Target-derived neurotrophins may influence the survival of adult retinal ganglion cells when local neurotrophic support is disrupted: Implications for glaucoma

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Summary Following target innervation, developing and neonatal retinal ganglion cells (RGCs) depend on neurotrophic factors from their target tissue for survival. This dependence is reduced for adult RGCs which rely primarily on trophic support from their local environment; however, some findings indicate that target tissue may play a role in the long-term survival of RGCs. We propose that a deficiency in neurotrophic factors from the target tissue may influence the survival of RGCs when local neurotrophic support is disrupted. Furthermore, we propose that this hypothesis may explain, at least in part, the progressive loss of RGCs in optic neuropathies such as glaucoma. Neurotrophic factors are present in the adult superior colliculus and they are trafficked to the retina; however, removal or lesioning of the adult target tissue results in little or no RGC loss for up to several months. In vitro, adult RGCs will survive when maintained by co-culturing these neurons with their target tissue. As well, the timing and pattern of adult RGC loss is consistent with that seen in glaucoma and in reports of delayed RGC loss following target-removal. Our hypothesis can be tested by selectively disrupting local neurotrophic support and evaluating RGC survival when target-derived neurotrophic support is maintained and when it is disrupted. Specifically, intravitreal injection of blocking antibodies could be used to disrupt local neurotrophic signaling, while aspiration of the superior colliculus will eliminate retrograde transport from the primary target tissue in rodents. The results of these experiments would provide valuable information concerning the influence of target-derived neurotrophic support when local neurotrophin signaling is disrupted. Specifically, this research could verify whether deficiencies in target-derived neurotrophic support play a role in RGC loss during glaucoma. A further understanding of this mechanism may lead to the development of effective neuroprotective strategies for treating glaucoma.

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Optic neuropathies

Optic neuropathies, such as glaucoma, are characterized by a progressive loss of retinal ganglion cells (RGCs) that, if undiagnosed and untreated, can result in irreversible blindness. The etiology of glaucoma is poorly understood and thus early detection and prevention of subsequent functional visual loss is often difficult. Increased intraocular pressure (IOP, >20 mm Hg) is the best known risk factor associated with glaucoma and the majority of therapeutics are aimed at reducing IOP (for reviews see [1,2]). Successful treatments include the use of topical pharmaceuticals (such as β-blockers or prostaglandin analogs), and laser therapy or surgery to alter the flow dynamics of aqueous humour. Despite effective therapeutic strategies aimed at the treatment of elevated IOP, neuronal loss can continue even after IOP is lowered. Thus, although RGCs in retinas with glaucoma may be more susceptible to damage when IOP is elevated, other mechanisms are likely also involved in the etiology of glaucoma [3,4]. Obstructed axonal transport along the optic nerve, that reduces the delivery of neurotrophins from targets in the brain to the retina, has been proposed as another possible mechanism that contributes to RGC loss in glaucoma [5,6]. In the current paper, we examine this theory further to clarify how this research may be related to studies which conclude that target-derived neurotrophins have little or no influence on the survival of adult RGCs, and to propose a method of testing this relationship.

The effectiveness of local and target-derived RGC trophic support is developmentally regulated

RGCs are dependent on the actions of endogenous neurotrophins such as brain-derived neurotrophic factor (BDNF) to maintain their survival (for reviews see [7,8]). The effectiveness of these factors differs depending on the site of delivery, either at the cell-body or distal-axon, and this location-dependent difference is developmentally regulated. During embryonic development, RGC survival is mediated by neurotrophins released from the local environment [9,10]. During neonatal development, as RGC axons extend into and innervate their appropriate targets within the brain, neurotrophins are secreted from these targets and trafficked to the retina, where they maintain RGC survival through the period of naturally occurring programmed cell death [11–14], and their dependence on local neurotrophic support is subsequently reduced. As RGCs mature, the target source becomes less influential for survival [12,15–17] and, therefore, it is thought that the survival of adult RGCs is maintained primarily by local sources of neurotrophins [18–24].

Evidence that the survival of adult RGCs is not maintained primarily by target-derived neurotrophic support comes from studies where RGC target tissue was lesioned [12,15–17]. RGC survival has been evaluated following unilateral aspiration of the superior colliculus, the primary RGC target tissue in rodents, at various ages [15]. In animals with a lesion at birth or at 5 days of age, there was a significant loss of RGCs in the contralateral eye 5 months later; however, in the group with a lesion at 30 days of age, no RGC loss occurred 5 months later. Another study produced unilateral superior colliculus lesions by kainic acid injection (to selectively destroy post-synaptic neurons but leave RGC axons nominally intact) in P4 and P10 rats and evaluated RGC survival 3 weeks later [12]. In rats lesioned at P4, approximately 50% fewer RGCs survived through the period of naturally occurring programmed cell death compared with no lesion. In lesioned P10 rats, no RGC loss was apparent when quantified 3 weeks later. Similar results have also been observed in adult cats (1–2 years of age), where RGC survival was evaluated after unilateral lateral geniculate nucleus lesion with kainic acid injection [16,17]. No RGC loss was evident at 4 months after the lesion; however, at 6 months a 32% and 42% loss of RGCs occurred in the central and peripheral retina, respectively. Together, these studies indicate that following removal of target tissue, most adult RGCs survive: RGC loss, if any, is not seen until several months after the lesion.

Theoretically, adult RGCs may survive after removal of their target tissue because sufficient neurotrophin levels are present in optic nerve axons to maintain a necessary amount of target-derived survival support. However, experimental evidence suggests this explanation is unlikely to account for long-term RGC survival. Radio-labeled BDNF applied to the colliculus of adult rodents typically reaches the retina by 6 hours after application [25]. Therefore, it is unlikely that BDNF trafficked from the colliculus could remain in the nerve for 5 months [12] and maintain long-term survival of RGCs in the absence of target tissue. As well, RGC survival rates in neonatal and adult rodents are different following axotomy performed at the same location, immediately posterior to the globe of the eye. In the neonatal rat, approximately
50% of RGCs are lost at 24 hours after optic nerve transection [26–28]. In the adult rodent, discernable RGC loss does not occur until 5 days after axotomy with 50% surviving at 7 days and 10% surviving at 14 days [29,30]. Therefore, neonatal RGCs are more sensitive to axotomy-induced RGC death. This vulnerability is likely due to the greater dependence neonatal RGCs have for target-derived neurotrophic support.

Studies investigating the effects of specific neurotrophins on RGC survival also provide evidence that a developmental switch occurs in the relative effectiveness of local versus target neurotrophic factors. In the adult, retinal BDNF protein levels are 4–5-fold higher, whereas BDNF protein levels in the adult superior colliculus are approximately a third lower, both compared to their respective levels during development [31]. As well, application of BDNF to the superior colliculus of neonatal hamsters during the period of developmental programmed cell death reduces the rate of RGC loss during this period [32] while antibodies against BDNF and NT-4/5 (the two ligands for the TrkB receptor) increase this death rate [11]. Despite evidence that factors from the target tissue do not maintain adult RGC survival, there is evidence that the trafficking of these factors persists in the adult. Although adult BDNF levels are reduced in comparison with those during development, BDNF is present in the adult colliculus [31,33–36] and is trafficked to the retina [25,37]. Taken together, these findings indicate that there is a developmentally regulated shift in the influence of local versus target-derived trophic support and, although the target tissue is not as critical for the survival of adult RGCs, the transport of neurotrophins from target tissue to retina does occur in adult RGCs.

Glaucma and target-derived neurotrophic support of RGCs

Since the loss of target-derived neurotrophic support does not influence the survival of adult RGCs until several months after its removal, and presumably local neurotrophic support is sufficient for their immediate survival, then the continuous transport of neurotrophins to the retina may be a redundant survival mechanism. In culture, co-culturing adult RGCs with their target tissue provides sufficient trophic support to maintain the survival of these RGCs, [23] indicating that target-derived neurotrophins can maintain the survival of adult RGCs even if they are not immediately needed. Consistent with this, we propose that target-derived neurotrophic support may exert an important influence on the survival of adult RGCs in situations where local neurotrophic support is disrupted. Neurotrophins trafficked from their target may act in a compensatory fashion in cases of injury where the effectiveness of local neurotrophins is impaired. This hypothesis could have significant implications for optic neuropathies such as glaucoma. If our hypothesis is valid, then we further propose that the loss of RGCs in glaucoma may be due, at least in part, to a deficiency in both target-derived and local trophic support.

Several research studies support our hypothesis. First, despite evidence that suggests adult RGCs are less dependent on their target tissue for survival (see above), studies indicate that neurotrophins continue to be transported from the brain to the retina in the adult and that the efficiency of this transport is significantly reduced after increased IOP [25]. The same researchers also demonstrated that after increased IOP, vesicles and TrkB accumulated at the optic nerve head [37]. As well, BDNF, nerve growth factor and its receptor (TrkA), are upregulated in retinal neurons of rodents with ocular hypertension-induced increased IOP, at times of RGC loss [38]. In situations of acute traumatic optic nerve injury such as an optic nerve crush or cut, an initial increase in retinal BDNF and TrkB levels occurs [39,40]; however, TrkB levels subsequently decrease significantly below normal levels during the peak time of RGC loss [39] thus indicating that the local neurotrophic signaling is reduced during the time of RGC loss. Another study found that BDNF and NT-4/5 immunolabeling were almost entirely absent in the optic nerve head and superior retina of rats with increased IOP during the period of RGC death [41]. Together, these studies demonstrate that local BDNF is altered and that its retrograde trafficking is impaired in cases of experimental glaucoma. Second, glaucoma is characterized by a progressive loss of RGCs that occurs over an extended period of time. RGC loss has been shown to occur at 6 months following target-removal in adult cats, but not at 4 months [16,17]. Therefore, at least experimentally, the temporal patterning of RGC loss after glaucoma and after RGC target-removal in adult cats are consistent. Third, visual field deficits arise first in peripheral vision while central visual field loss generally occurs later [1]. Interestingly, RGC loss was greater in the peripheral compared with the central retina at 6 months after removal of the lateral geniculate nucleus in cats [16]. Together, these findings demonstrate that our proposed hypothesis is consistent with the pattern of RGC loss during both glaucoma and the removal of RGC target tissue.
To test our hypothesis that the influence of target-derived neurotrophins on adult RGC survival is greater when local neurotrophic support is impaired, we would need to selectively disrupt neurotrophic support in the local environment and then evaluate RGC survival in situations where target neurotrophic support is maintained and where it is disrupted. Recent studies have employed blocking antibodies to specific neurotrophins to assess their importance for RGC survival in the neonatal rat [11, 42]. These agents act to disrupt retinal neurotrophic signaling in the local environment when injected intravitreally. In the neonate, this treatment induces a loss of RGCs [11], while presumably leaving the signaling along the optic nerve intact. In addition, the retrograde trafficking of neurotrophins from their target cells may be eliminated by aspiration of this tissue. In neonatal rodents, this procedure was used to demonstrate that RGCs survival is reliant on retrograde transport of neurotrophins [11–14]. Our proposed hypothesis could be tested with the use of these two techniques. A time-course of RGC loss would be determined following intravitreal injection of neurotrophin blocking antibodies in two groups: one with an aspiration lesion of the superior colliculus to disrupt transport along the optic nerve and one group with no colliculus lesion and thus intact optic nerve transport. If the loss of RGCs is accelerated in the group lacking transport along the optic nerve, then this finding would confirm that target-derived neurotrophic support influences the survival of adult RGCs when local neurotrophic support is disrupted. Elucidation of this mechanism ultimately may lead to the development of neuroprotective strategies for treating optic neuropathies such as glaucoma.

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References

[10] Armson PF, Bennett MR, Raju TR. Retinal ganglion cell survival and neurite regeneration requirements: the change from Muller cell dependence to superior colliculus dependence during development. Brain Res 1987;429:207–16.
[23] Raju TR, Rao MS, Nagaraja TN, Meti BL, Schulz M. Retinal ganglion cell survival and neurite regeneration in vitro after cell death period are dependent upon target derived...


