Preservation of Intrinsically Photosensitive Retinal Ganglion Cells (ipRGCs) in Late Adult Mice: Implications as a Potential Biomarker for Early Onset Ocular Degenerative Diseases

Anna Matynia,[1,2,*] Brandy S. Recio,[3] Zachary Myers,[3] Sachin Parikh,[1,2] Rajesh Kumar Goit,[1,2] Nicholas C. Brecha,[1–3,5] and Luis Pérez de Sevilla Müller[3]

[1]Department of Ophthalmology, Jules Stein Eye Institute, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, United States
[2]Brain Research Institute, University of California, Los Angeles, Los Angeles, California, United States
[3]Department of Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, United States
[4]VA Greater Los Angeles Healthcare System, Los Angeles, California, United States
[5]Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, United States

Correspondence: Luis Pérez de Sevilla Müller, Department of Neurobiology, David Geffen School of Medicine, University of California, Los Angeles, 10833 Le Conte Avenue, Los Angeles, CA 90095-1763, USA; luisperez@mednet.ucla.edu.

Current affiliation: *College of Optometry, University of Houston, Houston, Texas 77204, United States

Received: June 29, 2023
Accepted: November 27, 2023
Published: January 15, 2024

Citation: Matynia A, Recio BS, Myers Z, et al. Preservation of intrinsically photosensitive retinal ganglion cells (ipRGCs) in late adult mice: Implications as a potential biomarker for early onset ocular degenerative diseases. *Invest Ophthalmol Vis Sci.* 2024;65(1):28. https://doi.org/10.1167/iovs.65.1.28

Purpose. Intrinsically photosensitive retinal ganglion cells (ipRGCs) play a crucial role in non–image-forming visual functions. Given their significant loss observed in various ocular degenerative diseases at early stages, this study aimed to assess changes in both the morphology and associated behavioral functions of ipRGCs in mice between 6 (mature) and 12 (late adult) months old. The findings contribute to understanding the preservation of ipRGCs in late adults and their potential as a biomarker for early ocular degenerative diseases.

Methods. Female and male C57BL/6J mice were used to assess the behavioral consequences of aging to mature and old adults, including pupillary light reflex, light aversion, visual acuity, and contrast sensitivity. Immunohistochemistry on retinal wholemounts from these mice was then conducted to evaluate ipRGC dendritic morphology in the ganglion cell layer (GCL) and inner nuclear layer (INL).

Results. Morphological analysis showed that ipRGC dendritic field complexity was remarkably stable through 12 months old of age. Similarly, the pupillary light reflex, visual acuity, and contrast sensitivity were stable in mature and old adults. Although alterations were observed in ipRGC-independent light aversion distinct from the pupillary light reflex, aged wild-type mice continuously showed enhanced light aversion with dilation. No effect of sex was observed in any tests.

Conclusions. The preservation of both ipRGC morphology and function highlights the potential of ipRGC-mediated function as a valuable biomarker for ocular diseases characterized by early ipRGC loss. The consistent stability of ipRGCs in mature and old adult mice suggests that detected changes in ipRGC-mediated functions could serve as early indicators or diagnostic tools for early-onset conditions such as Alzheimer's disease, Parkinson's disease, and diabetes, where ipRGC loss has been documented.

Keywords: melanopsin, aging, mouse retina, function, biomarker

Visual function declines with age,[1] with aging affecting both the cornea and lens required for a clear light path, photoreceptors and their circuitry, and visual processing in the brain. Manifestations of normal aging include decreased visual acuity, contrast sensitivity and the onset of sleep and circadian dysfunction,[2,3] which are linked to both rod and cone photoreceptors and intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina.[4–10]

ipRGCs containing the photopigment melanopsin[8–10] are well conserved across species, including humans,[11–14] and are located in the retinal ganglion cell layer (GCL), with a large displaced population in the inner nuclear layer (INL) in humans and a small displaced population in mice.[15] ipRGCs are a class of photoreceptors that mediate both non–image-forming functions of the eye[16] and vision-forming pathways. ipRGC functions include photoentrainment of circadian rhythms, modulation of the sleep/wake cycle, masking

response, sleep regulation, control of pupillary light reflex, light-induced suppression of melatonin secretion, mood regulation,[17,18] and color pathway and brightness perception.[19–23]

With age, a number of extraretinal changes can alter visual function. For example, age-related changes in lens clarity and density[24,25] can reduce the transmission of blue light, which is known to suppress ipRGC-mediated melatonin secretion during the day.[26] A further age-related change in the eye that may contribute to reduced levels of light reaching the ipRGCs is the reduction in pupil size.[27] As a result, this diminished blue light input to the circadian clock has been shown to result in disturbed circadian rhythm and sleep in the elderly.[2,7,28,29] In addition to these nonspecific decreases in retinal illumination, ipRGCs are often disrupted in many neurodegenerative disorders including Alzheimer's disease (AD),[30–33] Parkinson's disease (PD),[34–37] Huntington's disease,[38,39] glaucoma,[40–42] and diabetes,[43,44] suggesting that ipRGC-mediated functions may be useful biomarkers of early onset disease. Therefore, it is crucial to study the effects of normal aging in mature and old adult control animals to facilitate identification of functional and anatomical retinal impairments resulting from early disease onset from younger adults. Given the reported correlation of ipRGC pathologies with ophthalmic diseases, there is a potential of ipRGCs to serve as biomarkers in many neurodegenerative diseases.

In this study, we present a comparison of ipRGC morphology in mature adult (6 months old) and old adult (12 months old) mice and its correlation with behavioral functions mediated by the melanopsin system. In addition, sex-related differences in most neurodegenerative diseases are increasingly recognized, particularly in early-onset conditions such as PD, that occur before the age of 40 or 50 years,[45,46] as well as early-onset AD, which affects individuals in their 30s and 40s.[47–49] Men tend to be more commonly affected by early-onset PD, possibly due to hormonal and genetic factors.[50,51] Conversely, early-onset AD exhibits a higher prevalence in women, potentially influenced by sex-specific genetic variants and hormonal fluctuations throughout their reproductive life.[52,53] Moreover, sex-related disparities are evident in nonarteritic anterior ischemic optic neuropathy occurring in the late 30s[54] and diabetic retinopathy, which can manifest as early as the age of 30.[55] The underlying mechanisms for these differences involve complex interactions among sex hormones, genetic predispositions, and immune responses. Recognizing and understanding these sex-specific aspects of neurodegenerative and ocular diseases are vital for developing tailored treatments and personalized approaches that consider the distinct needs of male and female patients, ultimately leading to improved therapeutic outcomes and enhanced quality of life. Therefore, we have included an analysis of the differences between male and female mice in ipRGC function and M1 ipRGC subtype morphology.

Materials and Methods

These studies were conducted under protocols approved by the University of California at Los Angeles (UCLA) Animal Research Committee. All experiments were carried out in accordance with guidelines for the welfare of experimental animals issued by the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the UCLA Animal Research Committee.

Animals

Female and male C57BL/6J mice (000664, bred at UCLA; The Jackson Laboratory, Bar Harbor, ME, USA) at 6 and 12 months of age (a total of 39 animals) were used in this study, and data analyzed from past experiments represented an additional 190 animals. All mice were housed in standard cages in a temperature-controlled room on a 12-hour light/dark cycle with free access to standard pellet food and water. Opn4^{DTA} mice (035927, Opn4tm3.1(DTA)Saha/J; The Jackson Laboratory) express the diphtheria toxin subunit alpha (DTA) sequence under the control of the Opn4 promoter (exons 1–9 of the opsin 4), resulting in ablation of the ipRGCs.[56] These mice were backcrossed into the C57Bl/6J background and bred heterozygous to heterozygous, with ipRGC ablation verified using a qualitative pupillary light reflex assay as reported previously.[57]

Behavioral Assays

The light-aversion assay was performed as previously described with modifications.[57,58] A two-chamber box with open, light and closed, dark sections was used to measure time spent in the light compartment. An overhead LED lighting system, with adjustable illumination from 0 to 1000 lux calibrated with a light meter (HHLM-2; Omega Engineering, Norwalk, CT, USA), a standard LED spectrum, and diffusers provided uniform illumination in the open, lit side of the chamber. Behavior was monitored using an infrared light source and video camera with white light filter, and automated tracking and analysis were performed with a video tracker (Med Associates, St. Albans, VT, USA) and an activity monitor (Med Associates), respectively. Mice were acclimated to a dimly lit room (less that 10 lux) for at least 15 minutes and dark adapted prior to testing for at least 10 minutes. Light aversion was tested at 0 and 1000 lux: the 0-lux test was used as baseline to calculate aversion indices. A 1% Atropine Sulfate Ophthalmic Solution (Akorn, Lake Forest, IL, USA) was used as a dilating agent where indicated.

Pupillary Light Reflex

Unanesthetized mice were used for pupillometry as previously described.[57] Before each experiment, mice were acclimated in a dimly lit room (less than 50 lux) for at least 10 minutes. The pupillary light reflex (PLR) was measured with a hand-held slit lamp (Kowa SL-15; Kowa Pharmaceuticals, Montgomery, AL, USA) using a semiquantitative scale, where 1 = pinpoint pupil and 3 = no constriction.

Visual Acuity and Contrast Sensitivity

Mice were acclimated in a dimly lit room (less than 50 lux) for at least 20 minutes prior to testing in a virtual automated optomotor system (OptoDrum; Striatech, Clearwater, FL, USA).[59–63] Briefly, a mouse was placed on the center platform of an enclosed chamber with four computer screens as walls that presented stripes of varying thickness and contrast. Head movement was captured by an overhead camera and tracking was computationally determined. Mice were tested for visual acuity using 99.7% contrast with spatial resolution between 0.056 and 0.50 cycles per degree. Contrast sensitivity was assessed at multiple spatial resolu-

Investigative Ophthalmology & Visual Science

tions with contrast between 0.9% and 99.7%, using the reciprocal of Michelson's contrast threshold. Light intensity at the mouse's cornea was in the mesopic range at approximately 90 to 110 lux.

Immunohistochemistry in Whole-Mounted Retinas

Following deep anesthesia with 1% to 3% isoflurane (Abbott Laboratories, Abbott Park, IL, USA), animals were euthanized by cervical dislocation or decapitation. The eyes were enucleated and dissected in Hibernate A (Invitrogen, Carlsbad, CA, USA) for fluorescence and immunohistochemical studies. The retinas were removed from the eyecups, and four small incisions were made on each retina to lay the tissue flat. Retinas were mounted onto nitrocellulose membrane filters (EMD Millipore, Billerica, MA, USA), with the GCL facing upward, and fixed for 15 minutes in 4% paraformaldehyde in 0.1-M phosphate buffer (PB) at room temperature.

Immunohistochemical labeling was performed using our published protocols.[64–67] After fixation, the whole-mounted retinas were subsequently washed in PB three times for a total of 90 minutes and incubated in 10% normal goat serum with 0.5% Triton X-100 at 4°C overnight. Retinas were removed from the blocking solution and subsequently incubated in anti-melanopsin primary antibodies (1:1000, AB-N39; Advanced Targeting Systems, San Diego, CA, USA) for 7 days at 4°C. They were then rinsed three times for 30 minutes each with 0.1-M PB and incubated with the corresponding secondary antibodies (1:1000, Goat anti-Rabbit IgG Alexa Fluor 488 or Alexa Fluor 594; Invitrogen) overnight at 4°C. The following day, the retinas were washed three times in 0.1-M PB for a total of 90 minutes and subsequently placed on a microscope slide with the GCL facing upward. Then, the samples were mounted in Aqua-Poly/Mount (Polysciences, Warrington, PA, USA), and the coverslips were sealed with nail polish. As a negative control, the omission of the primary antibody confirmed the elimination of specific labeling.

Fluorescent Image Acquisition

Labeling was assessed with a Zeiss laser scanning microscope (Zeiss LSM 880; Carl Zeiss Microscopy, Jena, Germany) with a Zeiss C-Apochromat 40×/1.2 corrected water immersion objective. The images were captured at a resolution of 1024 × 1024 pixels.

M1 ipRGC Morphological Analysis

M1 ipRGCs from all retinal quadrants (nasal, temporal, superior, and inferior) were reconstructed using Imaris 9.5.0 (Bitplane AG, Concord, MA, USA) with the Filament Tracer option. The Filament Tracer operates on three-dimensional images, which provides sufficient resolution to resolve the filaments to be studied in all three spatial directions. The Filament Tracer option automatically computes all the paths from a user-defined starting point (ipRGC body) to the end of the structure. The filaments were then manually traced by the user. Imaris provided the dendrite length, Sholl analysis, and total number of dendritic branch points. M1 densities and soma sizes were measured with Imaris software.

Statistics

All values are given as mean and standard error of the mean (SEM). Single statistical comparisons of a group versus its control group were performed using a two-tailed Student's *t*-test or two-way ANOVA as indicated in Prism 4.0 or 9.0 (GraphPad, Boston, MA, USA). If data were not normally distributed, non-parametric tests (Mann–Whitney *U* test) were used. $P \leq 0.05$ was considered statistically significant.

Results

Behavioral Consequences on Light Aversion and Pupil Dilation in Mice

To investigate whether aging results in detectable functional deficits in ipRGCs in mice, we tested light aversion behavior with and without pupil dilation (Fig. 1). Wild-type mice exhibited increased light aversion with atropine dilation for both sexes (6-month-old males, $n = 41$; 12-month-old males, $n = 3$; 6-month-old females, $n = 32$; 12-month-old females, $n = 6$) (Figs. 1A, 1B). Mice lacking ipRGCs (6-month-old males, $n = 20$; 12-month-old males, $n = 1$; 6-month-old females, $n = 14$; 12-month-old females, $n = 5$) exhibited decreased light aversion that did not increase with dilation (Fig. 1C), consistent with previous reports.[57,68,69] For both wild-type and mice lacking ipRGCs, there was an increase in light aversion without dilation at 12 months old compared to 6 months old and for wild-type mice with dilation (Figs. 1A, 1C). No age-dependent effect was observed in mice lacking ipRGCs with atropine dilation. There were no significant differences in pupil constriction to bright light in 6-month-old and 12-month-old wild-type mice or mice lacking ipRGCs for either sex (Figs. 1E, 1F). No effect of sex was observed in any of these metrics. As previously reported, mice lacking ipRGCs have significantly reduced pupil constriction compared to wild-type mice.[57,70,71]

ipRGCs have been shown to contribute to both visual acuity and contrast sensitivity. The M4 class of ipRGCs responds to contrast gradients by electrophysiological recordings, and functional deficits have been observed using an optomotor task.[56,72] To determine if mature adulthood impacts these functions, wild-type mice were tested at 6 months old or 12 months old ($n = 13$ males, $n = 23$ females) (Fig. 2B). Visual acuity remained stable even though more variability occurred in the older mice. Similarly, contrast sensitivity was not affected. However, a trend toward increased contrast sensitivity at lower spatial resolution in older adult mice was observed (Figs. 2C, 2D).

Morphological Analysis of M1 Cells

To investigate whether morphological changes occurred in ipRGCs in the GCL and INL in male and female retinas, we used an antibody that stains different types of ipRGCs.[73] We focused on the M1 ipRGCs, as they are the main type of ipRGCs with a high content of melanopsin. They are easily differentiated from other ipRGC types in the GCL due to their high content of melanopsin immunoreactivity and two to five primary dendrites that stratify in one single layer at the outmost part of the IPL as previously reported[73,74] (Figs. 3A, 3E). ipRGCs located in the INL are exclusively M1 cells[73,74] (Figs. 3B, 3F).

Investigative Ophthalmology & Visual Science

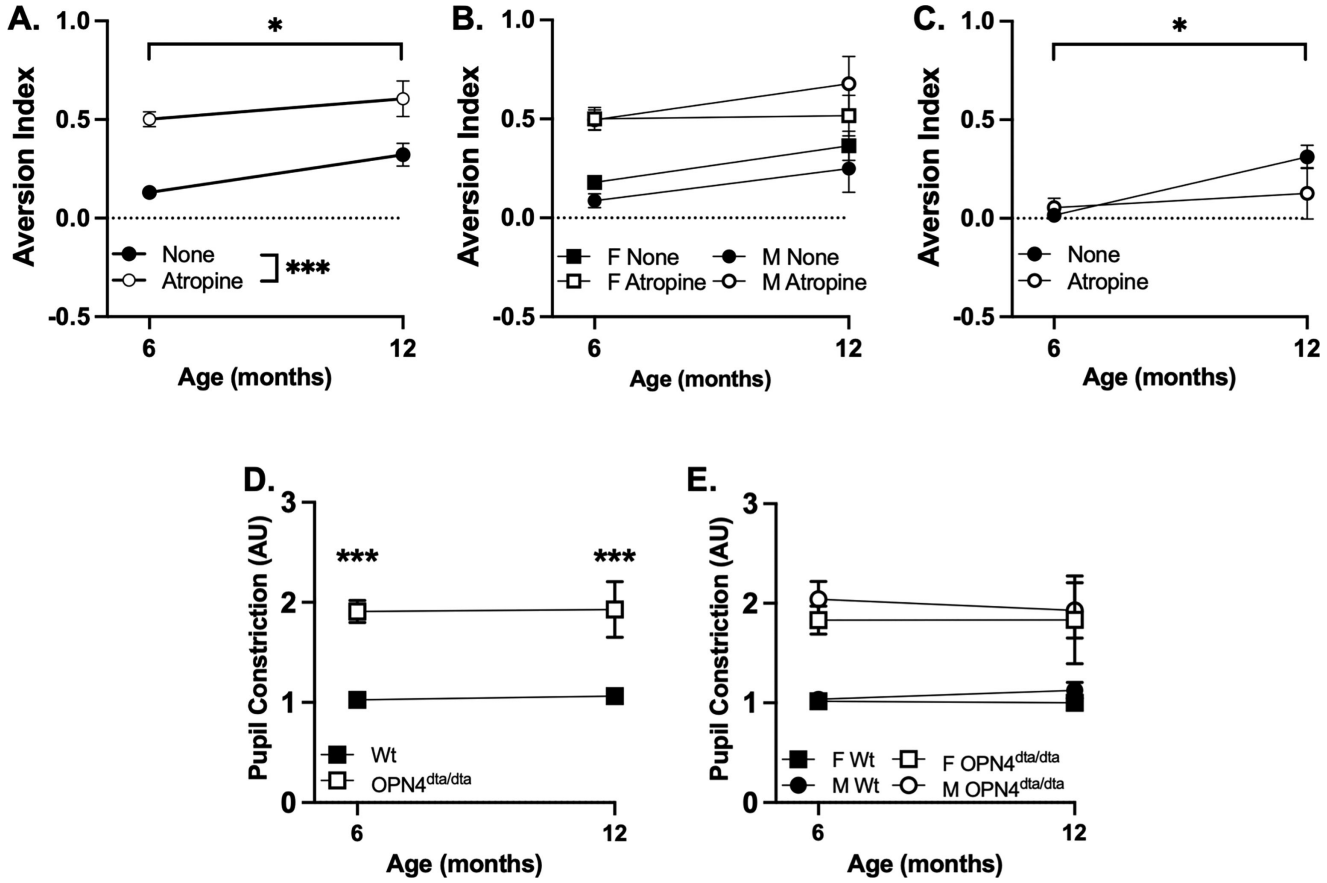

Figure 1. Light aversion and pupillary light reflex in mature and old adult mice. (**A**) Light aversion tested at 1000 lux with and without atropine eye drops in 6- and 12-month-old wild-type mice. (**B**) Light aversion tested at 1000 lux with and without atropine eye drops in 6- and 12-month-old wild-type mice by sex. (**C**) Light aversion tested at 1000 lux with and without atropine eye drops in 6- and 12-month-old mice lacking melanopsin-expressing neurons. (**D**) Pupillary light reflex in 6- and 12-month-old wild-type mice and mice lacking melanopsin-expressing neurons. (**E**) Pupillary light reflex in 6- and 12-month-old wild-type mice and mice lacking melanopsin-expressing neurons by sex. For light aversion in wild-type mice, there was a significant main effect of age ($P = 0.03$; $F_{1,166} = 5.02$) and of eye drops ($P < 0.0001$; $F_{1,166} = 24.68$), but no interaction ($P = 0.50$; $F_{1,166} = 0.45$). No effect of sex was observed. For light aversion in the OPN4$^{dta/dta}$ mice, there was a significant main effect of age ($P = 0.01$; $F_{1,76} = 6.38$) but not of eye drops ($P = 0.32$; $F_{1,76} = 1.01$) and no interaction ($P = 0.13$; $F_{1,76} = 2.39$). No effect of sex was observed. For pupillometry in wild-type mice, there was a significant main effect of genotype ($P < 0.00001$; $F_{1,90} = 84.58$) but not of age ($P = 0.91$; $F_{1,24} = 0.011$) and no interaction ($P = 0.76$; $F_{1,24} = 0.10$). No effect of sex was observed.

Soma sizes were evaluated in male mice 6 months and 12 months old, as well as in female mice of corresponding age groups. In the male 6-month-old group, soma sizes ranged from 11 to 18 µm, with an average of 13.4 ± 2.2 µm ($n = 83$ cells from three retinas from three male mice). Similarly, for male mice 12 months old, soma sizes ranged from 9 to 16 µm, with an average of 12.5 ± 1.6 µm ($n = 98$ cells from three retinas from three male mice). Among the female mice at 6 months, soma sizes ranged from 9 to 17 µm, with an average of 12.9 ± 1.9 µm ($n = 112$ cells from three retinas from three female mice). In 12-month-old females, soma sizes ranged from 9 to 17 µm, with an average of 12.2 ± 1.9 µm ($n = 85$ cells from three retinas from three female mice). No statistically significant differences were observed in soma sizes across the distinct age and sex groups. Morphological analysis of M1 ipRGCs was performed using Imaris 9.5.0, and information on dendritic length, total number of dendrite branch points, and Sholl analysis were determined (Figs. 3C, 3D, 3G, 3H).

Our data showed that M1 cells in the GCL and INL did not exhibit significant morphological differences in their dendritic complexity between 6 and 12 months of age in either males ($n = 19$ cells in the GCL and $n = 5$ cells in the INL from five retinas from three male mice at 6 months old; $n = 28$ cells in the GCL and $n = 8$ cells in the INL from four retinas from four male mice at 12 months old) (Figs. 3A–3D) or females ($n = 27$ cells in the GCL and $n = 10$ cells in the INL from four retinas from four female mice at 6 months old; $n = 29$ cells in the GCL and $n = 7$ cells in the INL from eight retinas from six female mice at 12 months old) (Figs. 3E–3H). There were no significant differences observed between male and female mice in any of these parameters. In both the 6-month-old and 12-month-old groups, the density of M1 cells per square millimeter exhibited no discernible disparity between males ($n = 5$ retinas at 6 months old; $n = 4$ retinas at 12 months old) and females ($n = 3$ retinas at 6 months old; $n = 4$ retinas at 12 months old) (Figs. 3C, 3D, 3G, 3H). Furthermore, no significant differences were detected within either age group or between genders. Although a decrease in M1 cell density was observed in both female and male retinas in the INL (Figs. 3D, 3H), this difference was not found to be statistically significant. These findings suggest that, in

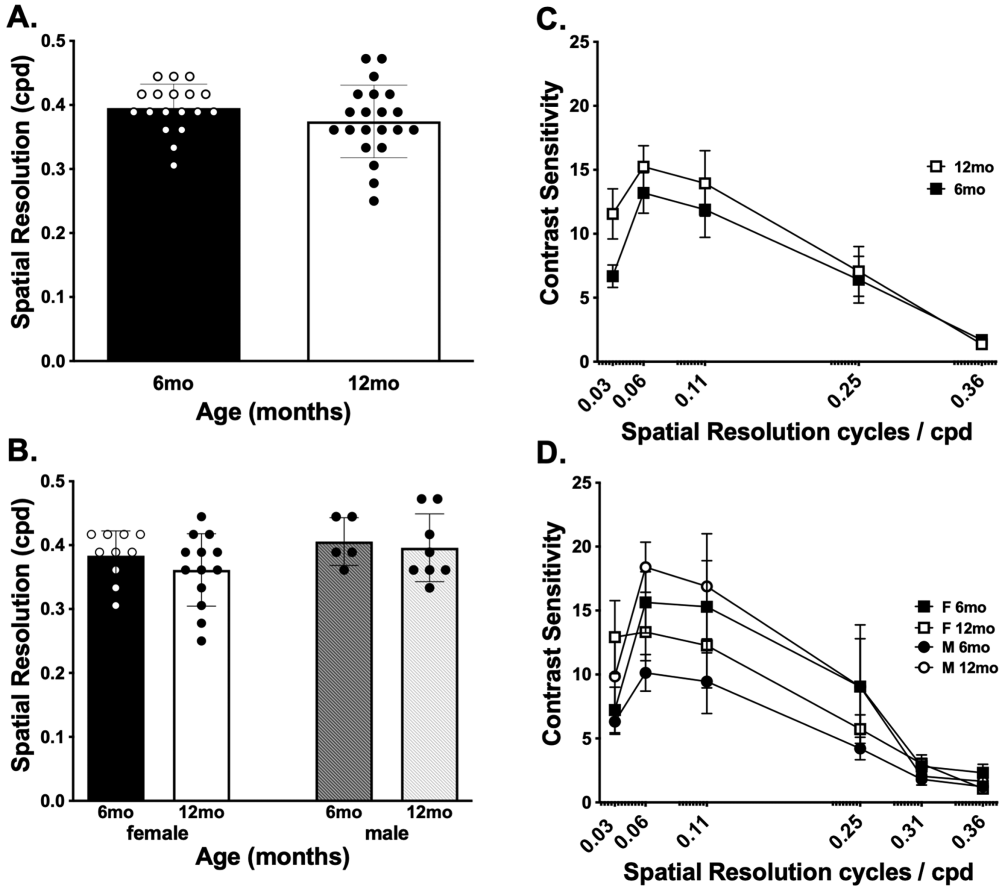

Figure 2. Visual acuity and contrast sensitivity in mature and old adult mice. (**A**) Spatial resolution in 6- and 12-month-old mice. (**B**) Spatial resolution in 6- and 12-month-old mice by sex. (**C**) Contrast sensitivity function in 6- and 12-month-old mice. (**D**) Contrast sensitivity function in 6- and 12-month-old mice by sex. No significant differences were found in visual acuity with age or sex using Student's *t*-test. There was a significant main effect of contrast ($P > 0.0001$; $F_{5,136} = 23.3$), but no main effect of age ($P = 0.10$; $F_{1,136} = 2.7$), and no interaction ($P = 0.72$; $F_{5,136} = 0.57$).

this study, age and sex did not have a significant impact on the measured structural characteristics of M1 neurons in the mice.

Discussion

Aging is associated with visual dysfunction, including reduced sensitivity of the circadian system to light, altered timing of circadian rhythms relative to nocturnal sleep, and increased sleep disturbances.[75–77] These functions are in part mediated by ipRGCs, although there are differences in the literature regarding RGC loss during aging, which may vary depending on the species and model studied. Although loss and/or morphological changes in RGCs have been observed in aged rodents and human,[78–80] other groups have not observed neuronal loss in the GCL in aged rats.[81–83]

Interestingly, human retinas show a relatively stable density of ipRGCs over time that is maintained in healthy subjects until the age of 70,[84] after which there is a decline of ipRGC density and atrophy of the dendritic arborizations in all ipRGC types.[84] This could explain the circadian rhythm desynchronization in the elderly.[3,85–88] Studies have also reported that ipRGC density and morphology are maintained in normal rats at 12 and 18 months of age,[81,89,90] which is consistent with our study in mice. However, the discrepancy between these studies could be attributed to the use of different animal models and their genetic backgrounds. For example, in Sprague Dawley rats, ipRGCs showed no significant morphological changes associated with age, but the mean density of ipRGCs in P23H rats showed a 67% decrease between 4 and 18 months of age.[89] Additionally, in 2-years-old rodless and coneless mice (*rd/rd cl*), the retinas showed normal levels of melanopsin expression, and immunocytochemistry assays demonstrated a maintained morphology of ipRGCs.[91]

ipRGC Function With Age

Retinal function is known to be affected with age,[92,93] and this can vary depending on biological sex, with female Sprague Dawley rats exhibiting better preserved retinal function at 18 months compared to males.[94] Aging also impacts circadian rhythms,[2,7,28,29] partly due to altered function of the ipRGC types. With aging, corneas and lenses undergo changes that result in less blue light reaching the retina, leading to reduced activation of blue light–sensitive ipRGCs.[26,27] As a result, it could be speculated that ipRGC-dependent functions such as pupil response to light, light aversion, visual acuity, and contrast sensitivity may be reduced in older individuals.[95–98] However, ipRGC-mediated circadian and pupillary responses to light are maintained in the absence of rods and cones,[91] and no age-related changes in

Investigative Ophthalmology & Visual Science

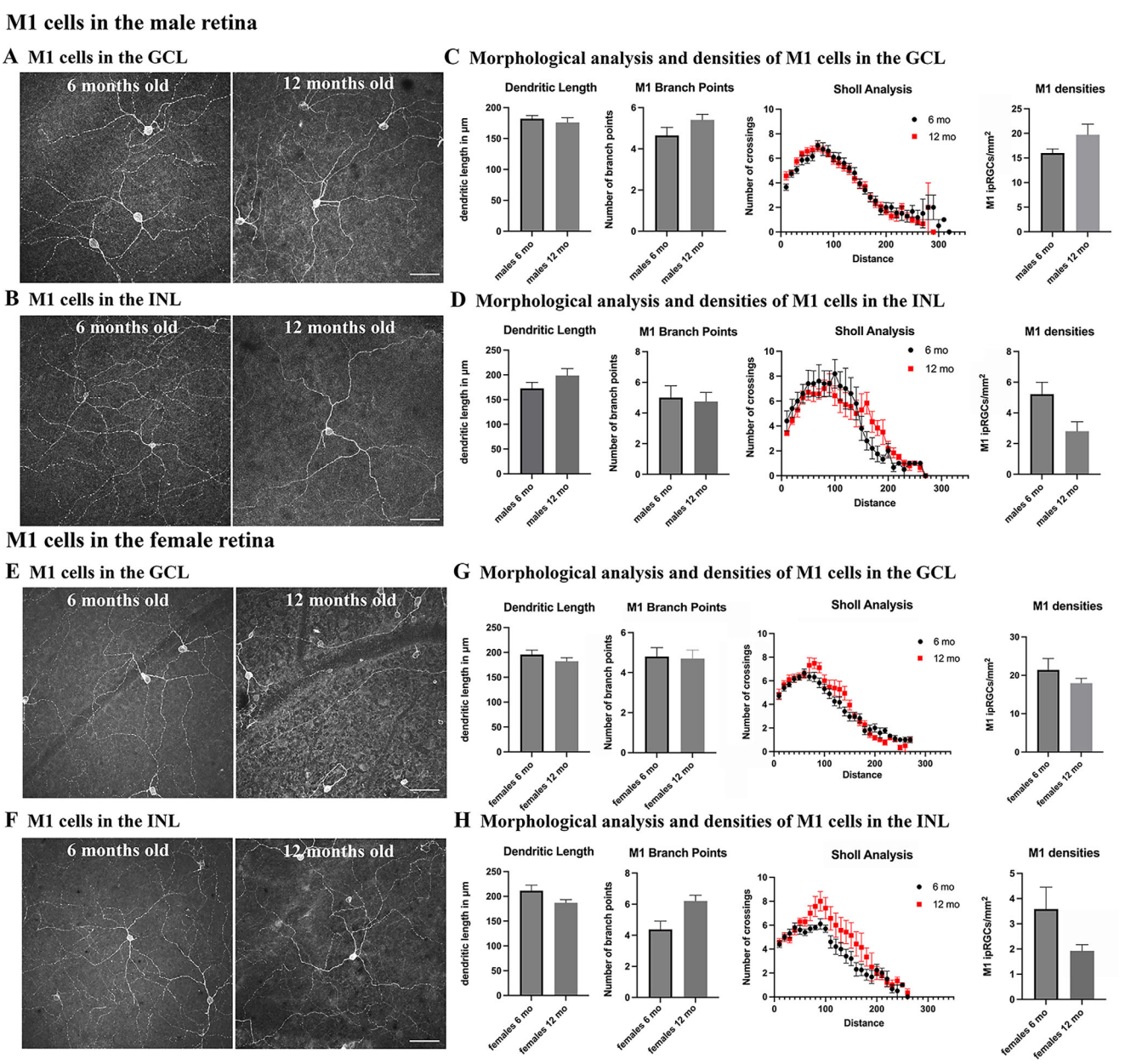

Figure 3. Dendritic structure and densities of M1 cells in male and female retinas in the GCL and INL. (**A**) Melanopsin staining in 6- and 12-month-old male wild-type retinas in the GCL. (**B**) Melanopsin staining in 6- and 12-month-old male wild-type retinas in the INL. (**C**) Quantification of morphological parameters examined for M1 cells at 6 and 12 months of age in the GCL. No significant differences were found in dendritic length, number of branch points, number of crossings in a Sholl analysis, or density. (**D**) Quantification of morphological parameters examined for M1 cells in 6- and 12-month-old retinas in the INL. No significant differences were found in dendritic length, number of branch points, number of crossings in a Sholl analysis, or density. ($P < 0.05$). (**E**) Melanopsin staining in 6- and 12-month-old female wild-type retinas in the GCL. (**F**) Melanopsin staining in 6- and 12-month-old female wild-type retinas in the INL. (**G**) Quantification of morphological parameters examined for M1 cells in 6- and 12-month-old retinas in the GCL. No significant differences were found in dendritic length, number of branch points, number of crossings in a Sholl analysis, or density. (**H**) Quantification of morphological parameters examined for M1 cells in 6- and 12-month-old retinas in the INL. No significant differences were found in dendritic length, number of branch points, number of crossings in a Sholl analysis, or density ($P < 0.05$). *Scale bar*: 50 µm (**A**, **B**, **E**, **F**).

pupil responses are found in humans.[99,100] These findings are also consistent with a study in which the magnitude of sustained pupillary constriction responses to blue-light and green-light stimuli did not exhibit significant changes between young and older human subjects,[101] as well as our studies in mice presented here.

Although the PLR responses of the Royal College of Surgeons (RCS) rats at 12 months of age were diminished compared to those of normal, non-dystrophic rats,[102] it seems that there are some age-dependent compensatory mechanisms to preserve PLR[99,102] and even to enhance pupil responses mediated by ipRGC in humans.[99] The improvement of the PLR at older ages may reflect some compensatory mechanisms in the inner retina, as well as in the central connections of the PLR pathway, to preserve the PLR responses. Taken together, the robustness of the PLR to

aging indicates a highly conserved and reliable mechanism that may reflect more than ipRGC function alone.

ipRGCs also mediate light aversion, which is clinically observed in conditions such as migraine as heightened sensitivity to light, known as photoallodynia or photophobia, and is defined as light-enhanced or -induced pain. In both humans and mice, the spectral properties of photoallodynia implicate ipRGCs,[103–108] a suggestion that was confirmed using mice lacking ipRGCs without pathophysiology and in specific disease models.[57,58,109] In migraine, rods, cones and ipRGCs[58] were shown to be equally essential in a rodent model, with ipRGCs selectively involved in photophobia between migraine episodes.[110] However, a role for green cone photoreceptors for amelioration of migraine symptoms in humans was also indicated.[111] In neonatal mice, photoaversion has been further mapped to a specific M1 class of ipRGCs,[112] and a specific photoreceptor pathway for photophobia in *Drosophila* larvae has been identified, which, due to their projection regions controlling circadian photoentrainment, is likely functionally related to ipRGCs.[113] ipRGCs persist during normal aging but are susceptible to degeneration even in mature adults in AD, PD, glaucoma, and diabetic retinopathy.[114] Our data indicate that ipRGCs are functionally maintained in light aversion in mice up to 12 months old, regardless of whether they are localized to the GCL or are displaced in the INL. Taken together, the role of ipRGCs in specific pathophysiologies is evident, and the loss of light aversion in mice lacking ipRGCs makes this differential a potential biomarker for ipRGC degeneration.

Visual acuity, a measure of sharpness and clarity of vision, diminishes with age and is a major health issue. In humans, the most common causes of reduced visual acuity are glaucoma and macular degeneration, including age-related macular degeneration and myopia-induced macular degeneration.[115–118] Similar age-related loss of visual acuity has been observed in rodent models, although loss of visual acuity usually occurs by around 1.5 to 2 years of age, consistent with our results indicating normal visual acuity in mice at 12 months old. Dysfunction or degeneration most frequently occurs in photoreceptors, retinal pigment epithelial cells or RGCs and can be traced to both genetic and environmental factors.[119–125]

Until recently, it was thought that rod and cone photoreceptor pathways exclusively mediate image-forming functions, where visual acuity and contrast sensitivity were firmly entrenched, and that ipRGCs exclusively mediated non–image-forming functions such as circadian photoentrainment and the PLR. Schmidt et al.[56] clearly demonstrated that the M4 class of ipRGCs is functionally equivalent to the alpha On RGCs, which are well known for their role in contrast sensitivity. This study showed a functional deficit in contrast sensitivity[56,87] in mice lacking M4 ipRGCs, which was further substantiated in mice lacking melanopsin but with the cells intact.[72] When compared to mice lacking rod or cone photoreceptors, the deficit in contrast sensitivity was greater than the loss in visual acuity, suggesting the relative role of ipRGCs in these different functions and the potential to serve as a biomarker for ipRGC function loss compared to rod and cone function. For context, the OPN4$^{dta/dta}$ mice generally lack all ipRGCs, but the possibility of a few remaining neurons cannot be ruled out.[126] The OPN4$^{dta/dta}$ mice are presented to illustrate the "floor effect"—that is, the maximal potential effect on outcomes if the ipRGCs were completely degenerated.

Like visual acuity, contrast sensitivity also decreases with age in humans, starting in the 50s for higher spatial resolution but eventually affecting all spatial resolutions and commencing with mesopic and proceeding to photopic vision loss.[97,127] In rodents, contrast sensitivity remains intact up to 18 months old[128] and decreases by 21 to 24 months old.[129] Our results are consistent with reported preserved visual acuity in mice up to 12 months old, with the observed small increase in contrast sensitivity at low spatial resolution possibly reflecting compensatory mechanisms or variability in small to medium-sized cohorts. Accelerated loss of contrast sensitivity is a hallmark of AD in humans and a mouse model.[128]

ipRGCs As a Biomarker in Ocular Diseases

The enduring integrity of ipRGC function throughout adulthood presents a promising biomarker for early neurodegenerative eye diseases. This is particularly relevant considering the widespread loss of ipRGCs in numerous ocular conditions. Aging is one of the main risk factors associated with glaucoma, PD, AD, and diabetes, among others.[30–44] Circadian clock disruption also triggers or accelerates the pathology progression in neurodegenerative diseases. For example, in AD, PD, and Huntington's diseases, circadian rhythm alterations seem to trigger or accelerate the pathology progression.[130,131] Other authors have reported that alterations of the circadian rhythm include a gradual decrease in nocturnal melatonin secretion[132] and alterations in sleep.[133]

It has been reported that a loss of circadian rhythms and impairment of pupillary constriction in diseases such as glaucoma, AD, PD, and Huntington's diseases[40,134–137] could also be linked to the ipRGC pathology loss, as well as a loss of the compensatory mechanisms observed in aging. In AD and other neurodegenerative diseases, early circadian rhythm alterations[138–142] indicate significant disruptions in the rod and cone photoreceptor pathways, as well as the ipRGC signaling pathways. The reported damage or loss of ipRGCs in the human retina[137,140–143] might account for many related visual dysfunctions, including impaired ocular motility, a reduction in amplitude of the PLR,[135,136,143,144] and circadian alterations of melatonin.[32,145,146] It is essential to recognize that, while ipRGCs hold promise as potential early biomarkers in certain ocular diseases, their utility might not be consistent across all conditions. Indeed, some diseases have exhibited remarkable resilience in ipRGCs,[147–150] showing limited or delayed alterations in these cells despite significant pathological changes occurring elsewhere in the retina. This underscores the complexity of ocular pathophysiology and the need for cautious interpretation when considering ipRGCs as biomarkers. The effectiveness of ipRGCs as diagnostic indicators would likely depend on the specific disease context and the interplay of various underlying factors. As such, the use of ipRGCs as biomarkers demands careful consideration of the unique characteristics of a disease and the role of ipRGCs in the pathogenesis of that disease. To improve our understanding and optimize the diagnostic value of ipRGCs, future research endeavors should focus on exploring the disease-specific roles of ipRGCs and thoroughly investigating the potential limitations they may pose as biomarkers in these early-onset diseases, understanding the dynamics of ipRGC loss to elucidate the specific underlying mechanisms, and assessing their diagnostic and prognostic relevance. More in-depth investigations into the contributions of different ipRGC subtypes, sex-specific differences,

Investigative Ophthalmology & Visual Science

and age-related factors are essential to establish ipRGCs as reliable biomarkers for early detection and monitoring of ocular diseases. By gaining a more nuanced understanding of their contributions to various ocular conditions, we can refine their diagnostic applicability and develop tailored approaches for leveraging ipRGCs as valuable tools in the early detection and management of ocular diseases.

Acknowledgments

The authors thank Arlene Hirano, PhD, for her insightful comments on the manuscript.

Supported by grants from the Department of Defense–Department of the Army–U.S. Army Medical Research Acquisition Activity (W81XWH-22-1-0294 to LPSM) and from the National Institutes of Health (1R01 EY034715-01 to NCB; 1R01 EY030864-01 to AM). This work was also supported by Veterans Affairs Senior Career Scientist Award (NCB).

Author Contributions: LPS conceived the project and supervised the project, LPS and AM designed and performed the experiments, and analyzed the data. BR and ZM reconstructed the M1 ipRGCs, SP and RKG performed behavioral experiments. LPS, AM, and NCB wrote the article. All authors contributed to the article and approved the submitted version.

Disclosure: **A. Matynia**, None; **B.S. Recio**, None; **Z. Myers**, None; **S. Parikh**, None; **R.K. Goit**, None; **N.C. Brecha**, None; **L. Pérez de Sevilla Müller**, None

References

1. Spear PD. Neural bases of visual deficits during aging. *Vision Res*. 1993;33(18):2589–2609.
2. Harper DG, Volicer L, Stopa EG, McKee AC, Nitta M, Satlin A. Disturbance of endogenous circadian rhythm in aging and Alzheimer disease. *Am J Geriatr Psychiatry*. 2005;13(5):359–368.
3. Cajochen C, Münch M, Knoblauch V, Blatter K, Wirz-Justice A. Age-related changes in the circadian and homeostatic regulation of human sleep. *Chronobiol Int*. 2006;23(1–2):461–474.
4. Wahl S, Engelhardt M, Schaupp P, Lappe C, Ivanov IV. The inner clock—blue light sets the human rhythm. *J Biophotonics*. 2019;12(12):e201900102.
5. Sletten TL, Revell VL, Middleton B, Lederle KA, Skene DJ. Age-related changes in acute and phase-advancing responses to monochromatic light. *J Biol Rhythms*. 2009;24(1):73–84.
6. Baba K, Tosini G. Aging alters circadian rhythms in the mouse eye. *J Biol Rhythms*. 2018;33(4):441–445.
7. La Morgia C, Carelli V, Sadun AA. Retina and melanopsin neurons. *Handb Clin Neurol*. 2021;179:315–329.
8. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. *Nat Neurosci*. 2001;4(12):1165–1165.
9. Hattar S, Liao HW, Takao M, Berson DM, Yau KW. Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. 2002;295(5557):1065–1070.
10. Provencio I, Rollag MD, Castrucci AM. Photoreceptive net in the mammalian retina. *Nature*. 2002;415(6871):493.
11. Nasir-Ahmad S, Lee SCS, Martin PR, Grünert U. Melanopsin-expressing ganglion cells in human retina: morphology, distribution, and synaptic connections. *J Comp Neurol*. 2019;527(1):312–327.
12. Chandra AJ, Lee SCS, Grünert U. Melanopsin and calbindin immunoreactivity in the inner retina of humans and marmosets. *Vis Neurosci*. 2019;36:E009.
13. Hannibal J, Christiansen AT, Heegaard S, Fahrenkrug J, Kiilgaard JF. Melanopsin expressing human retinal ganglion cells: subtypes, distribution, and intraretinal connectivity. *J Comp Neurol*. 2017;525(8):1934–1961.
14. Mure LS. Intrinsically photosensitive retinal ganglion cells of the human retina. *Front Neurol*. 2021;12:636330.
15. Rodriguez AR, de Sevilla Müller LP, Brecha NC. The RNA binding protein RBPMS is a selective marker of ganglion cells in the mammalian retina. *J Comp Neurol*. 2014;522(6):1411–1443.
16. Do MTH, Yau KW. Intrinsically photosensitive retinal ganglion cells. *Physiol Rev*. 2010;90(4):1547–1581.
17. Hattar S, Kumar M, Park A, et al. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J Comp Neurol*. 2006;497(3):326–349.
18. Ksendzovsky A, Pomeraniec IJ, Zaghloul KA, Provencio JJ, Provencio I. Clinical implications of the melanopsin-based non-image-forming visual system. *Neurology*. 2017;88(13):1282–1290.
19. Schmidt TM, Do MTH, Dacey D, Lucas R, Hattar S, Matynia A. Melanopsin-positive intrinsically photosensitive retinal ganglion cells: from form to function. *J Neurosci*. 2011;31(45):16094–16101.
20. Spitschan M, Lucas RJ, Brown TM. Chromatic clocks: color opponency in non-image-forming visual function. *Neurosci Biobehav Rev*. 2017;78:24–33.
21. Cao D, Chang A, Gai S. Evidence for an impact of melanopsin activation on unique white perception. *J Opt Soc Am A Opt Image Sci Vis*. 2018;35(4):B287–B291.
22. Woelders T, Wams EJ, Gordijn MCM, Beersma DGM, Hut RA. Integration of color and intensity increases time signal stability for the human circadian system when sunlight is obscured by clouds. *Sci Rep*. 2018;8(1):15214.
23. Zele AJ, Feigl B, Adhikari P, Maynard ML, Cao D. Melanopsin photoreception contributes to human visual detection, temporal and colour processing. *Sci Rep*. 2018;8(1):3842.
24. Smith VC, Pokorny J. Spectral sensitivity of the foveal cone photopigments between 400 and 500 nm. *Vision Res*. 1975;15(2):161–171.
25. Weale RA. The post-mortem preservation of the transmissivity of the human crystalline lens. *Exp Eye Res*. 1985;41(5):655–659.
26. Reiter RJ, Richardson BA. Some perturbations that disturb the circadian melatonin rhythm. *Chronobiol Int*. 1992;9(4):314–321.
27. Verriest G. Influence of age on visual functions in humans. *Bull Acad R Med Belg*. 1971;11(8):527–578.
28. Turek FW, Penev P, Zhang Y, van Reeth O, Zee P. Effects of age on the circadian system. *Neurosci Biobehav Rev*. 1995;19(1):53–58.
29. Touitou Y, Haus E. Alterations with aging of the endocrine and neuroendocrine circadian system in humans. *Chronobiol Int*. 2000;17(3):369–390.
30. Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-nerve degeneration in Alzheimer's disease. *N Engl J Med*. 1986;315(8):485–487.
31. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science*. 2016;354(6315):1004–1008.
32. Wu YH, Swaab DF. Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. *Sleep Med*. 2007;8(6):623–636.
33. Toljan K, Homolak J. Circadian changes in Alzheimer's disease: neurobiology, clinical problems, and therapeutic opportunities. *Handb Clin Neurol*. 2021;179:285–300.

34. Gros P, Videnovic A. Overview of sleep and circadian rhythm disorders in Parkinson disease. *Clin Geriatr Med*. 2020;36(1):119–130.
35. Breen DP, Vuono R, Nawarathna U, et al. Sleep and circadian rhythm regulation in early Parkinson disease. *JAMA Neurol*. 2014;71(5):589.
36. De Lazzari F, Bisaglia M, Zordan M, Sandrelli F. Circadian rhythm abnormalities in Parkinson's disease from humans to flies and back. *Int J Mol Sci*. 2018;19(12):3911.
37. Zuzuárregui JRP, During EH. Sleep issues in Parkinson's disease and their management. *Neurotherapeutics*. 2020;17(4):1480–1494.
38. Lin MS, Liao PY, Chen HM, Chang CP, Chen SK, Chern Y. Degeneration of ipRGCs in mouse models of Huntington's disease disrupts non-image-forming behaviors before motor impairment. *J Neurosci*. 2019;39(8):1505–1524.
39. Ouk K, Hughes S, Pothecary CA, Peirson SN, Jennifer Morton A. Attenuated pupillary light responses and down-regulation of opsin expression parallel decline in circadian disruption in two different mouse models of Huntington's disease. *Hum Mol Genet*. 2016;25(24):5418–5432.
40. Adhikari P, Zele AJ, Thomas R, Feigl B. Quadrant field pupillometry detects melanopsin dysfunction in glaucoma suspects and early glaucoma. *Sci Rep*. 2016;6(1):33373.
41. Gao J, Provencio I, Liu X. Intrinsically photosensitive retinal ganglion cells in glaucoma. *Front Cell Neurosci*. 2022;16:992747.
42. Jean-Louis G, Zizi F, Lazzaro DR, Wolintz AH. Circadian rhythm dysfunction in glaucoma: a hypothesis. *J Circadian Rhythms*. 2008;6:1.
43. Bhatwadekar AD, Rameswara V. Circadian rhythms in diabetic retinopathy: an overview of pathogenesis and investigational drugs. *Expert Opin Investig Drugs*. 2020;29(12):1431–1442.
44. Peng X, Fan R, Xie L, et al. A growing link between circadian rhythms, type 2 diabetes mellitus and Alzheimer's disease. *Int J Mol Sci*. 2022;23(1):504.
45. Brüggemann N, Klein C. Parkin type of early-onset Parkinson disease. In: Adam MP, Mirzaa GM, Pagon RA, et al., eds. *GeneReviews*. Seattle, WA: University of Washington; 1993.
46. Riboldi GM, Frattini E, Monfrini E, Frucht SJ, Di Fonzo A. A practical approach to early-onset Parkinsonism. *J Parkinsons Dis*. 2022;12(1):1–26.
47. Sirkis DW, Bonham LW, Johnson TP, La Joie R, Yokoyama JS. Dissecting the clinical heterogeneity of early-onset Alzheimer's disease. *Mol Psychiatry*. 2022;27(6):2674–2688.
48. Mendez MF. Early-onset Alzheimer disease and its variants. *Continuum (Minneap Minn)*. 2019;25(1):34–51.
49. Jia J, Zhang Y, Shi Y, et al. A 19-year-old adolescent with probable Alzheimer's disease. *J Alzheimers Dis*. 2023;91(3):915–922.
50. Shulman JM, De Jager PL, Feany MB. Parkinson's disease: genetics and pathogenesis. *Annu Rev Pathol Mech Dis*. 2011;6(1):193–222.
51. Zappia M, Annesi G, Nicoletti G, et al. Sex differences in clinical and genetic determinants of levodopa peak-dose dyskinesias in Parkinson disease: an exploratory study. *Arch Neurol*. 2005;62(4):601.
52. Neu SC, Pa J, Kukull W, et al. Apolipoprotein E genotype and sex risk factors for Alzheimer disease: a meta-analysis. *JAMA Neurol*. 2017;74(10):1178.
53. Riedel BC, Thompson PM, Brinton RD. Age, APOE and sex: triad of risk of Alzheimer's disease. *J Steroid Biochem Mol Biol*. 2016;160:134–147.
54. Tian GH, Jia N, Lu CF, Zhang XJ. Clinical characteristics of non-arteritic anterior ischemic optic neuropathy. *Zhonghua Yan Ke Za Zhi*. 2009;45(12):1064–1067.
55. Reddy NG, Venkatesh R, Jayadev C, et al. Diabetic retinopathy and diabetic macular edema in people with early-onset diabetes. *Clin Diabetes*. 2022;40(2):222–232.
56. Schmidt TM, Alam NM, Chen S, et al. A role for melanopsin in alpha retinal ganglion cells and contrast detection. *Neuron*. 2014;82(4):781–788.
57. Matynia A, Parikh S, Chen B, et al. Intrinsically photosensitive retinal ganglion cells are the primary but not exclusive circuit for light aversion. *Exp Eye Res*. 2012;105:60–69.
58. Matynia A, Nguyen E, Sun X, et al. Peripheral sensory neurons expressing melanopsin respond to light. *Front Neural Circuits*. 2016;10:60.
59. Benkner B, Mutter M, Ecke G, Münch TA. Characterizing visual performance in mice: an objective and automated system based on the optokinetic reflex. *Behav Neurosci*. 2013;127(5):788–796.
60. Lupi D, Semo M, Foster RG. Impact of age and retinal degeneration on the light input to circadian brain structures. *Neurobiol Aging*. 2012;33(2):383–392.
61. Vugler A, Semo M, Ortín-Martínez A, et al. A role for the outer retina in development of the intrinsic pupillary light reflex in mice. *Neuroscience*. 2015;286:60–78.
62. Subramanian K, Weigert M, Borsch O, et al. Rod nuclear architecture determines contrast transmission of the retina and behavioral sensitivity in mice. *eLife*. 2019;8:e49542.
63. Carido M, Zhu Y, Postel K, et al. Characterization of a mouse model with complete RPE loss and its use for RPE cell transplantation. *Invest Ophthalmol Vis Sci*. 2014;55(8):5431.
64. Pérez de Sevilla Müller L, Solomon A, Sheets K, Hapukino H, Rodriguez AR, Brecha NC. Multiple cell types form the VIP amacrine cell population. *J Comp Neurol*. 2019;527(1):133–158.
65. Pérez de Sevilla Müller L, Shelley J, Weiler R. Displaced amacrine cells of the mouse retina. *J Comp Neurol*. 2007;505(2):177–189.
66. Pérez de Sevilla Müller L, Dedek K, Janssen-Bienhold U, et al. Expression and modulation of connexin 30.2, a novel gap junction protein in the mouse retina. *Vis Neurosci*. 2010;27(3–4):91–101.
67. Pérez de Sevilla Müller L, Do MTH, Yau KW, He S, Baldridge WH. Tracer coupling of intrinsically photosensitive retinal ganglion cells to amacrine cells in the mouse retina. *J Comp Neurol*. 2010;518(23):4813–4824.
68. Thompson S, Recober A, Vogel TW, et al. Light aversion in mice depends on nonimage-forming irradiance detection. *Behav Neurosci*. 2010;124(6):821–827.
69. Semo M, Gias C, Ahmado A, et al. Dissecting a role for melanopsin in behavioural light aversion reveals a response independent of conventional photoreception. *PLoS One*. 2010;5(11):e15009.
70. Keenan WT, Rupp AC, Ross RA, et al. A visual circuit uses complementary mechanisms to support transient and sustained pupil constriction. *eLife*. 2016;5:e15392.
71. Wang HB, Zhou D, Luk SHC, et al. Long wavelength light reduces the negative consequences of dim light at night. *Neurobiol Dis*. 2023;176:105944.
72. Schroeder MM, Harrison KR, Jaeckel ER, et al. The roles of rods, cones, and melanopsin in photoresponses of M4 intrinsically photosensitive retinal ganglion cells (ipRGCs) and optokinetic visual behavior. *Front Cell Neurosci*. 2018;12:203.
73. Berson DM, Castrucci AM, Provencio I. Morphology and mosaics of melanopsin-expressing retinal ganglion cell types in mice. *J Comp Neurol*. 2010;518(13):2405–2422.
74. Sondereker KB, Onyak JR, Islam SW, Ross CL, Renna JM. Melanopsin ganglion cell outer retinal dendrites: morphologically distinct and asymmetrically distributed

Investigative Ophthalmology & Visual Science

in the mouse retina. *J Comp Neurol*. 2017;525(17):3653–3665.
75. Duffy JF, Dijk DJ, Klerman EB, Czeisler CA. Later endogenous circadian temperature nadir relative to an earlier wake time in older people. *Am J Physiol*. 1998;275(5):R1478–R1487.
76. Duffy JF, Zeitzer JM, Czeisler CA. Decreased sensitivity to phase-delaying effects of moderate intensity light in older subjects. *Neurobiol Aging*. 2007;28(5):799–807.
77. Duffy JF, Zeitzer JM, Rimmer DW, Klerman EB, Dijk DJ, Czeisler CA. Peak of circadian melatonin rhythm occurs later within the sleep of older subjects. *Am J Physiol*. 2002;282(2):E297–E303.
78. Cano J, Machado A, Reinoso-Suárez F. Morphological changes in the retina of ageing rats. *Arch Gerontol Geriatr*. 1986;5(1):41–50.
79. Gao H, Hollyfield JG. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 1992;33(1):1–17.
80. Neufeld A. The inherent, age-dependent loss of retinal ganglion cells is related to the lifespan of the species. *Neurobiol Aging*. 2003;24(1):167–172.
81. García-Ayuso D, Di Pierdomenico J, Esquiva G, et al. Inherited photoreceptor degeneration causes the death of melanopsin-positive retinal ganglion cells and increases their coexpression of Brn3a. *Invest Ophthalmol Vis Sci*. 2015;56(8):4592.
82. Harman AM, MacDonald A, Meyer P, Ahmat A. Numbers of neurons in the retinal ganglion cell layer of the rat do not change throughout life. *Gerontology*. 2003;49(6):350–355.
83. Nadal-Nicolás FM, Sobrado-Calvo P, Jiménez-López M, Vidal-Sanz M, Agudo-Barriuso M. Long-term effect of optic nerve axotomy on the retinal ganglion cell layer. *Invest Ophthalmol Vis Sci*. 2015;56(10):6095.
84. Esquiva G, Lax P, Pérez-Santonja JJ, García-Fernández JM, Cuenca N. Loss of melanopsin-expressing ganglion cell subtypes and dendritic degeneration in the aging human retina. *Front Aging Neurosci*. 2017;9:79.
85. Myers BL, Badia P. Changes in circadian rhythms and sleep quality with aging: mechanisms and interventions. *Neurosci Biobehav Rev*. 1995;19(4):553–571.
86. Tales A, Troscianko T, Lush D, Haworth J, Wilcock GK, Butler SR. The pupillary light reflex in aging and Alzheimer's disease. *Aging (Milano)*. 2001;13(6):473–478.
87. Schmidt C, Peigneux P, Cajochen C. Age-related changes in sleep and circadian rhythms: impact on cognitive performance and underlying neuroanatomical networks. *Front Neurol*. 2012;3:118.
88. Hood S, Amir S. The aging clock: circadian rhythms and later life. *J Clin Invest*. 2017;127(2):437–446.
89. Esquiva G, Lax P, Cuenca N. Impairment of intrinsically photosensitive retinal ganglion cells associated with late stages of retinal degeneration. *Invest Ophthalmol Vis Sci*. 2013;54(7):4605.
90. Lax P, Esquiva G, Fuentes-Broto L, et al. Age-related changes in photosensitive melanopsin-expressing retinal ganglion cells correlate with circadian rhythm impairments in sighted and blind rats. *Chronobiol Int*. 2016;33(4):374–391.
91. Semo M, Peirson S, Lupi D, Lucas RJ, Jeffery G, Foster RG. Melanopsin retinal ganglion cells and the maintenance of circadian and pupillary responses to light in aged rodless/coneless (*rd /rd cl*) mice: melanopsin ganglion cells and light responses. *Eur J Neurosci*. 2003;17(9):1793–1801.
92. Zeng Y, Yang K. Sirtuin 1 participates in the process of age-related retinal degeneration. *Biochem Biophys Res Commun*. 2015;468(1–2):167–172.
93. Geng Y, Chen H, Liu L, Lin H, Zhang M. Comparison on photopic electroretinogram negative response between young and old Lewis rats. *Eye Sci*. 2011;26(3):171–172.
94. Chaychi S, Polosa A, Lachapelle P. Differences in retinal structure and function between aging male and female Sprague-Dawley rats are strongly influenced by the estrus cycle. *PLoS One*. 2015;10(8):e0136056.
95. Saftari LN, Kwon OS. Ageing vision and falls: a review. *J Physiol Anthropol*. 2018;37(1):11.
96. Jackson GR, Owsley C. Visual dysfunction, neurodegenerative diseases, and aging. *Neurol Clin*. 2003;21(3):709–728.
97. Owsley C. Vision and aging. *Annu Rev Vis Sci*. 2016;2(1):255–271.
98. Packer M, Ginsburg AP. Contrast sensitivity and aging. *Ophthalmology*. 2007;114(8):1589.
99. Herbst K, Sander B, Lund-Andersen H, et al. Intrinsically photosensitive retinal ganglion cell function in relation to age: a pupillometric study in humans with special reference to the age-related optic properties of the lens. *BMC Ophthalmol*. 2012;12(1):4.
100. Rukmini AV, Milea D, Aung T, Gooley JJ. Pupillary responses to short-wavelength light are preserved in aging. *Sci Rep*. 2017;7(1):43832.
101. Daneault V, Vandewalle G, Hébert M, et al. Does pupil constriction under blue and green monochromatic light exposure change with age? *J Biol Rhythms*. 2012;27(3):257–264.
102. Whiteley SJO, Young MJ, Litchfield TM, Coffey PJ, Lund RD. Changes in the pupillary light reflex of pigmented Royal College of Surgeons rats with age. *Exp Eye Res*. 1998;66(6):719–730.
103. Newman LA, Walker MT, Brown RL, Cronin TW, Robinson PR. Melanopsin forms a functional short-wavelength photopigment. *Biochemistry*. 2003;42(44):12734–12738.
104. Stringham JM, Fuld K, Wenzel AJ. Action spectrum for photophobia. *J Opt Soc Am A Opt Image Sci Vis*. 2003;20(10):1852–1858.
105. Noseda R, Kainz V, Jakubowski M, et al. A neural mechanism for exacerbation of headache by light. *Nat Neurosci*. 2010;13(2):239–245.
106. Main A, Vlachonikolis I, Dowson A. The wavelength of light causing photophobia in migraine and tension-type headache between attacks. *Headache*. 2000;40(3):194–199.
107. Johnson J, Wu V, Donovan M, et al. Melanopsin-dependent light avoidance in neonatal mice. *Proc Natl Acad Sci USA*. 2010;107(40):17374–17378.
108. Amini A, Digre K, Couldwell WT. Photophobia in a blind patient: an alternate visual pathway. Case report. *J Neurosurg*. 2006;105(5):765–768.
109. Matynia A, Parikh S, Deot N, et al. Light aversion and corneal mechanical sensitivity are altered by intrinsically photosensitive retinal ganglion cells in a mouse model of corneal surface damage. *Exp Eye Res*. 2015;137:57–62.
110. McAdams H, Kaiser EA, Igdalova A, et al. Selective amplification of ipRGC signals accounts for interictal photophobia in migraine. *Proc Natl Acad Sci USA*. 2020;117(29):17320–17329.
111. Noseda R, Bernstein CA, Nir RR, et al. Migraine photophobia originating in cone-driven retinal pathways. *Brain*. 2016;139(7):1971–1986.
112. Caval-Holme FS, Aranda ML, Chen AQ, et al. The retinal basis of light aversion in neonatal mice. *J Neurosci*. 2022;42(20):4101–4115.
113. Sorkaç A, Savva YA, Savaş D, Talay M, Barnea G. Circuit analysis reveals a neural pathway for light avoidance in *Drosophila* larvae. *Nat Commun*. 2022;13(1):5274.
114. Georg B, Ghelli A, Giordano C, et al. Melanopsin-expressing retinal ganglion cells are resistant to cell injury, but not always. *Mitochondrion*. 2017;36:77–84.

115. Saw SM, Gazzard G, Shih-Yen EC, Chua WH. Myopia and associated pathological complications. *Ophthalmic Physiol Opt*. 2005;25(5):381–391.
116. Quigley HA. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol*. 2006;90(3):262–267.
117. Weinreb RN, Aung T, Medeiros FA. The pathophysiology and treatment of glaucoma: a review. *JAMA*. 2014;311(18):1901.
118. Chakravarthy U, Evans J, Rosenfeld PJ. Age related macular degeneration. *BMJ*. 2010;340:c981.
119. Janssen SF, Gorgels TGMF, Ramdas WD, et al. The vast complexity of primary open angle glaucoma: disease genes, risks, molecular mechanisms and pathobiology. *Prog Retin Eye Res*. 2013;37:31–67.
120. Kaarniranta K, Uusitalo H, Blasiak J, et al. Mechanisms of mitochondrial dysfunction and their impact on age-related macular degeneration. *Prog Retin Eye Res*. 2020;79:100858.
121. Van Lookeren Campagne M, LeCouter J, Yaspan BL, Ye W. Mechanisms of age-related macular degeneration and therapeutic opportunities: pathology, genetics, animal models, and therapeutic rationale of AMD. *J Pathol*. 2014;232(2):151–164.
122. Faktorovich E, Steinberg R, Yasumura D, Matthes M, LaVail M. Basic fibroblast growth factor and local injury protect photoreceptors from light damage in the rat. *J Neurosci*. 1992;12(9):3554–3567.
123. Liu MM, Zack DJ. Alternative splicing and retinal degeneration: alternative splicing and retinal degeneration. *Clin Genet*. 2013;84(2):142–149.
124. Quigley HA. Neuronal death in glaucoma. *Prog Retin Eye Res*. 1999;18(1):39–57.
125. Vrabec JP, Levin LA. The neurobiology of cell death in glaucoma. *Eye (Lond)*. 2007;21(suppl 1):S11–S14.
126. Chew KS, Renna JM, McNeill DS, et al. A subset of ipRGCs regulates both maturation of the circadian clock and segregation of retinogeniculate projections in mice. *eLife*. 2017;6:e22861.
127. Zhuang X, Tran T, Jin D, Philip R, Wu C. Aging effects on contrast sensitivity in visual pathways: a pilot study on flicker adaptation. *PLoS One*. 2021;16(12):e0261927.
128. Vit JP, Fuchs DT, Angel A, et al. Color and contrast vision in mouse models of aging and Alzheimer's disease using a novel visual-stimuli four-arm maze. *Sci Rep*. 2021;11(1):1255.
129. Sugita Y, Yamamoto H, Maeda Y, Furukawa T. Influence of aging on the retina and visual motion processing for optokinetic responses in mice. *Front Neurosci*. 2020;14:586013.
130. Videnovic A, Lazar AS, Barker RA, Overeem S. 'The clocks that time us'—circadian rhythms in neurodegenerative disorders. *Nat Rev Neurol*. 2014;10(12):683–693.
131. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. *Science*. 2016;354(6315):1004–1008.
132. Karasek M, Reiter RJ. Melatonin and aging. *Neuro Endocrinol Lett*. 2002;23(suppl 1):14–16.
133. Neikrug AB, Ancoli-Israel S. Sleep disorders in the older adult – a mini-review. *Gerontology*. 2010;56(2):181–189.
134. Feigl B, Mattes D, Thomas R, Zele AJ. Intrinsically photosensitive (melanopsin) retinal ganglion cell function in glaucoma. *Invest Ophthalmol Vis Sci*. 2011;52(7):4362.
135. Kankipati L, Girkin CA, Gamlin PD. The post-illumination pupil response is reduced in glaucoma patients. *Invest Ophthalmol Vis Sci*. 2011;52(5):2287.
136. Nissen C, Sander B, Milea D, et al. Monochromatic pupillometry in unilateral glaucoma discloses no adaptive changes subserved by the ipRGCs. *Front Neurol*. 2014;5:15.
137. Obara EA, Hannibal J, Heegaard S, Fahrenkrug J. Loss of melanopsin-expressing retinal ganglion cells in severely staged glaucoma patients. *Invest Ophthalmol Vis Sci*. 2016;57(11):4661.
138. Oh AJ, Amore G, Sultan W, et al. Pupillometry evaluation of melanopsin retinal ganglion cell function and sleep-wake activity in pre-symptomatic Alzheimer's disease. *PLoS One*. 2019;14(12):e0226197.
139. Mattis J, Sehgal A. Circadian rhythms, sleep, and disorders of aging. *Trends Endocrinol Metab*. 2016;27(4):192–203.
140. La Morgia C, Ross-Cisneros FN, Sadun AA, Carelli V. Retinal ganglion cells and circadian rhythms in Alzheimer's disease, Parkinson's disease, and beyond. *Front Neurol*. 2017;8:162.
141. La Morgia C, Ross-Cisneros FN, Hannibal J, Montagna P, Sadun AA, Carelli V. Melanopsin-expressing retinal ganglion cells: implications for human diseases. *Vision Res*. 2011;51(2):296–302.
142. La Morgia C, Carelli V, Carbonelli M. Melanopsin retinal ganglion cells and pupil: clinical implications for neuro-ophthalmology. *Front Neurol*. 2018;9:1047.
143. La Morgia C, Ross-Cisneros FN, Koronyo Y, et al. Melanopsin retinal ganglion cell loss in Alzheimer disease. *Ann Neurol*. 2016;79(1):90–109.
144. Tales A, Snowden RJ, Phillips M, et al. Exogenous phasic alerting and spatial orienting in mild cognitive impairment compared to healthy ageing: study outcome is related to target response. *Cortex*. 2011;47(2):180–190.
145. Iseri PK, Altinaş O, Tokay T, Yüksel N. Relationship between cognitive impairment and retinal morphological and visual functional abnormalities in Alzheimer disease. *J Neuroophthalmol*. 2006;26(1):18–24.
146. Tranah GJ, Blackwell T, Stone KL, et al. Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. *Ann Neurol*. 2011;70(5):722–732.
147. Hannibal J, Vrang N, Card JP, Fahrenkrug J. Light-dependent induction of cFos during subjective day and night in PACAP-containing ganglion cells of the retinohypothalamic tract. *J Biol Rhythms*. 2001;16(5):457–470.
148. Robinson GA, Madison RD. Axotomized mouse retinal ganglion cells containing melanopsin show enhanced survival, but not enhanced axon regrowth into a peripheral nerve graft. *Vision Res*. 2004;44(23):2667–2674.
149. Nadal-Nicolás FM, Sobrado-Calvo P, Jiménez-López M, Vidal-Sanz M, Agudo-Barriuso M. Long-term effect of optic nerve axotomy on the retinal ganglion cell layer. *Invest Ophthalmol Vis Sci*. 2015;56(10):6095.
150. Pérez De Sevilla Müller L, Sargoy A, Rodriguez AR, Brecha NC. Melanopsin ganglion cells are the most resistant retinal ganglion cell type to axonal injury in the rat retina. *PLoS One*. 2014;9(3):e93274.

Investigative Ophthalmology & Visual Science