A novel viewpoint in glaucoma therapeutics: enriched environment

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Abstract
Glaucoma is one of the world’s most frequent visual impairment causes and leads to selective damage to retinal ganglion cells and their axons. Despite glaucoma’s most accepted risk factor is increased intraocular pressure (IOP), the mechanisms behind the disease have not been fully elucidated. To date, IOP lowering remains the gold standard; however, glaucoma patients may still lose vision regardless of effective IOP management. Therefore, the exclusive IOP control apparently is not enough to stop the disease progression, and developing new resources to protect the retina and optic nerve against glaucoma is a goal of vast clinical importance. Besides pharmacological treatments, environmental conditions have been shown to prevent neurodegeneration in the central nervous system. In this review, we discuss current concepts on key pathogenic mechanisms involved in glaucoma, the effect of enriched environment on these mechanisms in different experimental models, as well as recent evidence supporting the preventive and therapeutic effect of enriched environment exposure against experimental glaucomatous damage. Finally, we postulate that stimulating vision may become a non-invasive and rehabilitative therapy that could be eventually translated to the human disease, preventing glaucoma-induced terrible sequelae resulting in permanent visual disability.

Key Words: brain-derived neurotrophic factor; chondroitin sulfate; enriched environment; glaucoma; optic nerve axons; optic nerve glia; retinal ganglion cells; visual stimulation

Introduction
Glaucoma is a multifactorial, progressive, and neurodegenerative disorder, characterized by the death of retinal ganglion cells (RGCs), and optic nerve (ON) axon loss (Nucci et al., 2016). With more than 70 million people, and approximately 10% of them suffering from bilateral blindness, glaucoma is recognized as a leading cause of irreversible blindness worldwide (Weinreb et al., 2014; Nucci et al., 2016). In fact, glaucoma is predicted to strike more than 110 million people by 2040, with strong Public Health implications (Tham et al., 2014). Due to the lack of symptoms during the disease early stages, half of those affected by glaucoma are estimated to not be aware of their condition; by the time vision loss has developed, significant and irreversible damage has occurred already. Glaucomatous visual field defects usually start in the periphery, and progress to central vision, with devastating consequences to the patient’s quality of life (Cesareo et al., 2015). Increased intraocular pressure (IOP) is the main risk factor for the onset and progression of neuronal damage within glaucoma (Nucci et al., 2016). Thus, glaucoma therapy is currently based on IOP reduction by medical, surgical or parasurgical treatments. A considerable percentage of patients experiences disease progression despite a satisfactory IOP control (Nucci et al., 2016). In fact, it is estimated that one in eight patients will eventually become blind in at least one eye as a result of glaucoma progression, even in populations with access to the best available treatments (Khatib and Martin, 2017). Therefore, although the current management of glaucoma is mainly focused on IOP control, a therapy that prevents RGC death and ON axon loss should be the main goal of glaucoma treatment. Unfortunately, the mechanisms that lead to RGC loss in glaucoma are still unclear. Since glaucoma is a complex and multifactorial disease, several molecular pathways are likely to converge, inducing structural and functional alterations. Signals that promote RGC death in glaucoma might be exacerbated by risks factors, tilting the neuron’s fate towards dysfunction and demise. In recent years, there has been a considerable progress in our understanding of multiple pathways that lead to RGC degeneration and ON injury. In this vein, several factors such as a reduced antioxidant defense system activity (Kimura et al., 2017), glutamate excitotoxicity (Moreno et al., 2005b), and sustained glial reactivity (Weinreb et al., 2016; Baudouin et al., 2020), among others, have been suggested as possible additional causes for early and/or advanced stages of glaucomatous damage. The contribution of different players to the pathogenesis of glaucoma supports the necessity of developing therapeutic strategies that ideally may act on several molecular and cellular targets, with the sustainability of treatments over time and their degree of invasiveness, being important points to consider.

Here, we will discuss advances in key factors contributing to glaucomatous neurodegeneration, with a particular focus on the advantages of the enriched environment as a non-invasive therapeutic strategy against glaucoma.
Experimental Models of Glaucoma

The development of clinically relevant models for glaucoma research has greatly improved our understanding of the pathobiological mechanisms of the disease, as well as the possibility of searching for neuroprotective agents. In recent years, rat and mouse glaucoma models have become key tools due to many advantages, such as their potential for experimental manipulation, low cost, and relatively human-like ocular anatomy and biology (Pang and Clark, 2020).

Ocular pressure-dependent models generally address the most important risk factor, although strategies for inducing ocular hypertension vary considerably. Several groups have managed to increase IOP in rodents by impeding the outflow of aqueous humor through laser photoagulation, episcleral veins cataractization or injection of hypertonic saline, as well as by intracameral injections of microbeads, or viscoelastics (Biswas and Wan, 2019). Furthermore, mouse genetic models, in which the standardized DBA2J mouse strain that develops pigment dispersal iris disease by spontaneous mutations leading to elevated IOP (Fernandes et al., 2015) are frequently used. All these models have both advantages and disadvantages.

We have developed a model of glaucoma through weekly injections of chondroitin sulfate (CS) in the eye anterior chamber of Wistar rats. Weekly intracameral injections of CS significantly increase IOP as compared with vehicle-injected contralateral eye. Although sustained ocular hypertension requires multiple intracameral injections, we have shown that the maneuver itself remains IOP, retinal function, or histology unaffected. Several advantages support this model: 1) highly consistent and moderate ocular hypertension, 2) reasonably long course, 3) low cost, and 4) easy to perform. The use of viscoelastics (hyaluronic acid or CS) to induce ocular hypertension has been validated by several groups (Foureaux et al., 2015; Mayordomo-Febrer et al., 2015; Pirhan et al., 2016; Chan et al., 2019; Yu et al., 2020). Chondroitin sulfate chronic administration significantly decreases the scotopic electroretinographic response and induces a significant RGCs and ON fibers loss (Moreno et al., 2005a; Belforte et al., 2010). Moreover, ocular hypertension induced by CS-injections decreases visual evoked potentials (VEPs), retinal arteriographic transport from RGCs to main central targets, retinal and proximal ON glial reactivity, and even induces alterations in non-image-forming visual functions (i.e., pupil light reflex, and locomotor activity daily rhythms) (Belforte et al., 2010; De Zavalia et al., 2011; Bordone et al., 2017; González Fleitas et al., 2020b). Taken together, these results support intracameral injections of CS in the anterior chamber of the rat eye reproduce the hallmarks of primary open-angle glaucoma, which is the most common form of the disease. Therefore, this model may be a useful tool to understand the cellular and molecular mechanisms involved in glaucomatous damage and to develop new therapeutic strategies.

Enriched Environment

The concept of the enriched environment (EE) emerged as an experimental approach to studying the effects of experience in the brain. EE was first defined as a complex combination of social and unanimated stimulation that was able to induce profound effects on brain morphology, chemistry, and physiology (Rosenzweig et al., 1960). In EE, animals are reared in wide cages, in the presence of a variety of objects with different shapes, colors, and textures regularly changed, and running wheels, which achieve a boost in exploratory, sensory, and cognitive activity, social interaction, and voluntary physical exercise. The exposure to EE triggers cellular and molecular plasticity across different brain areas, as compared to animals housed in standard laboratory conditions (standard environment (SE)) (Kempermann, 2019). EE beneficial effects were found across the lifespan in different species, as well as in the context of experimental models of brain damage (Nithianantharajah and Hannan, 2006; Hui et al., 2011; Sale, 2018). At the cellular level, EE potentiates dendritic length and spine density in several neuronal populations and promotes syn-aptogenesis and neurogenesis in the hippocampus (Kondo, 2017). At the molecular level, these effects are supported by alterations in gene expression, histone acetylation, and chromatin remodeling (Griñán-Ferré et al., 2018; Wang et al., 2020). Moreover, EE housing increases neurotrophins levels (particularly brain-derived neurotrophic factor (BDNF), and nerve growth factor), which play essential roles in neuronal trophic signaling (Nithianantharajah and Hannan, 2006; Kondo, 2017). Age is not a limitation for EE consequences. In aging, EE positively impacts neuronal physiology, for example, by modulating the dynamics of several neurotransmitter systems. In fact, it has been shown that EE has similar effects in the early post-natal stage and adulthood (Sale et al., 2014). Macroscopically, there is evidence that EE modifies behavior (Sale et al., 2014), increasing learning and memory, and reducing age-related cognitive decay (Kondo, 2017; Mandolesi et al., 2017).

Extensive research has explored the neuroprotective effects of EE in the context of central nervous system diseases. In this line, it has been shown that EE has protective effects in different animal models of neurodegenerative disorders, and different types of brain damage in adults (Nithianantharajah and Hannan, 2006), such as stroke, Parkinson’s disease, Alzheimer’s disease, epilepsy, and traumatic brain injury (Sale et al., 2014; Kline et al., 2016). Additionally, EE mediates axonal regeneration (Hutson et al., 2019), functional white matter recovery (Forbes et al., 2020), and neuroinflammation inhibition (Zhang et al., 2020). As already mentioned, BDNF plays a major role in EE-mediated neuroprotection (Kondo, 2017). EE housing increases the levels of BDNF and its receptor TrkB in the superior colliculus (SC), the visual cortex (Franklin et al., 2006), and the hippocampus (Sale et al., 2014). In addition, TrkB expression knockdown impairs EE-mediated neuroprotection and spatial memory during hypobaric hypoxia (Jain et al., 2013).

The early-life visual system is significantly influenced by the environment. Postnatal EE housing not only accelerates retinal acuity maturation, but also counteracts the effects of dark rearing on RGC dendritic stratification, through a BDNF-dependent mechanism (Sale et al., 2014). Prenatal exposure to EE accelerates neural progenitor migration, and spontaneous apoptosis in retinal neurons (Sale, 2018). The impact of EE in visual brain areas has been also analyzed in experimental amblyopia (Consorti et al., 2019), a permanent loss of visual acuity in one deprived eye, due to various causes, which provokes a binocular functional mismatch that results in abnormal early visual experience. This neurodevelopmental, purely cortical, visual deficit is mainly characterized by a loss of visual acuity, and low contrast sensitivity. Although it is widely accepted that normal visual function can only be recovered by the reestablishment of a “correct” visual experience within a narrow period (i.e., the critical period) during visual development, it has been demonstrated that exposure to EE for 2–3 weeks promotes full visual acuity, and ocular dominance recovery, both at the electrophysiological and behavioral level in adult rats with experimental amblyopia (Baroncelli et al., 2012). The adult retina has long been considered less plastic than the brain cortex or the hippocampus, the canonical sites of experience-dependent plasticity; however, in recent years, the neuroprotective role of EE has gained relevance in rodent adult retina and ON (Dorfman et al., 2013, 2014, 2015; Aranda et al.,
Table 1 | Summary of protocols of EE used in experimental models of retinal diseases

| Experimental model | EE protocol | EE’s exposure period | EE preserves | Reference |
|--------------------|-------------|----------------------|--------------|-----------|
| Dark rearing in Thy-1-mGFP single mice | Cage dimensions: 44 x 62 x 28 cm³. EE cages contained several food hoppers, a running wheel and differently shaped objects (tunnels, shelters, stairs) that were completely substituted once a week. | Pregnant females exposed 7 d before delivery and pups exposed from birth up to P30 | -Retinal function (ERG) | Landi et al., 2007 |
| Glutamate excitotoxicity in Wistar rats | Cage dimensions: 88 x 50 x 44 cm³. EE cages contained different toys, objects, running tunnels and rotating rods. Half of the objects were changed daily, while the other half was left unchanged. | From birth up to 5 wk | -Retinal structure | Szabadfi et al., 2009 |
| Retinitis pigmentosa in rd10 mutants and wild-type mice (C57BL/6J) | Cage dimensions: 60 x 38 x 20 cm³. Running wheels were repositioned once a week and tunnels and objects were replaced with the same frequency. 6-8 pups per cage. | Whole pregnancy occurred in EE and pups remained in EE from birth up to 210 d | -Photoreceptor survival and morphology | Barone et al., 2012 |
| Retinal ischemia in Wistar rats | Cage dimensions: 46.5 x 78 x 95 cm³. Food hoppers, water bottles, running wheels, tubes, ramps, and differently shaped objects (balls, ropes, stones) were repositioned once a day and fully substituted once a week. | 3 wk after ischemia | -Retinal function (ERG) | Dorfman et al., 2013 |
| Diabetic retinopathy in Wistar rats | Cage dimensions: 46.5 x 78 x 95 cm³. Food hoppers, water bottles, running wheels, tubes, ramps, and differently shaped objects (balls, ropes, stones) were repositioned once a day and fully substituted once a week. | 3 wk before ischemia | -Retinal function (ERG) | Gonzalez Fleitas et al., 2019 |
| Optic neuritis in Wistar rats | Cage dimensions: 46.5 x 78 x 95 cm³. Food hoppers, water bottles, running wheels, tubes, ramps, and differently shaped objects (balls, ropes, stones) were repositioned once a day and fully substituted once a week. | 3 wk after optic nerve inflammation | -Visual function (PLR/VEPs) | Aranda et al., 2019 |
| Glaucoma in Wistar rats | Cage dimensions: 46.5 x 78 x 95 cm³. Food hoppers, water bottles, running wheels, tubes, ramps, and differently shaped objects (balls, ropes, stones) were repositioned once a day and fully substituted once a week. | 10 wk after first CS-intracameral injection | -Visual function (VEPs) | Gonzalez Fleitas et al., 2020 |

BDNF: Brain-derived neurotrophic factor; B-R: blood-retina; CNFT: cilary neurotrophic factor; COX-2: cyclooxygenase-2; CS: chondroitin sulfate; EE: enriched environment; ERG: electroretinogram; GCL: ganglion cell layer; GFAP: glial fibrillary acidic protein; IL-1β: interleukin-1β; IPL: inner plexiform layer; mTOR: mammalian target of rapamycin; NOS-2: inducible nitric oxide synthase; ON: optic nerve; ONL: outer nuclear layer; PLR: pupil light reflex; RGCs: retinal ganglion cells; TBARS: thiobarbituric acid reactive substances; TNFα: tumor necrosis factor; VEGF: vascular endothelial growth factor; VEPs: visual evoked potentials.
Oxidative Stress in Glaucoma and the Effect of Enriched Environment

Notwithstanding the local antioxidant high levels, the retina is particularly sensitive to oxidative stress due to several factors, such as high oxygen consumption, light exposure, oxidation of polyunsaturated fatty acids, and photoreceptor phagocytosis (Nishimura et al., 2017). Oxidative stress critically impacts the RGC apoptotic pathway and disrupts the antioxidant capacity of surrounding glial cells (Rohowetz et al., 2018). Growing evidence obtained from clinical and experimental studies over the past decade highlights the involvement of oxidative stress in glaucomatous neurodegeneration (Moreno et al., 2004; Pinazo-Durán et al., 2015). Oxidative stress markers and lipid peroxidation products increase in the aqueous humor and the trabecular meshwork from glaucomatous eyes (Nucci et al., 2013; Goyal et al., 2014; Tang et al., 2019). In agreement, the ocular antioxidant defense system decreases in the anterior chamber of patients with advanced glaucoma (Goyal et al., 2014; Liu et al., 2021). We have demonstrated that retinal superoxide dismutase and catalase activities significantly decrease in eyes with chronic ocular hypertension, consistently with a significant increase in retinal lipid peroxidation (Moreno et al., 2004). In addition, experimental glaucoma induced by photobleaching of the trabecular meshwork, increases superoxide radical levels in the retina and ON head with alterations in antioxidant enzymes (Chidlow et al., 2017). Based on these results, it seems possible that a decrease in antioxidant enzyme activities may surpass RGC resistance against oxidative damage, supporting the involvement of oxidative stress in glaucomatous damage. In this sense, we have demonstrated that exposure to EE protects lipid peroxidation in experimental optic neuritis (Aranda et al., 2019), and diabetic retinopathy (Dorffman et al., 2014). These results suggest that the exposure to EE is beneficial against oxidative damage to the retina and ON and could potentially prevent oxidative stress in glaucoma.

Excitotoxicity in Glaucoma and the Effect of Enriched Environment

Glutamate is the main excitatory neurotransmitter in the retina. After its release from presynaptic terminals, glutamate binds to postsynaptic receptors, inducing the influx of sodium and calcium and, consequently, membrane depolarization. As a result of several insults, like oxidative stress, inappropriate electrical activity or energy supply, ischemia/reperfusion injury, or decreased neurotrophins levels, glutamate can accumulate and provoke toxicity to retinal neurons (Baudouin et al., 2020). Retinal glutamatergic synapse is surrounded by glial cells, mainly astrocytes and Müller glia that removes extracellular glutamate through specific transporters and metabolizes it by glutamine synthetase to the nontoxic amino acid glutamine (Ishikawa, 2013). It has been shown that excitotoxicity plays a key role in glaucoma-induced RGC degeneration, through the overactivation of both non-NMDA and NMDA receptors (Nucci et al., 2016). Many subtypes of RGCs are highly susceptible to glutamatergic excitotoxicity due to the abundance of non-NMDA glutamate receptors (Opere et al., 2018). An elevation of glutamate concentration in the vitreous humor was reported in several species with glaucoma, whereas other authors show no significant increase of this parameter in samples from human or experimental glaucoma (Ishikawa, 2013). Glutamate concentrations in vitreous samples could not be necessarily correlated with the local concentration of glutamate at RGC membrane receptors. For experimental measurement, vitreous humor must be removed using a method that will disturb its state, possibly altering the measured amount of glutamate. Glutamate levels are likely to increase eventually in localized areas of the retina during glaucomatous neurodegeneration (Ishikawa, 2013), although there are no available tools to assess this parameter in vivo. Notwithstanding, the synaptic concentrations of glutamate can be estimated by analyzing the mechanisms regulating retinal glutamate clearance and recycling. It has been suggested that Müller cells may increase RGC susceptibility to stress signals in response to elevated IOP, reducing retinal ability to regulate glutamate homeostasis (Bringmann et al., 2009). In rats subjected to elevated IOP, we have demonstrated that the retinal glutamate/glutamine cycle activity is significantly altered (Moreno et al., 2005b). Due to the lack of extracellular enzymes that degrade glutamate, the responsibility for the maintenance of low glutamate synaptic concentrations is of glutamate transporters. Retinal glutamate uptake significantly decreases in hypertensive eyes (Moreno et al., 2005b), consistently with excitatory amino acid transporters dysfunction (Nucci et al., 2016). In fact, a downregulation of excitatory amino acid transporter type 1 transporter was observed in glaucoma patient Müller cells (Naskar et al., 2000). Müller cells quickly convert glutamate to glutamine, which favors glutamate uptake by these cells versus retinal neurons. Then, glutamine is released from Müller cells as a precursor for glutamate synthetase by neuronal glutaminase. We have shown that ocular hypertension decreases glutamine synthetase activity and increases glutaminase activity, contributing to glutamate production and synaptic glutamate accumulation (Moreno et al., 2005b). Moreover, increased glutamine uptake in hypertensive eyes could potentiate substratum availability for glutamate synthesis, also contributing to glaucomatous RGC loss (Moreno et al., 2005b). These results disclose the relevance of glutamatergic excitotoxicity for retinal alterations in glaucoma. We have analyzed the effect of EE against retinal glutamatergic excitotoxicity in adult rats (Dorffman et al., 2013). EE housing prevents the decrease in retinal glutamate uptake and glutamine synthetase activity induced by ischemia/reperfusion (Dorffman et al., 2013). Moreover, when supraphysiological concentrations of glutamate were intravitreally injected, the exposure to EE protects not only the retinal function and histology, but also reduces the loss of RGCs induced by glutamate (Dorffman et al., 2013). Therefore, the exposure to EE could be an effective strategy against glutamatergic excitotoxicity in different scenarios, eventually including glaucoma.

Glia Response in Glaucoma and the Effect of Enriched Environment

Glial cells are currently considered fundamental players in the progression of glaucomatous neurodegeneration. Microglia and macroglia, comprising astrocytes, Müller cells, and oligodendroglial lineage cells, adopt specific configurations in the visual system, that make each compartment unique and distinctively affected by glaucoma (Williams et al., 2017). Microglial cells take part in neuroinflammatory processes, showing signs of increased reactivity in the retina and ON of human and experimental glaucoma (Rolle et al., 2021). In fact, microglial activation is considered one of the first events in glaucomatous neural damage occurring prior to RGC loss (Bordone et al., 2017; Wei et al., 2019). In experimental glaucoma, increased levels of cytokines and chemokines, such as complement factors, tumor necrosis factor-alpha (TNF-α), and interleukin-6 that amplify the response and promote morphological changes in microglial cells, has been shown (Russo et al., 2016; Rolle et al., 2021). Under pathological conditions, microglia communicates via signaling proteins to Müller cells and astrocytes, which also undergo a pronounced transformation termed “reactive gliosis” (Liddelow et al., 2017). It has been recently reported that blocking the reactive astrocyte phenotype preserves the electrical activity and number of RGCs in a microbead occlusion model of glaucoma,
and that an injury is a necessary condition for RGCs to become susceptible to astrocyte-mediated toxicity (Guttenplan et al., 2020). Reactive Müller cells, detected by the upregulation of intermediate filament glial fibrillary acidic protein (GFAP) and extracellular signal-regulated kinase, were identified in glaucoma patients and experimental models (Russo et al., 2016; Bordone et al., 2017). Moreover, subtle and severe reactivity of astrocytes from the ON head is observed during glaucoma, opening the question whether these changes occur before microglial reactivity (Williams et al., 2017). In addition, ON oligodendrocytes, which are essential for axonal maintenance and correct transmission of visual information from the retina to brain targets, are also affected in experimentally induced glaucoma (Son et al., 2010; Bordone et al., 2017).

Consistent data obtained by our group supports EE housing positive effect against glial alterations in the retina and ON in experimental models of optic neuritis and diabetic retinopathy (Dorfman et al., 2014; Aranda et al., 2019). The exposure to EE prevents Müller cells’ reactivity against retinal ischemic damage (Dorfman et al., 2013). Furthermore, EE housing significantly reduces microglial/macrophages reactivity, astrogliosis, and myelin alterations, and avoided the increase in TNFα and interleukin-1β mRNA levels in the proximal region of the ON induced by experimental optic neuritis (Aranda et al., 2019), supporting EE’s impact in reducing neuroinflammation, and glial alterations (Aranda et al., 2019).

Environmental Enrichment and Glaucoma

As already mentioned, oxidative damage, excitoxicity, decreased neurotrophins, and glial alterations play key roles in glaucoma pathogenesis. Based on the evidence supporting the antioxidant, antiinflammatory, and pro-BDNF properties of EE housing, together with its effect against glutamate toxicity, we have studied the therapeutic effect of exposure to EE in an experimental model of glaucoma (González Fleitas et al., 2020b). For this purpose, male Wistar rats in which one eye received weekly intracamerale injections of CS were housed in SE or EE for 10 weeks, as shown in Figure 1. Flash VEPs are clinically used to analyze different ophthalmologic diseases, as an index of the visual function from the retina to the visual cortex. Concomitantly with data from human and experimentally induced glaucoma (Belforte et al., 2010; Hines-Beard et al., 2016; Jha et al., 2017), CS-induced ocular hypertension provokes a decrease in VEPs, which is prevented by EE housing (Figure 1). Evidence supports the axonal compartment as a preference location of glaucomatous alterations, and a misconnection between the retina and its primary central targets has been shown in different glaucoma models (Crish et al., 2010; Bordone et al., 2017), and histological changes and metabolic adaptations add to glaucomatous damage susceptibility (Yang et al., 2018; Cooper et al., 2020).

EE housing avoids the deficit induced by ocular hypertension in the anterograde transport from the retina to its main central targets (i.e., the SC, and the lateral geniculate nucleus), as shown in Figure 1. Based on several reports showing axonal alterations in different regions of the ON in different glaucoma models (i.e., prelaminar, laminar, and proximal post-laminar) (Son et al., 2010; Bordone et al., 2017), the levels of phosphorylated neuropilin heavy chain (PNFH), involved in neuronal morphology, axonal transport, and plasticity, were assessed. The exposure to EE prevents the decrease in PNFH immunoreactivity and the number of axons in hypertensive eyes, which is compatible with the preservation of axonal transport, and VEP amplitude (González Fleitas et al., 2020b).

EE neuroprotection is not exclusively restricted to the axonal compartment in glaucoma, as it significantly impacts on the retina and ON glial reactivity. Ocular hypertension triggers microglial/macrophage and astrocyte reactivity in the proximal ON that is also prevented by EE (Figure 1). Moreover, EE housing prevents myelination alterations in this region of the ON induced by experimental glaucoma (Bordone et al., 2017; González Fleitas et al., 2020b). At the retinal level, EE lessens microglial/macrophages in the inner retina and avoided central and peripheral RGC loss in hypertensive eyes (Figure 1). These data suggest that the preservation of RGCs could be due to the protection of axons and the decreased microglial reactivity induced by EE both in the ON and the retina. In order to analyze whether EE housing, apart from preventing glaucoma development, is able to reduce glaucomatous damage progression, SE-housed animals with 6 weeks of ocular hypertension (a time-point at which histological and functional alterations are already evident), were transferred to EE (delayed EE). Delayed EE notably reduces functional and structural alterations in the retina and ON of glaucomatous eyes (González Fleitas et al., 2020b), supporting a therapeutic effect of EE on ongoing glaucomatous damage.

Emerging strategies of neuroprotection in the context of glaucoma focus on neurotrophin-dependent mechanisms on the basis of their well-documented roles in neuronal survival (Kimura et al., 2016). Treatments based on BDNF have gained relevance over the last years because of its powerful neuroprotective function, especially for the retina (González Fleitas et al., 2013). BDNF shows retinal and ON beneficial effects in different injury models, such as light-induced photoreceptor...
degeneration, optic neuritis, and anotomy, among others (Cerri et al., 2015; Brandli et al., 2016; Aranda et al., 2019), supporting that BDNF could constitute a common protective mechanism against different visual deleterious conditions. BDNF levels and its signaling through TrkB receptors are significantly reduced in the ON head of glaucoma patients (Gupta et al., 2014). Moreover, decreased BDNF levels in the aqueous humor from early human glaucoma have also been reported (Shpak et al., 2018), although another study did not find changes in this parameter (Zhang et al., 2017). In animal models of glaucoma, several studies have shown that BDNF supplementation is successful at slowing the progression of RGC degeneration (Nysson et al., 2017). Nevertheless, the therapeutic use of BDNF in glaucoma is greatly limited by the fact that BDNF does not cross the blood-brain barrier (Kimura et al., 2016). Gene therapies that increase retinal BDNF or its signaling by delivering adenoviruses in the vitreous humor demonstrate promising results against glaucomatous damage (Chitranshi et al., 2019; Wojcik-Gryciuk et al., 2020). However, many of these strategies present translational limitations to human glaucoma due to their grade of invasiveness, as repeated injections are required for sustained effects, and the difficulty in setting doses that do not downregulate TrkB receptor expression. In this line, a better approach would be to locally enhance endogenous BDNF expression and signaling. We have demonstrated that a long-term exposure to EE significantly increases BDNF protein content in glaucomatous retinas (González Fleitas et al., 2020b). In addition, EE housing increases ON BDNF levels, and the administration of an antagonist of TrkB receptor abolishes the protective effect of EE in an experimental model of optic neuritis (Aranda et al., 2019), supporting a key role of the BDNF/TrkB receptor pathway in neuroprotection induced by EE. In conclusion, our results support that EE housing preserves visual functions, provides neuroprotection to RGCs and their axons, and avoids glial cell alterations following the exposure to chronic ocular hypertension, probably through a BDNF-dependent mechanism. Notwithstanding, the involvement of the antioxidant, and antiinflammatory factors involved in the EE-induced neuroprotection cannot be ruled out.

Apart from hypertensive glaucoma, there is a condition that shows glaucomatous optic neuropathy without elevation of IOP, termed normal tension glaucoma (NTG). It has been postulated that IOP-independent risk factors, such as vascular dysregulation, higher transalaminar pressure gradient due to impaired cerebrospinal fluid dynamics, immune-related disorders, and myopia-related biomechanical factors, play important roles in the development of NTG (Kim and Park, 2016; Trivié et al., 2016; Zhang et al., 2019). In addition, recent studies have shown the possible involvement of oxidative stress, and glutamate neurotoxicity in the pathogenesis of NTG (Trivié et al., 2019; Harada et al., 2020), supporting that there is a close pathogenic link between primary open-angle glaucoma and NTG (Bulut et al., 2016; Zhang et al., 2019). Since EE behaves as antioxidant, and antiexcitotoxic therapy, and is beneficial against a disruption in ocular blood flow (as shown in retinal ischemia/reperfusion), it may be expected that EE could have a positive impact also against NTG.

New Perspectives: Visual Stimulation for Recovering Vision in Glaucoma?

In contrast to the original concept indicating that the adult retina is less susceptible than cortical areas to experience-dependent plasticity, the already mentioned data highlights the possibility of inducing retinal plasticity even in the adult stage. As already mentioned, EE preserves retinal function and structure not only against experimental glaucoma, but also against retinal ischemia, diabetic retinopathy, and optic neuritis (Dorfman et al., 2013, 2014, 2015; Aranda et al., 2019; González Fleitas et al., 2020b). Furthermore, the previous exposure to EE increases retinal resilience against acute ischemic damage (González Fleitas et al., 2019).

A natural question that emerges is about the translational feasibility of the EE paradigm application to human glaucoma. Although evidence suggests that at least for the brain, the greatest benefits are obtained from the sum synergic effects of the full repertoire of EE (Faherty et al., 2003; Szeda et al., 2010), one step forward to EE paradigm clinical application is identifying the role of independent EE components (e.g., social, sensory, motor) in eliciting the beneficial effects evoked by the full enriched experience, followed by the design of therapeutic strategies based on the most promising and effective variables (Baroncelli et al., 2012; Sale et al., 2014). In that context, we isolated the components (cognitive, social, and visual) of EE condition, and analyzed their particular effect on the retinal protection against acute ischemic damage in adult rats. For this purpose, animals submitted to unilateral retinal ischemia were exposed to modified environments in order that several components of the usual EE could be separately studied (González Fleitas et al., 2020a). The contribution of the social component was evaluated by housing five animals at a time in standard laboratory cages, and the outcome was compared with animals housed in standard cages containing two animals per cage. The exposure to social environment does not reproduce the neuroprotective effects of EE on retinal function and structure, suggesting that isolated social stimulation does not contribute to the retinal protection against acute retinal ischemia. The cognitive component of EE was tested by using SE cages containing objects that were daily changed for novelty maintenance purposes (novelty environment). It has been demonstrated that the interaction with complex objects apart from social interaction successfully improves functional recovery after brain lesion in rodents (Will and Kelche, 1992). However, in animals housed in a novelty environment no significant retinal protection was observed, suggesting that different mechanisms from those acting in retinal protection could act in brain plasticity. As EE is visually complex compared with standard laboratory housing, we analyzed the contribution of the visual input to EE neuroprotection in animals submitted to bilateral retinal ischemia. In bilateral retinal ischemia, EE housing did not induce retinal protection against acute ischemia. As it has been suggested that the neuroprotection achieved by EE can exclusively rely on the physical component of environmental enrichment (Kobilo et al., 2011), we analyzed if bilaterally induced ischemia could affect locomotor activity thus the altered physical activity be the cause of loss of EE-induced retinal neuroprotection. Nevertheless, locomotor activity shows no differences among intact animals, and after unilateral or bilateral ischemia, which demonstrates that the abolishment of EE protective effect in animals bilaterally submitted to ischemia cannot be explained by altered locomotor activity, and that at least visual input in one eye may be a necessary condition for protection induced by EE in the contralateral (ischemic) eye. Thus, we hypothesized that visual stimulation could protect retinal alterations induced by ischemia. In order to analyze the effect of visual stimulation, animals were housed as follows: (i) standard visual environment: cages similar to SE (two animals/cage) surrounded by four PC monitors, which projected a 50% gray image during the total light phase, and (ii) visual environment (VE): cages equal to SE (two animals/cage) surrounded by four PC monitors, which projected a 100% contrast black/white pattern for 6 seconds, before a 50% gray image for 12 seconds, also during the entire light phase. VE achieved a significant retina protective function and structural protection, and preserves RGC number in ischemic retinas, indicating that visual stimulation (but not social interaction or cognitive stimulation) reproduces EE protective effect. How visual stimulation is able to avoid damage against
Since acute retinal ischemic damage shares some of the pathogenic mechanisms involved in chronic glaucoma, it is tempting to speculate that visual stimulation could also protect the retina and ON against glaucomatous injury, a hypothesis that is currently under investigation. There is evidence in humans showing that experimental paradigms analogous to EE, such as playing video games or being trained in perceptual learning tasks, can be quite successful in eliciting amblyopia recovery in adult subjects (Foss, 2017). Although amblyopia involves an alteration that mainly impacts on the visual cortex, whereas glaucoma primarily affects retinal and ON cells, it seems that visual stimulation could be a rehabilitative therapy for retinal disorders in adulthood that eventually may translate to the human condition, averting glaucoma-induced dreaded sequelae that result in permanent visual disability.

One of the many complications of glaucoma is its chronicity, which implies that treatments (of any kind) should be considered in the long-term. For mimicking enriched environments (as described for rodents), glaucomatous patients could be advised to change several aspects of their lifestyle, such as increasing social interactions, physical exercise, and cognitive challenges. In the case of visual overstimulation, a very frequent application (e.g., playing video games once or twice a day) would be useful. In this way, preservation of the visual functions in glaucomatous patients through an intensified use of the sensory and motor systems, and/or social interactions could be a novel, harmless, and non-invasive strategy to promote visual recovery.

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Corrigendum

Corrigendum: Maternally expressed gene 3 regulates retinal neovascularization in retinopathy of prematurity
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In the article titled “Maternally expressed gene 3 regulates retinal neovascularization in retinopathy of prematurity” published on pages 1364–1368, Issue 6, Volume 17 of *Neural Regeneration Research* (Di et al., 2022), the first lane of protein bands from western blot was incorrectly written as “p-A13K” due to a typesetting error, it should read “p-Pi3K”. The publisher apologizes for the error.

The online version of the original article can be found under doi: 10.4103/1673-5374.327358