Cell Tissue Res
DOI 10.1007/s00441-005-0025-z

REGULAR ARTICLE

Eun-Jin Lee · Myoung-Chul Song · Hyun-Ju Kim · Eun-Jin Lim · In-Beom Kim · Su-Ja Oh · Jung-IL Moon · Myung-Hoon Chun

Brain-derived neurotrophic factor modulates the dopaminergic network in the rat retina after axotomy

Received: 19 March 2005 / Accepted: 1 June 2005
© Springer-Verlag 2005

Abstract Dopaminergic cells in the retina express the receptor for brain-derived neurotrophic factor (BDNF), which is the neurotrophic factor that influences the plasticity of synapses in the central nervous system. We sought to determine whether BDNF influences the network of dopaminergic amacrine cells in the axotomized rat retina, by immunocytochemistry with an anti-tyrosine hydroxylase (TH) antiserum. In the control retina, we found two types of TH-immunoreactive amacrine cells, type I and type II, in the inner nuclear layer adjacent to the inner plexiform layer (IPL). The type I amacrine cell varicosities formed ring-like structures in contact with AII amacrine cell somata in stratum 1 of the IPL. In the axotomized retinas, TH-labeled processes formed loose networks of fibers, unlike the dense networks in the control retina, and the ring-like structures were disrupted. In the axotomized retinas treated with BDNF, strong TH-immunoreactive varicosities were present in stratum 1 of the IPL and formed ring-like structures. Our data suggest that BDNF affects the expression of TH immunoreactivity in the axotomized rat retina and may therefore influence the retinal dopaminergic system.

Keywords Tyrosine hydroxylase · AII amacrine cells · BDNF · Optic nerve transection · Immunocytochemistry · Rat (Sprague Dawley, adult male, albino)

E.-J. Lee and M.-C. Song contributed equally to this work.
This work was supported by Korea Research Foundation (grant no. E00004, 2004).

E.-J. Lee · M.-C. Song · H.-J. Kim · E.-J. Lim · I.-B. Kim · S.-J. Oh · M.-H. Chun (✉)
Department of Anatomy, College of Medicine, The Catholic University of Korea,
505 Banpo-dong, Socho-gu,
Seoul, 137-701, South Korea
e-mail: mhchun@catholic.ac.kr
Tel.: +82-2-5901152
Fax: +82-2-363110

J.-I. Moon
Department of Ophthalmology, College of Medicine
The Catholic University of Korea,
505 Banpo-dong, Socho-gu,
Seoul, 137-701, South Korea

Introduction

The nerve growth factor (NGF) gene family, referred to as the neurotrophins, comprises largely characterized neurotrophic factors, including NGF itself, brain-derived neurotrophic factor (BDNF), neurotrophin-3, and neurotrophin-4/5 (Ibanez 1994). Neurotrophins and their cognate receptors (TrkA, TrkB, and TrkC) are expressed primarily in neurons, where they mediate diverse effects and promote the differentiation, maturation, and survival of neurons in both the peripheral and central nervous systems (Davies 1994; Barbacid 1995; Cellerino and Maffei 1996; Lewin and Barde 1996). In the retina, BDNF promotes the survival of ganglion cells in vitro (Johnson et al. 1986; Rodriguez-Tebar et al. 1989; Thanos et al. 1989; Cohen-Cory and Fraser 1994) and in vivo (Mey and Thanos 1993; Mansour-Robaey et al. 1994; Weibel et al. 1995). In addition, neurotrophin isoforms of the Trk receptors are expressed throughout phenotypic differentiation by both ganglion cells and numerous cells in the inner nuclear layer (INL; Rickman and Brecha 1995). The majority of these cells remains unidentified, but a recent study (Cellerino and Kohler 1997) has revealed that, in a wide range of species, most dopaminergic cells express the BDNF receptor, TrkB. Furthermore, in the rabbit retina, BDNF increases dopamine release (Neal et al. 2003).

Dopaminergic amacrine cells are a particularly well-described subtype of retinal neurons and of the wide-field amacrine cells that receive input from the cone bipolar cells (Hokoc and Mariani 1987; Voigt and Wässle 1987; Dacey 1990). Dopaminergic amacrine and interplexiform cells in the retina establish synapses on AII amacrine cells (Pourcho 1982; Voigt and Wässle 1987; Kolb et al. 1990, 1991), a neuronal cell type involved in the rod pathway that carries dim-light signals to ganglion cells. The balance between rod and cone inputs to the ganglion cells is under the

control of AII amacrine cells, which are inhibited by dopaminergic amacrine cells (Müller et al. 1988; Witkovsky and Schutte 1991). Light increases dopamine release, and this light-induced release of dopamine is believed to play a role in the inhibitory mechanisms underlying light adaptation (Witkovsky and Schutte 1991). Thus, dopamine acts as a neurotransmitter and modulator in the retinas of all vertebrates.

Previous studies have shown that dopaminergic neurons in the vertebrate retina express the BDNF receptor TrkB (Barbacid 1994) and have suggested that BDNF controls the development of the retinal dopaminergic network (Cellerino et al. 1998). In this study, we have examined the expression of TH-immunoreactive amacrine cells in the rat retina after optic nerve transection (ONT) and by means of antisera to tyrosine hydroxylase (TH) to determine whether removal of ganglion cells (the expected source of neurotrophic support in the INL) affects the network of dopaminergic amacrine cells. In addition, we have investigated whether BDNF is required to form the ring-like structures in stratum 1 of the inner plexiform layer (IPL); these structures constitute a major conventional synaptic output onto the interneuronal AII amacrine cells of the rod pathway in mammalian retinas.

Materials and methods

Tissue preparation

Twenty adult male albino Sprague–Dawley rats weighing 200–250 g were used. Six were designated as controls, and seven were used for each of two experimental groups. The animals were treated according to the regulations of the Catholic Ethics Committee of the Catholic University of Korea, Seoul and to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications, no. 80-23, revised 1996).

The animals in the experimental groups were deeply anesthetized by intraperitoneal injection of 4% chloral hydrate (1 ml/100 g body weight). Optic nerve transection (ONT) was performed 5 mm from the posterior pole of the eye without damaging the retinal blood supply. At 7 and 14 days after ONT, the animals were sacrificed by an overdose of chloral hydrate, and the eyes were enucleated. The anterior segments were removed, and the eyecups were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2–3 h. Following fixation, the retinas were carefully dissected, transferred to 30% sucrose in PB for 24 h at 4°C, and frozen in liquid nitrogen for storage. They were thawed and rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.4) before use.

Immunocytochemistry

For fluorescence immunocytochemistry, 50-μm-thick vibratome sections were cut from the frozen retinas, thawed, and rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.4). The sections were incubated in PBS containing 10% normal goat serum (NGS) and 1% Triton X-100 for 1 h at room temperature to block nonspecific binding sites and then overnight at 4°C with a mouse anti-TH monoclonal antibody (1:1,000; Chemicon International, Temecula, Calif.) in PBS containing 0.5% Triton X-100, followed by three washes in PBS (each 15 min) and incubation with fluorescein isothiocyanate (FITC)-conjugated affinity-purified anti-mouse IgG (1:100; Jackson ImmunoResearch Laboratories, West Grove, Pa.) for 2 h at room temperature. After further washes in 0.1 M PB for 30 min, the sections were mounted with 10% glycerol in 0.1 M PB.

For double-labeling studies, sections were incubated with a mixture of rabbit anti-TH polyclonal antibody (1:1,000; Protos Biotech, New York) and mouse anti-parvalbumin monoclonal antibody (1:500; Sigma, St. Louis, Mo.) in 0.1 M PBS containing 0.5% Triton X-100 overnight at 4°C, rinsed for 30 min with 0.1 M PBS, and incubated with FITC-conjugated affinity-purified anti-rabbit IgG (1:100; Jackson ImmunoResearch) and Cy3-conjugated anti-mouse IgG (1:100; Jackson ImmunoResearch) for 1–2 h at room temperature. After further washes in 0.1 M PB for 30 min, the sections were mounted with 10% glycerol in 0.1 M PB.

To confirm that the secondary antibody did not cross-react with an inappropriate primary antibody, some sections were incubated with rabbit polyclonal primary antibody followed by anti-mouse secondary antibody, and other sections were incubated with mouse primary antibody followed by anti-rabbit secondary antibody. These sections showed no immunostaining.

Confocal laser scanning microscopy

The sections were analyzed by using a Bio-Rad Radiance Plus confocal scanning microscope (Bio-Rad, Hemel Hempstead, UK) installed on a Nikon Eclipse E600 fluorescence microscope (Nikon, Tokyo, Japan). The FITC and Cy3 signals were detected separately. The FITC label was excited by using the 488-nm line of an argon ion laser and was detected after passage through an HQ513/30 (Bio-Rad) emission filter. For the detection of the Cy3 signal, the 543-nm line of a green HeNe laser was used in combination with a 605/32 (Bio-Rad) emission filter. All images were scaled to their final size, adjusted for contrast and brightness, labeled, and formatted by using Adobe Photoshop version 5.5 (Adobe Systems, Mountain View, Calif.).

Intravitreal BDNF treatment

For one group of experimental animals, human recombinant BDNF (5 μg in 5 μl sterile saline; Regeneron Pharmaceuticals, Tarrytown, N.Y.) was injected into the vitreal chamber of each eye immediately after ONT, by using a Hamilton syringe with a 30-gauge needle. The injection was made over a 30-s period, and the needle was left in position for an additional 12 min to allow for the diffusion

of BDNF from the injection site and to minimize backflow. Sham injections, for controls, consisted of 5 μl sterile saline. In all cases, we took care to avoid the lens and ciliary body, as these structures are potential sources of endogenous neurotrophic factors (Bennett et al. 1999; Leon et al. 2000) that might enhance retinal ganglion survival (Mansour-Robaey et al. 1994).

Measurement of the immunostained area

The area percent of labeled processes per unit area (1 mm^2) was measured in stratum 1 of the IPL of the central region near the optic disc by using an image analysis system, BMI-PLUS (Bummi, Ansan, Korea). Images were captured via a 40× objective and 10× eyepieces and then processed. The TH-immunoreactive area was expressed as the percentage of labeled area per unit area (1 mm^2), and the data were recorded as mean values±standard deviation. Statistical evaluation was based on ANOVA *F*-test and multiple comparison, with $P<0.05$ indicating significance.

Results

TH immunoreactivity

A distinct subpopulation of amacrine cells (Fig. 1a–c) displayed TH immunoreactivity. Two subpopulations of TH-immunoreactive cells containing dopamine (Nguyen-Legros et al. 1994) were originally demonstrated in the rat retina. As a goal of our study was to identify the effect of BDNF on the ring-like structures of dopaminergic cells, we focused on the morphological changes in the processes of type I TH-immunoreactive cells. In the control retinas, type I cells had a large soma, which was highly branched, mainly in the outermost sublayer (stratum 1) of the IPL (Fig. 1a) in which these fibers formed the so-called dopaminergic rings (Pourcho 1982; Voigt and Wässle 1987; Kolb et al. 1990). Some fibers ascended to the outer plexiform layer (OPL) of the retina. Occasionally processes in stratum 1 ran into more internal sublayers (stratum 3) of the IPL (Kolb et al. 1990). In the experimental retinas at 7 days (Fig. 1b) and 14 days (Fig. 1c) after ONT, the type I cell varicosities in stratum 1 of the IPL displayed less-dense immunoreactivity (Fig. 1b,c). In whole-mount retinal preparations, where we focused on the INL adjacent to the IPL, TH-immunoreactive amacrine cell somata were distributed throughout the retina (Fig. 2a–c). In the control retinas (Fig. 2a), three primary dendrites emerged from the type I cells, and a dense plexus of varicose fibers was observed in stratum 1. This dendritic network was not homogeneous: circular holes surrounded by dendritic rings were clearly visible (Fig. 2a). The presence of ring-like structures in the rat retina agreed with observations in other mammalian retinas (Brecha et al. 1984; Voigt and Wässle 1987; Nguyen-Legros 1988). In the rat retina, these holes are occupied by AII amacrine cell bodies (Voigt and Wässle 1987). In the experimental retinas at 7 days (Fig. 2b) and 14 days (Fig. 2c) after ONT, primary dendrites emerging from type I cells were hardly visible, and the dense network of labeled processes was disrupted in stratum 1 (Fig. 2b,c). The area percent of labeled processes per unit area (1 mm^2) was calculated (summarized in Table 1). In the normal retina, the percent area of the TH-labeled processes corresponded to 22.5±3.7%. The percent area decreased significantly up to 2 weeks after ONT. At 1 and 2 weeks, the labeled area occupied 16.1±1.5% and 13.4±1.2% of the unit area, respectively. These results suggested that the removal of ganglion cells by ONT changed the dendritic network of TH-immunoreactive amacrine cells because of the lack of neurotrophic support delivered by the ganglion cell axons.

Double immunofluorescence for TH and parvalbumin

The dopaminergic amacrine or interplexiform neurons of the retina establish synapses on AII amacrine cells (Voigt

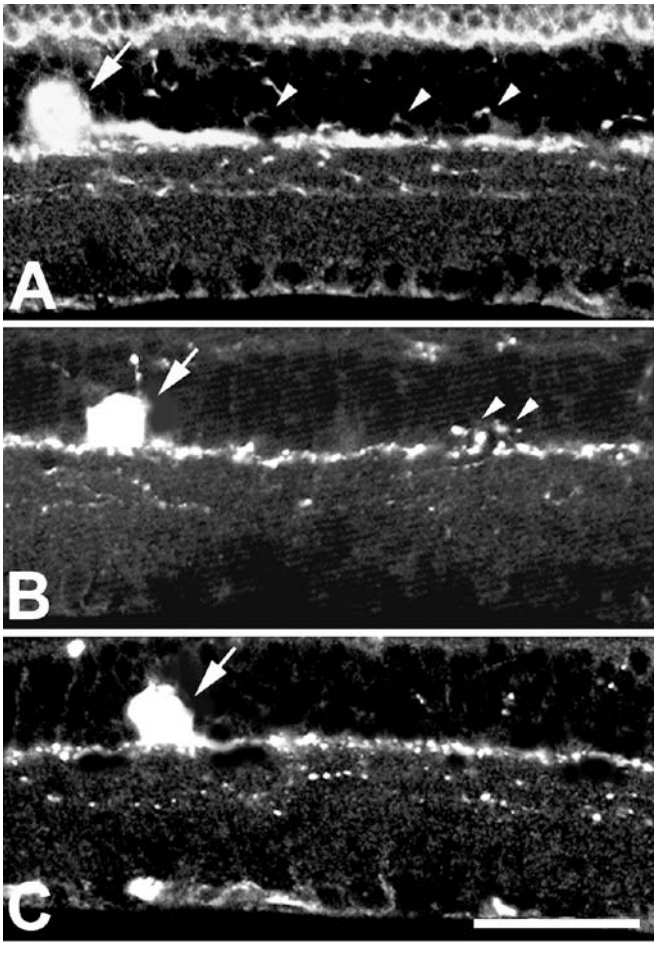

Fig. 1 Light micrographs taken from 50-μm-thick vertical vibratome sections processed for TH immunoreactivity in the control retina (**a**) and experimental retinas at 7 days (**b**) and 14 days (**c**) after optic nerve transection (ONT). **a**–**c** A TH-immunoreactive type I amacrine cell (*arrows*) with a larger-sized soma is located in the inner nuclear layer (INL) adjacent to the inner plexiform layer (IPL). At 7 (**b**) and 14 (**c**) days after ONT, the type I amacrine cell varicosities forming ring-like structures in stratum 1 of the IPL (*arrowheads*) are reduced. *Bar* 50 μm

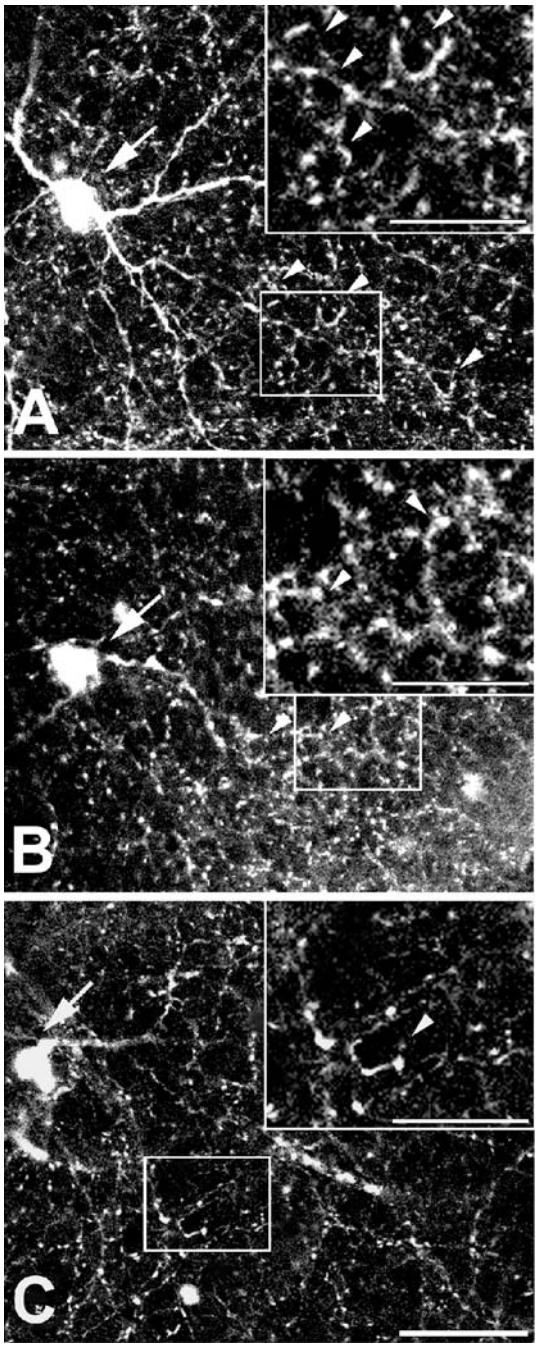

Fig. 2 Light micrographs of whole-mount preparations processed for TH immunoreactivity in the control retina (**a**) and experimental retinas at 7 days (**b**) and 14 days (**c**) after ONT. **a**–**c** INL adjacent to IPL. TH-immunoreactive type I amacrine cell bodies (*arrows*). At 7 (**b**) and 14 (**c**) days after ONT, primary processes originating from the type 1 TH-labeled amacrine cell are barely visible, and the labeled processes form a loose network in stratum 1 of the IPL (*arrowheads*). *Insets* Higher magnification of the adjacent *boxes*. *Bar* 50 μm

and Wässle 1987), a neuronal type inserted in series along the pathway that carries dim-light signals to ganglion cells. Therefore, we have investigated whether ONT affects the ring-like structure that surrounds the AII amacrine cells at the border of the INL and IPL.

Table 1 Area percent of labeled processes calculated from central region of the retina processed for TH immunoreactivity in experimental groups of the rat retina

Experimental groups	Area percent of labeled profiles (%)
Control (*n*=6)	25.5±3.7
Axotomized retina	
1 week (*n*=3)	16.1±1.5*
2 weeks (*n*=4)	13.4±1.2*
BDNF-treated axotomized retina	
1 week (*n*=3)	28.5±1.5
2 weeks (*n*=4)	31.6±5.3

*P<0.05

Double-labeling experiments with antisera against TH (Fig. 3a,d,g) and parvalbumin (Fig. 3b,e,h) were performed with control retinas (Fig. 3a–c) and experimental retinas at 7 days (Fig. 3d–f) and 14 days (Fig. 3g–i) after ONT. In the control retinas (Fig. 3a,c), the type I cell dendrites formed an extensive network at the INL–IPL border, as was seen in Fig. 2a. The type I cell processes contacted other fibers and coalesced into a dense network of ring-like structures. Regularly distributed somata of parvalbumin-immunoreactive amacrine cells were evident (Fig. 3b). Merger of the micrographs shown in Fig. 3a,b showed that the parvalbumin-immunoreactive cell bodies were surrounded by ring structures (Fig. 3c). In the experimental retinas at 7 days (Fig. 3d–f) and 14 days (Fig. 3g–i) after ONT, a loose network of ring-like structures formed by TH-immunoreactive type I cells was seen (Fig. 3d,g). Merger of the micrographs in Fig. 3d,e or g,h showed that fewer ring-like structures surrounded the parvalbumin-labeled amacrine cell somata than in the control retina. These findings demonstrated that the network of TH-immunoreactive amacrine cell processes made fewer synaptic outputs onto AII amacrine cells in the axotomized rat retina than in the control retina.

BDNF affects TH expression in the axotomized retina

To determine whether BDNF affected TH immunoreactivity in the axotomized retinas, BDNF (5 μg in 5 μl) was injected intravitreally after ONT in one group of experimental animals. In the BDNF-treated retinas at 7 days (Fig. 4b) and 14 days (Fig. 4c) after ONT, the distibution patterns of TH-labeled somata and processes were similar to those of the control retinas (Fig. 4b,c).

A comparison of Fig. 2b,c with Fig. 4b,c reveals differences in the TH-immunoreactive patterns between the BDNF-treated axotomized retinas and untreated axotomized retinas. The processes of the type I amacrine cells are more densely arborized and the TH-labeled varicosities appear to be more numerous in the BDNF-treated retinas.

As shown in Table 1, the percent area of the TH-labeled processes in the BDNF-treated axotomized retinas was

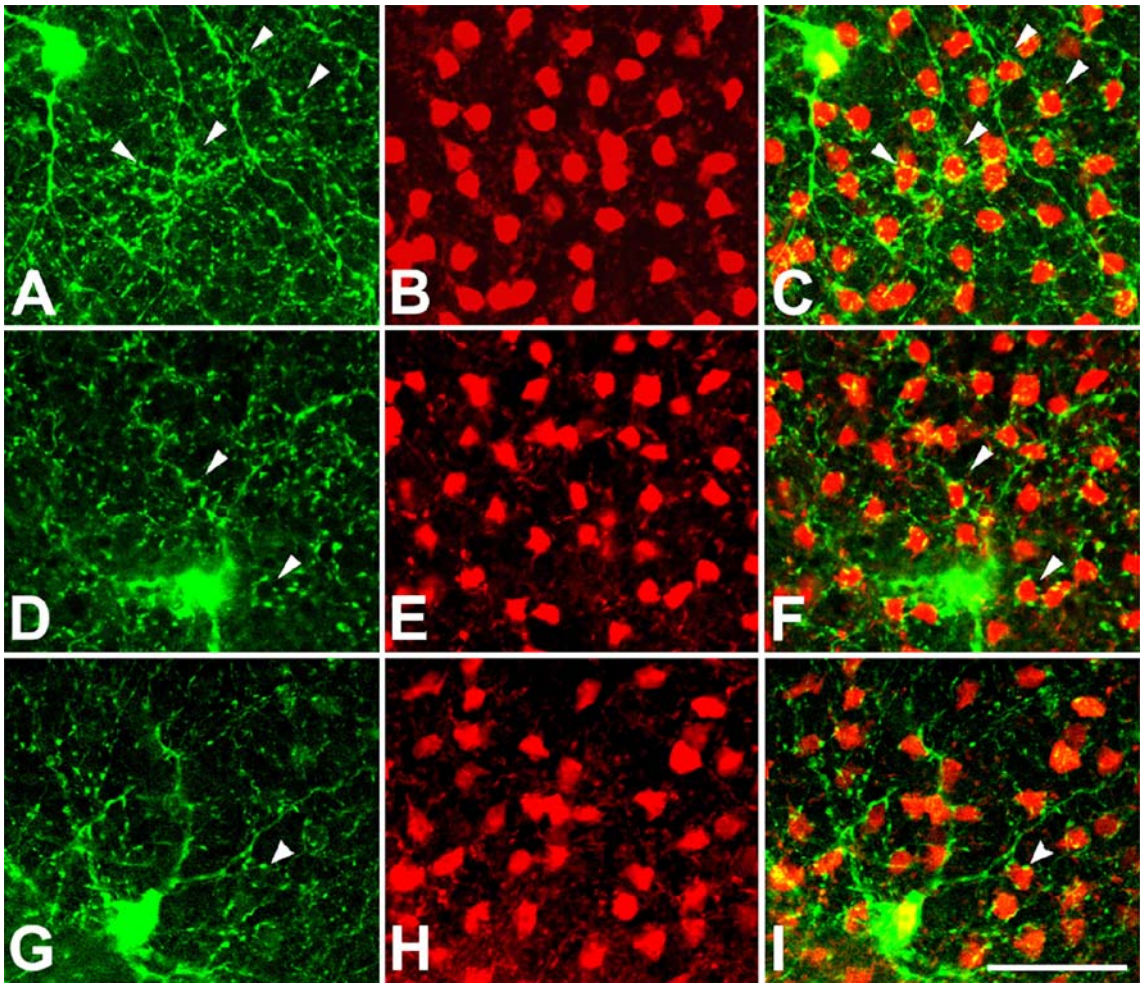

Fig. 3 Confocal micrographs of whole-mount preparations processed for TH (**a**, **d**, **g**) and parvalbumin (**b**, **e**, **h**) immnoreactivity for the control retina (**a**–**c**) and experimental retinas at 7 days (**d**–**f**) and 14 days (**g**–**i**) after ONT. TH and parvalbumin immunoreactivity was visualized with an FITC-conjugated secondary antibody and a Cy3-conjugated secondary antibody, respectively. **a**, **d**, **g** A TH-immnoreactive type I amacrine cell and its processes. **b**, **e**, **h** Parvalbumin-immunoreactive amacrine cell bodies and lobular appendages. **c** Merged image of TH and parvalbumin processes around an AII amacrine cell body in the control retina. **f**, **i** Merged images showing that the TH-labeled processes contacting AII amacrine cell bodies are visibly reduced at 7 days (**f**) and 14 days (**i**) after ONT (*arrowheads*). *Bar* 50 μm

similar to that of the control retina and unlike untreated axotomized retinas. At 1 and 2 weeks, the labeled area occupied 28.5±1.5% and 31.6±5.3% of the unit area, respectively. These results clearly indicated that BDNF was essential for the expression of TH within dopaminergic amacrine cells.

BDNF affects the ring-like structures formed by dopaminergic amacrine cells

To determine whether BDNF affects the ring-like structures that surround the somata of the glycinergic AII amacrine cells, double-labeling experiments with antisera against TH (Fig. 5a,d,g) and parvalbumin (Fig. 5b,e,h) were performed in control retinas (Fig. 5a–c) and in experimental retinas at 7 days (Fig. 5d–f) and 14 days (Fig. 5g–i) after ONT. In BDNF-treated retinas at 7 and 14 days after ONT, the plexus of dopaminergic fibers localized in stratum 1 (Fig. 5d,g) of the IPL was denser than that in untreated axotomized retinas. Similarly, in the merged images (Fig. 5c,f,i), more densely packed parvalbumin-immunoreactive amacrine cell somata in contact with dopaminergic cell dendrites were visible at the INL–IPL border in the BDNF-treated retinas than in the untreated axotomized retinas. Our results clearly indicated that BDNF was essential for the maintenance of the dopaminergic amacrine cell network.

Discussion

In this study, we have demonstrated the expression of dopaminergic amacrine cells in axotomized rat retinas by using antisera against TH. In addition, BDNF has been injected intravitreally into the axotomized retinas to determine the effect of BDNF on the dopaminergic network. In unlesioned control retinas, TH immunoreactivity has been

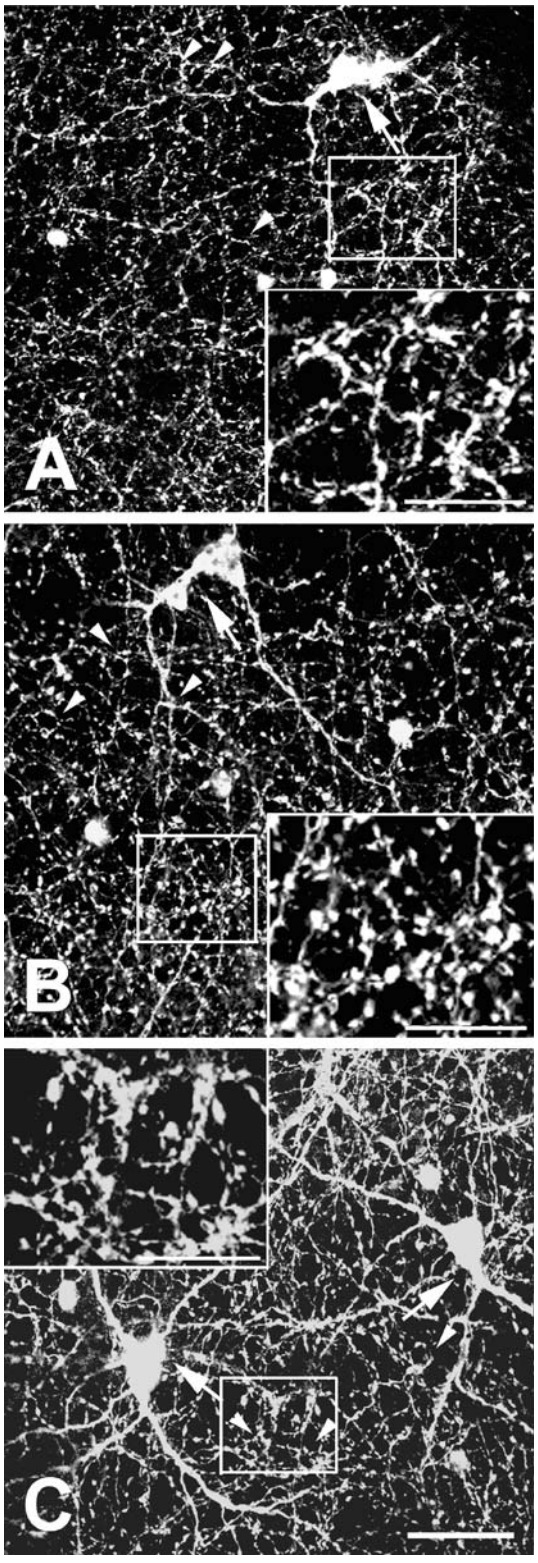

Fig. 4 Light micrographs of whole-mounted preparations processed for TH immunoreactivity in the control retina (**a**) and intravitreal BDNF-treated experimental retinas at 7 days (**b**) and 14 days (**c**) after ONT (*arrows* TH-immunoreactive type I amacrine cell bodies, *arrowheads* labeled processes). **b**, **c** INL adjacent to the IPL. The distribution patterns of TH-labeled processes are similar in the BDNF-treated and control retinas. *Insets* Higher magnification of the adjacent *boxes*. *Bar* 50 μm

seen in a specific population of amacrine cells with large somata (type I) and processes ramifying mainly in stratum 1, where they form ring-like structures, as reported previously for several mammalian retinas (Voigt and Wässle 1987; Mitrofanis and Provis 1990; Casini and Brecha 1992). Processes originating from TH-labeled amacrine cells descend into stratum 3 of the IPL. In addition, interplexiform cells have been found among the type I cells (Savy et al. 1991; Nguyen-Legros et al. 1997). TH immunoreactivity has also been noted in the small-body (type II) cells, the processes of which descend through the IPL and ramify in stratum 3 of the IPL. These observations agree with previous studies showing a similar distribution of TH immunoreactivity in the retinas of primates (Crooks and Kolb 1992) and rats (Mitrofanis et al. 1988; Crooks and Kolb 1992; Young 1994). However, at 7 and 14 days after ONT, TH immunoreactivity in the type I cells shows a different pattern. The processes of TH-labeled amacrine cells are disrupted, and the ring structures formed by the processes are less frequently observed. Dopaminergic ring-like structures are known to establish synapses onto the somata of AII amacrine cells (Pourcho 1982; Voigt and Wässle 1987; Kolb et al. 1990, 1991), a neuronal cell involved in the rod pathway that carries dim-light signals to ganglion cells. The difference in the phenotypic expression of TH immunoreactivity between the control and axotomized rat retinas stems from the elimination of ganglion cells; this deprives amacrine cells of their BDNF source and of other potential neurotrophic factors derived from ganglion cells. Further evidence that ganglion cells may be responsible for the expression pattern of TH-immunoreactive cells in the axotomized retina comes from studies showing that more than 90% of TH-immunoreactive cells die by 14 days after ONT (Berkelaar et al. 1994; Huxlin et al. 1995; Villegas-Perez et al. 1993). Our findings are also consistent with a previous report showing that natural cell death of developing amacrine cells in the vertebrate retina is accentuated by the early removal of retinal ganglion cells (Cusato et al. 2002). In addition, because dopaminergic neurons respond to several factors, the removal of both NT-3 and BDNF impairs their development to an even greater degree (Cellerino and Kohler 1997).

The intravitreal injection of BDNF to the axotomized retinas produces dramatic changes in the expression of TH. The distribution pattern of TH-labeled processes in stratum 1 is similar to that of the control retina. This finding is consistent with a previous report showing that BDNF increases dopamine uptake activity and the survival of cells containing TH, a catecholamine biosynthetic enzyme, in neuron-enriched cultures of embryonic rat ventral mesencephalon (Knusel et al. 1991; Hyman et al. 1991). An interesting characteristic of dopaminergic retinal neurons is their use of gamma-aminobutyric acid (GABA) as a co-neurotransmitter (Wässle and Chun 1988). Dopaminergic cells containing GABA are also known to be affected by BDNF in the cortex and hippocampus of the brain (Nawa et al. 1994; Cellerino et al. 1996). In addition, dopaminergic neurons express the BDNF receptor, TrkB, in the vertebrate retina (Barbacid 1994). Thus, BDNF may con-

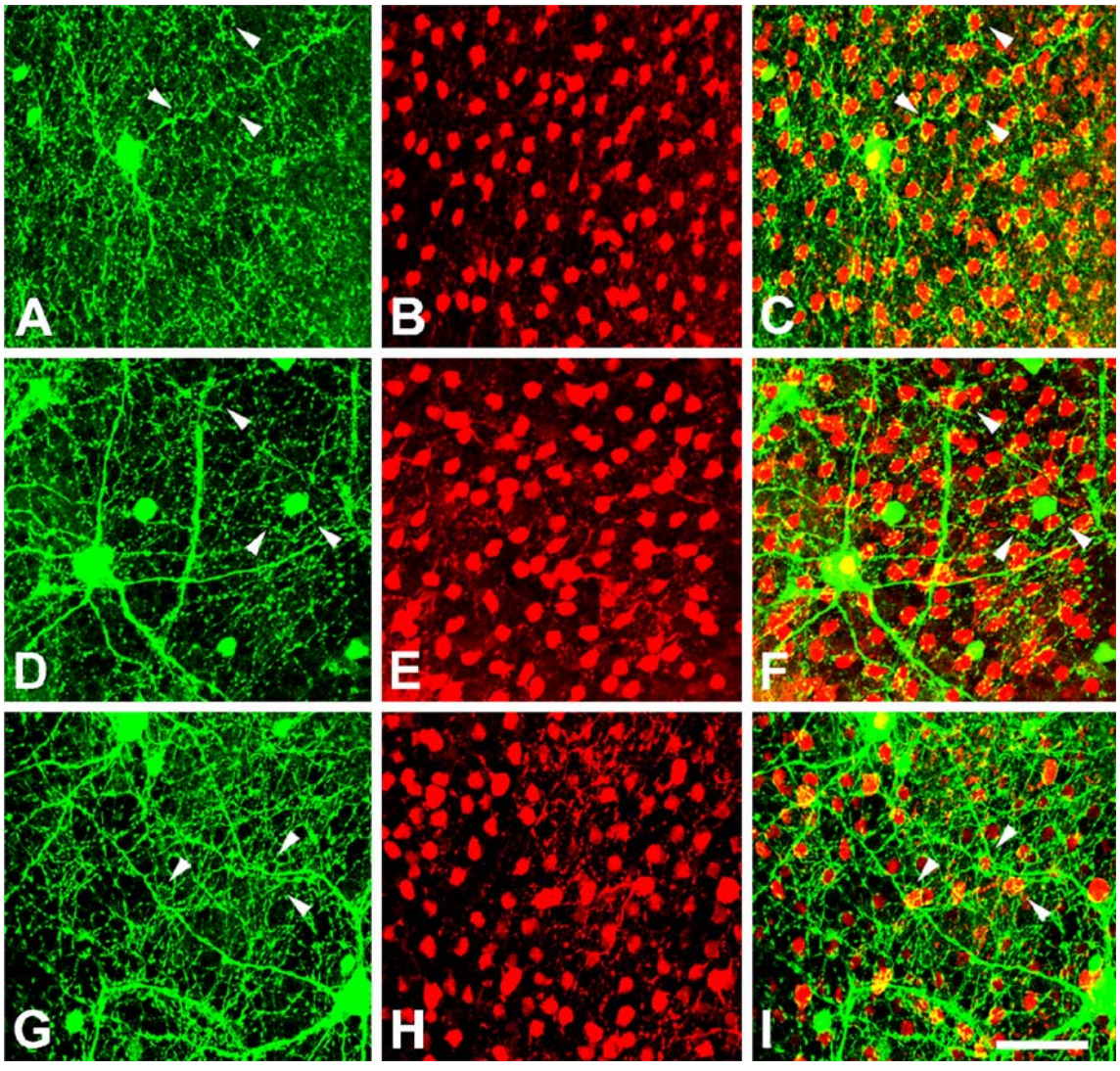

Fig. 5 Confocal micrographs of whole-mount preparations processed for TH (**a**, **d**, **g**) and parvalbumin (**b**, **e**, **h**) immunoreactivities and the merged images (**c**, **f**, **i**) for the control retina (**a**–**c**) and intravitreal BDNF-treated experimental retinas at 7 days (**d**–**f**) and 14 days (**g**–**i**) after ONT. TH and parvalbumin immnoreactivity was visualized by using an FITC-conjugated secondary antibody and a Cy3-conjugated secondary antibody, respectively. **a**, **d**, **g** Strong TH immunoreactivity and numerous TH-immunoreactive type I varicosities are visible. **b**, **e**, **h** Parvalbumin-immunoreactive amacrine cell bodies and lobular appendages are visible. **c**, **f**, **i** Merged images of TH and parvalbumin showing that similar ring-like structures contacting AII amacrine cell somata are present in the control retina (**c**) and the BDNF-treated axotomized retinas at 7 days (**f**) and 14 days (**i**) after ONT (*arrowheads*). *Bar* 50 μm

trol the retinal dopaminergic system and influence a modulatory action in the retina, even though retinal BDNF mRNA is not dramatically reduced when retinal ganglion cells are eliminated by section of the optic stalk (Herzog and von Bartheld 1998). Furthermore, our study suggests that, although the small reduction in the level of BDNF induced by an ONT may not affect cell survival in the INL, the reduced level of BDNF may influence synaptogenesis, activity-dependent synaptic plasticity, and the synthesis of neurotransmitters and neuromodulators (for reviews, see Thoenen 1995; Lo 1995; Bonhoeffer 1996; Cellerino and Maffei 1996).

In the retina, dopaminergic amacrine cells are in contact with many of the neurons in the INL (Voigt and Wässle 1987), and AII amacrine cells are a major target of the dopaminergic synaptic output of amacrine cells. The distinct ring-like clusters of varicosities observed by using catecholamine histofluorescence (Mariani et al. 1984) or TH immunoreactivity (Brecha et al. 1984) are the sites of dense synaptic input to the soma and proximal dendrites of the AII amacrine cell type (Pourcho 1982; Voigt and Wässle 1987). The disruption of the ring-like structures in the axotomized retina may disturb the balance between rod and cone inputs to the ganglion cells. In the present study, the disrupted ring-like structures in the axotomized retina were re-formed following BDNF treatment. Dopamine has been reported to modulate the permeability of the gap junctions of AII amacrine cell to tracers (Hampson et al. 1992; Mills and Massey 1995). Taken together, these findings indicate that BDNF may control dopamine release, thereby balancing the rod and cone pathway in the vertebrate retina.

References

Barbacid M (1994) The Trk family of neurotrophin receptors. J Neurobiol 25:1386–1403

Barbacid M (1995) Neurotrophic factors and their receptors. Curr Opin Cell Biol 7:148–155

Bennett JL, Zeiler SR, Jones KR (1999) Patterned expression of BDNF and NT-3 in the retina and anterior segment of the developing mammalian eye. Invest Ophthalmol Vis Sci 40:2996–3005

Berkelaar M, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Axotomy results in delayed death and apoptosis of retinal ganglion cells in adult rats. J Neurosci 14:4368–4374

Bonhoeffer T (1996) Neurotrophins and activity-dependent development of the neocortex. Curr Opin Neurobiol 6:119–126

Brecha NC, Oyster CW, Takahashi ES (1984) Identification and characterization of tyrosine hydroxylase immunoreactive amacrine cells. Invest Ophthalmol Vis Sci 25:66–70

Casini G, Brecha NC (1992) Postnatal development of tyrosine hydroxylase immunoreactive amacrine cells in the rabbit retina. II. Quantitative analysis. J Comp Neurol 326:302–313

Cellerino A, Kohler K (1997) Brain-derived neurotrophic factor/neurotrophin-4 receptor TrkB is localized on ganglion cells and dopaminergic amacrine cells in the vertebrate retina. J Comp Neurol 386:149–160

Cellerino A, Maffei L (1996) The action of neurotrophins in the development and plasticity of the visual cortex. Prog Neurobiol 49:53–71

Cellerino A, Maffei L, Domenici L (1996) The distribution of brain-derived neurotrophic factor and its receptor TrkB in parvalbumin-containing neurons of the rat visual cortex. Eur J Neurosci 8:1190–1197

Cellerino A, Pinzon-Duarte G, Carroll P, Köhler K (1998) Brain-derived neurotrophic factor modulates the development of the dopaminergic network in the rodent retina. J Neurosci 18:3351–3362

Cohen-Cory S, Fraser SE (1994) BDNF in the development of the visual system of *Xenopus*. Neuron 12:747–761

Crooks J, Kolb H (1992) Localization of GABA, glycine, glutamate and tyrosine hydroxylase in the human retina. J Comp Neurol 315:287–302

Cusato K, Bosco A, Linden R, Reese BE (2002) Cell death in the inner nuclear layer of the retina is modulated by BDNF. Dev Brain Res 139:325–330

Dacey DM (1990) The dopaminergic amacrine cell. J Comp Neurol 301:461–489

Davies AM (1994) The role of neurotrophins in the developing nervous system. J Neurobiol 25:1334–1348

Hampson EC, Vaney DI, Weiler R (1992) Dopaminergic modulation of gap junction permeability between amacrine cells in mammalian retina.J Neurosci 12:4911–4922

Herzog KH, Bartheld CS von (1998) Contributions of the optic tectum and the retina as sources of brain-derived neurotrophic factor for retinal ganglion cells in the chick embryo. J Neurosci 18:2891–2906

Hokoc JN, Mariani AP (1987) Tyrosine hydroxylase immunoreactivity in the rhesus monkey retina reveals synapses from bipolar cells to dopaminergic amacrine cells. J Neurosci 7:2785–2793

Huxlin KR, Dreher B, Schulz M, Sefton AJ, Bennett MR (1995) Effect of collicular proteoglycan on the survival of adult rat retinal ganglion cells following axotomy. Eur J Neurosci 7:96–107

Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD, Squinto SP, Lindsay RM (1991) BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. Nature 350:230–232

Ibanez CF (1994) Structure-function relationships in the neurotrophin family. J Neurobiol 25:1349–1361

Johnson JE, Barde YA, Schwab M, Thoenen H (1986) Brain-derived neurotrophic factor supports the survival of cultured rat retinal ganglion cells. J Neurosci 6:3031–3038

Knusel B, Winslow JW, Rosenthal A, Burton LE, Seid DP, Nikolics K, Hefti F (1991) Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin 3. Proc Natl Acad Sci U S A 88:961–965

Kolb H, Cuenca N, Wang HH, Dekorver L (1990) The synaptic organization of the dopaminergic amacrine cell in the cat retina. J Neurocytol 19:343–366

Kolb H, Cuenca N, Dekorver L (1991) Postembedding immunocytochemistry for GABA and glycine reveals the synaptic relationships of the dopaminergic amacrine cell of the cat retina. J Comp Neurol 310:267–284

Leon S, Yin Y, Nguyen J, Irwin N, Benowitz LI (2000) Lens injury stimulates axon regeneration in the mature rat optic nerve. J Neurosci 20:4615–4626

Lewin GR, Barde YA (1996) Physiology of the neurotrophins. Annu Rev Neurosci 19:289–317

Lo DC (1995) Neurotrophic factors and synaptic plasticity. Neuron 15:979–981

Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A 91:1632–1636

Mariani AP, Kolb H, Nelson R (1984) Dopamine-containing amacrine cells of rhesus monkey retina parallel rods in spatial distribution. Brain Res 19:1–7

Mey J, Thanos S (1993) Intravitreal injections of neurotrophic factors support the survival of axotomized retinal ganglion cells in adult rats in vivo. Brain Res 5:304–317

Mills SL, Massey SC (1995) Differential properties of two gap junctional pathways made by AII amacrine cells. Nature 377: 734–737

Mitrofanis J, Provis JM (1990) A distinctive soma size gradient among catecholaminergic neurons of human retinae. Brain Res 527:69–75

Mitrofanis J, Maslim J, Stone J (1988) Catecholaminergic and cholinergic neurons in the developing retina of the rat. J Comp Neurol 276:343–359

Müller F, Wässle H, Voigt T (1988) Pharmacological modulation of the rod pathway in the cat retina. J Neurophysiol 59:1657–1672

Nawa H, Pelleymounter MA, Carnahan J (1994) Intraventricular administration of BDNF increases neuropeptide expression in newborn rat brain. J Neurosci 14:3751–3765

Neal M, Cunningham J, Lever I, Pezet S, Malcangio M (2003) Mechanism by which brain-derived neurotrophic factor increases dopamine release from the rabbit retina. Invest Ophthalmol Vis Sci 44:791–798

Nguyen-Legros J (1988) Functional neuroarchitecture of the retina: hypothesis on the dysfunction of retinal dopaminergic circuitry in Parkinson's disease. Surg Radiol Anat 10:137–144

Nguyen-Legros J, Krieger M, Simon A (1994) Immunohistochemical localization of L-dopa and aromatic L-amino acid-decarboxylase in the rat retina. Invest Ophthalmol Vis Sci 35: 2906–2915

Nguyen-Legros J, Simon A, Caille I, Bloch B (1997) Immunocytochemical localization of dopamine D1 receptors in the retina of mammals. Vis Neurosci 14:545–551

Pourcho RG (1982) Dopaminergic amacrine cells in the cat retina. Brain Res 252:101–109

Rickman DW, Brecha NC (1995) Expression of the proto-oncogene, Trk, receptors in the developing rat retina. Vis Neurosci 12:215–222

Rodriguez-Tebar A, Jeffrey PL, Thoenen H, Barde YA (1989) The survival of chick retinal ganglion cells in response to brain-derived neurotrophic factor depends on their embryonic age. Dev Biol 136:296–303

Savy C, Simon A, Nguyen-Legros J (1991) Spatial geometry of the dopamine innervation in the avascular area of the human fovea. Vis Neurosci 7:487–498

Thanos S, Bähr M, Barde YA, Vanselow J (1989) Survival and axonal elongation of adult rat retinal ganglion cells. Eur J Neurosci 1:19–26

Thoenen H (1995) Neurotrophins and neuronal plasticity. Science 270:593–598

Villegas-Perez MP, Vidal-Sanz M, Rasminsky M, Bray GM, Aguayo AJ (1993) Rapid and protracted phases of retinal ganglion cell loss follow axotomy in the optic nerve of adult rats. J Neurobiol 24:23–36

Voigt T, Wässle H (1987) Dopaminergic innervation of A II amacrine cells in mammalian retina. J Neurosci 7:4115–4128

Wässle H, Chun MH (1988) Dopaminergic and indoleamine-accumulating amacrine cells express GABA-like immunoreactivity in the cat retina. J Neurosci 8:3383–3394

Weibel D, Kreutzberg GW, Schwab ME (1995) Brain-derived neurotrophic factor (BDNF) prevents lesion-induced axonal die-back in young rat optic nerve. Brain Res 679:249–254

Witkovsky P, Schutte M (1991) The organization of dopaminergic neurons in vertebrate retinas. Vis Neurosci 7:113–124

Young HM (1994) Co-localization of GABA- and tyrosine hydroxylase-like immunoreactivities in amacrine cells of the rabbit retina. Vis Res 34:995–999