, immunostaining appeared no different from sense-treated or uninjected controls. The level of suppression of immunostaining was variable, but in most instances very faint or no trk_B-IR was observed in any retinal region. On the other hand, the pattern of trk_A-IR was not affected.

To assess the effects of intraocular application of the trk_B antisense oligonucleotide on gene transcription, we analyzed the levels of trk_B mRNA at posttreatment intervals by Northern blot analysis (Fig. 2). At 24 hr after treatment, no differences in RNA levels were seen between trk_B antisense-

Table 1. Experimental paradigms

Injection schedule	Day of retinal isolation	Type of analysis
PND-0	PND-1, PND-2, PND-3, PND-4, PND-5	RNA, immunohistochemistry
PND-0, PND-3, PND-7	PND-10	RNA, immunohistochemistry
PND-5, PND-10	PND-15	Immunohistochemistry

PND, postnatal day.

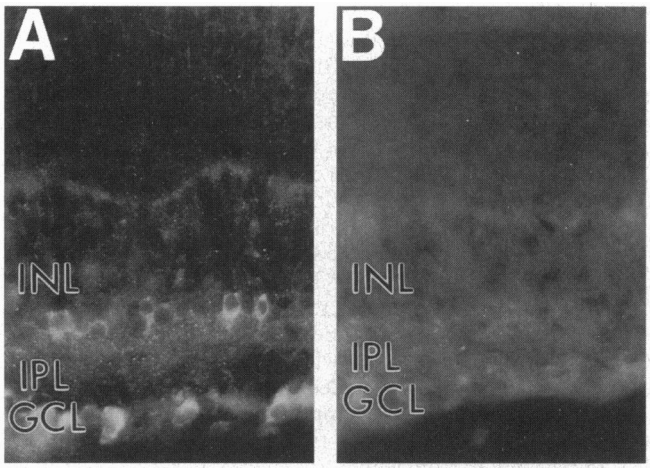

FIG. 1. Fluorescence photomicrographs showing normal and antisense-suppressed *trk$_B$-IR in the PND-10 rat retina.* (*A*) *trk*$_B$-IR in a retina that received intraocular injection of a *trk*$_B$ sense strand of DNA on PND-0, PND-3, and PND-7. Immunostaining is localized to cells in both the GCL and the INL. (*B*) *trk*$_B$-IR in the contralateral retina that received intraocular injection (10 μM) of a *trk*$_B$ antisense strand of DNA on PND-0, PND-3, and PND-7. No specific immunostaining was observed.

treated retinas and retinas that received no injection. After 48 hr, both groups displayed small but identical increases in mRNA levels. These values continued to increase, and by PND-10, substantial, identical increases were observed in both groups, even following multiple injections of the antisense oligonucleotide.

To assess the effect of *trk*$_B$ suppression on the development of specific neurochemical phenotypes, we examined the immunostaining patterns of two calcium-binding proteins (PV and CaB) in PND-10 retinas that displayed no, or suppressed, *trk*$_B$-IR and in PND-15 retinas that had previously received *trk*$_B$ antisense injections on PND-5 and PND-10 but now displayed positive *trk*$_B$-IR.

Fig. 3 *A* and *B* shows the patterns of CaB-IR in *trk*$_B$ sense- and antisense-treated retinas, respectively, at PND-10. In both cases, as in the untreated retina (data not shown), CaB-IR was prominent in horizontal cells. It was also present in numerous somata at various strata of the INL, a few cells in the ganglion cell layer (GCL) and in a thin band of stained processes in the distal inner plexiform layer (IPL), as earlier reported (29).

PV-IR, however, was altered following *trk*$_B$ antisense treatment. Fig. 4 *A* and *C* shows the distribution of PV-IR cells in sense-treated controls at PND-10 and PND-15, respectively. Immunostained somata were densely arrayed at the INL/IPL border, and occasional cells were present in the GCL. Furthermore, a fine plexus of processes was present in the IPL. Following *trk*$_B$ antisense treatment (Fig. 4 *B* and *D*), the numbers and staining intensity of PV-IR cells were markedly reduced. Fig. 4*B* shows the pattern of PV-IR at PND-10 following multiple injections of *trk*$_B$ antisense oligonucleotide during the first postnatal week (Table 1). The number of well-stained somata in the INL was reduced by ≈56%. In addition, only occasional processes were observed in the IPL. At PND-15, a similar pattern was observed following *trk*$_B$ antisense treatment (Fig. 4*D*). Again, the numbers of immunostained somata and processes were greatly reduced. The number of well-stained somata in the INL was reduced by ≈53%.

DISCUSSION

It is likely that BDNF plays an important role in retinal development. These studies demonstrate that the expression of the high-affinity BDNF receptor *trk*$_B$ can be selectively suppressed in the postnatal rat retina. This is important because a great deal of retinal neuronal differentiation, synaptogenesis, and apoptotic cell death occurs postnatally. Overall, the histological appearance of the retina is not affected by *trk*$_B$ antisense treatment, nor is there any gross alteration in the thickness of retinal layers. There are, however, effects on specific populations of neurochemically-identified neurons, as evidenced by the demonstrated alterations in PV-IR.

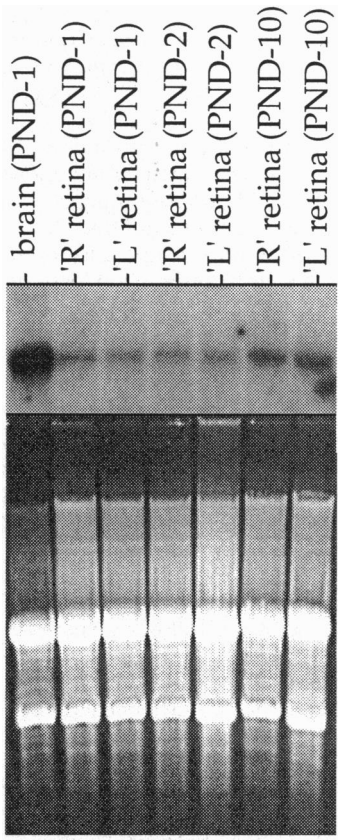

FIG. 2. Northern blot (*Upper*) and denaturing agarose gel (*Lower*) of rat total RNA (10 μg/lane). Rat tissue from which total RNA was isolated and age at time of isolation is indicated at the top of each lane. In each case, the right eye ('R') received *trk*$_B$ antisense oligonucleotide injection and the left eye ('L') received no injection on postnatal day (PND)-0. Retinas were then isolated at PND-1 or PND-2. The last two lanes show RNA from retinas that received subsequent injections on PND-3 and PND-7. These retinas were isolated at PND-10. The Northern blot was probed with a pcr-derived, ^{32}P-labeled fragment of *trk*$_B$ cDNA (nucleotides 1362 to 1558 of full-length *trk*$_B$). There was no difference in size or relative abundance of *trk*$_B$ mRNA 24 or 48 hr following antisense treatment, nor following multiple antisense injections.

NT Redundancy. In the rat, populations of retinal ganglion cells express *trk*$_A$- and *trk*$_B$-IRs during the perinatal period (17). Presumably, multiple *trk* isoforms are coexpressed within the same neuron (17). These patterns of receptor expression correspond to the expression of different NTs in central retinorecipient targets. For example, BDNF mRNA has been localized to the superficial layers of the superior colliculus (6, 37), whereas nerve growth factor mRNA has been localized to the lateral geniculate nucleus (6, 38). Axonal projections of retinal ganglion cells terminate primarily in the contralateral superior colliculus (39). Collateral branches of these axons may also terminate in the contralateral dorsal lateral geniculate nucleus (40). Presumably, these two NTs activate different cascades of regulation of gene expression within the same cell, affecting different pathways of phenotypic differentiation.

Both BDNF and nerve growth factor contribute to neuronal survival of retinal ganglion cells, as evidenced by the effect of their intraocular injection following optic nerve transection

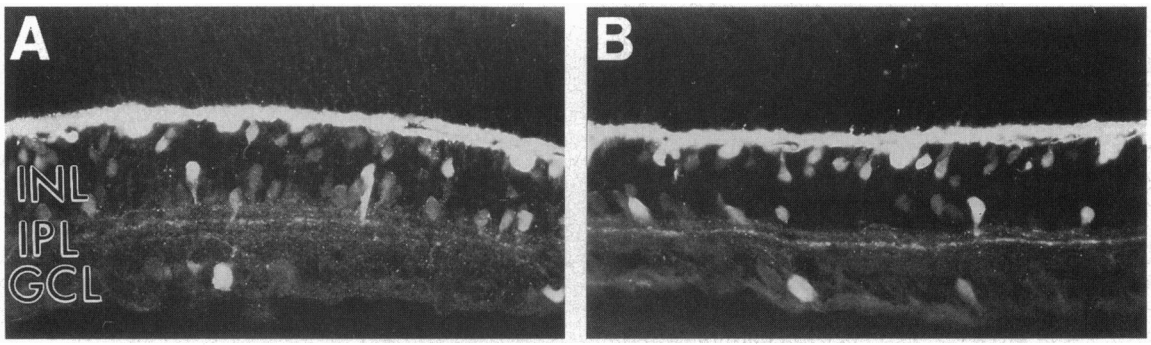

FIG. 3. Fluorescence photomicrographs showing the localization of CaB-IR in the PND-10 rat retina. (*A*) CaB-IR in the PND-10 rat retina that received intraocular injection of trk_B sense oligonucleotide on PND-0, PND-3, and PND-7. CaB-IR is localized, predominantly, to horizontal cells and to numerous cells scattered throughout the INL and GCL. (*B*) CaB-IR in the PND-10 rat retina that received intraocular injection of trk_B antisense oligonucleotide on PND-0, PND-3, and PND-7. The pattern and intensity of immunolabeling is unchanged.

(20, 21, 41). They may, however, have quite different effects on morphological differentiation, such as the development of patterns of dendritic arborization, or on the expression of specific neurochemical phenotypes. Thus the numbers of retinal neurons may not be expected to be attenuated following antisense suppression of a specific *trk* isoform. Rather, more subtle phenotypic changes may be observed. The effects of NTs on ganglion cell dendritic development requires further study. Indeed, the NT-dependent laminar distribution of developing dendrites has been demonstrated for pyramidal cells of the visual cortex (42).

The PV-IR AII Amacrine Cell. In the rat retina, PV immunoreactivity labels numerous amacrine, displaced amacrine, and ganglion cells. In the INL, the predominant population of labeled neurons is a narrow-field, bistratified amacrine cell, the AII (27, 30, 31, 43, 44). This interneuron is a central element of the rod (scotopic) pathway. It receives input from rod bipolar cells and makes glycinergic synapses with OFF-cone bipolar cells and OFF-ganglion cells, and it contacts ON-cone bipolar cells by gap junctions (42, 45–47).

In the rat retina, PV-IR appears within the first postnatal week (ref. 45; D.W.R. unpublished observations). By the time

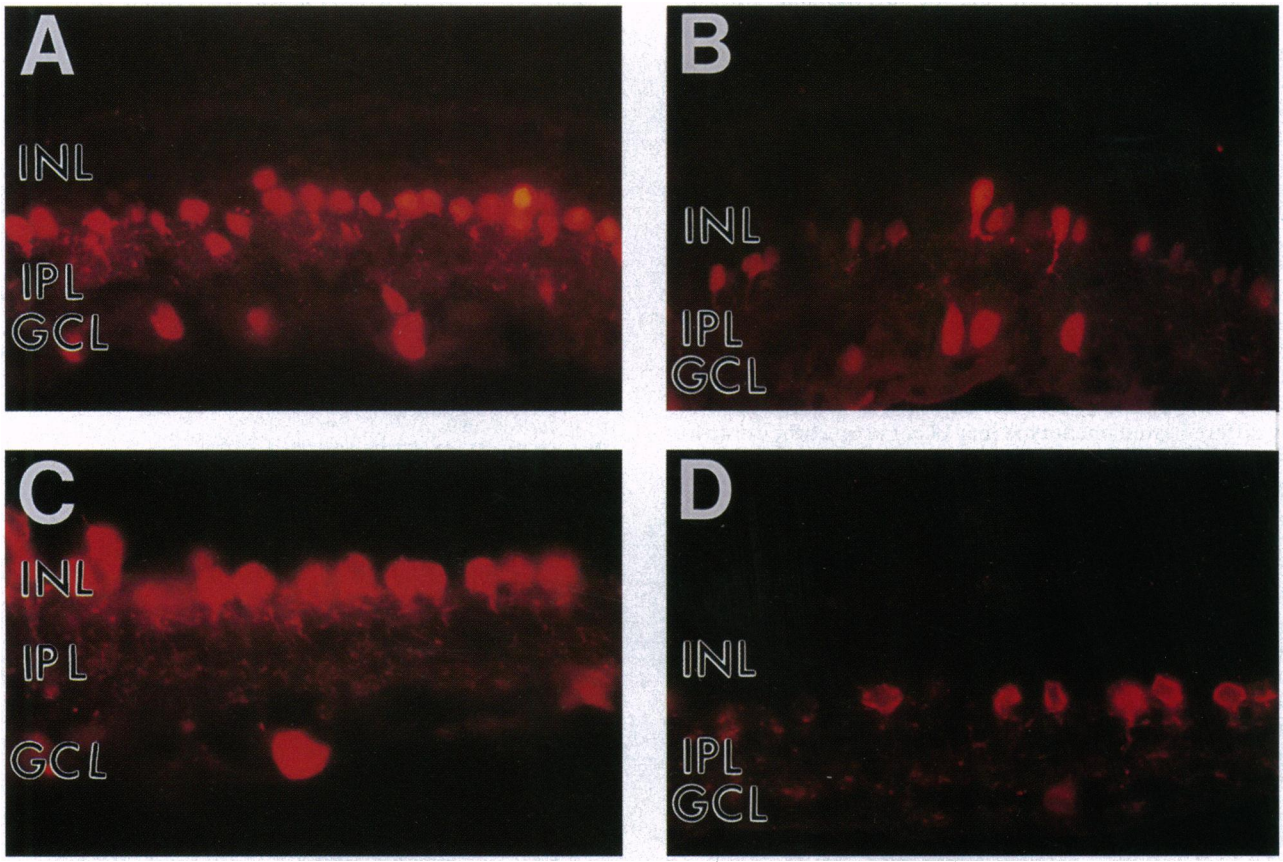

FIG. 4. Fluorescence photomicrographs showing the localization of PV-IR in the rat retina. (*A*) The pattern of PV-IR in the PND-10 rat retina that received intraocular injection of trk_B sense oligonucleotide on PND-0, PND-3, and PND-7. PV-IR is localized primarily to numerous cells at the border of the INL and IPL (presumed AII amacrine cells), and to some cells in the GCL. (*B*) The pattern of PV-IR in the PND-10 rat retina that received intraocular injection of trk_B antisense oligonucleotide on PND-0, PND-3, and PND-7. The number of PV-IR cells in the INL is greatly reduced. In addition, most labeled cells are only faintly stained. (*C*) The pattern of PV-IR in the PND-15 rat retina that received intraocular injection of trk_B sense oligonucleotide on PND-5 and PND-10. At this time (around eye opening), the pattern of PV-IR approximates the pattern of immunostaining in the mature retina. (*D*) The pattern of PV-IR in the PND-15 rat retina that received intraocular injection of trk_B antisense oligonucleotide on PND-5 and PND-10. Again, note that both the number of PV-IR cells and the intensity of immunostaining are reduced.

of eye-opening (around PND-15), the predominant population of PV-IR cells (i.e., the AII amacrines) appears mature. At the INL/IPL border, these cells give rise to a single, stout process that descends into the IPL. In the distal IPL, radially-oriented dendritic branches end in characteristic swellings (so-called "lobular appendages"), while in the proximal IPL, dendrites form a fine plexus.

Following trk_B antisense treatment, PV-IR was markedly reduced, even after trk_B-IR had returned to detectable levels (at PND-15). This suggests a disruption in the neurochemical differentiation of the PV-IR phenotype. It was not possible to determine, from this analysis, if the noted decrease in PV-IR reflected both a morphological and neurochemical alteration in the predominant PV-IR population, the AII amacrine cell. For instance, a reduction in PV protein within a cell may merely render the finer dendritic processes devoid of immunostaining. This determination will require further study.

It is difficult to state conclusively whether the observed decrease in PV-IR is a direct effect of trk_B suppression by PV-IR cells or an indirect effect resulting from an alteration in the ganglion cell targets of PV-IR cells. Since many cells in the INL express only low levels of trk_B-IR, definitive double-label immunofluorescence studies are impossible. Another observation, however, suggests that the effect may be indirect. Namely, the number of PV-IR ganglion cells is not affected by trk_B antisense treatment. Therefore we propose that a morphological alteration in the dendritic arborization of the target ganglion cell, resulting from trk_B suppression, perturbs the development of the PV-IR, AII amacrine cell. Furthermore, this hypothesis provides a rationale for the observed variability in PV-IR in different brain regions in BDNF and trk_B knockout mice.

CaB-IR. It is noteworthy that the pattern and intensity of CaB-IR was unchanged following trk_B antisense treatment, particularly in light of the fact that both PV and CaB are reduced in the brains of BDNF knockout mice (25). Several factors may account for this discrepancy. First of all, factors that influence calcium-binding protein expression (e.g., trophic redundancy) may be quite different in the rat and mouse nervous systems. Secondly, CaB-IR apparently has a more heterogeneous distribution in the retina than in the cerebral cortex. In the retina, CaB-IR is localized to a wide variety of cell types in the inner retina. The targets and, hence, trophic support for these cells may provide a variety of soluble differentiation factors. Next, commitment to a neurochemically differentiated phenotype may occur at an earlier time for these neurons. Perhaps by targeting trk_B mRNA in the embryonic rat retina, we could affect CaB expression as well. Finally, it should be noted that CaB is not decreased in all brain regions of the BDNF-deficient mouse (25). This suggests that the expression of calcium-binding proteins is dependent upon different factors in different neuronal populations. Nevertheless, the present studies demonstrate that the effects of trk_B ligands (presumably BDNF, exclusively, *in vivo*) are quite specific on the regulation of neuronal phenotypic expression.

In summary, we have demonstrated the suppression of a *trk* receptor isoform, trk_B, in the neonatal retina using an antisense oligonucleotide directed to the trk_B mRNA. This technique has afforded us the opportunity to explore the effects of limiting, or "knocking down," the expression of this high-affinity BDNF receptor during the period of postnatal retinal development. These studies confirm an observation from BDNF knockout mice—that is, the decrease in PV expression. In the retina, PV-IR is present, predominantly, in a population of amacrine cells, the AIIs, that represents a central interneuron of the rod pathway. We suggest that BDNF has a role in the neurochemical differentiation of the AII amacrine cell and, therefore, in the development of a well-characterized retinal neuronal circuit.

We thank Nha Le for his excellent technical assistance and David McKinnon for the trk_B cDNA. These studies were supported, in part, by Research to Prevent Blindness, Inc.

1. Eide, F. F., Lowenstein, D. H. & Reichardt, L. F. (1993) *Exp. Neurol.* **121,** 200–214.
2. Götz, R., Köster, R., Winkler, C., Raulf, F., Lottspeich, F., Schartl, M. & Thoenen, H. (1994) *Nature (London)* **372,** 266–269.
3. Höfer, M., Pagliusi, S. R., Hohn, A., Leibrock, J. & Barde, Y. A. (1990) *EMBO J.* **9,** 2459–2464.
4. Maisonpierre, P. C., Bellusicio, L., Friedman, B., Alderson, F. R., Wiegand, S. J., Furth, M. E., Lindsay, R. M. & Yancopolos, G. D. (1990) *Science* **247,** 1446–1451.
5. Schecterson, L. C. & Bothwell, M. (1992) *Neuron* **9,** 449–463.
6. Rickman, D. W., Lauterborn, J. C., Brecha, N. C. & Gall, C. M. (1992) *Soc. Neurosci. Abstr.* **18,** 225.
7. Cohen-Cory, S. & Fraser, S. E. (1994) *Neuron* **12,** 747–761.
8. Martin-Zanca, D., Hughes, S. H. & Barbacid, M. (1986) *Nature (London)* **319,** 743–748.
9. Hempstead, B. L., Dionisio, M.-Z., Kaplan, D. R., Parada, L. F. & Chao, M. V. (1991) *Nature (London)* **319,** 743–748.
10. Klein, R., Nanduri, V., Jing, S., Lamballe, F., Tapley, P., Bryant, S., Cordon-Cardo, C., Jones, K. R., Reichardt, L. F. & Barbacid, M. (1991) *Cell* **66,** 395–403.
11. Lamballe, F., Klein, R. & Barbacid, N. (1991) *Cell* **66,** 967–979.
12. Ross, A. H. (1991) *Cell Regul.* **2,** 685–690.
13. Klein, R., Lamballe, F., Bryant, S. & Barbacid, M. (1992) *Neuron* **8,** 947–956.
14. Takahashi, J. B., Hoshimaru, M., Kikuchi, H. & Hatanaka, M. (1993) *Neurosci. Lett.* **151,** 174–177.
15. Ernfors, P., Merlio, J. P. & Persson, H. (1992) *J. Neurosci.* **4,** 1140–1158.
16. Zanellato, A., Comelli, M. C., Dal Toso, R. & Carmignoto, G. (1993) *Dev. Biol.* **159,** 105–113.
17. Rickman, D. W. & Brecha, N. C. (1995) *Visual Neurosci.* **12,** 215–222.
18. Johnson, J. E., Barde, Y.-A., Schwab, M. & Thoenen, H. (1986) *J. Neurosci.* **6,** 3031–3038.
19. Castillo, B., del Cerro, M., Breakefield, X. O., Frim, D. M., Barnstable, C. J., Dean, D. O. & Bohn, M. C. (1994) *Brain Res.* **647,** 30–36.
20. Mey, J. & Thanos, S. (1993) *Brain Res.* **602,** 302–317.
21. Mansour-Robaey, S., Clarke, D. B., Wang, Y.-C., Bray, G. M. & Aguayo, A. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 1632–1636.
22. Cohen, A., Bray, G. M. & Aguayo, A. J. (1994) *J. Neurobiol.* **25,** 953–959.
23. Perez, M.-T. R. & Caminos, E. (1995) *Neurosci. Lett.* **183,** 96–99.
24. Klein, R., Smeyne, R. J., Wurst, W., Long, L. K., Auerbach, B. A., Joyner, A. L. & Barbacid, M. (1993) *Cell* **75,** 113–122.
25. Jones, K. R., Farinas, I., Backus, C. & Reichardt, L. F. (1994) *Cell* **76,** 989–999.
26. Snider, W. D. (1994) *Cell* **77,** 627–638.
27. Endo, T., Kobayashi, M., Kobayashi, S. & Onaya, T. (1986) *Cell Tissue Res.* **243,** 213–217.
28. Hamano, K., Kiyama, H., Emson, P. C., Manabe, R., Nakauchi, M. & Tohyama, M. (1990) *J. Comp. Neurol.* **302,** 417–424.
29. Pasteels, B., Rogers, J., Blachier, F. & Pochet, R. (1990) *Visual Neurosci.* **5,** 1–16.
30. Sanna, P. P., Keyser, K. T., Celio, M. R., Karten, H. J. & Bloom, F. E. (1993) *Brain Res.* **600,** 141–150.
31. Wässle, H., Grünert, U. & Röhrenbeck, J. (1993) *J. Comp. Neurol.* **332,** 407–420.
32. Eng, L. F. (1993) in *Molecular and Cellular Approaches to the Treatment of Neurological Diseases*, ed. Waxman, S. G. (Raven, New York), pp. 293–310.
33. Wahlestedt, C. (1994) *Trends Pharmacol. Sci.* **15,** 42–46.
34. Dixon, J. E. & McKinnon, D. (1994) *Dev. Brain Res.* **77,** 177–182.
35. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
36. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137,** 266 (addendum).
37. Gall, C. M., Gold, S. J., Isackson, P. J. & Seroogy, K. B. (1992) *Mol. Cell. Neurosci.* **3,** 56–63.
38. Lauterborn, J. C. & Gall, C. M. (1994) *Mol. Cell. Neurosci.* **5,** 46–62.
39. Bunt, A. H., Lund, R. D. & Lund, J. S. (1974) *Brain Res.* **73,** 215–228.

40. Kondo, Y., Takada, M., Honda, Y. & Mizuno, N. (1993) *Brain Res.* **608,** 204–215.
41. Carmignoto, G., Comelli, M. C., Candeo, P., Cavicchioli, L., Yan, Q., Merighi, A. & Mafei, L. (1991) *Exp. Neurol.* **111,** 302–311.
42. McAllister, A. K., Lo, D. C. & Katz, L. C. (1995) *Neuron* **15,** 791–803.
43. Boos, R., Schneider, H. & Wässle, H. (1993) *J. Neurosci.* **13,** 2874–2888.
44. Chun, M. H., Han, S. H., Chung, J. W. & Wässle, H. (1993) *J. Comp. Neurol.* **332,** 421–432.
45. Famiglietti, E. V. & Kolb, H. (1975) *Brain Res.* **84,** 293–300.
46. Sterling, P. (1983) *Annu. Rev. Neurosci.* **6,** 149–185.
47. Strettoi, E., Raviola, E. & Dacheux, R. F. (1992) *J. Comp. Neurol.* **325,** 152–168.
48. Guo, Q. X., Yu, M. C., Garey, L. J. & Jen, L. S. (1992) *Exp. Brain Res.* **90,** 359–368.