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Neurotrophic regulation of retinal ganglion cell synaptic connectivity: from axons and dendrites to synapses

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ABSTRACT This review highlights important events during the morphological development of retinal ganglion cells (RGCs), focusing on mechanisms that control axon and dendritic arborization as a means to understand synaptic connectivity with special emphasis on the role of neurotrophins during structural and functional development of RGCs. Neurotrophins and their receptors participate in the development of visual connectivity at multiple levels. In the visual system, neurotrophins have been shown to exert various developmental influences, from guiding the morphological differentiation of neurons to controlling the functional plasticity of visual circuits. This review article examines the role of neurotrophins, and in particular of BDNF, during the morphological development of RGCs, and discusses potential interactions between activity and neurotrophins during development of neuronal connectivity.

KEY WORDS: *retina, optic tectum, arborization, BDNF, visual system*

Retinal ganglion cells (RGCs), the sole projection neurons from the retina, integrate, process, and convey all visual information transmitted to the brain (Dowling, 1987). Within the retina, RGCs attain their fates and differentiate early, preceding other types of retinal neurons (Fig. 1). RGCs reside near the lens in the ganglion cell layer (GCL) and exhibit distinctive, characteristic morphologies. Different morphological subtypes of mature RGCs are characterized by soma size and dendritic arborization patterns. To receive synaptic inputs from amacrine and bipolar neurons, each RGC elaborates several branched dendrites into the retina's inner plexiform layer (IPL) and to provide synaptic input to the central targets, each RGC extends a single axon that exits the retina via the optic nerve to synapse on neurons in the brain (the optic tectum in lower vertebrates or superior colliculus, pretectum, and lateral geniculate nucleus in higher vertebrates). When the RGC growth cone has reached and recognized its target region, the growth cone transforms into an actively branching axon terminal, extending and retracting branches it forms synapses with target neurons in the brain. The events of RGC axon extension, growth cone pathfinding, and target recognition all coincide with RGC dendritic arborization in the retina. RGCs begin to extend primary dendrites as their axons course towards their targets, and continue to elaborate complex dendritic arbors as their axon terminals innervate and branch within the target (Holt, 1989).

This review highlights important events during the morphological development of RGC that influence both the presynaptic (dendritic) and postsynaptic (axon) connectivity of RGC projection neurons. The elucidation of the events that guide development and differentiation of RGCs is due in part to recent advances in *in vivo* imaging techniques that have permitted visualization of cellular events that underlie the establishment of RGC synaptic connectivity (Cohen-Cory, 2002; Debski and Cline, 2002). The frog and fish retinotectal projections have provide particularly accessible systems to study the molecular basis of visual connectivity. While many environmental factors modulate various aspects of RGC development, this review emphasizes the roles that the neurotrophins play during the morphological development of RGCs and during the establishment of neuronal connectivity in the visual system, with emphasis on insights learned from studies of lower vertebrates.

Neurotrophins and visual system development

The neurotrophin family of neuronal growth factors modulates multiple aspects of the development, differentiation, and function of many neurons in the peripheral and central nervous systems, including RGCs (Huang and Reichardt, 2001). The neurotrophin

Abbreviations used in this paper: BDNF, Brain Derived Neurotrophic Factor; CAMKII, Calmodulin-dependent kinase II; GFP, Green Fluorescent Protein; NMDA, N-methyl-D-Aspartate ; NT-3, Neurotrophin 3; PI3K, Phosphatidylinositol 3 kinase; RGC, Retinal Ganglion Cell.

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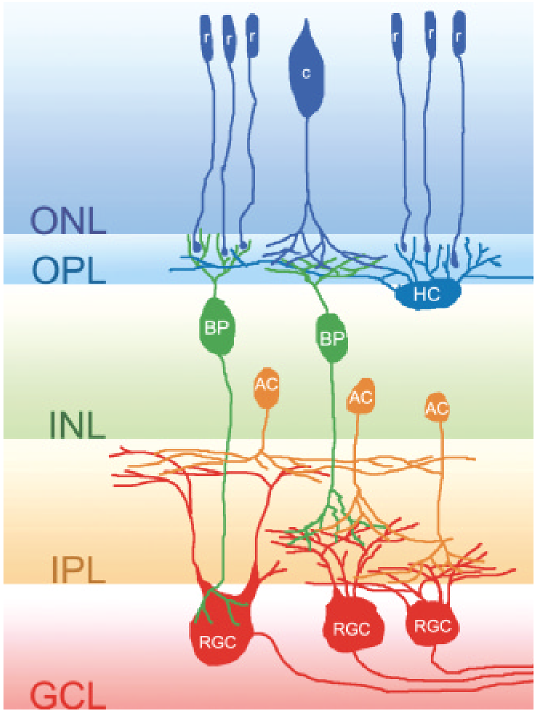

Fig. 1. Neural connectivity in the vertebrate retina. *The vertebrate retina is organized in five distinct layers. Information flows from the photoreceptive rods (r) and cones (c) in the outer nuclear layer (ONL) via synapses in the outer plexiform layer (OPL) with horizontal cells (HC) and bipolar cells (BP). The axons of bipolar and amacrine cells (AC) in the inner nuclear layer (INL) then transmit information to retinal ganglion cells (RGC) by synapsing on RGC dendrites in the inner plexiform layer (IPL). RGC soma reside in the ganglion cell layer and RGC axons exit the retina together via the optic nerve.*

ligands include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5). Neurotrophins exert most of their biological functions by binding to members of the Trk family of specific high-affinity tyrosine kinase receptors (Patapoutian and Reichardt, 2001). TrkA is the specific receptor for NGF, TrkB is a shared receptor for BDNF and NT-4/5, and TrkC is a specific receptor for NT-3. Ligand binding induces Trk receptor dimerization and autophosphorylation that initiates several signal transduction cascades commonly implicated in growth factor signaling (Chao, 2003). Neurotrophins also bind nonspecifically to the low-affinity p75 receptor, which lacks an intracellular kinase domain and signals through ceramide pathways that play important roles in regulating cell survival (Kaplan and Miller, 2000). Neurotrophins were initially identified for their ability to promote neuronal survival, but a large body of evidence now demonstrates that neurotrophins also participate in many aspects of neuronal development and function (Poo, 2001). *In vivo* expression patterns indicate that developing neurons are frequently exposed to multiple neurotrophic sources that exert spatial and temporal control over their morphological differentiation (Huang and Reichardt, 2001). Neurotrophins can retrogradely influence the development of presynaptic neurons and anterogradely influence the development of postsynaptic cells (von Bartheld *et al.*, 2001). Moreover, neurotrophins secreted by one neuron can exert both autocrine influences on that neuron itself as well as paracrine influences on nearby neurons. A large body of experimental evidence indicates that the morphologies of many types of vertebrate neurons are sculpted by neurotrophins both *in vitro* and *in vivo* (Cohen-Cory *et al.*, 1991; Horch and Katz, 2002; Lom *et al.*, 2002; Lom and Cohen-Cory, 1999; McAllister *et al.*, 1995; Morrison and Mason, 1998; Purves *et al.*, 1988; Snider, 1988; Xu *et al.*, 2000; Yacoubian and Lo, 2000).

While each neurotrophin has been reported to influence visual system development to some extent, BDNF has repeatedly emerged as a particularly important neurotrophic signal that influences multiple phases of vertebrate RGC development including survival, morphological differentiation of axons and dendrites, synapse formation, and regeneration (Bahr, 2000; Frost *et al.*, 2001; Isenmann *et al.*, 2003; von Bartheld, 1998). The temporal and spatial expression patterns of BDNF and TrkB receptors within the developing visual system indicate that BDNF is available to influence important aspects of RGC differentiation, including their morphological maturation. BDNF and its receptor TrkB are highly expressed in the visual system of most vertebrate species examined, from fish to mammals (Cellerino and Kohler, 1997; Cohen-Cory *et al.*, 1996; Cohen-Cory and Fraser, 1994; Duprey-Diaz *et al.*, 2002; Frost *et al.*, 2001; Garcia *et al.*, 2003; Hallbook *et al.*, 1996; Hashimoto and Heinrich, 1997; Herzog *et al.*, 1994; Herzog and von Bartheld, 1998; Jelsma *et al.*, 1993; Perez and Caminos, 1995). BDNF was initially characterized for its ability to promote survival of cultured RGCs (Cohen-Cory and Fraser, 1994; Johnson *et al.*, 1986; Rodriguez-Tebar *et al.*, 1989), but BDNF does not modulate RGC programmed cell death *in vivo* because RGC numbers in TrkB- or BDNF-deficient mice are similar to wild types (Cellerino *et al.*, 1997; Pollock *et al.*, 2003; Rohrer *et al.*, 2001). BDNF, however, as been shown to be a potent survival agent for axotomized and injured RGCs (Di Polo *et al.*, 1998; Mansour-Robaey *et al.*, 1994; Mey and Thanos, 1993; Peinado-Ramon *et al.*, 1996). BDNF also enhances retinal neurite extension and regeneration *in vitro* (Goldberg *et al.*, 2002a; Lom *et al.*, 1998; Takahashi *et al.*, 1993; Takano *et al.*, 2002).

In vivo, developing RGCs are exposed to two distinct sources of BDNF that spatially and temporally coincide with the differentiation of their axonal and dendritic arbors. BDNF is expressed both by target neurons in the tectum where RGC axons actively arborize and locally by retinal neurons in regions where RGC dendrites arborize (Fig. 2). Within the retina, most but not all neurons in the ganglion cell layer express BDNF and TrkB, indicating that RGCs are capable of producing and responding to BDNF (Cohen-Cory *et al.*, 1996; Cohen-Cory and Fraser, 1994; Hallbook *et al.*, 1996; Perez and Caminos, 1995). A small population of neurons in the inner nuclear layer also expresses TrkB, suggesting that a subset of amacrine and/or bipolar neurons have the ability to respond to the BDNF produced by RGCs (Cohen-Cory *et al.*, 1996; Garner *et al.*, 1996). Thus, BDNF is spatiotemporally available within the retina and at the RGC axon targets during periods when BDNF can significantly influence the morphological differentiation of RGCs.

Retinal ganglion cell axon arborization and synaptogenesis in the brain

The retinotectal projection has served as a powerful model system to elucidate the molecular mechanisms that control the formation of topographically ordered maps in the brain. In frogs and fish, functional visual connections are formed through the initial targeting of RGC axon growth cones within the optic tectum, where neighbor to neighbor relations are maintained to create a topographically inverted visual map. Sperry (1963) first proposed, in his "chemoaffinity hypothesis", that molecular gradients along the optic tectum interacted with complementary gradients of molecules expressed by RGC growth cones to guide each RGC axon to its appropriate, topographically inverted position along the anterior-posterior axis of the developing tectum. More than 30 years after Sperry's proposal, two groups identified a family of receptors (Eph receptors) expressed in a positional gradient by RGC axons and their corresponding ligands (ephrins) expressed in a complementary gradient in the target that act as topographic guidance molecules responsible for establishing the retinotopic map (O'Leary and Wilkinson, 1999). Since then, additional axon guidance molecules have been identified as participants in the topographic organization of the retinotectal projection of a number of species, including frogs and fish (Demyanenko and Maness 2003; Mann *et al.,* 2002; Webber *et al.,* 2003; Erkman *et al.,* 2000; Ringstedt *et al.,* 2000).

In most vertebrate species, a required step in the establishment of precisely ordered visual connections is a gradual process in which afferent axonal arbors initially branch widely over their target area and then gradually refine their arbors by withdrawing branches from topographically inappropriate areas and strengthening connection with the correct targets (Antonini and Stryker, 1993b; Katz and Shatz, 1996; Roskies *et al.*, 1995). Imaging studies in lower vertebrates have revealed that this gradual remodeling of axonal arbors is a highly dynamic process in which axonal branches are constantly added and eliminated, until stable synaptic connections are retained (Nakamura and O'Leary, 1989; O'Rourke *et al.*, 1994; O'Rourke and Fraser, 1990). While it is known that molecular guidance cues are essential for the initial establishment of topographic maps (O'Leary and Wilkinson, 1999; Mann *et al., this issue*), significantly less is known about the identities of molecular factors that control RGC axon arborization. Roles for ephrin ligands and Eph receptors have recently been proposed as modulators of topographic axon branching (Hindges *et al.*, 2002; Yates *et al.*, 2001), where ephrin ligands restrict axon branching to topographically appropriate retinotectal sites (Sakurai *et al.*, 2002). Similar roles for another well-characterized family of repulsive axon guidance molecules, the semaphorins, have been proposed based on their ability to promote RGC axon back branching while inducing growth cone collapse (Campbell *et al.*, 2001). Cell adhesion molecules have also been implicated in both initiating and shaping RGC axon arbors (Elul *et al.*, 2003; Inoue and Sanes, 1997; Riehl *et al.*, 1996).

Considerable evidence indicates that activity-mediated processes control the branching and refinement of RGC axon terminals (Cohen-Cory, 1999; Muir-Robinson *et al.*, 2002; Penn *et al.*, 1998; Reh and Constantine-Paton, 1985; Ruthazer *et al.*, 2003; Sretavan *et al.*, 1988). As the visual system becomes functional, patterned neuronal activity guides the branching of axon terminals and therefore is thought to guide the development and refinement of precise visual connections (Debski and Cline, 2002; Katz and Shatz, 1996). For example, in the absence of action potential or synaptic activity axons projecting to their target regions elaborate arbors that are more complex than those developing with normal visual input and fail to segregate in retinotopic specific lamina (Antonini and Stryker, 1993a; Kobayashi *et al.*, 1990; Reh and Constantine-Paton, 1985; Ruthazer *et al.*, 2003; Sretavan *et al.*, 1988). It remains controversial, however, to what extent activity-dependent axon arbor branching influences the establishment of precisely ordered retinotopic maps (Eglen *et al.*, 2003; Sur and Leamey, 2001). Some evidence indicates that activity plays a permissive role, being required for axons to interpret molecular cues present in the environment (Crowley and Katz, 2002), whereas most evidence indicates that activity plays an instructive role, guiding the competition between axonal inputs for common post-synaptic sites (Eglen *et al.*, 2003, Grubb *et al.*, 2003, McLaughlin *et al.*, 2003).

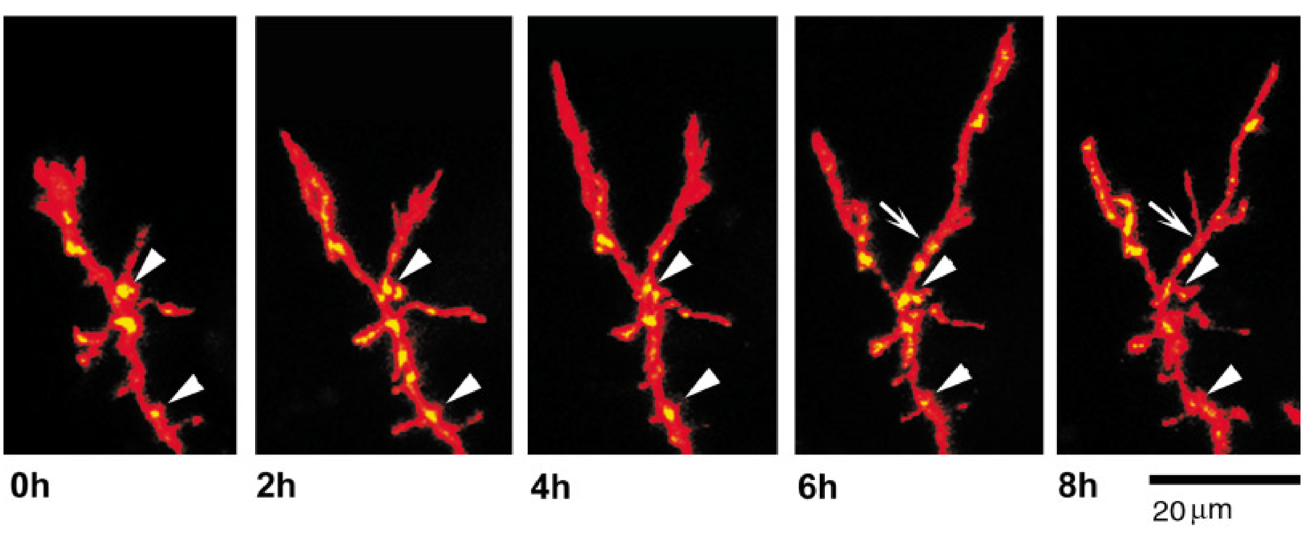

Fig. 2. Visualizing synapse formation in arborizing RGC axons *in vivo*. *Dual-color imaging of* Xenopus *RGC axon arbors branching in the optic tectum illustrates the relationship between synapse formation and distribution, and axon branch dynamics (Alsina* et al.*, 2001). Time-lapse confocal microscope images of a GFP-synaptobrevin and DsRed2-expressing RGC axon arbor show that new branches originate at axon arbor sites rich in GFP-synaptobrevin clusters (arrowheads and arrow at 6 and 8 hrs).*

Fig. 3. BDNF enhances RGC axon arborization and synaptogeneis at the target. *The optic tectum expresses BDNF during periods when RGCs actively arborize and form synapses with tectal neuron dendrites.* In vivo *imaging of RGC axonal arbor morphology and GFP-tagged presynaptic proteins over time reveal that BDNF levels are directly related to axon arbor morphology and synaptic density. This cartoon illustrates how BDNF influences branch addition, arbor length, synapse density, and synapse stability (Cohen Cory and Fraser, 1995; Alsina* et al., *2001; Hu & Cohen-Cory, 2003).*

One molecular mechanism that has been used to explain the involvement of neuronal activity during the establishment of precisely ordered visual maps is the activity-dependent competition for neurotrophic substances (Katz and Shatz, 1996; Snider and Lichtman, 1996). Evidence that neuronal activity directly regulates the production and release of neurotrophins (Lessmann *et al.*, 2003), and that the development of topographically ordered and functional visual maps in the cortex rely on endogenous neurotrophin function (Cabelli *et al.*, 1997; Huang *et al.*, 1999; Maffei *et al.*, 1992), support the idea that neurotrophins influence visual connectivity in an activity-dependent manner. Although no direct evidence exists to date to prove that neurotrophins are the object of competition between axonal inputs, several lines of evidence indicate that at least one neurotrophin, BDNF, significantly influences synaptic connectivity at multiple levels (Berardi *et al.*, 2003; Vicario-Abejon *et al.*, 2002). Evidence that directly demonstrates a significant role for BDNF during the morphological development of RGC axon arbors was initially provided by experiments that used the *Xenopus* visual system as an *in vivo* model (Alsina *et al.*, 2001; Cohen-Cory and Fraser, 1995). When recombinant BDNF was applied at the target optic tectum, arborizing RGC axons specifically responded to BDNF by adding more branches and increasing their total arbor length (Cohen-Cory and Fraser, 1995). Correspondingly, when endogenous tectal BDNF levels were reduced with BDNF function blocking antibodies, the complexity of RGC axon arbors and axon branch dynamics were reduced. Thus, target-derived BDNF promotes RGC axon arbor growth and has the potential to impact the synaptic opportunities between RGC axons and tectal neurons.

More direct evidence supporting a role for BDNF during the development of synaptic connectivity has been obtained by visualizing pre-synaptic structures in RGC axon terminals branching *in vivo*. The expression of green fluorescent protein (GFP)-labeled synaptic components combined with real-time imaging of neurons has provided a powerful tool to reveal dynamic events that take place during synaptogenesis in the developing CNS (Ahmari and Smith, 2002; Cohen-Cory, 2002). Real-time imaging of a GFP-tagged version of the synaptic vesicle protein synaptobrevin II within individual fluorescently labeled *Xenopus* RGC axon arbors has revealed that synaptogenesis in the retinotectal system is dynamic (Fig. 2), and is directly correlated to the branching and remodeling of RGC axon terminal arbors (Alsina *et al.*, 2001; Cohen-Cory and Fraser, 1995; Rajan *et al.*, 1999). As RGC axon arbors branch over the tectum, new candidate synapses are formed while others are eliminated; yet a large proportion of synapses remain stable (Alsina *et al.*, 2001). BDNF significantly influences RGC synaptogenesis in multiple ways: BDNF enhances axon arbor complexity therefore increasing total synaptic territory (Cohen-Cory, 1999; Cohen-Cory and Fraser, 1995), while also increasing the density of GFP-synaptobrevin identified presynaptic sites on RGC axonal branches (Fig. 3; Alsina *et al.*, 2001). More recent evidence indicates that BDNF modulates synapse number not only by promoting the addition of new candidate synapses but also by stabilizing existing synapses. Specifically, when endogenous BDNF levels were decreased by injection of neutralizing antibodies into the tadpole optic tectum, a significant number of GFP-synaptobrevin identified synaptic sites in individual RGC axon terminals were dismantled (Hu and Cohen-Cory, 2003). Furthermore, active dismantling of presynaptic sites in BDNF-depleted tadpoles was coupled with destabilization and retraction of RGC axon branches, again demonstrating that BDNF strongly influences the stability and synaptic complexity of developing RGCs. An axon branch stabilization role for BDNF during visual system development is consistent with observations that increasing BDNF levels in the developing superior colliculus prevents normal pruning of ipsilateraly projecting axons (Isenmann *et al.*, 1999).

A more direct measure of a potential role for BDNF during axon branch and synapse stabilization comes from studies in which BDNF effects on RGC axon arbors were tested after experimentally increasing the probability of axon branch destabilization during the active period of axon arborization. Synapse stability and potentially axon branch stability can be altered by treatment with pharmacologic agents that alter synaptic transmission in the optic tectum (Debski and Cline, 2002). Injection of the NMDA receptor antagonists into the tectum transiently destabilized GFP-synaptobrevin identified synapses, inducing their elimination (Hu and Cohen-Cory, 2003). When BDNF was injected into the optic tectum simultaneously with the NMDA receptor antagonists, BDNF rescued synapses affected by altering synaptic transmission. BDNF maintained synapse density by rapidly inducing their addition and stabilization (Hu and Cohen-Cory, 2003). Thus, BDNF influences RGC synaptic connectivity in multiple ways, promoting not only the morphological maturation of RGC axon arbors but also their stabilization, by a mechanism that stabilizes synapses. A role for BDNF during early stages of synapse formation and stabilization is consistent with observations that genetic alterations in BDNF or TrkB expression significantly influence axon arbor morphology and synaptic connectivity in other areas of the developing brain (Martinez *et al.*, 1998; Rico *et al.*, 2002; Vicario-Abejon *et al.*, 2002).

The expression patterns of BDNF and TrkB in the developing visual system suggest that BDNF can act directly on RGCs to modulate axon arbor morphology and synaptic connectivity. BDNF's primary site(s) of action, however, is not yet known. The time course of BDNF action supports the potential of BDNF to act directly on developing RGC axons: BDNF applications in the target optic tectum rapidly influence the dynamic morphology of arborizing RGC axon terminals within 1-2 hours of treatment, even in the absence of neuronal activity (Cohen-Cory, 1999; Cohen-Cory and Fraser, 1995; Hu and Cohen-Cory, 2003). BDNF's rapid influence on RGC axon morphology is dependent on the site of BDNF application because similar manipulations in retinal BDNF levels do not affect RGC axon arborization (Lom *et al.*, 2002). These observations suggest that tectal BDNF specifically regulates the maturation of RGC axons at the target. It is also possible that BDNF influences the morphological development of postsynaptic tectal neurons directly and that this in turn influences RGC presynaptic arbors, but preliminary studies indicate that BDNF influences postsynaptic specializations on tectal neurons only 24 hours after BDNF treatment (Sanchez *et al.,* 2003). Changes in tectal neuron synaptic connectivity induced by BDNF appear then to be secondary to the enhanced stability and growth of presynaptic axons. It remains possible that BDNF may affect the differentiation of both retinal axons and tectal neuron dendrites, but influences presynaptic and postsynaptic neurons independently. BDNF may affect retinotectal synaptic connectivity by acting anterogradely on tectal neurons. BDNF is anterogradely transported by RGC axons to the optic tectum in chicks and mammals (von Bartheld *et al.*, 2001) and can rescue neurons in superior colliculus from programmed cell death (Spalding *et al.*, 2002). In addition, substance P-expressing tectal neurons are capable of responding to BDNF (Tu and Debski, 2001). It is therefore possible that anterogradely derived BDNF may influence synaptic connectivity by increasing synapse number, vesicle density, and number of docked vesicles as demonstrated for anterogradely derived NT-3 (Wang *et al.*, 2003). Each of these possibilities needs further evaluation before BDNF's primary site of action in the developing tectum can be ascertained.

Retinal ganglion cell dendritic arborization

Like axonal arborization at the target, RGC dendritic arborization within the retina is highly dynamic (Scott and Luo, 2001; Sernagor *et al.*, 2001; Wong and Ghosh, 2002). RGC dendritic development begins with the extension of several primary dendrites that project out from the RGC soma, toward the inner plexiform layer. After initiation from the soma, RGC primary dendrites then branch actively, adding and retracting branches to remodel the RGC's dendritic arbor. Such dynamic structural rearrangements of dendritic architecture occur both early in RGC dendritogenesis and later in visual system development, when the axons of amacrine and bipolar cells form synaptic contacts with RGC dendrites. Like their axons, the morphology of RGC dendrites continues to be modified by pruning mechanisms that persist long after initial synaptic contacts are formed in the visual system. Even after RGCs can functionally respond to visual stimuli RGC dendritic arbors continue to be pruned.

RGCs establish and refine dendritic morphology and synaptic connectivity in response to both intrinsic growth programs and environmental signals. It is generally thought that intrinsic growth programs regulate early dendritic arborization and environmental signals are involved in later dendritic development. RGC dendritic interactions within the local retinal environment as well interactions between RGC axon terminals and their central targets can influence the morphological maturation of RGC dendrites. The extent and form of a RGC's dendritic arbor is modulated within the retina by afferent input mediated through classic neurotransmitters (reviewed in Sernagor *et al.*, 2001) as well as by RGC density (Bahr *et al.*, 1992; Perry and Maffei, 1988; Troilo *et al.*, 1996). Recent time-lapse imaging experiments have revealed that RGCs rapidly turn over dendritic filopodia (Lohmann *et al.*, 2002; Wong *et al.*, 2000). By extending and retracting dendritic filopodia it is thought that RGCs take an active role in sampling the local retinal environment and establish functional synaptic contacts with amacrine and bipolar neurons. The motility of dendritic filopodia, as occurs during early RGC dendritogenesis, has recently been associated with activity-dependent synapse formation in other neuronal systems (Portera-Cailliau *et al.*, 2003). While neither visual stimulation nor action potential activity appear to be required for the early events of normal RGC dendritic arbor development, neurotransmitter-dependent synaptic activity does influence RGC dendritogenesis and dendritic filopodial motility (Lohmann *et al.*, 2002; Wong *et al.*, 2000). Alterations in cholinergic or glutamatergic transmission modify RGC total dendritic length and branch numbers, suggesting that activity-mediated interactions between RGCs and amacrine cells are important signals for coordinating RGCs dendritic arborization (Lohmann *et al.*, 2002). Additionally, visual experience regulates the later pruning of RGC dendritic arbors into discrete ON or OFF sublamina (Tian and Copenhagen, 2003), indicating that RGC dendritic architecture is sculpted by numerous types of factors.

It is well known that in the peripheral nervous system target tissues retrogradely influence the morphological development of dendrites (Purves *et al.*, 1988; Voyvodic, 1989; Yin and Oppenheim, 1992). The influence of target-derived factors on RGC dendritic arborization, however, is considerably less well understood. Experiments in *Xenopus* and chick suggested that early phases of RGC dendritic arborization are independent of target-derived cues. When RGC axons were prevented from interacting with the tectum by transplanting the retina to ectopic locations or when the target specificity or size were altered, early RGC dendritic arbor development was morphologically unaffected, demonstrating that the target exerts relatively little control over the early phase of RGC dendritic differentiation (Campbell *et al.*, 1997; Ramoa and Yamasaki, 1996; Sakaguchi, 1989; Vanselow *et al.*, 1990). In support of early intrinsic mechanisms of RGC dendritogenesis is the observation that isolated RGCs, when placed *in vitro,* extend dendrites that cover territories similar to RGCs in intact retinas (Montague and Friedlander, 1991). Other experiments, however, indicate that RGC dendritic branch complexity can be influenced by the target. Altering the environment of aberrantly projecting RGC axons (which normally simplify their morphology) can significantly alter the morphology of RGCs dendritic arbors, mainly by influencing the later stages of dendritic terminal remodeling that occur after the initial stages of dendritic elaboration (Wingate and Thompson, 1994). Both dark rearing and monocular enucleation prevent this dendritic remodeling suggesting that activity-mediated retrograde signals from RGC axon arbors interacting with target neurons can modulate dendritic form. Moreover, *in vivo* studies in *Xenopus* indicated that developing RGC dendrites are also sensitive to target-derived neurotrophic signals (Lom *et al.*, 2002).

In vivo experiments demonstrate that BDNF can exert multiple roles during RGC dendritic differentiation, BDNF's action being critically dependent on the site of application. In the *Xenopus* retina, RGCs begin to express BDNF and TrkB at the onset of dendritic arborization and both the neurotrophin and its specific receptor are expressed maximally during the period when RGC axons and dendrites branch actively (Cohen-Cory *et al.*, 1996; Cohen-Cory and Fraser, 1994). *In vivo* experiments that altered BDNF levels within the developing *Xenopus* retina revealed that retinal BDNF levels are inversely related to the morphological complexity of RGC dendritic arbors (Lom and Cohen-Cory, 1999). When exogenous BDNF was applied to RGCs at the onset of dendritic development, morphological analysis of retrogradely labeled RGC dendritic arbors revealed that excess retinal BDNF reduced RGC dendritic arborization (Fig. 4). Correspondingly, when endogenous retinal BDNF was neutralized with BDNF function blocking antibodies, RGCs elaborated more complex dendritic arbors. Together these results indicate that retinal BDNF exerts a significant, local influence on the morphological differentiation of RGC dendritic arbors. The question that still remains is how retinal BDNF restricts RGC dendritogenesis. Time-lapse analysis has not yet been conducted to determine if retinal BDNF prevents dendritic initiation and branching or retinal BDNF enhances excessive and/or premature pruning. In the experiments performed in *Xenopus*, RGCs were exposed *in vivo* to high levels of retinal BDNF before endogenous BDNF expression normally peaks within the retina. Because pruning of RGC dendrites occurs in most species examined, the possibility exists that presenting exogenous BDNF prematurely may bypass potential BDNF-induced refinement events that occur when dendrites branch.

The effects of altering retinal BDNF on RGC dendritic arbor morphologies may be caused by BDNF directly influencing RGCs through TrkB or by BDNF indirectly influencing TrkB-expressing amacrine cells. Several recent studies indicate that amacrine cells can regulate the development of RGC dendritic connectivity. Early

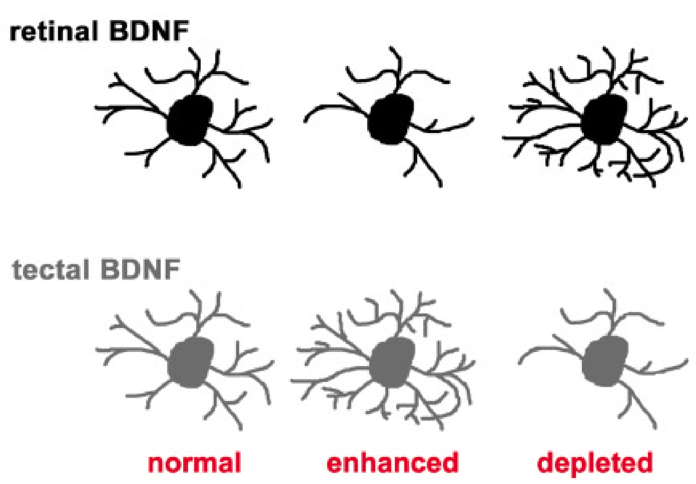

Fig. 4. Local and target-derived BDNF differentially influence RGC dendritic arborization. *During periods of active dendritic arborization BDNF is expressed both at the target and within the retina. Retinal and tectal BDNF levels were independently altered* in vivo *during dendritic arborization and the resulting morphologies of RGC dendritic arbors analyzed. Local (retinal) BDNF reduced dendritc branching, while target (tectal) BDNF enhanced dendritic branching (Lom & Cohen-Cory, 1999; Lom et al., 2002).*

interactions between amacrine cells and RGCs may provide a signal to initiate RGC dendritogenesis, as interactions between amacrine cells and RGCs allows for a switch in RGC axon growth versus dendritic growth (Goldberg *et al.*, 2002b). In addition to influencing RGC development, BDNF also modulates multiple aspects of amacrine cell function and differentiation. BDNF has been demonstrated to regulate amacrine cell morphological phenotype, neuropeptide expression, and neurotransmitter release (Cellerino *et al.*, 2003; Cellerino *et al.*, 1998; Neal *et al.*, 2003). Consequently, it is plausible that BDNF may influence RGC dendritic arborization indirectly by modulating amacrine cell development and/or function. Additionally, it is possible that BDNF's effects on RGC dendritic arborization are mediated through local autocrine or paracrine interactions between neighboring RGCs because RGCs express both BDNF and TrkB during the same developmental period (Cohen-Cory *et al.*, 1996). Local, contact mediated inhibition between RGCs has been suggested as a mechanism that limits RGC dendritic differentiation and regulates the overlap of RGC dendritic fields. Developing RGCs exhibit dendro-dendritic contacts with other RGCs of the same subtype and these specific dentritic interactions may regulate the formation of ON and OFF mosaics within the retina (Lohmann and Wong, 2001). Finally, because the expression and release of BDNF depend on activity (particularly visual activity), the effects of altering BDNF on RGC dendritic arborization could also be due to modifications in neuronal connectivity at the target (see above) that in turn influence RGCs dendritic complexity. For example, BDNF expression in RGCs is significantly decreased by visual deprivation (Seki *et al.*, 2003).

RGC dendritic arbors react in differing manners to retinal- and tectal-derived BDNF (Lom *et al.*, 2002). While retinal BDNF reduces RGC dendritic arborization, tectal BDNF stimulates RGC dendritic arborization. Tectal neurons normally express increasing amounts of BDNF during the period when RGC dendritic arbors branch in the retina and RGC axonal arbors are forming contacts with these BDNF-expressing tectal neurons. When endogenous tectal BDNF levels were enhanced by application of recombinant BDNF, RGCs with axons that successfully reached the tectum exhibited more elaborate dendritic arbors and correspondingly, when tectal BDNF levels are reduced with BDNF function blocking antibodies, RGCs elaborate simpler dendritic arbors, indicating that tectal BDNF signals promote RGC dendritic arborization (Lom *et al.*, 2002). The effects that altering tectal BDNF exerts on RGC dendritic morphology may be explained by BDNF influencing RGC axons and their synaptic connectivity (Alsina *et al.*, 2001). Alternatively, because neurotrophins are powerful molecular cues that can transmit multiple signals that are interpreted differentially by developing neurons (Heerssen and Segal, 2002; MacInnis and Campenot, 2002; Miller and Kaplan, 2002), it is also possible that developing RGCs directly integrate opposing BDNF signals from both tectal- and retinal-derived BDNF to modulate the arborization of their dendrites. BDNF can promote as well as inhibit dendritic arborization in other types of neurons. For example, in cultured cortical neurons bath application of BDNF differentially influences the branching of basal versus apical dendrites, indicating that BDNF can exert multiple and opposing effects during dendritic arborization (Horch and Katz, 2002; McAllister *et al.*, 1995, 1997). *In vitro* experiments in cultured neurons demonstrate that the spatial location of neurotrophin stimulation determines the intracellular signaling pathway activated (Heerssen and Segal, 2002; MacInnis and Campenot, 2002; Miller and Kaplan, 2002). Retrograde neurotrophin signaling at the axon initiates a PI3-kinase mediated retrograde survival signal to the nucleus mediate via ERK5, while neurotrophic stimulation of the cell body initiates non-survival signals via ERK1 and ERK2 activity (Watson *et al.*, 2001). These *in vitro* signaling data provide a new spatial view of neurotrophic regulation that could potentially explain the differential effects of retinal versus tectal BDNF on RGC dendritic differentiation. Differential integration of spatially discrete local versus target-derived neurotrophic signals within an individual RGC may therefore serve to finely tune both axonal and dendritic morphology and ultimately affect both afferent and efferent neuronal connectivity.

As for dendritic branching, the signaling mechanisms involved in axon branching and synapse number are just beginning to be elucidated. In zebrafish, downregulation of glycogen synthase kinase-3β (GSK-3β) activity decreases RGC axon arbor size and synapse number (Tokuoka *et al.*, 2002). GSK-3β is involved in PI3K growth factor mediated signaling, a pathway activated by BDNF (Casaccia-Bonnefil *et al.*, 1998; Kaplan and Miller, 2000). CaMKII, another target of BDNF signaling has been implicated in axon branching and synapse stabilization (Pratt *et al.*, 2003; Zou and Cline, 1996). Rho GTPases, that control both RGC dendritic and axonal branching (Li *et al.*, 2000; Ruchhoeft *et al.*, 1999; Sin *et al.*, 2002; Wong *et al.*, 2000) have also been implicated in the signaling mechanisms induced by the binding of neurotrophins to the p75 neurotrophin receptor (Yamashita *et al.*, 1999). Thus, the multiple signaling mechanisms that neurotrophins employ to modulate the distinct aspects of RGC structural differentiation, synaptic connectivity, and ultimately visual function remain open to further investigation.

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