INTRODUCTION

Nonexudative age-related macular degeneration (NE-AMD) is the leading cause of irreversible blindness in the elderly worldwide, affecting ~9% of the global population, and thought to almost duplicate by 2040.1,2 Within NE-AMD, the atrophy of the retinal pigment epithelium (RPE) and photoreceptors (PR) occurs, exclusively localized at the central retinal region (ie the macular area). Clinically, NE-AMD appears as a progressive defect in central vision, leading to legal blindness. Many factors are related to the development and progression of NE-AMD; female gender, age, diet, cigarette smoking and...
Caucasian race among others have been implicated in NE-AMD development and progression.\textsuperscript{1,3,4} Moreover, factors such as choroidal blood flow impairment, the accumulation of lipofuscin at the RPE, oxidative stress and inflammation have been related to the ethiopathogenesis of NE-AMD.\textsuperscript{5,7} Several studies indicate that oxidative stress damaging the RPE is an early phenomenon in NE-AMD.\textsuperscript{1,7-12} So far, based on the AREDS I and II clinical trials, the only available therapeutic approach is the supplementation with high doses of antioxidants, vitamins and zinc (reviewed in\textsuperscript{13}). However, both the complex formulation and the high antioxidants and vitamins doses make their long-term use unsafe and, on top of that, their use does not achieve conclusive results.\textsuperscript{14}

We have recently developed a new model of NE-AMD induced by superior cervical ganglionectomy (SCGx) in C57Bl/6J mice, which reproduces the central features of human NE-AMD. SCGx induces a decline in PR function and ubiquitous alterations in the choroid and choriocapillaris, whereas the increase in Bruch’s membrane (BrM) thickness, the decrease in the content of RPE melanin and retinoid isomerohydrolase (RPE65)-immunoreactivity, the occurrence of drusen-like deposits, and the atrophy of the RPE and PR are localized exclusively at the temporal region of the optic nerve head.\textsuperscript{15} Moreover, SCGx induces an increase in several markers of oxidative stress, and a decrease both in the central temporal (without any nasal alterations) RPE mitochondria mass and endogenous antioxidant system enzymes.\textsuperscript{16} Melatonin is a molecule with proven antioxidant and anti-inflammatory activities, which reduces oxidative stress and inflammatory cell damage often underlying neurodegenerative disorders (reviewed in\textsuperscript{17-19}). Evidence strongly supports that melatonin and its metabolites act both as direct and indirect antioxidants by free radicals scavengers, antioxidant enzymes stimulators and other antioxidants activities enhancers.\textsuperscript{20-24} Melatonin may also act as a shield in other ocular afflictions such as cataract, photokeratitis, prematurity retinopathy and injury due to ischaemia/reperfusion,\textsuperscript{25-27} as well as refractory central serous chorioretinopathy in humans.\textsuperscript{28} We have previously exposed the benefits of the treatment with melatonin against glaucoma,\textsuperscript{29} uveitis,\textsuperscript{30-32} type-2 diabetes mellitus-induced retinal damage \textsuperscript{33} and optic neuritis.\textsuperscript{34} In this context, the purpose of our work was analysing melatonin therapeutic effect on the RPE and outer retina damage in SCGx-induced NE-AMD.

2 | MATERIAls AND METHODS

2.1 | Animals

For all experiments, adult male C57BL/6J mice (weighting an average of 27 ± 3 g and ageing an average of 2.5 ± 0.5 months) were kept in a standard animal room and fed with food and water ad libitum under controlled conditions of temperature, light and humidity, in a 12-hour light/12-hour dark cycle. A total number of 138 animals were used, as follows: 30 for electroretinogram (ERG), melanin content and immunofluorescence studies, 10 for electron microscopy, 16 for MitoTracker and MitoSOX studies, 32 for Western blot, 10 behaviour visual tests and 40 for analysing a delayed treatment with melatonin. This study was approved by the ethics committee of the School of Medicine, University of Buenos Aires (Institutional Committee for the Care and Use of Laboratory Animals, (CICUAL), and animal suffering was minimized with all efforts. Intramuscular injection of 100 mg/kg ketamine hydrochloride and 2 mg/kg xylazine hydrochloride was used for every experimental procedure anaesthesia.

2.2 | Superior cervical ganglionectomy

The left superior cervical ganglion (SCG) was aseptically removed through a ventral midline incision in the neck, as previously described.\textsuperscript{15} For all experiments but the behaviour visual tests, we conducted a simulated procedure (ie non-removing the right SCG), thus considering the right eye as control (from then on named sham). No differences between eyes from sham-treated animals and naïve animals were found along our studies.\textsuperscript{15,16}

2.3 | Melatonin treatment

Using a bronzed cylinder of 2.5 mm diameter and 1 mm length, we achieved a pellet of melatonin (20 mg in 3% w/v vegetable oil), which was subcutaneously implanted 48 h or 4 weeks post-SCGx, and replaced every 10 days, based on previous reports.\textsuperscript{29,31,34} Another group of animals was sham-operated (without the pellet implant (further named control).

2.4 | ERG recording

Standard scotopic electroretinograms were assessed using a HMxERG model 2000 (Ocuscience LLC) equipped with a Ganzfeld dome. Light stimulus was done with a fitted white light–emitting diode at an eye distance of 2 cm, as previously described.\textsuperscript{15,16} Briefly, 15 (2 ms each) full-field 10 cd/sm\textsuperscript{2} flashes at a frequency of 0.25 Hz were averaged for each animal, and the mean of 10 animals was taken as representative for each group.

2.5 | Behaviour visual tests

A looming and visual cliff tests were used to evaluate visual functions. The looming and visual cliff tests were performed
as described by Lim et al. The freezing latency and response to stimulus were analysed. The virtual visual cliff test was performed as described by Gu et al. Animals were subjected to unilateral SCGx or sham procedure, while the contralateral eye remained intact. After 48 h or 4 weeks (delayed treatment), some animals received melatonin, whereas others were operated without the pellet implant (control). A number of 10 mice/group were used for each test, which in the case of the looming test was performed only once for each mouse to avoid habituation.

### 2.6 Electron microscopy

Using an ultramicrotome Ultracut E (Reichert-Jung, Vienna, Austria), we obtained ultrathin retinal sections (50 nm) from the central nasal and temporal region (at 800 µm from the optic nerve head (ONH)), as previously described. For orientation (nasal/temporal axis), nictitating membrane was left attached to the eye, and a clean cut was made through the horizontal meridian including

2.7 RPE melanin content quantification

After deparaffination and dehydration, sections were mounted in slides covered with slips using Canada balsam as previously described. Light microscopic images

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**FIGURE 1** Effect of melatonin on visual function alterations at 10 weeks post-SCGx. Panel (A) The average amplitudes of scotopic ERG a-wave are shown. SCGx induced a significant decrease in ERG a-wave amplitude, whereas a treatment with melatonin starting 48 h post-SCGx, which had no effect per se, completely prevented the decrease in this parameter. Data are mean ± SEM (n: 10 animals per group), **P < .01** vs sham-treated eyes from control (without melatonin) animals; a: P < .01, and b: P < .05 vs SCGx-treated eyes from control animals, by Tukey’s test. Panel (B) Representative scotopic ERG traces of sham- and SCGx-eyes in control- and melatonin-treated animals, by Tukey’s test. Panel (C) The average responses in three behaviour visual tasks are shown. SCGx induced a significant increase in the freezing latency of the looming test and a significant decrease both in the percentage of time spent on the shallow side in the virtual visual cliff test and the fraction of shallow side selected trials in the visual cliff test, which were prevented by melatonin treatment. Data are mean ± SEM (n: 10 animals per group), **P < .01** vs control animals with sham-treated eyes; a: P < .01, and b: P < .05 vs control animals with SCGx-treated eyes, by Tukey’s test.
(×1000) from the total RPE section were obtained at equi-
distant eccentricities from the ONH (taken as zero) to the
nasal or temporal periphery each 200 μm long. The content
of RPE melanin was quantified at 800 μm nasally and tem-
porally of the ONH using ImageJ software version 1.42q
(NIH). The average from four separate sections per eye and
the mean of five eyes were recorded as the representative
value for each group.

2.8 | Immunofluorescence studies

Animals were intracardially perfused with saline, followed
by 4% paraformaldehyde in PBS and paraffin sections were
obtained and mounted in superfrost microscope slides (Erie
Scientific Company, Portsmouth, New Hampshire, USA),
as previously described.15,16 Sections were pre-incubated
with 5% normal horse serum for 1 h and then were incubated
overnight at 4°C with a mouse polyclonal anti-RPE65 antibody (1:500; EMD Millipore, MAB5428), a mouse monoclonal anti-4-hydroxy-2-nonenal (4HNE) (1:250, R&D Systems), or a mouse monoclonal anti-carboxymethyl-lysine (CML) (1:250, R&D Systems). The following secondary antibodies were added and incubated for 2 h at room temperature: a goat anti-mouse IgM secondary antibody conjugated to Alexa Fluor 568 (1:500; Invitrogen, Molecular Probes, **P < .01 vs sham-treated eyes from animals control (without melatonin); b: **P < .01 vs SCGx-treated eyes from control animals, by Tukey’s test
A11031). Nuclei were stained with Hoechst (1 μg/mL Sigma Chemical Co.) and observed under an epifluorescence microscope (BX50; Olympus) with a video camera attached to a computer running image analysis software (Image-Pro Plus, Media Cybernetics Inc). All retinal images shown were oriented with the vitreous up. The RPE65 (+) area was quantified using ImageJ software version 1.42q (NIH). For each eye, we analysed 4 separate sections from the RPE at the central nasal and temporal region and the mean from 5 different eyes/group was considered the representative value.

2.9 | Ex vivo flat-mounted RPE mitochondrial labelling and superoxide detection

We obtained flat-mounts of the RPE incubated them either with MitoTracker-Red CMXRos (500 nM) (Molecular Probes, Eugene, OR, USA) for 15 min in mammalian ringer (MR) buffer or with MitoSOX-Red (5 µM) (Molecular Probes) for 30 min in MR buffer at 37°C under red dim light, as we described.15,16 Samples were observed under an epifluorescent microscope (BX-50, Olympus). The (+) area of both markers was quantified using ImageJ software version 1.42q (NIH, Bethesda, MD, USA). For each group, we analysed 4 images from the RPE at the central nasal and temporal region and the mean from 4 different eyes/group was considered the representative value.

2.10 | Tissue harvesting for SDS-PAGE and Western blotting

After enucleation, the retina was delicately detached from the RPE followed by the separately homogenization of each tissue (retina and RPE/choroid) as we have previously described.16 Using bovine serum albumin as the standard, the protein content was determined following the method of Lowry et al37 Proteins (50 µg/sample) were separated by SDS-PAGE 12%. Proteins were then transferred to polyvinylidene difluoride membranes (60 min at 100 V in a Bio-Rad Trans-Blot SD system (Bio-Rad Laboratories)). Membranes were then incubated (overnight at 4°C) with the following antibodies: a mouse monoclonal

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**FIGURE 6** Effect of melatonin on the RPE and PR ultrastructure alterations induced by SCGx. Representative transverse ultrathin RPE and PR sections from sham-treated eyes, and eyes at 10 weeks post-SCGx from control- and melatonin-treated animals. Superior panel: Melatonin prevented the vacuolization of the temporal RPE (arrow), the thickening of the RPE basal infoldings (arrowhead). Shown are representative photomicrographs from 5 eyes/group. OS, photoreceptors outer segments; RPE, retinal pigment epithelium; BI, basal infoldings. Scale bar = 500 nm. Lower panel: SCGx induced focal losses of the temporal (but not nasal) PR discs and blebs (asterisk), which were not observed in eyes from animals treated with melatonin. Shown are representative photomicrographs from 5 eyes/group. Scale bar = 100 nm. Data are mean ± SEM (n: 5 eyes per group), **P < .01 vs sham-treated eyes from control animals (without melatonin); a: P < .01 vs SCGx-treated eyes from control animals, by Tukey’s test
anti-cytochrome c (1:1000, Santa Cruz Biotechnology), a rabbit polyclonal anti-translocase of the outer membrane (TOM20, 1:500, Santa Cruz Biotechnology), a rabbit polyclonal anti-voltage-dependent selective-anion channel (VDAC) (1:300, Santa Cruz Biotechnology) and a mouse anti-β-actin (1:1000, Santa Cruz Biotechnology). The following secondary antibodies were used: a donkey anti-mouse (1:2000, Jackson Laboratory) and a donkey anti-rabbit (1:2000, Jackson Laboratory). Enhanced chemiluminescence Western blotting detection reagents were used to visualize immunoblots (Amersham Biosciences). ImageQuant software was used to quantify densitometric signals adjusted by the density of β-actin. For each group, the mean of 4 homogenates was averaged and taken as the representative value.

2.11 | Statistical analysis

Statistical analysis of results was made by a two-way analysis of variance (ANOVA) followed by Tukey’s test, as stated, and met the necessary assumptions. The assumption of equal variances was tested by the F test. In every statistical analysis, $P < .05$ was considered statistically significant.

3 | RESULTS

Visual functions were assessed by ERG recording, and three visual tests (ie looming, virtual visual cliff, and visual cliff) in sham and SCGx animals, and in the absence or presence of melatonin starting at 48 h post-SCGx. SCGx induced a significant decrease in scotopic ERG a-wave amplitude and in the looming, virtual visual cliff and visual cliff test responses at 10 weeks postsurgery. The treatment with melatonin, which had no effect per se, completely prevented the visual dysfunctions (Figure 1). Figure 2 shows representative ultraphotomicrographs of the central nasal and temporal choriocapillaris at 10 weeks postsurgery. The treatment with melatonin had no effect on the ubiquitous increase in choriocapillaris lumen height increase at either region, both in sham-treated or SCGx-subjected eyes (Figure 2). However, melatonin prevented the SCGx-induced alterations in BrM ultrastructure, showing dense fibrillary collagen deposits within the elastic layer, an increase in RPE and endothelial cell basal membrane thickness, and BrM thickening only at the temporal region (Figure 3). Photomicrographs and ultraphotomicrographs representative of each group at the central nasal and temporal RPE are shown in Figure 4. Melatonin prevented the decrease in RPE melanin and melanosome content in the temporal region induced by

**FIGURE 7** Effect of melatonin on the outer retina/RPE region-dependent oxidative damage at 6 weeks post-SCGx. 4HNE- and CML-immunostaining at the nasal and temporal outer retina and RPE. Melatonin prevented the SCGx-induced increase in 4HNE- and CML-immunoreactivity at the temporal outer retina/RPE (arrow), and RPE (arrowhead), respectively. Shown are photomicrographs representative from 5 eyes/group. ONL, outer nuclear layer; IS, photoreceptor inner segments; OS, photoreceptor outer segments; RPE, retinal pigment epithelium; Chr, choroid. Scale bar: 50 μm
SCGx at 10 weeks postsurgery. In addition, SCGx induced a significant decrease in RPE65-immunoreactivity circumscribed to the temporal RPE, which was completely prevented by melatonin (Figure 5). The ultrastructural analysis showed RPE vacuolization and a clear disorganization of the PR membranous discs, blebs and complete disc loss, replaced by amorphous electron-dense material localized at the temporal region at 10 weeks post-SCGx, which were not observed in SCGx-submitted eyes from melatonin-treated animals, as shown in Figure 6. Oxidative stress markers were analysed at 6 weeks postsurgery. The treatment with melatonin completely prevented the increase in 4HNE- and CML-immunoreactivity at the temporal RPE and PR outer segments and RPE, respectively, induced by SCGx (Figure 7). In addition, melatonin significantly prevented the SCGx-induced increase in the temporal RPE mitochondria superoxide content (assessed with MitoSOX-Red probe) and the decrease in MitoTracker-Red-labelled mitochondria (an index of mitochondrial mass) (Figure 8). Moreover, melatonin prevented the SCGx-induced decrease in the levels of intrinsic mitochondrial proteins (i.e., cytochrome c, VDAC and TOM20) exclusively at the temporal RPE, as shown in Figure 9. The results shown above demonstrate that the treatment with melatonin starting at 48 h post-SCGx prevented the damage induced by experimental NE-AMD. When melatonin treatment started at 4 weeks post-SCGx, it completely reversed the visual dysfunctions (ERG and visual tests) induced by SCGx at 10 weeks postsurgery, as shown in Figure 10, as well as the decrease in temporal RPE melanin content and RPE65-immunoreactivity (Figure 11).

4 | DISCUSSION

For the first time, the foregoing results demonstrate that melatonin provided functional and structural protection to the RPE and outer retina in experimental NE-AMD. In particular, melatonin preserved the ERG a-wave amplitude, the visual test performances, and the BrM, the RPE and the PR ultrastructure. In accordance with previous studies both in rats and mice, SCGx induces ubiquitous choroid alterations. However, all but...
the choriocapillaris alterations found within NE-AMD induced by SGCx were exclusively located at the central temporal area of the outer retina/RPE, an area that according to Volland et al. shows human-like cone/rod ratio, the highest concentration of cones and the specialization of the RPE and Bruch’s membrane (BrM) in C57BL/6J mice, making it comparable to the human macula. Moreover, biochemical and structural differences between the nasal and temporal region could account for the localized damage induced by SCGx. Experimental NE-AMD induced by SCGx can be divided in two phases: an early phase (up to 6 weeks post-SCGx), characterized by PR dysfunction (ERG a-wave), BrM thickening, decreased RPE melanin content, and RPE65-immunoreactivity, subtle ultrastructural RPE and PR alterations, increase in oxidation markers (4HNE, CML, MitoSOX) and mitochondria mass decrease, followed by a late phase (at 10 weeks post-SCGx), at which severe RPE ultrastructural damage and PR loss become evident. These alterations cannot be attributed to a denervation-related decrease in pineal gland-synthesized melatonin, since SCGx induces ubiquitous alterations in the choroid and choriocapillaris, while exclusively circumscribed changes in the PR, BrM and RPE localized at the central temporal region, and because no differences between the contralateral eye and naïve eyes are observed.

In the present report, we chose to analyse the effect of melatonin on NE-AMD outcomes at 10 weeks post-SCGx so as to maximize the damage in the RPE/outer retina function and histology, thus allowing us to discard the possibility of melatonin inducing a transient protection. Melatonin was administered as subcutaneous pellets, which secured reaching continuous dosing and reduced the animal manipulation associated with a daily administration. Moreover, we have already shown in previous reports the success of this dosing and administration way to achieve retinal and optic nerve protection against several experimental visual diseases.

Electroretinography is a method to objectively evaluate the retinal function. The treatment with melatonin prevented the decrease in the scotopic ERG a-wave amplitude induced by SCGx, supporting that melatonin protected PR function. Since ERG only provides reliable information on the electrical retinal response to a flash of light, vision being a much more complex function, we assessed the effect of SCGx and melatonin on three behavioural tests, thus evaluating visual functions from a broader perspective. The looming response is a reflex relying exclusively on visual cues that evaluates the integrity of the retino-colicular pathway, while both the visual and the virtual visual cliff tests evaluate binocular

**FIGURE 9** Effect of melatonin on the decreased levels of intrinsic mitochondria proteins at 6 weeks post-SCGx. Left panel: Representative Western blots for the assessment of mitochondria intrinsic proteins. SCGx, which had no effect on the nasal RPE, induced a decrease in the levels of cytochrome c, VDAC, and TOM20 at the temporal RPE. Melatonin, which did not have effects per se, prevented these alterations. The densitometric analysis is shown on the right. Data are mean ± SEM (n: 4 homogenates per group), **P < .01, *P < .05 vs sham-treated eyes from control animals; a: P < .01, b: P < .05 vs SCGx-treated eyes from control animals, by Tukey’s test
depth perception as a result of the functional integrity of the retino-geniculo-cortical pathway.\textsuperscript{36,41} SCGx induced a decrease in the performance of these behavioural tests, while the treatment with melatonin preserved visual functions.

Although melatonin was unable to modify the choriocapillaris lumen height increase induced by SCGx, it significantly prevented alterations in BrM thickness and ultrastructure in SCGx-eyes. Bruch’s membrane, formerly considered a passive structure lying between the RPE and the choroid, actually constitutes altogether with the choriocapillaris and the RPE a single functional cluster,\textsuperscript{42} strategically located, and allowing hydraulic homeostasis, metabolic waste disposal and nutrient transport between systemic circulation, and avascular outer retina. It has been demonstrated that BrM thickening leads to RPE and PR damage.\textsuperscript{43,44} Regardless it is not yet clear whether BrM alterations lead to RPE damage or vice versa, the treatment with melatonin protected the BrM either as a primary effect or as a consequence of RPE protection.

Melanin, a pigment concentrated in the choroid and the RPE, has a central role in removing radiation excess, and reactive oxygen species (ROS) derived from RPE metabolism.\textsuperscript{45} Thus, a decrease in the RPE melanin content might lead to a deficit in quenching both high-energy photons and ROS, and in turn, to an increase in oxidative stress. RPE65, an isomerohydrolase specifically located in the RPE, produces 11-cis-retinol from all-trans-retinyl esters, is a key player in the visual cycle. Melatonin prevented the decrease in RPE melanin content and RPE65-immunoractivity at the central temporal region induced by SCGx.

The mechanisms involved in the therapeutic effect of melatonin remain to be established. NE-AMD is a complex disease involving several pathological mechanisms; still, oxidative stress, particularly acting at the central temporal RPE, seems to play a pivotal role in its onset and progression. Excess of mitochondria-derived ROS due to mitochondria malfunction has been considered an early event in human and experimental NE-AMD.\textsuperscript{1,16,46,47} Moreover, gene polymorphisms related to oxidized base repair proteins\textsuperscript{48} and increased mitochondrial DNA damage\textsuperscript{49} have been found in AMD patients. Furthermore, decreased mitochondrial mass and mitochondrial ultrastructure alterations in AMD specimens,\textsuperscript{46} and retinal degeneration in a RPE sod2 mutant mouse\textsuperscript{11} have been described. According to our results, melatonin prevented the increase in lipid peroxidation, mitochondria superoxide levels, and the decrease in mitochondria mass induced by SCGx, supporting that melatonin protected RPE/PR function and

![Figure 10](image-url) Effect of the delayed treatment with melatonin on the visual function alterations induced by SCGx. Panel (A) The average amplitudes of scotopic ERG a-wave are shown. Melatonin treatment starting at 4 weeks post-SCGx completely reversed the decrease in the ERG a-wave amplitude. Data are mean ± SEM (n: 10 animals per group), **P < .01 vs sham-treated eyes from control animals; a: P < .01 vs SCGx-treated eyes from control animals, by Tukey’s test. Panel (B) Representative scotopic ERG traces of sham- and SCGx-eyes from control- and melatonin-treated animals. Panel (C) The average responses in three behaviour visual tasks are shown. The delayed treatment with melatonin reversed the visual behaviour responses at 10 weeks post-SCGx. Data are mean ± SEM (n: 10 animals per group), **P < .01, and b: P < .05 vs control animals with sham-treated eyes; a: P < .01, and b: P < .05 vs control animals with SCGx-treated eyes, by Tukey’s test.
structure through its antioxidant activity. Consistently with our results, it has been reported that melatonin can exert protection against RPE cells oxidative damage and cell death induced by ischaemia.\textsuperscript{50-53} In addition, melatonin has shown to rescue mitochondria functionality by reducing oxidative stress and increasing mitochondria biogenesis in diabetic myocardial ischaemia/reperfusion damage.\textsuperscript{54} There are reports showing melatonin exerts its protective effects against oxidative stress through MT1 and/or MT2 receptors;\textsuperscript{55,56} however, receptor-independent antioxidant effects of melatonin have also been described.\textsuperscript{57,58} At present, we cannot ascertain whether the protective effect of melatonin against NE-AMD involves receptor-dependent or receptor-independent mechanisms, an issue that will be analysed in the near future.

The fact that the treatment with melatonin started 48 h after SCGx could limit the potential clinical translation of these results. Therefore, in order to analyse whether melatonin could not only prevent, but also slow or reverse NE-AMD progression, in another set of animals, we started melatonin treatment at 4 weeks post-SCGx, a time point at which functional and structural alterations are already evident.\textsuperscript{15} The delayed treatment with melatonin reversed functional damage (ERG and behaviour visual tests) and achieved a complete protection of RPE melanin content and RPE65-immunoreactivity, thus being capable of actively suppressing ongoing pathological mechanisms in experimental NE-AMD. In line with our results, in a single nonrandomized clinical trial, it has been shown that the treatment with melatonin decreased the progression of AMD,\textsuperscript{59} reverses macular oedema and improves visual acuity within human refractory central serous chorioretinopathy,\textsuperscript{28} a complex disease associated with an RPE dysfunction.
Overall, our results demonstrate that melatonin is capable of significantly preserve visual functions and retinal structure in experimental NE-AMD. Therefore, despite the well-known differences between the mouse and human retina, the treatment with melatonin, a very safe compound which lacks proven adverse effects even at high doses in humans, could become a promising therapy to prevent, slow down or even reverse RPE/retinal damages caused by human NE-AMD.

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AUTHOR CONTRIBUTIONS
Hernán H. Diéguez involved in design/concept, data acquisition and interpretation/data analysis; María F. González Fleitas, Marcos L. Aranda, Juan S. Calanni, María I. Keller Sarmiento, Mónica S. Chianelli, Pablo H. Sande and Horacio E. Romeo involved in data acquisition; Ruth E. Rosenstein involved in design/concept, interpretation/data analysis and manuscript drafting; Damián Dorfman involved in design/concept, interpretation/data analysis, manuscript drafting and article final approval.

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