Neuropharmacology xxx (2017) 1–12

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Therapeutic benefit of environmental enrichment on optic neuritis

Marcos L. Aranda, María F. González Fleitas, Hernán H. Dieguez, Georgia A. Milne, Julián D. Devouassoux, María I. Keller Sarmiento, Mónica Chianelli, Pablo H. Sande, Damián Dorfman, Ruth E. Rosenstein*

Laboratory of Retinal Neurochemistry and Experimental Ophthalmology, Department of Human Biochemistry, School of Medicine/CEFyBO, University of Buenos Aires/CONICET, Paraguay 2155, 5th Floor, 1121, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 14 July 2017
Received in revised form
13 November 2017
Accepted 9 December 2017
Available online xxx

Keywords:
Optic neuritis
Environmental enrichment
Neuroinflammation
Retinal ganglion cells

ABSTRACT

Optic neuritis (ON) is an inflammatory, demyelinating, neurodegenerative, and presently untreatable condition of the optic nerve which might induce blindness. We analyzed the effect of environmental enrichment (EE) on visual pathway damage provoked by experimental ON induced by a microinjection of bacterial lipopolysaccharide (LPS) into the optic nerve. For this purpose, LPS was microinjected into the optic nerve from male *Wistar* rats. After injection, one group of animals was submitted to EE, and another group remained in standard environment (SE) for 21 days. EE prevented the decrease in pupil light reflex (PLR), visual evoked potentials, retinal anterograde transport, phosphorylated neurofilament immunoreactivity, myelination (luxol fast blue staining), and axon (toluidine blue staining) and retinal ganglion cell (Brn3a-immunoreactivity) number. EE also prevented microglial/macrophage reactivity (Iba-1- and ED1-immunoreactivity), and astrocytosis (glial fibrillary acidic protein-immunostaining) induced by experimental ON. LPS-injected optic nerves displayed oxidative damage and increased inducible nitric oxide synthase, cyclooxygenase-2, and interleukin-1β and TNFα mRNA levels which were prevented by EE. EE increased optic nerve brain-derived neurotrophic factor levels. When EE started at 4 (but not 7) days post-injection of LPS, a preservation of the PLR was observed at 21 days post-LPS, which was blocked by the daily administration of ANA-12 from day 4 to day 7 post-LPS. Moreover, EE from day 4 to day 7 post-LPS significantly preserved the PLR at 21 days post-injection. Taken together, our data suggest that EE preserved visual functions and reduced neuroinflammation of the optic nerve.

1. Introduction

Optic neuritis (ON), a dysfunction of the optic nerve, is the most common cause of optic neuropathy among young adults. ON involves primary inflammation, demyelination, and axonal injury of the optic nerve, which leads to visual dysfunction and retinal ganglion cell (RGC) loss (Hickman et al., 2002; Toosy et al., 2014). The clinical hallmarks of ON include subacute, central vision loss in one eye that may progress over 1-2 weeks, accompanied by pain with eye movements, impairment of color vision out of proportion to visual acuity, a relative afferent pupillary defect, and abnormal visual evoked potentials (VEPs) (Hickman et al., 2002; Toosy et al., 2014). Many of the patients with ON can self-recover over 1 - 3 months; however, varying degree of permanent visual dysfunction can occur in ~50% of patients. In that sense, several studies have found evidence of persistent retinal thinning, optic nerve atrophy, and VEP alterations which are consistent with ON-induced demyelination and neuroaxonal loss (Galetta et al., 2015). These alterations can impair patients' abilities to perform daily activities (e.g., driving, working), so they have important implications for their life quality (Galetta et al., 2015).

ON has many causes; it may be associated to a broad range of infectious or autoimmune diseases (Eggenberger, 2001; Frigui et al., 2011; Hickman et al., 2002; Horwitz et al., 2014;

Abbreviations used: BDNF, brain derived neurotrophic factor; COX-2, cyclooxygenase-2; CTB, cholera toxin β-subunit; EE, environmental enrichment; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium binding adaptor molecule 1; IL-1β, interleukin-1β; LPS, bacterial lipopolysaccharide; MS, multiple sclerosis; pNFH, phosphorylated neurofilament heavy chain; NOS-2, inducible nitric oxide synthase; ON, optic neuritis; PLR, pupil light reflex; RGCs, retinal ganglion cells; SC, superior colliculus; SE, standard environment; TBARS, thiobarbituric acid reactive substances; TNFα, tumor necrosis factor α; VEPs, visual evoked potentials.

* Corresponding author. Departamento de Bioquímica Humana, Facultad de Medicina/CEFyBO, Universidad de Buenos Aires/CONICET, Paraguay 2155, 5°P, 1121, Buenos Aires, Argentina.
E-mail address: ruthr@fmed.uba.ar (R.E. Rosenstein).

https://doi.org/10.1016/j.neuropharm.2017.12.017
0028-3908/

Kallenbach and Frederiksen, 2008), and it is closely associated with multiple sclerosis (MS) (Optic Neuritis Study Group, 2008). In fact, ON is the initial symptom of MS in ~25% of cases, and may occur during the disease in about 70%, usually in the relapsing-remitting phase (Hickman et al., 2002; Toosy et al., 2014). On the other hand, acute ON often occurs as an isolated clinical event, without contributory systemic abnormalities, and it is retrospectively diagnosed as idiopathic (or primary) ON (Hickman et al., 2002; Optic Neuritis Study Group, 2008). Unraveling which are the most critical mechanisms in ON is unlikely to be achieved in studies which are limited to the clinically observable changes to the optic nerve and retina that are seen in human ON. Far more detailed and invasive studies are required, preferably in a readily available animal model. Various rodent models representing different etiologies have been developed for ON studies. The most commonly used immune-mediated animal model for ON, is experimental autoimmune encephalomyelitis (Wekerle et al., 1994), a validated model for human MS (Gold et al., 2006), which can be induced by active immunization with different myelin proteins or spontaneous transgenic models (Robinson et al., 2014). Recently, we have developed a new experimental model of primary ON in rats through a single microinjection of bacterial lipopolysaccharide (LPS) directly into the optic nerve (Aranda et al., 2015). The local injection of LPS induces a significant and persistent decrease in VEP amplitude and pupil light reflex (PLR), without changes in retinal function (electroretinogram). Moreover, LPS induces a deficit in the anterograde transport from the retina to its central targets, increases optic nerve microglial/macrophage reactivity, and induces astrocytosis, demyelination, and axon and RGC loss, without signs of systemic inflammation or cerebral involvement (Aranda et al., 2015), supporting that the microinjection of LPS into the optic nerve may serve as a new experimental model of primary ON which could be a useful tool for developing new therapeutic strategies.

High-dosage methylprednisolone treatment has been established as the standard therapy of acute ON. However, although methylprednisolone accelerates visual recovery, it does not influence visual outcome, lesion length or atrophy of the optic nerve (Gal et al., 2015). In addition, methylprednisolone even increases RGC degeneration in an experimental model of ON (Diem et al., 2003). Therefore, currently there are no therapeutic strategies able to improve the visual outcome in ON, and the development of therapies with the potential to prevent neuroaxonal loss following ON remains a significant unmet clinical need.

Environmental enrichment (EE) consists of a manipulation in which animals are exposed to complex conditions through adaptations in the physical and social environment. This complex environment is composed by nesting materials, running wheels for voluntary exercise, tunnels, ladders, and toys with different textures, colors, shapes, and sizes, which are moved around daily to stimulate novelty, and provide continuous opportunity for exploration and stimulating sensory, cognitive, and physical activity (Bondi et al., 2014; Sale et al., 2014). EE induces changes in neuron morphology and synaptogenesis during development, adulthood and aging, due to increased brain derived neurotrophic factor (BDNF) expression, among other mechanisms (Berardi et al., 2015; Landi et al., 2007, 2015; Sale et al., 2014), supporting that brain neural plasticity in response to environmental experiences lasts throughout the lifespan. It has been demonstrated that EE increases hippocampal levels of BDNF with consequent activation of TrkB receptors (Ambrogini et al., 2013), and that knockdown of TrkB expression impairs EE mediated neuroprotection of spatial memory during hypobaric hypoxia (Jain et al., 2013).

In addition to morphological changes, several report show that EE provides a better recovery from different neuropathologies such as stroke, Parkinson's disease, Alzheimer's disease, epilepsy and traumatic brain injury (reviewed by Hannan, 2014; Kline et al., 2016; Sale et al., 2014). As for retinal development, it has been demonstrated that EE accelerates rat retinal acuity maturation (Landi et al., 2007), and prevents retinal degeneration in rats induced by a neonatal treatment with glutamate (Szabadfi et al., 2009). In addition, it has been shown that the exposure of mice to EE from birth enhances visual acuity (Prusky et al., 2000). In contrast, the adult retina and optic nerve have long been considered “less plastic” than the brain cortex or hippocampus, the canonical sites of experience-dependent plasticity. However, we have demonstrated that EE significantly protects retinal function and histology from ischemia/reperfusion, excitotoxicity (Dorfman et al., 2013), and experimental diabetes in adult animals (Dorfman et al., 2014, 2015). Taken together, these results support that environmental stimuli can significantly modify the extent of visual pathway damage, even in the adult stage. Since the impact of EE on brain inflammatory processes has been scarcely examined, the present study sought to determine if the deleterious effects of ON on the visual pathway could be prevented or attenuated by exposure to EE. Moreover, since inflammatory response and oxidative stress play crucial roles in the progression of ON (Aranda et al., 2016; Li et al., 2014; Qi et al., 2007), we analyzed the variation in inducible nitric oxide synthase (NOS-2) and cyclooxygenase-2 (COX-2) protein levels, as well as interleukin-1β (IL-1β) and TNFα mRNA levels, and oxidative stress. In addition, a possible contribution of BDNF/TrkB pathway in the effects of EE was analyzed by the assessment of BDNF levels in the optic nerve, and the effect of ANA-12 (an inhibitor of TrkB receptors) on the optic nerve dysfunction in animals exposed to standard laboratory conditions or EE.

2. Materials and methods

2.1. Animals

All animal use procedures were in strict accordance with the NIH Guide for Care and Use of Laboratory Animals. The ethics committee of the School of Medicine, University of Buenos Aires (Institutional Committee for the Care and Use of Laboratory Animals (CICUAL)) approved this study. Adult male *Wistar* rats (2 months old, average weight, 350 ± 50 g) from our own colony derived from a stock supplied by Charles River Breeding Laboratories (Wilmington, MA, U.S.A.) were housed in a standard animal room. Before the experiments, four rats at a time were housed in standard laboratory cages (33.5 × 45 × 21.5 cm), with food and water *ad libitum*, under controlled conditions of humidity and temperature (21 ± 2 °C). The room was lighted by fluorescent lights (200 lux) that were turned on and off automatically every 12 h. Animals from the control group (standard environment, SE) were housed in standard cages with two animals per cage. For EE, six animals at a time were housed in big cages (46.5 × 78 × 95 cm), containing four floors and several food hoppers, water bottles, running wheels, tubes, ramps and differently shaped objects (balls, ropes, stones) repositioned once a day and fully substituted once a week as previously described (Dorfman et al., 2013). Animals were continuously exposed to SE or EE during different intervals (21, 17, 14, or 3 days), as indicated in each case. In order to evaluate the involvement of running wheels in the effect of EE, running wheels were locked in some experiments.

2.2. Experimental model of ON

Experimental ON was induced as previously described (Aranda

Please cite this article in press as: Aranda, M.L., et al., Therapeutic benefit of environmental enrichment on optic neuritis, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.12.017

et al., 2015). Briefly, animals were anesthetized with ketamine hydrochloride (150 mg/kg) and xylazine hydrochloride (2 mg/kg) administered intraperitoneally. A lateral canthotomy was made to perform an incision of 2–3 mm. The optic nerve was exposed, a 30G needle was inserted into the optic nerve as superficially as possible, 2 mm posterior to the globe, and 1 μl of 4.5 μg/μl *Salmonella typhimurium* LPS (Sigma Chemical Co. St Louis, MO, USA) in pyrogen-free saline or vehicle were injected. After injections, the skin incision was sutured and antibiotics were topically administered to prevent infection. After recovery from anesthesia, animals were randomly assigned to standard or enriched cages, as described above.

2.3. Pupillary light reflex

Animals were injected with vehicle or LPS into one optic nerve, while the contralateral optic nerve remained intact. After 2 h of darkness adaptation, the eye whose optic nerve was injected with vehicle or LPS was stimulated with high intensity light (1200 lux) for 30 s and the PLR was recorded in the contralateral (intact) eye. The recordings were made under infrared light with a digital video camera (Sony DCR-SR60, Tokyo, Japan), as previously described (Fernandez et al., 2013). The sampling rate was 30 frames per second. Images were acquired with OSS Video Decompiler Software (One Stop Soft, New England, USA). The results were expressed as the percentage of the pupil contraction before (steady state) and 30 s after the light pulse.

2.4. Visual evoked potential recording

Animals were anesthetized as described above. Under stereotaxic control, two stainless steel electrodes, used as positive electrodes, were surgically placed 3 mm lateral to the interhemispheric suture and 5.6 mm behind bregma penetrating the cortex to approximately 0.5 mm. Reference electrodes were placed 2 mm lateral to the midline and 2 mm anterior to bregma, as previously described (Aranda et al., 2015). After 5 days of electrode implantation, animals were dark-adapted for 6 h, and anesthetized as already described. Recordings were obtained as previously described (Aranda et al., 2015).

2.5. Cholera toxin β-subunit transport studies

At 18 days post-injection of vehicle or LPS, rats were anesthetized, and a drop of 0.5% proparacaine (Anestalcon, Alcon Laboratories, Buenos Aires, Argentina) was topically administered for local anesthesia. Using a 30G Hamilton syringe (Hamilton, Reno, NV, USA), 4 μl of 0.1% cholera toxin β-subunit (CTB) conjugated to Alexa 488 dye (Molecular Probes Inc. Eugene, OR, USA) in 0.1 mol/L PBS (pH 7.4) was injected into the vitreous, as previously described (Dorfman et al., 2013). Three days after injection, rats were anesthetized and intracardially perfused. Cerebral coronal sections (40 μm) were obtained using a freezing microtome (CM, 1850; Leica, Leica Microsystems, Buenos Aires, Argentina), mounted in glasses and viewed under an epifluorescence microscope (BX50, Olympus, Duarte, CA, USA). Every other coronal section was used for the superior colliculus (SC) retinorecipient area reconstruction using Matlab (The Math- Works Inc. Natick, MA, USA), as previously described (Dorfman et al., 2013). The quantification of CTB(+) area was performed by a morphometric analysis, as described below.

2.6. Histological evaluation

Animals were deeply anesthetized and intracardially perfused with saline, followed by a fixative solution (4% paraformaldehyde in phosphate buffer (PBS), pH 7.2). Eye cups and optic nerves were obtained as previously described (Aranda et al., 2015). Serial transversal sections (5 μm) were obtained using a microtome (Leica, Leica Microsystems, Buenos Aires, Argentina). After deparaffinization, some sections were hydrated and immersed overnight at 60 °C in 0.1% luxol fast blue (LFB, Biopack, Buenos Aires, Argentina) in acidified 95% ethanol. Differentiation and counterstaining were carried out as previously described (Aranda et al., 2015). Light microscopic images (200×) were digitally captured via a Nikon Coolpix S10 camera (Nikon, Tokyo, Japan).

2.7. Semi-thin section processing

Anesthetized rats were intracardially perfused with saline solution, followed by a fixative solution containing 4% formaldehyde and 2% glutaraldehyde in 0.1 mol/L PBS (pH 7.4). Optic nerves were carefully removed, and portions (2 mm) near the eye were obtained and embedded in the same fixative solution for 24 h. Semi-thin sections (0.5 μm) obtained with an ultramicrotome (Ultracut E, Reichert-Jung, Austria) were stained with toluidine blue, and used for morphometric analysis, as previously described (Aranda et al., 2015).

2.8. Immunohistochemical studies

Antigen retrieval was performed by heating slices at 90 °C for 30 min in citrate buffer (pH 6.3). The following antibodies were used: a goat anti-ionized calcium binding adaptor molecule 1 (Iba-1) antibody (AB Registry ID: AB_2224402, 1:500; Abcam Inc. Buenos Aires, Argentina), a mouse anti-ED1 antibody (AB Registry ID: AB_1141557, 1:500; Abcam Inc. Buenos Aires, Argentina), a mouse monoclonal anti-glial fibrillary acidic protein (GFAP) antibody conjugated to Cy3 (AB Registry ID: AB 476889, 1:1200; Sigma Chemical Co. St Louis, MO, USA), a mouse pNFH antibody (AB Registry ID: AB_448147, 1:1000; Abcam, MA, USA), a donkey anti-mouse secondary antibody conjugated to Alexa 488 (Cat.#: ab150105, 1:500; Molecular Probes, Buenos Aires, Argentina), and a donkey anti-goat secondary antibodies conjugated to Alexa 568 (AB Registry ID: AB_2636995, 1:500; Molecular Probes, Buenos Aires, Argentina). Samples were incubated with secondary antibodies as previously described (Aranda et al., 2015). For each nerve, results obtained from four separate sections (taken at a 10 μm-distance) were averaged, and the mean of 6 nerves was recorded as the representative value for each group. The variability among sections inside each group was lower than 10%. Whole-mount retinas were incubated overnight at 4 °C with a goat anti-Brn3a antibody (AB Registry ID: AB_2167511, 1:500; Santa Cruz Biotechnology, Buenos Aires, Argentina). After several washes, a donkey anti-goat secondary antibody conjugated to Alexa 568 (1:500; Molecular Probes, Buenos Aires, Argentina) was added, and incubated for 2 h at room temperature. Finally, retinas were mounted with fluorescent mounting medium (Dako, Glostrup, Denmark), and observed under an epifluorescence microscope (BX50; Olympus, Tokyo, Japan) connected to a video camera (3CCD; Sony, Tokyo, Japan), attached to a computer running image analysis software (Image-Pro Plus; Media Cybernetics Inc. Bethesda, MD, USA). For each retina, results obtained from five separate quadrants were averaged as previously described (Aranda et al., 2015).

2.9. Morphometric analysis

All the images obtained were assembled and processed using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA, USA) to adjust brightness and contrast. No other adjustments were made. For all morphometric image processing and analysis, digitalized captured

Please cite this article in press as: Aranda, M.L., et al., Therapeutic benefit of environmental enrichment on optic neuritis, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.12.017

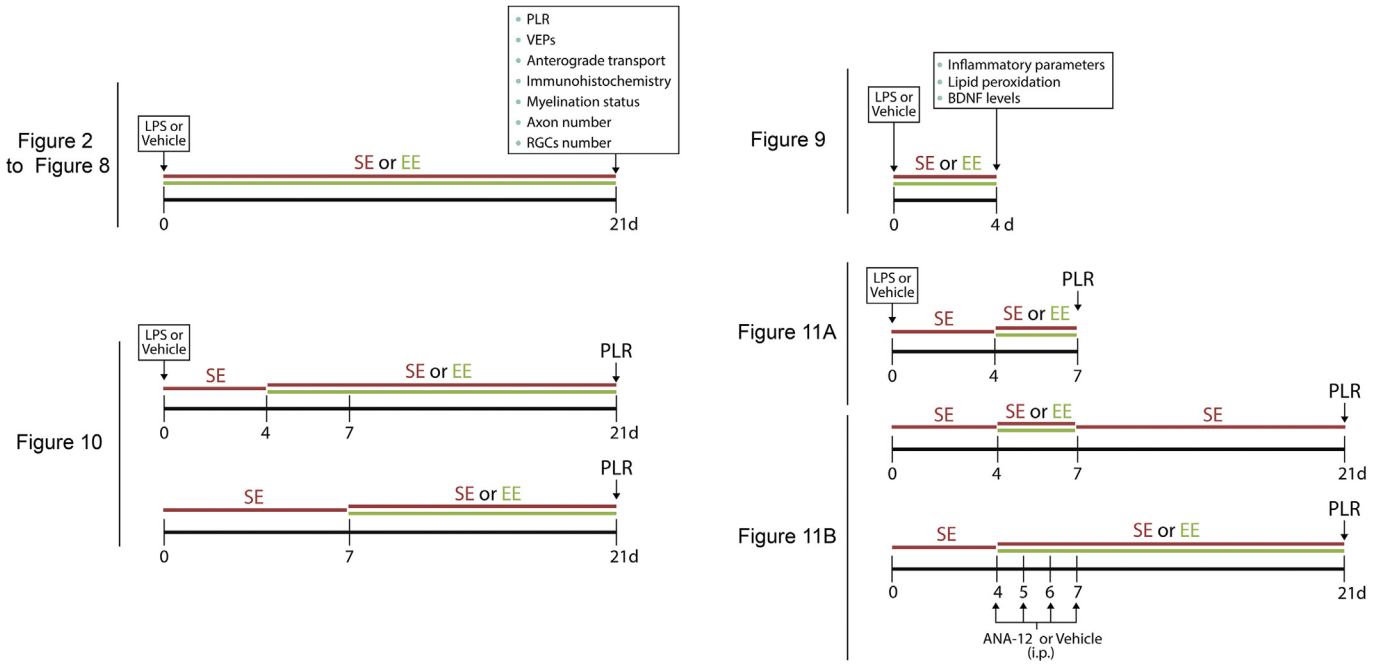

Fig. 1. Experimental groups and protocols for all the experiments.

TIFF images were transferred to ImageJ software (National Institutes of Health, Bethesda, Maryland, USA; http://imagej.nih.gov/ij/).

2.10. Measurement of thiobarbituric acid reactive substances (TBARS) levels

Optic nerves were homogenized in 250 μl of 15 mM potassium buffer plus 60 mM KCl, pH 7.2, and TBARS levels were analyzed as previously described (Aranda et al., 2016). The reaction mixture contained: optic nerve homogenate (100 μl), 25 μl 10% SDS, and 465 μl ml 0.8% thiobarbituric acid dissolved in 10% acetic acid (pH 3.5). This solution was heated to 100 °C for 60 min. After cooling, the precipitate was removed by centrifugation at 3200*g* for 10 min. The absorbance of the organic layer was measured at an emission wavelength of 553 nm by using an excitation wavelength of 515 nm with a Jasco FP 770 fluorescence spectrophotometer (Japan Spectroscopic Co. Ltd. Tokyo, Japan). The range of the standard curves of malondialdehyde bis-dimethyl acetal (MDA) was 10–2000 pmol. Results were expressed as nanomol MDA/mg prot.

2.11. RNA isolation and quantitative real-time PCR

Total optic nerve-RNA was isolated using TRI reagent (Genbiotech SRL, Buenos Aires, Argentina) according to the manufacturer's instructions. Reverse transcription was performed using Moloney Murine Leukemia Virus reverse transcriptase (Life Technologies, Carlsbad, CA, USA). Expression analysis of Il-1β and TNF was performed using specific primers (for Il-1β: Fw: 5′-TGAGTGACACTGCCTTCCTG-3′, Rv: 5′-AGGCTTCCTTGTGCAAGTGT-3'; for TNF: Fw: 5′-TCAGCCTCTTCTCATTCCTGC-3′, Rv: 5′-TTGGTGGTTTGCTACGACGTG-3′). Optic nerve-cDNA was amplified by real-time PCR in a Rotor-Gene 6000 Corbett Life Science Real Time Thermal Cycler (Corbett Research, Mortlake, Australia) and quantified with the Rotor Gene 6000 Series Software (version 1.7 Build 40). PCR products were analyzed and normalized to *Actb* as an internal control, using the ΔΔCt relative quantification method (Livak and, Schmittgen, 2001).

2.12. Western blotting

Optic nerves were homogenized in 250 μl buffer containing 10 mM HEPES, 1 mM EDTA, 1 mM EGTA, 10 mM KCl, 0.5% (v/v) Triton, pH 7.9, supplemented with a cocktail of protease inhibitors (Sigma Chemical Co. St Louis, MO). Proteins were separated by electrophoresis transferred to polyvinylidene difluoride membranes, as previously described (Aranda et al., 2016). After blockage, membranes were incubated overnight at 4 °C with a mouse polyclonal anti-COX-2 antibody (Cat#: 160126, 1:200, Cayman Chemical, MI, USA) and a rabbit polyclonal anti–NOS–2 antibody (Cat#: 160862, 1:200, Cayman Chemical, MI, USA) as previously described (Aranda et al., 2016). Membranes were washed and then incubated for 1 h with a horseradish peroxidase-conjugated secondary antibody and visualized by enhanced chemiluminescence Western blotting detection reagents (Amersham Biosciences, Buenos Aires, Argentina). Densitometry was quantified by using ImageQuant software and adjusted by the density of β-actin.

2.13. BDNF level assessment

Optic nerves were homogenized in 100 μL of a sample buffer from the BDNF E_{max} ImmunoAssay System (Promega Corporation, Madison, WI, USA) and a cocktail of protease inhibitors. Samples were cleared by centrifugation for 10 min at 15700 g. BDNF levels were determined using a specific rat enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions. The reaction was stopped, and absorbance was immediately read at 450 nm on a microplate reader (Model 3550, BIO-RAD Laboratories, Hercules, CA, USA).

2.14. ANA-12 treatment

ANA-12 (Sigma-Aldrich Corp. St. Louis, MO, USA) or vehicle (0.2 mg/kg body weight in 1% DMSO) were daily (i.p.) administered, from day 4 to day 7 post-injection of LPS, at 1 h before lights turn on. The administration way and dosage of ANA-12 were selected on

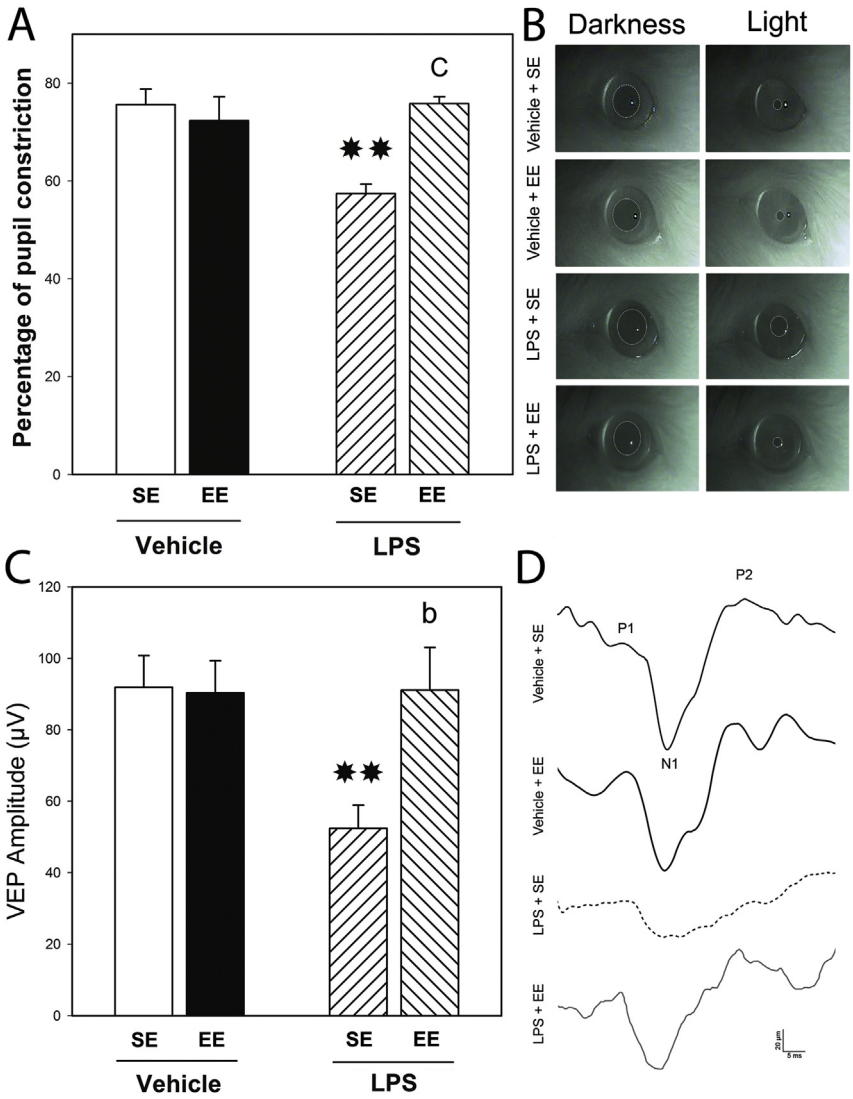

Fig. 2. Effect of EE and experimental ON on PLR and VEPs. Panel A: The rat pupil diameter (relative to the limbus diameter) was assessed at 21 days post-injection of vehicle or LPS into the optic nerve, and the percentage of pupil constriction was calculated in the contralateral eye whose optic nerve remained intact. The LPS-induced decrease in PRL was completely prevented by EE. Panel B: Representative images of the consensual PLR in all experimental groups. Panel C: Average amplitude of VEP N1—P2 component recorded at 21 days post-injections. In SE, LPS induced a significant decrease in VEP amplitude, whereas EE completely prevented the effect of LPS on this parameter. Panel D: Representative VEP traces. For panels A and C, data are the mean ± SEM (n = 12 eyes/group). Two ways ANOVA, *panel A:* $F_{interaction\ (1,44)}$ 11.8, $P < 0.01$; *panel C:* $F_{interaction\ (1,44)}$ 4.74, $P < 0.05$. $^{**}P < 0.01$ vs. vehicle-injected optic nerves in SE, b: $P < 0.01$, c: $P < 0.001$ vs. LPS-injected optic nerves in SE, by Tukey's test.

the basis of a previous report (Cazorla et al., 2011).

2.15. Protein level assessment

Protein content was determined by the method of Lowry et al. (1951) using BSA as the standard.

2.16. Statistical analysis

Statistical analysis of results was performed by two-way analysis of variance (ANOVA) with two levels of environment (SE and EE) and two levels of treatment (vehicle and LPS), followed by Tukey's test. Significance was set at P values below 0.05 for all analyses, and values are expressed as mean ± standard error (SE).

2.17. Experimental design

In the experiments depicted in Fig. 2 to Fig. 8, animals were continuously exposed to SE or EE for 21 days post-LPS. For the results depicted in Fig. 9, animals were sacrificed after 4 days of exposure to SE or EE. For the experiments depicted in Fig. 10, the exposure to SE or EE started at 4 or 7 days post-injection of LPS and finished at 21 days post-injection. Fig. 11 shows the effect of the following treatments: i) exposure to EE from day 4 to day 7 post-LPS, and PLR assessment at day 7 post-LPS; ii) exposure to EE from day 4 to day 7 post-LPS, and PLR assessment at day 21 post-LPS, and iii) similar conditions than in (ii), with injections of vehicle or ANA-12 (once a day for four days, starting at 4 days post-LPS), in order to analyze the involvement of TrkB receptors in the effect of EE. The experimental design for all the experiments is depicted in Fig. 1.

3. Results

To analyze the effect of EE on the visual dysfunction induced by experimental ON, the PLR and VEPs were analyzed at 21 days post-

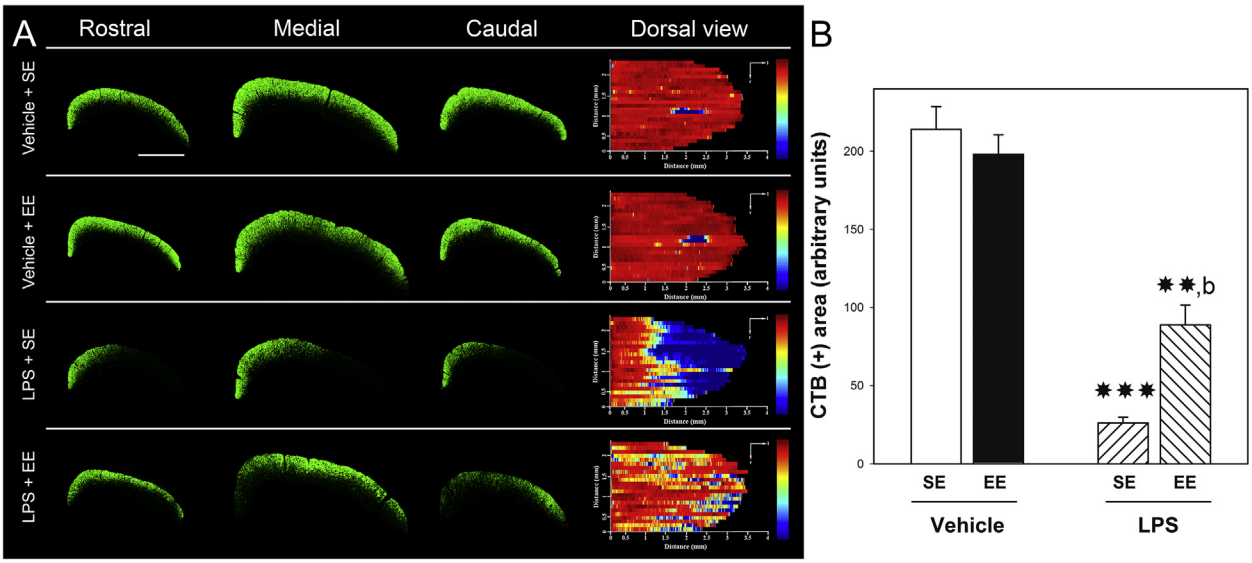

Fig. 3. Effect of EE and experimental ON on retinal anterograde transport to the SC. Panel A: Representative photomicrographs showing CTB staining patterns in the retinotopic layers of the SC from rats injected with vehicle in one optic nerve and LPS in the contralateral optic nerve and exposed to SE or EE. Three representative sections (rostral, medial, and caudal) and the dorsal views of a retinotopic SC map reconstruction are shown. Scale bar = 500 μm. Panel B: Analysis of CTB (+) area. EE partly preserved retinal anterograde transport through LPS-injected optic nerves. Data are mean ± SEM (n = 6 animals per group). Two ways ANOVA $F_{\text{interaction (1,20)}}$ 11,51, $P < 0.01$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle-injected optic nerves in SE, b: $P < 0.01$ vs. LPS-injected optic nerves in SE, by Tukey's test.

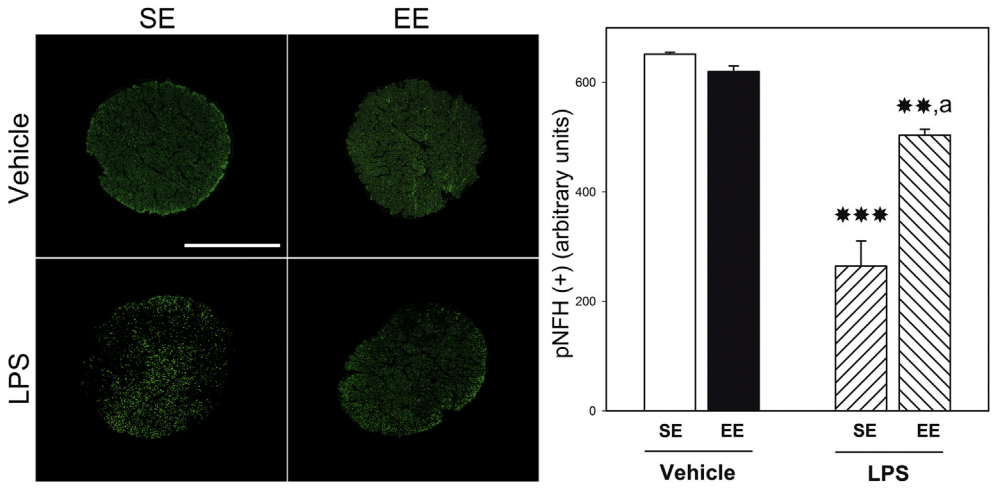

Fig. 4. PNFH-immunoreactivity. Left panel: Representative photomicrographs showing pNFH-immunostaining in transverse sections of vehicle- or LPS-injected optic nerves from animals exposed to SE or EE. Scale bar = 200 μm. Right panel: Quantification of pNFH(+) area in the ON. In SE-housed animals, LPS induced a significant decrease in this parameter which was significantly preserved in animals exposed to EE. Data are mean ± SEM (n = 6 animals per group). Two ways ANOVA $F_{\text{interaction (1,20)}}$ 31.04, $P < 0.001$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle-injected optic nerves in SE, a: $P < 0.05$ vs. LPS-injected optic nerves in SE, by Tukey's test.

microinjection of vehicle or LPS into the optic nerve. When eyes from SE-housed animals whose optic nerves received LPS were stimulated with light, a significant decrease in the pupil contraction of the contralateral intact eye was observed, whereas the EE completely prevented the effect of LPS on the PLR (Fig. 2A and B). Fig. 2C shows VEP amplitude average recorded in animals housed in SE or exposed to EE in which one optic nerve was injected with vehicle, and the contralateral optic nerve received LPS. In SE-housed animals, LPS induced a significant decrease in VEP amplitude, which was completely prevented by EE. Representative waveforms of VEPs from all experimental groups are shown in Fig. 2D. No significant differences in VEP amplitudes were evident in vehicle-injected optic nerves from animals housed in SE or exposed to EE, and EE did not affect VEPs in vehicle-injected optic nerves. No differences in P1, N1, and P2 latencies were observed among groups (data not shown). Since running wheel locking did not modify the protective effect of EE on PLR and VEPs (data not shown), running wheels were locked in the following experiments.

To assess retinal anterograde transport to the SC, CTB was intravitreally administered to animals in which one optic nerve was injected with vehicle and the contralateral optic nerve received LPS. In SE-housed animals, LPS induced a reduction in CTB staining of retinotopic projections to the contralateral SC, whereas EE partly prevented the deficit in the anterograde transport induced by LPS (Fig. 3). EE did not affect this parameter in vehicle-treated optic nerves. As shown in Fig. 4, a decrease in pNFH-immunoreactivity was observed in transversal sections of LPS-injected optic nerves from animals housed in SE, whereas EE prevented the effect of LPS microinjection on pNFH-immunostaining.

Microglia/macrophages were analyzed by Iba-1 and ED1

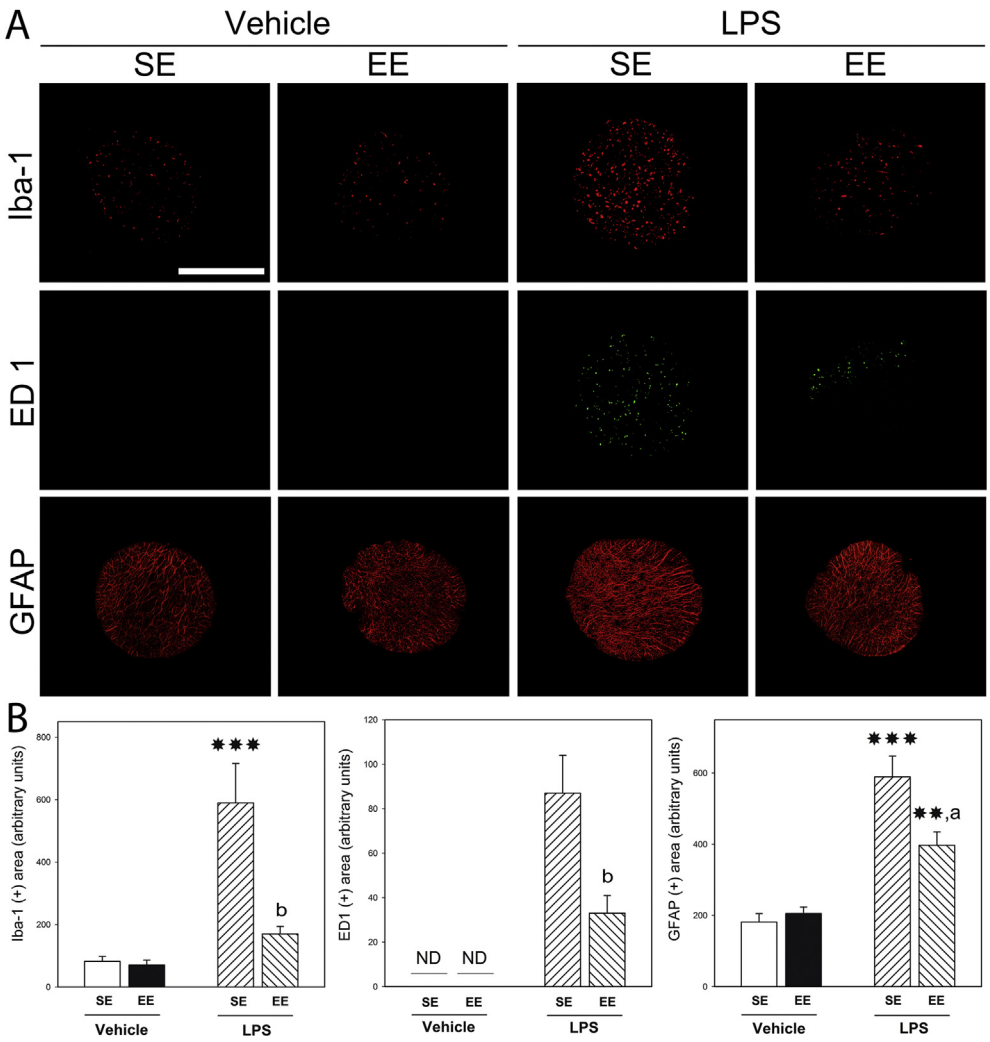

Fig. 5. Microglia/macrophage and astrocyte analysis in vehicle- and LPS-injected optic nerves from animals exposed to SE or EE. Panel A: Representative photomicrographs showing Iba-1 (upper panel), ED1 (middle panel) and GFAP (lower panel) immunostaining. Scale bar = 200 μm. Panel B: Analysis of Iba-1 (+), ED1 (+), and GFAP (+) area. Data are the mean ± SEM (n = 6 optic nerves per group). Two ways ANOVA *Iba-1 (+) area:* $F_{\text{interaction }(1,20)}$ 9.73, $P < 0.01$; *GFAP (+) area:* $F_{\text{interaction }(1,20)}$ 8.15, $P < 0.001$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle-injected optic nerves in SE, a: $P < 0.05$, b: $P < 0.01$ vs. LPS-injected optic nerves in SE, by Tukey's test.

immunostaining, and astrocyte reactivity was assessed by GFAP-immunoreactivity in optic nerve cross-sections. In SE-housed animals, a significant increase in Iba-1 (+), ED1 (+), and GFAP (+) area was observed at 21 days post-injection of LPS, whereas EE significantly prevented the effect of LPS on Iba-1-, ED1-, and GFAP-immunoreactivity, as shown in Fig. 5. EE did not affect these parameters in vehicle-injected optic nerves.

In SE-housed animals, a decrease in LFB staining was evident in LPS-injected optic nerves, which was prevented by EE (Fig. 6). In SE-housed animals, LPS induced a significant decrease in axon number, which was significantly prevented in animals exposed to EE (Fig. 7). RGCs were analyzed by Brn3a-immunostaining in flat-mounted retinas. EE prevented the decrease in Brn3a (+) cell number observed in LPS-injected optic nerves from animals housed in SE, as shown in Fig. 8.

Fig. 9 shows NOS-2, COX-2, lipid peroxidation, and IL-1β and TNFα mRNA levels, in vehicle or LPS-injected optic nerves from animals housed in SE or exposed to EE. EE significantly prevented the increase in these parameters induced by LPS. In addition, EE increased BDNF levels in vehicle- and LPS-injected optic nerves (Fig. 9F). When EE started at 4 days after vehicle or LPS injection, a complete preservation of the PLR against damage induced by experimental ON was observed at 21 days post-injection of LPS (Fig. 10). In contrast, when EE started at 7 days post-injection, no protection of the PLR was observed (Fig. 10). The beneficial effect on the PLR of a delayed EE starting at 4 days post-injection, was already evident after 3 days (i.e., at 7 days post-injection of LPS) of EE, as shown in Fig. 11A. When EE lasted 4 days (starting at day 4 and finishing at day 7 post-LPS), a complete preservation of PLR was observed at day 21 (Fig. 11B), whereas the injection of ANA-12, a TrkB receptor antagonist, once a day for four days, starting at 4 days post-LPS, completely inhibited the effect of EE on the PLR at 21 days post-injection of LPS (Fig. 11B). ANA-12 did not affect this parameter in SE-housed animals whose optic nerves were injected with vehicle or LPS.

4. Discussion

These novel results indicate that EE, a non-invasive, and biologically significant stimulation of the sensory and motor pathways, protected the visual system against experimental ON, supporting that EE may prevent the consequences of neuroinflammation even in a central nervous system component which was originally considered “less plastic”, as the optic nerve from adult rats. LPS

Please cite this article in press as: Aranda, M.L., et al., Therapeutic benefit of environmental enrichment on optic neuritis, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.12.017

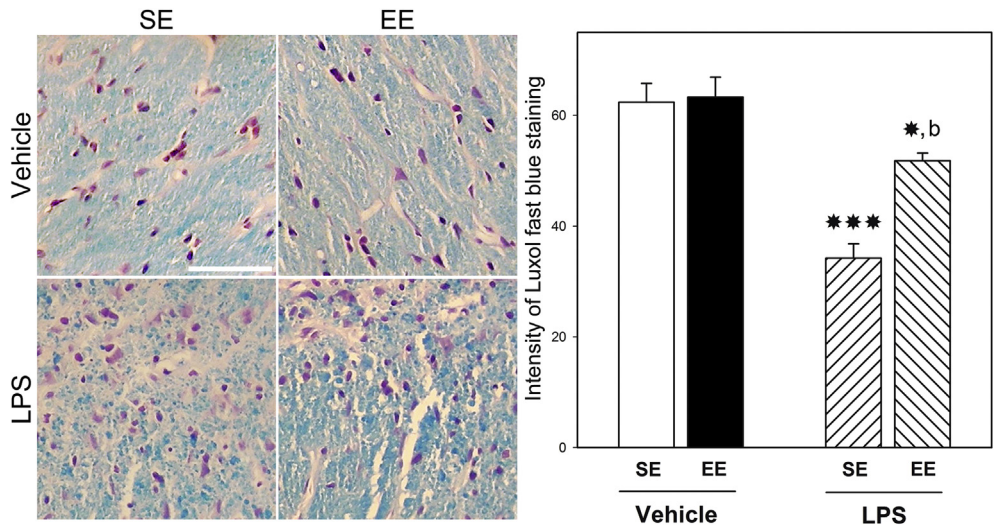

Fig. 6. Effect of EE on optic nerve demyelination induced by experimental ON. Left panel: Representative cross-sections of optic nerves at 21 days post-injection of vehicle or LPS, stained with LFB. Scale bar = 50 μm. Right panel: Quantification of the staining intensity. In SE-housed animals, LPS induced a significant decrease in LFB staining which was prevented in animals exposed to EE. Two ways ANOVA $F_{interaction\ (1,20)}$ 8.39, $P < 0.001$. Data are mean ± SEM (n: 6 optic nerves/group). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. vehicle-injected optic nerves in SE, b: $P < 0.01$ vs. LPS-injected optic nerves in SE, by Tukey's test.

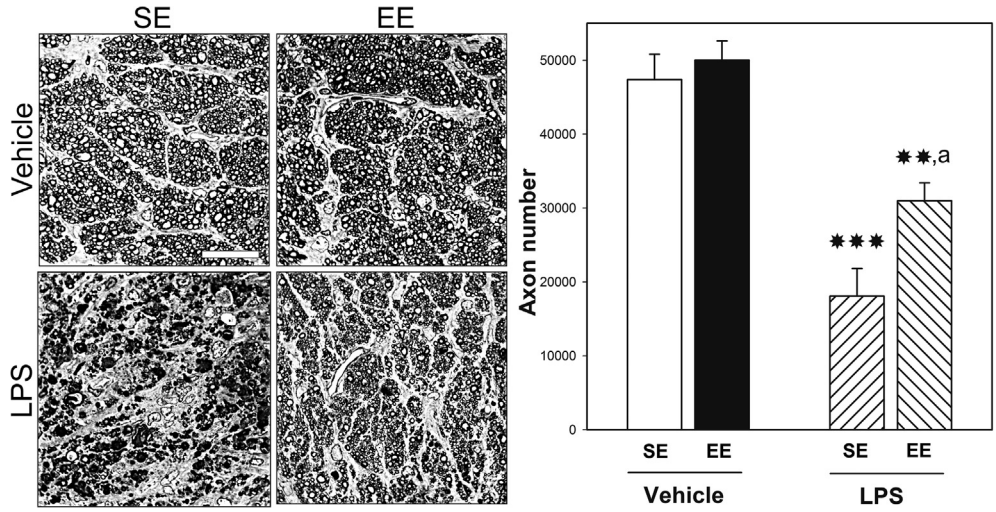

Fig. 7. Optic nerve axons. Left panel: Representative images of cross-sections of vehicle- or LPS-injected optic nerves from animals exposed to SE or EE for 21 days, stained with toluidine blue. Scale bar = 25 μm. Right panel: In SE-housed animals, the injection of LPS into the optic nerve induced a significant decrease in axon number, which was significantly prevented by EE. Data are mean ± SEM (n: 6 optic nerves/group). Two ways ANOVA $F_{interaction\ (1,20)}$ 5.09, $P < 0.05$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. vehicle-injected optic nerves in SE, a: $P < 0.05$ vs. LPS-injected optic nerves in SE, by Tukey's test.

microinjection into the rat optic nerve, which does not affect retinal function (electroretinogram), induces early and late changes in the visual pathway: up to 7 days post-injection, LPS induces functional alterations (PLR and VEPs), and increased microglial/macrophage reactivity in the optic nerve, and at 21 days post-injection (a time-point at which functional alterations and microglial/macrophage reactivity persist), LPS provokes reactive gliosis, demyelination, and axon and RGC loss (Aranda et al., 2015). In order to maximize visual pathway damage, and to discard the possibility of a transient protection, animals were exposed to EE for 21 days post-injection of LPS.

VEP and PLR alterations are characteristic signs of ON, that closely correlate with optic nerve damage (Naismith et al., 2009; Shindler et al., 2012; Toosy et al., 2014; You et al., 2015). EE completely prevented PLR and VEP alterations provoked by LPS-induced ON. In SE-housed animals, experimental ON induced a deficit in the anterograde transport of CTB from the retina to the superficial layers of the SC (the primary termination site of most of RGC axons in rodents), whereas EE partly prevented the "misconnection" between the retina and its main synaptic target. Concomitantly with the functional improvement, EE protected the optic nerve from histopathologic alterations induced by experimental ON. NFH phosphorylation seems to be a major mechanism of neurofilament cross-bridges formation, and it is deeply involved in neuronal morphology and axonal transport (Nixon and Sihag, 1991); thus, the effect of EE on pNFH-immunoreactivity seems to be consistent with the preservation of the retina-SC communication.

Although the beneficial effects of EE have primarily focused on changes in neuronal morphology, synaptic plasticity, and neurogenesis, it has been shown that EE also induces changes in non-neuronal components of the central nervous system such as

Please cite this article in press as: Aranda, M.L., et al., Therapeutic benefit of environmental enrichment on optic neuritis, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.12.017

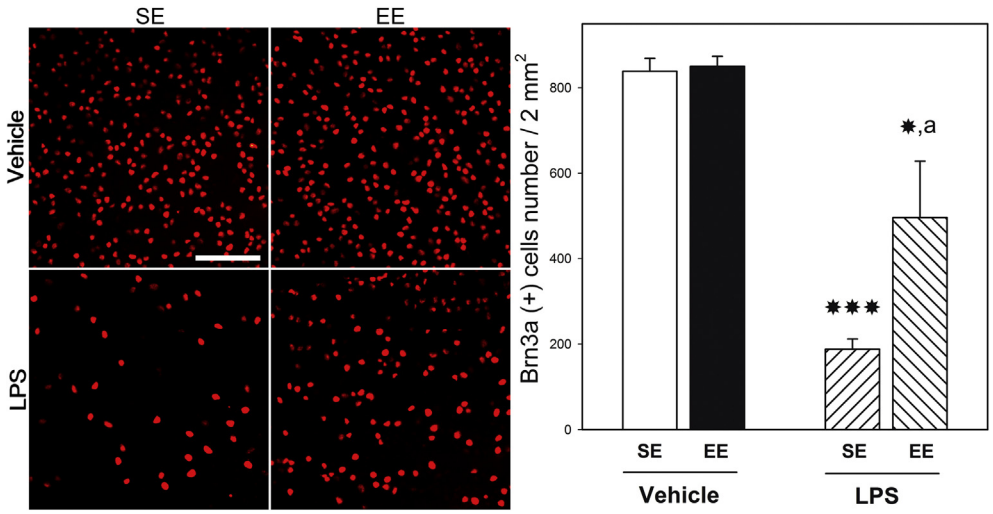

Fig. 8. Effect of EE on RGC number. Left panel: Representative photomicrographs of flat-mounted retinas from animals exposed to SE or EE in which one optic nerve was injected with vehicle and the contralateral optic nerve received LPS. Right panel: In SE, LPS injection into the optic nerve provoked a significant decrease in Brn3a (+) RGC number which was significantly prevented in animals exposed to EE. Scale bar = 100 μm. Data are the mean ± SEM (n = 6 retinas/group). Two ways ANOVA $F_{interaction\ (1,20)}$ 4.51, $P < 0.05$. *$P < 0.05$, ***$P < 0.001$ vs. vehicle-injected optic nerves in SE, a: $P < 0.05$ vs. LPS-injected optic nerves in SE, by Tukey's test.

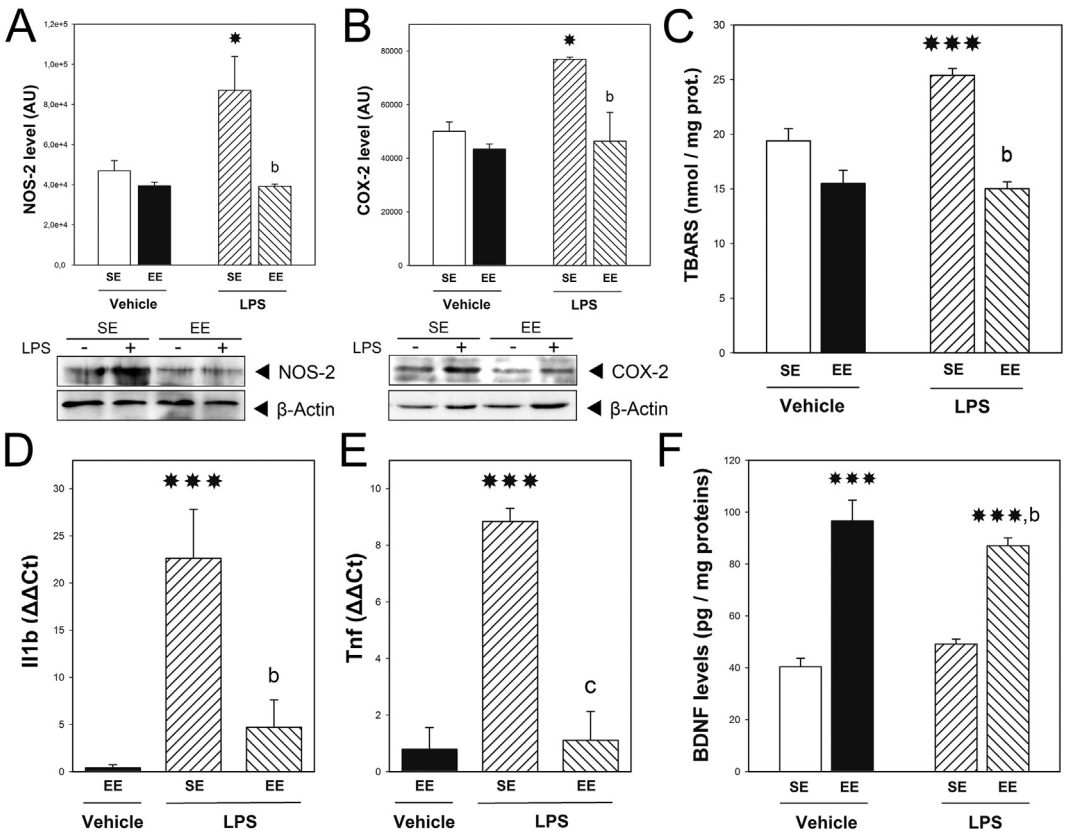

Fig. 9. Effect of EE on NOS-2, COX-2, lipid peroxidation, IL1β mRNA, TNFα mRNA, and BDNF optic nerve levels. EE for 4 days significantly prevented the increase in NOS-2 (panel A), COX-2 (panel B), lipid peroxidation (panel C), IL1β (panel D), and TNFα (panel E) mRNA levels induced by LPS. EE for 4 days significantly increased BDNF levels (panel F) in vehicle- and LPS-injected optic nerves. Data are the mean ± SEM (n = 6 optic nerves/group). Two ways ANOVA *panel A:* $F_{interaction\ (1,20)}$ 5.21, $P < 0.01$; *panel B:* $F_{interaction\ (1,20)}$ 5.17, $P < 0.05$; *panel C:* $F_{interaction\ (1,20)}$ 11.66, $P < 0.05$; *panel D:* $F_{interaction\ (1,20)}$ 8.34, $P < 0.01$; *panel E:* $F_{interaction\ (1,20)}$ 21.3, $P < 0.001$; *panel F:* $F_{interaction\ (1,20)}$ 6.18, $P < 0.05$. *$P < 0.05$, ***$P < 0.001$ vs. vehicle-injected optic nerves in SE, b: $P < 0.01$, c: $P < 0.001$ vs. LPS-injected optic nerves in SE, by Tukey's test.

microglia, astrocytes, and oligodendrocytes. In this line, it has been demonstrated that EE reduces microglial reactivity, astrocytosis and demyelination in the optic nerve from diabetic rats (Dorfman et al., 2015), and decreases microgliosis in the brain from mice submitted to viral encephalitis (de Sousa et al., 2011). In addition, EE reverses reactive gliosis in the dentate gyrus from mice with experimental Alzheimer's disease (Rodríguez et al., 2013), and induces an increase in myelinated fibers in the white matter of aging

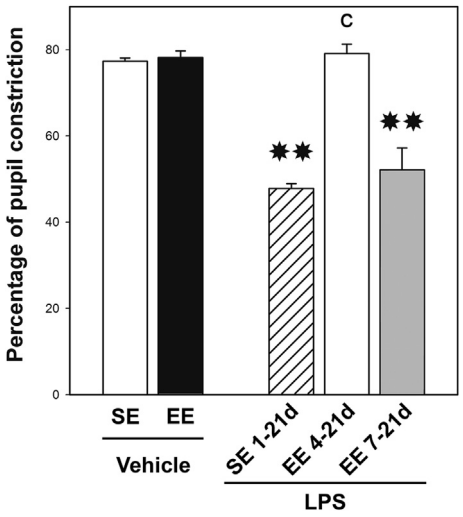

Fig. 10. Effect of a delayed EE on PLR. When EE started at 4 (but not 7) days post-injection, a significant preservation of the PLR against LPS-induced damage was observed. Data are the mean ± SEM (n = 6 eyes/group). Two ways ANOVA $F_{interaction\ (2,30)}$ 22.78, $P < 0.001$. $^{**}P < 0.01$ vs. vehicle in SE, c: $P < 0.001$ vs. LPS-injected optic nerves in SE, by Tukey's test.

rats (Yang et al., 2013). In LPS-injected optic nerves, EE prevented the increase in Iba-1 (+) area (a microglial/macrophage density index), and ED1 (+) area (an index of phagocytic activity), suggestive of reduced microglia/macrophages reactivity and/or infiltration, as well as changes in their phenotype. Moreover, EE prevented astrogliosis and demyelination induced by LPS microinjection into the optic nerve. Although current data remain incapable of addressing whether the protection induced by EE primarily occurred in axons or glial cells, our results could suggest that the altered axon-glia crosstalk could be positively impacted by EE which might also benefit the neural circuitry by modulating axonal conductance velocity.

ON is not merely an inflammatory condition, but also involves neurodegenerative mechanisms. In fact, RGC loss seems to be the main cause of permanent visual deficit in both experimental (Horstmann et al., 2013; Smith et al., 2016) and human (Toosy et al., 2014; Walter et al., 2012) ON. In this line, EE significantly prevented RGC loss induced by LPS microinjection. Since axonal damage often results in permanent loss of the cell body (Horstmann et al., 2013), the preservation of RGC number is coherent with axon loss prevention observed in animals exposed to EE.

Inflammatory signals and oxidative stress have been involved in ON pathophysiology (Aranda et al., 2016; Das et al., 2013; Guy, 2008; Qi et al., 2007). EE significantly prevented the effect of experimental ON on NOS-2 and COX-2 protein levels, IL-1β and TNFα mRNA levels, and lipid peroxidation, indicating that EE favors an anti-inflammatory and anti-oxidant state in the optic nerve. Many lines of evidence strongly support that BDNF is a key mediator in the brain beneficial effects of EE (reviewed by Landi et al., 2007; Sale et al., 2014). In agreement, EE induced a significant increase in BDNF levels both in vehicle- and LPS-injected optic nerves. With some exceptions (Campos et al., 2016; Nowacka-Chmielewska et al., 2017), reductions in BDNF protein levels in rodents systemically challenged with LPS have been reported (Guan and Fang, 2006; McGuiness et al., 2016; Tanaka et al., 2006), while in our experimental conditions, LPS injection did not affect this parameter in the optic nerve from SE-housed animals. Currently, we do not have any explanation for this discrepancy; however, we cannot entirely exclude the involvement of tissue-, dose-, and time post-injection-dependent effects. In fact, to the best of our knowledge, the effect of LPS on BDNF levels in the optic nerve has not been previously analyzed.

Understanding the relation between inflammation and neurodegeneration is of key importance in developing therapeutic strategies for ON. In ON models, some neuroprotective drugs (e.g., resveratrol) fail to show anti-inflammatory effects (Fonseca-Kelly et al., 2012), whereas some anti-inflammatory agents (e.g., 17α-ethynyl-5-androstene-3β,7β,17β-triol), do not prevent RGC loss (Khan et al., 2014), indicating that an optimal therapeutic strategy may

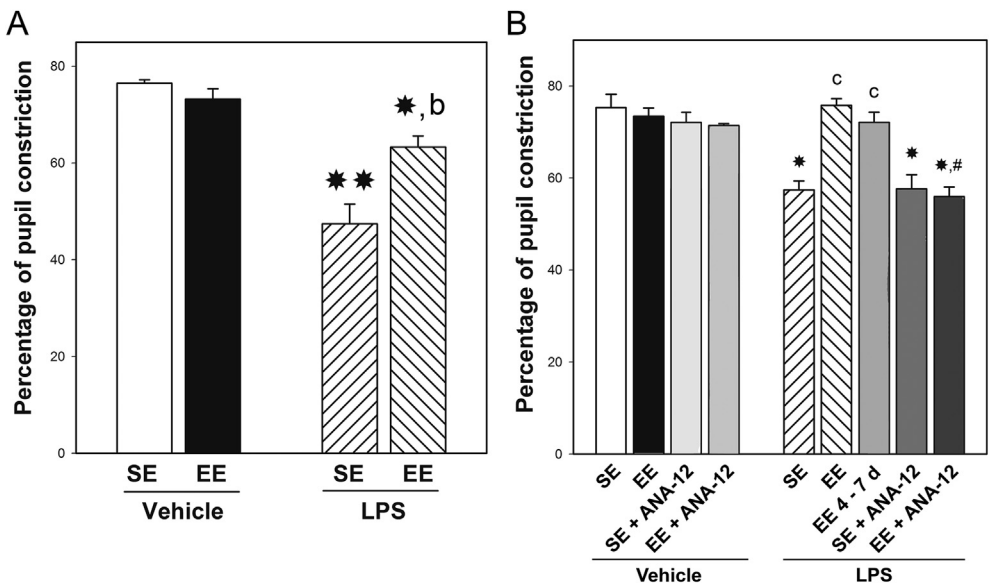

Fig. 11. Effect of a short term exposure to EE and ANA-12 on PLR. Panel A: In animals exposed to EE at day 4 post-LPS, the PLR was significantly preserved at day 7 post-injection. Panel B: At day 4 post-LPS, a group of animals was moved to EE, and at day 7 they were returned to SE. This short term exposure to EE significantly protected the PLR assessed at day 21 days post-injection. Another group of animals that remained in EE from day 4 until day 21 post-injection received a daily injection of ANA-12 from day 4 to day 7. The treatment with ANA-12 completely abolished the protective effect of EE on PLR assessed at 21 days post-LPS. Data are the mean ± SEM (n = 6 eyes/group). Two ways ANOVA *panel A:* $F_{interaction\ (1,20)}$ 13.65, $P < 0.01$; *panel B:* $F_{interaction\ (4,50)}$ 8.95, $P < 0.001$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. vehicle in SE, b: $P < 0.01$, c: $P < 0.001$ vs. LPS in SE, #: $P < 0.05$ vs. LPS in EE, by Tukey's test.

Please cite this article in press as: Aranda, M.L., et al., Therapeutic benefit of environmental enrichment on optic neuritis, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.12.017

require a combination of an anti-inflammatory with a neuroprotective agent, requirements that may be achieved by EE (Kline et al., 2016). Other candidates were analyzed as potential therapeutic strategies for experimental ON, such as erythropoietin (Sättler et al., 2004), glatiramer acetate (Maier et al., 2006), or reduction of oxidative stress (Qi et al., 2007), among others. However, most of these therapies were only effective when administered before or concurrently with ON induction. Since ON patients do not present with symptoms until inflammation begins, the therapeutic benefit of these strategies may have limited clinical implications. As a significant decrease in the PLR is already evident at 1 day post-injection of LPS and persists for at least 21 days post-injection (Aranda et al., 2015), we exposed animals to EE at 4 days post-injection of LPS. This delayed EE significantly reduced the effect of experimental ON on the PLR, supporting that EE not only prevented, but also reduced the progression of the optic nerve dysfunction. In agreement, it has been shown that a delayed EE induces functional improvement after experimental traumatic brain injury in rats (Hoffman et al., 2008; Matter et al., 2011). Notably, after only 3 days (i.e., at 7 days post-LPS) from the delayed housing, the PLR was already protected. The fact that EE was ineffective when started at 7 days post-LPS could suggest that there is a temporal window (likely between 4 and 7 days post-LPS) in which protection may be achieved. In favor of this hypothesis, a short term exposure to EE (i.e., from day 4 to day 7 post-LPS) significantly protected the PLR at 21 days post-LPS. BDNF actions are dependent on binding to transmembrane receptor systems (Trk and the p75 neurotrophin receptors), but it has preferential binding for TrkB receptors (reviewed by Mitre et al., 2017). As shown herein, a treatment with ANA-12 (a TrkB receptor antagonist) during the putative temporal window (i.e., once a day, from day 4–7 post-LPS) abolished the protective effect of EE on the PLR assessed at 21 days post-LPS. These results support the existence of a temporal window in the EE-induced protection, along which the BDNF/TrkB receptor pathway seems to play a key role. In agreement, it has been shown that blocking TrkB receptors nullifies the beneficial effect of EE on hypobaric hypoxia-induced memory impairment (Jain et al., 2013), and that ANA-12 blocks the effects of EE on postoperative cognitive dysfunction (Fan et al., 2016). Ongoing studies are in progress to elucidate in more detail the events that occur during this temporal window.

Voluntary wheel running exercise protects the retina in an inherited retinal degeneration mouse model (Hanif et al., 2015). The fact that in our experimental setting running wheel locking did not affect the beneficial effect of EE on visual functions, does not completely rule out the involvement of physical activity which, despite running wheel locking, could be increased by the big size of enriched cages, as compared with standard cages. In any case, while additional studies should identify the extent to which individual components of enrichment (i.e., sensorial, social or cognitive stimulation, and physical activity) are responsible for the visual pathway protection against ON, evidence suggests that at least in terms of the brain, the greatest benefits are gained from additive or synergistic effects of the full EE repertoire (Faherty et al., 2003; Jurgens and Johnson, 2012; Sozda et al., 2010). Recently, Sale et al. (2014) have raised the view of EE as an “endogenous pharmacotherapy” in which neural plasticity is not obtained by external administration of active substances, but using the environmental stimulation to enhance the spontaneous reparative potential held by the brain. In this line, the further elucidation of the mechanisms underlying the beneficial effects of EE could help in implementing more specific interventions aimed at protecting the visual system against ON.

5. Conclusions

The present results show that EE protected the visual pathway function and structure against experimental ON. Although care must be taken when extrapolating data obtained in experimental models to humans, the protective effect of EE could reflect a scenario in which a physically and mentally active lifestyle promotes the visual pathway resiliency to disruption induced by neuroinflammation.

Conflicts of interest

None.

Acknowledgements

This research was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica [PICT 1563]; The University of Buenos Aires [20020130100564]; Consejo Nacional de Investigaciones Científicas y Técnicas [PIP 0707], Buenos Aires, Argentina. These funding sources have no involvement in study design, the collection, analysis and interpretation of data, the writing of the report, and in the decision to submit the article for publication.

References

Ambrogini, P., Lattanzi, D., Ciuffoli, S., Betti, M., Fanelli, M., Cuppini, R., 2013. Physical exercise and environment exploration affect synaptogenesis in adult-generated neurons in the rat dentate gyrus: possible role of BDNF. Brain Res. 1534, 1–12. https://doi.org/10.1016/j.brainres.2013.08.023.

Aranda, M.L., Dorfman, D., Sande, P.H., Rosenstein, R.E., 2015. Experimental optic neuritis induced by the microinjection of lipopolysaccharide into the optic nerve. Exp. Neurol. 266, 30–41. https://doi.org/10.1016/j.expneurol.2015.01.010.

Aranda, M.L., González Fleitas, M.F., De Laurentiis, A., Keller Sarmiento, M.I., Chianelli, M., Sande, P.H., Dorfman, D., Rosenstein, R.E., 2016. Neuroprotective effect of melatonin in experimental optic neuritis in rats. J. Pineal Res. 60, 360–372. https://doi.org/10.1111/jpi.12318.

Berardi, N., Sale, A., Maffei, L., 2015. Brain structural and functional development: genetics and experience. Dev. Med. Child Neurol. 57, 4–9. https://doi.org/10.1111/dmcn.12691.

Bondi, C.O., Klitsch, K.C., Leary, J.B., Kline, A.E., 2014. Environmental enrichment as a viable neurorehabilitation strategy for experimental traumatic brain injury. J. Neurotrauma 31, 873–888. https://doi.org/10.1089/neu.2014.3328.

Campos, A.C., Rocha, N.P., Nicoli, J.R., Vieira, L.Q., Teixeira, M.M., Teixeira, A.L., 2016. Absence of gut microbiota influences lipopolysaccharide-induced behavioral changes in mice. Behav. Brain Res. 312, 186–194. https://doi.org/10.1016/j.bbr.2016.06.027.

Cazorla, M., Prémont, J., Mann, A., Girard, N., Kellendonk, C., Rognan, D., 2011. Identification of a low-molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. J. Clin. Invest. 121, 1846–1857. https://doi.org/10.1172/JCI43992.

Das, A., Guyton, M.K., Smith, A., Wallace 4th, G., McDowell, M.L., Matzelle, D.D., Ray, S.K., Banik, N.L., 2013. Calpain inhibitor attenuated optic nerve damage in acute optic neuritis in rats. J. Neurochem. 124, 133–146. https://doi.org/10.1111/jnc.12064.

de Sousa, A.A., Reis, R., Bento-Torres, J., Trévia, N., Lins, N.A., Passos, A., Santos, Z., Diniz, J.A., Vasconcelos, P.F., Cunningham, C., Perry, V.H., Diniz, C.W., 2011. Influence of enriched environment on viral encephalitis outcomes: behavioral and neuropathological changes in albino Swiss mice. PLos One 6, e15597. https://doi.org/10.1371/journal.pone.0015597.

Diem, R., Hobom, M., Maier, K., Weissert, R., Storch, M.K., Meyer, R., Bähr, M., 2003. Methylprednisolone increases neuronal apoptosis during autoimmune CNS inflammation by inhibition of an endogenous neuroprotective pathway. J. Neurosci. 23, 6993–7000.

Dorfman, D., Fernandez, D.C., Chianelli, M., Miranda, M., Aranda, M.L., Rosenstein, R.E., 2013. Post-ischemic environmental enrichment protects the retina from ischemic damage in adult rats. Exp. Neurol. 240, 146–156. https://doi.org/10.1016/j.expneurol.2012.11.017.

Dorfman, D., Aranda, M.L., González Fleitas, M.F., Chianelli, M.S., Fernandez, D.C., Sande, P.H., Rosenstein, R.E., 2014. Environmental enrichment protects the retina from early diabetic damage in adult rats. PLos One 9, e101829. https://doi.org/10.1371/journal.pone.0101829.

Dorfman, D., Aranda, M.L., Rosenstein, R.E., 2015. Enriched environment protects the optic nerve from early diabetes-induced damage in adult rats. PLos One 10, e0136637. https://doi.org/10.1371/journal.pone.0136637.

Eggenberger, E.R., 2001. Inflammatory optic neuropathies. Ophthalmol. Clin. North Am. 14, 73–82.

Faherty, C.J., Kerley, D., Smeyne, R.J., 2003. A Golgi-Cox morphological analysis of neuronal changes induced by environmental enrichment. Brain Res. Dev. Brain Res. 141, 55–61.

Fan, D., Li, J., Zheng, B., Hua, L., Zuo, Z., 2016. Enriched environment attenuates

surgery-induced impairment of learning, memory, and neurogenesis possibly by preserving BDNF expression. Mol. Neurobiol. 53, 344–354. https://doi.org/10.1007/s12035-014-9013-1.

Fernandez, D.C., Sande, P.H., de Zavalía, N., Belforte, N., Dorfman, D., Casiraghi, L.P., Golombek, D., Rosenstein, R.E., 2013. Effect of experimental diabetic retinopathy on the non-image-forming visual system. Chronobiol. Int. 30, 583–597. https://doi.org/10.3109/07420528.2012.754453.

Fonseca-Kelly, Z., Nassrallah, M., Uribe, J., Khan, R.S., Dine, K., Dutt, M., Shindler, K.S., 2012. Resveratrol neuroprotection in a chronic mouse model of multiple sclerosis. Front. Neurol. 3, 84. https://doi.org/10.3389/fneur.2012.00084.

Frigui, M., Frikha, F., Sellemi, D., Chouayakh, F., Feki, J., Bahloul, Z., 2011. Optic neuropathy as a presenting feature of systemic lupus erythematosus: two case reports and literature review. Lupus 20, 1214–1218. https://doi.org/10.1177/0961203311403344.

Gal, R.L., Vedula, S.S., Beck, R., 2015. Corticosteroids for treating optic neuritis. Cochrane Database Syst. Rev. 8, CD001430. https://doi.org/10.1002/14651858.CD001430.pub4.

Galetta, S.L., Villoslada, P., Levin, N., Shindler, K., Ishikawa, H., Parr, E., Cadavid, D., Balcer, L.J., 2015. Acute optic neuritis: unmet clinical needs and model for new therapies. Neurol. Neurophysiol. Neurosci. 2, e135 https://doi.org/10.1212/NXI.0000000000000135.

Gold, R., Linington, C., Lassmann, H., 2006. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain 129, 1953–1971. https://doi.org/10.1093/brain/awl075.

Guan, Z., Fang, J., 2006. Peripheral immune activation by lipopolysaccharide decreases neurotrophins in the cortex and hippocampus in rats. Brain Behav. Immun. 20, 64–71. https://doi.org/10.1016/j.bbi.2005.04.005.

Guy, J., 2008. Optic nerve degeneration in experimental autoimmune encephalomyelitis. Ophthalmic Res. 40, 212–216. https://doi.org/10.1159/000119879.

Hanif, A.M., Lawson, E.C., Prunty, M., Gogniat, M., Aung, M.H., Chakraborty, R., Boatright, J.H., Pardue, M.T., 2015. Neuroprotective effects of voluntary exercise in an inherited retinal degeneration mouse model. Invest. Ophthalmol. Vis. Sci. 56, 6839–6846. https://doi.org/10.1167/iovs.15-16792.

Hannan, A.J., 2014. Environmental enrichment and brain repair: harnessing the therapeutic effects of cognitive stimulation and physical activity to enhance experience-dependent plasticity. Neuropathol. Appl. Neurobiol. 40, 13–25. https://doi.org/10.1111/nan.12102.

Hickman, S.J., Dalton, C.M., Miller, D.H., Plant, G.T., 2002. Management of acute optic neuritis. Lancet 360, 1953–1962.

Hoffman, A.N., Malena, R.R., Westergom, B.P., Luthra, P., Cheng, J.P., Aslam, H.A., Zafonte, R.D., Kline, A.E., 2008. Environmental enrichment-mediated functional improvement after experimental traumatic brain injury is contingent on task-specific neurobehavioral experience. Neurosci. Lett. 431, 226–230.

Horstmann, L., Schmid, H., Heinen, A.P., Kurschus, F.C., Dick, H.B., Joachim, S.C., 2013. Inflammatory demyelination induces glia alterations and ganglion cell loss in the retina of an experimental autoimmune encephalomyelitis model. J. Neuroinflammation 10, 120. https://doi.org/10.1186/1742-2094-10-120.

Horwitz, H., Friis, T., Modvig, S., Roed, H., Tsakiri, A., Laursen, B., Frederiksen, J.L., 2014. Differential diagnoses to MS: experiences from an optic neuritis clinic. J. Neurol. 261, 98–105. https://doi.org/10.1007/s00415-013-7166-x.

Jain, V., Baitharu, I., Prasad, D., Ilavazhagan, G., 2013. Enriched environment prevents hypobaric hypoxia induced memory impairment and neurodegeneration: role of BDNF/PI3K/GSK3β pathway coupled with CREB activation. PLos One 8, e62235. https://doi.org/10.1371/journal.pone.0062235.

Jurgens, H.A., Johnson, R.W., 2012. Environmental enrichment attenuates hippocampal neuroinflammation and improves cognitive function during influenza infection. Brain Behav. Immun. 26, 1006–1016. https://doi.org/10.1016/j.bbi.2012.05.015.

Kallenbach, K., Frederiksen, J.L., 2008. Unilateral optic neuritis as the presenting symptom of human immunodeficiency virus toxoplasmosis infection. Acta Ophthalmol. 86, 459–460. https://doi.org/10.1111/j.1600-0420.2007.01034.x.

Khan, R.S., Dine, K., Luna, E., Ahlem, C., Shindler, K.S., 2014. HE3286 reduces axonal loss and preserves retinal ganglion cell function in experimental optic neuritis. Invest. Ophthalmol. Vis. Sci. 55, 5744–5751. https://doi.org/10.1167/iovs.14-14672.

Kline, A.E., Leary, J.B., Radabaugh, H.L., Cheng, J.P., Bondi, C.O., 2016. Combination therapies for neurobehavioral and cognitive recovery after experimental traumatic brain injury: is more better? Prog. Neurobiol. 142, 45–67. https://doi.org/10.1016/j.pneurobio.2016.05.002.

Landi, S., Sale, A., Berardi, N., Viegi, A., Maffei, L., Cenni, M.C., 2007. Retinal functional development is sensitive to environmental enrichment, a role for BDNF. Faseb. J. 21, 130–139. https://doi.org/10.1096/fj.06-6083com.

Li, K., Du, Y., Fan, Q., Tang, C.Y., He, J.F., 2014. Gypenosides might have neuroprotective and immunomodulatory effects on optic neuritis. Med. Hypotheses. https://doi.org/10.1016/j.mehy.2014.02.030, 82,636–638.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−ΔΔC(T)) method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Maier, K., Kuhnert, A.V., Taheri, N., Sättler, M.B., Storch, M.K., Williams, S.K., Bähr, M., Diem, R., 2006. Effects of glatiramer acetate and interferon-beta on neurodegeneration in a model of multiple sclerosis: a comparative study. Am. J. Pathol. 169, 1353–1364.

Matter, A.M., Folweiler, K.A., Curatolo, L.M., Kline, A.E., 2011. Temporal effects of environmental enrichment-mediated functional improvement after experimental traumatic brain injury in rats. Neurorehabilitation Neural Repair 25, 558–564. https://doi.org/10.1177/1545968310397206.

McGuiness, B., Gibney, S.M., Beumer, W., Versnel, M.A., Sillaber, I., Harkin, A., Drexhage, H.A., 2016. Exaggerated increases in microglia proliferation, brain inflammatory response and sickness behaviour upon lipopolysaccharide stimulation in non-obese diabetic mice. Neuroimmunomodulation 23, 137–150. https://doi.org/10.1159/000446370.

Mitre, M., Mariga, A., Chao, M.V., 2017. Neurotrophin signalling: novel insights into mechanisms and pathophysiology. Clin. Sci. 131, 13–23.

Naismith, R.T., Tutlam, N.T., Xu, J., Shepherd, J.B., Klawiter, E.C., Song, S.K., Cross, A.H., 2009. Optical coherence tomography is less sensitive than visual evoked potentials in optic neuritis. Neurology 73, 46–52. https://doi.org/10.1212/WNL.0b013e3181aaea32.

Nixon, R.A., Sihag, R.K., 1991. Neurofilament phosphorylation, a new look at regulation and function. Trends Neurosci. 14, 501–506.

Nowacka-Chmielewska, M.M., Kasprowska, D., Paul-Samojedny, M., Bielecka-Wajdman, A.M., Barski, J.J., Małecki, A., Obuchowicz, E., 2017. The effects of desipramine, fluoxetine, or tianeptine on changes in bulbar BDNF levels induced by chronic social instability stress and inflammation. Pharmacol. Rep. 69, 520–525. https://doi.org/10.1016/j.pharep.2017.01.004.

Optic Neuritis Study Group, 2008. Multiple sclerosis risk after optic neuritis: final optic neuritis treatment trial follow-up. Arch. Neurol. 65, 727–732. https://doi.org/10.1001/archneur.65.6.727.

Prusky, G.T., Reidel, C., Douglas, R.M., 2000. Environmental enrichment from birth enhances visual acuity but not place learning in mice. Behav. Brain Res. 114, 11–15.

Qi, X., Sun, L., Lewin, A.S., Hauswirth, W.W., Guy, J., 2007. Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis. Invest. Ophthalmol. Vis. Sci. 48, 681–691. https://doi.org/10.1167/iovs.06-0553.

Robinson, A.P., Harp, C.T., Noronha, A., Miller, S.D., 2014. The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. Handb. Clin. Neurol 122, 173–189. https://doi.org/10.1016/B978-0-444-52001-2.00008-X.

Rodríguez, J.J., Terzieva, S., Olabarria, M., Lanza, R.G., Verkhratsky, A., 2013. Enriched environment and physical activity reverse astrogliodegeneration in the hippocampus of AD transgenic mice. Cell Death Dis. 4, e678 https://doi.org/10.1038/cddis.2013.194.

Sale, A., Berardi, N., Maffei, L., 2014. Environment and brain plasticity: towards an endogenous pharmacotherapy. Physiol. Rev. 94, 189–234. https://doi.org/10.1152/physrev.00036.2012.

Sättler, M.B., Merkler, D., Maier, K., Stadelmann, C., Ehrenreich, H., Bähr, M., Diem, R., 2004. Neuroprotective effects and intracellular signaling pathways of erythropoietin in a rat model of multiple sclerosis. Cell Death Differ. 11, S181–S192. https://doi.org/10.1093/brain/awh365.

Shindler, K.S., Revere, K., Dutt, M., Ying, G.S., Chung, D.C., 2012. In vivo detection of experimental optic neuritis by pupillometry. Exp. Eye Res. 100, 1–6. https://doi.org/10.1016/j.exer.2012.04.005.

Smith, A.W., Rohrer, B., Wheless, L., Samantaray, S., Ray, S.K., Inoue, J., Azuma, M., Banik, N.L., 2016. Calpain inhibition reduces structural and functional impairment of retinal ganglion cells in experimental optic neuritis. J. Neurochem. 139, 270–284. https://doi.org/10.1111/jnc.13770.

Sozda, C.N., Hoffman, A.N., Olsen, A.S., Cheng, J.P., Zafonte, R.D., Kline, A.E., 2010. Empirical comparison of typical and atypical environmental enrichment paradigms on functional and histological outcome after experimental traumatic brain injury. J. Neurotrauma 27, 1047–1057. https://doi.org/10.1089/neu.2010.1313.

Szabadfi, K., Atlasz, T., Horváth, G., Kiss, P., Hamza, L., Farkas, J., Tamás, A., Lubics, A., Gábriel, R., Reglodi, D., 2009. Early postnatal enriched environment decreases retinal degeneration induced by monosodium glutamate treatment in rats. Brain Res. 1259, 107–112. https://doi.org/10.1016/j.brainres.2009.01.004.

Tanaka, S., Ide, M., Shibutani, T., Ohtaki, H., Numazawa, S., Shioda, S., Yoshida, T., 2006. Lipopolysaccharide-induced microglial activation induces learning and memory deficits without neuronal cell death in rats. J. Neurosci. Res. 83, 557–566. https://doi.org/10.1002/jnr.20752.

Toosy, A.T., Mason, D.F., Miller, D.H., 2014. Optic neuritis. Lancet Neurol. 13, 83–99. https://doi.org/10.1016/S1474-4422(13)70259-X.

Walter, S.D., Ishikawa, H., Galetta, K.M., Sakai, R.E., Feller, D.J., Henderson, S.B., Wilson, J.A., Maguire, M.G., Galetta, S.L., Frohman, E., Calabresi, P.A., Schuman, J.S., Balcer, L.J., 2012. Ganglion cell loss inrelation to visual disability in multiple sclerosis. Ophthalmology 119, 1250–1257. https://doi.org/10.1016/j.ophtha.2011.11.032.

Wekerle, H., Kojima, K., Lannes-Vieira, J., Lassmann, H., Linington, C., 1994. Animal models. Ann. Neurol. (36 Suppl. l), S47–S53.

Yang, S., Li, C., Qiu, X., Zhang, L., Lu, W., Chen, L., Zhao, Y.Y., Shi, X.Y., Huang, C.X., Cheng, G.H., Tang, Y., 2013. Effects of an enriched environment on myelin sheaths in the white matter of rats during normal aging: a stereological study. Neuroscience 234, 13–21. https://doi.org/10.1016/j.neuroscience.2013.01.003.

You, Y., Gupta, V.K., Chitranshi, N., Reedman, B., Klistorner, A., Graham, S.L., 2015. Visual evoked potential recording in a rat model of experimental optic nerve demyelination. J. Virtual Explor. 101, e52934 https://doi.org/10.3791/52934.

Please cite this article in press as: Aranda, M.L., et al., Therapeutic benefit of environmental enrichment on optic neuritis, Neuropharmacology (2017), https://doi.org/10.1016/j.neuropharm.2017.12.017