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REVIEW

Melanopsin-Expressing Intrinsically Photosensitive Retinal Ganglion Cells in Retinal Disease

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ABSTRACT

Melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are a class of photoreceptors with established roles in non–image-forming processes. Their contributions to image-forming vision may include the estimation of brightness. Animal models have been central for understanding the physiological mechanisms of ipRGC function and there is evidence of conservation of function across species. Intrinsically photosensitive retinal ganglion cells can be divided into five ganglion cell subtypes that show morphological and functional diversity. Research in humans has established that ipRGCs signal environmental irradiance to entrain the central body clock to the solar day for regulating circadian processes and sleep. In addition, ipRGCs mediate the pupil light reflex (PLR), making the PLR a readily accessible behavioral marker of ipRGC activity. Less is known about ipRGC function in retinal and optic nerve disease, with emerging research providing insight into their function in diabetes, retinitis pigmentosa, glaucoma, and hereditary optic neuropathy. We briefly review the anatomical distributions, projections, and basic physiological mechanisms of ipRGCs and their proposed and known functions in animals and humans with and without eye disease. We introduce a paradigm for differentiating inner and outer retinal inputs to the pupillary control pathway in retinal disease and apply this paradigm to patients with age-related macular degeneration (AMD). In these cases of patients with AMD, we provide the initial evidence that ipRGC function is altered and that the dysfunction is more pronounced in advanced disease. Our perspective is that with refined pupillometry paradigms, the PLR can be extended to AMD assessment as a tool for the measurement of inner and outer retinal dysfunction.
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Key Words: melanopsin-containing intrinsically photosensitive retinal ganglion cells, pupil light reflex, age-related macular degeneration, ipRGC, PIPR

Melanopsin-expressing intrinsically photosensitive retinal ganglion cells (ipRGCs) are the third photoreceptor class in the eye.[1–3] Intrinsically photosensitive retinal ganglion cells are an atypical photoreceptor type separate from the rod and cone photoreceptor classes that have an intrinsic photoresponse and extrinsically transmit outer retinal photoreception. They signal locally within the retina and distribute light information across more than a dozen distinct brain regions.[1,2,4–8] The primary function of ipRGCs is for non–image-forming photoreception, but emerging evidence indicates that they have roles in image-forming vision.[8,9] The non–image-forming functions include the signaling of environmental irradiance level to entrain the central body clock located in the suprachiasmatic nucleus (SCN) to the solar day to maintain the circadian rhythm to near a 24-hour day-and-night cycle and for mediating the pupil light reflex (PLR) via signaling to the olivary pretectal nucleus (OPN).[1,2,10–12] The most prominent ipRGC contribution to the PLR is the postillumination pupil response (PIPR), the sustained constriction after offset of high-irradiance, short-wavelength light; this characteristic affords the direct measurement of ipRGC function in humans.[11,13,14] Although there is a long history of research of conventional retinal ganglion cell morphology, physiology, connectivity, function, and central projections,[3,15] ipRGC research into their subtypes, central projections, and function is still in its infancy. Moreover, our knowledge is predominantly derived from transgenic animal models (for comprehensive reviews, see Refs.[3,14,16]) and

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new areas of investigations are beginning to define the functional roles of ipRGCs in humans,[11,17–19] with important reference to applications in the detection and monitoring of inner and outer retinal disease.[14,20–25] This review will consider the effect of retinal disease on ipRGC function and will introduce new paradigms for measuring inner and outer retinal function in age-related macular degeneration (AMD).

ipRGCs: THE "NOVEL-OLD" CELL

The initial evidence of a third photoreceptor class was available as early as 1927 when Keeler[26] demonstrated that mice with severe outer retinal photoreceptor loss retained a pupil light response. More recently, normal photoentrainment and pupil responses were observed in humans who were blind owing to extensive outer retinal damage.[27] The photopigment melanopsin (opn4) was first discovered in the frog (*Xenopus laevis*) skin melanophores, deep brain nuclei, the iris and retina,[4] and then in a distinct ganglion cell population in humans, the ipRGCs.[8,28] The melanopsin photopigment is diffusely expressed along the dendrites and soma of ipRGCs (~3 molecules μm^{-2})[7] and is lower in density compared with rod and cone photopigments (~25,000 molecules μm^{-2}), but the melanopsin signal amplification is higher. Whereas rods and cones signal with graded membrane voltages, melanopsin phototransduction shows different electrophysiological responses to light; ipRGCs signal to the brain using action potentials (spikes), the single photon absorption response is larger than in rods,[7] and their response is sluggish in onset and slow in termination,[1] lasting some 10 seconds, which is about 100-fold longer than that in cones and 20-fold longer than that in rods.[7] Recent measurements show that 10 hours of constant light activation of ipRGCs continuously evoke action potentials, such that irradiance changes can feasibly be tracked during the day.[29] The long operational timescales and slow kinetics of ipRGCs increase sensitivity through long temporal summation.

Evidence from studies of the human PLR indicates that melanopsin is a bistable photopigment, and unlike conventional photopigments that are dependent on exogenous supply of a chromophore, melanopsin is thought to regenerate from photoconversion.[17] The intrinsic ipRGC response gives maximum depolarization in response to short-wavelength (blue appearing light), high–retinal irradiance (>~11.5 log quanta s^{-1} cm^{-2}) lights with a λ_{max} of about 482 nm.[1,8,11] The data in Fig. 1 show that the peak opn4 ipRGC spectral sensitivity derived from a criterion PIPR in humans is positioned in the short-wavelength region of the spectrum between the S-cone and rod nomograms. The half-maximal PIPR occurs at a retinal irradiance of about 13.7 log quanta s^{-1} cm^{-2} in humans. The ipRGCs also receive extrinsic input from rods and cones as shown in mice and primates,[8,30,31] presumably via bipolar (excitatory) and amacrine (inhibitory) cells[16,31] that subserve a faster temporal response than the melanopsin-elicited intrinsic response.[32] Intrinsically photosensitive retinal ganglion cells are thought to have unmyelinated axons consistent with the slow conduction velocities of fibers within the retinohypothalamic tract as shown in studies of primates.[33]

MORPHOLOGICAL DIVERSITY OF ipRGCs

Since their initial discovery, five ipRGC subtypes (M1 to M5) have been identified using transgenic mouse models. They are defined based on the stratification of their dendrites within the extreme outer and inner laminae of the inner plexiform layer (IPL). Three subtypes have been identified in rats, with dendrites stratifying in either the outer margin (M1) or inner side (M2) of the IPL, or stratifying in both the outer and inner plexuses (M3).[34] Similar dendritic stratifications have been described for M1 and

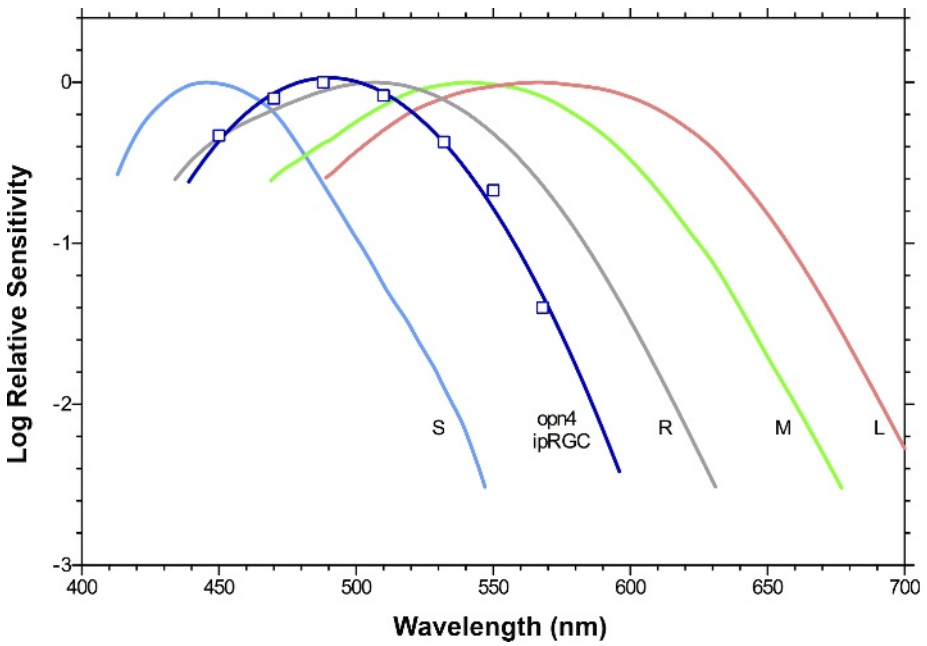

FIGURE 1.

Visual pigment nomogram of the opn4 melanopsin derived from a criterion PIPR (square symbols) in a human participant closely matches measurements of ipRGCs from *in vitro* primate retinal preparations.[8] The rod (R) and S-, M-, and L-cone corneal spectral sensitivities of Smith and Pokorny are also shown. Modified after Markwell et al.[14] A color version of this figure is available online at www.optvissci.com.

M2 cells in primate retinae.[31] Intrinsically photosensitive retinal ganglion cells have the largest identified retinal ganglion cell dendritic fields (~400 to 1200 μm) but have small somata and represent only a small subset (~3000 cells or ~0.2 to 4%) of the total ganglion cell population.[8] The dendritic fields form a photoreceptive network that is concentrated parafoveally as evidenced in macaque retinas.[8] The ipRGC subclasses show morphological and functional diversity and the different cortical projections are thought to evoke different behaviors (for a review, see Ref. [16]). Given that there appears to be some conservation of ipRGC subtypes and pathways between species,[35] the quantification of ipRGC structure and function in animal models will promote development of new methods for observing ipRGC activity in humans. In brief, M1 cell's dendrites stratify in the OFF sublamina (outer IPL), whereas M2 subclasses stratify in the inner IPL (ON sublamina). M3 cells are bistratified and extend their dendrites into both sublaminae. There are discrepancies in the estimates of the relative proportions of these three subclasses, with the proportion of M1 cells varying between 22 and 68%, the proportion of M2 cells varying between 40 and 53%, and the proportion of M3 cells varying between 7 and 26%,[36,37] the variation possibly arising because of methodological differences in their labeling. Moreover, dendritic arbors of M1 and M2 subtypes show a large amount of overlap[38] with M2 subtypes having larger and complex dendritic fields and larger somata compared with M1 subtypes[16,39]; M1 cells display larger membrane depolarization compared with M2 and are about 10-fold more sensitive to light.[40] Primate M1 cells also show intraretinal branching to provide synaptic feedback, an atypical morphological feature of ganglion cells exiting the retina.[35] The M3 subtype is morphologically comparable to M2,[40] but its dendrites are absent in some areas of the retina[38]; hence, M3 subtypes might only play a role in non–image-forming visual processes because complete coverage of the visual field by ganglion cells is important for image-forming vision.[40]

In mammals, M1 cells have the highest expression of the opn4 melanopsin photopigment followed by M2 and M3. In mice, M1 and M2 cells primarily receive excitatory inputs from ON pathways and M1 exhibiting much larger synaptic responses than M2 cells.[40] There is evidence in mice that M1 and M2 convey light information differently with M2 being more reliant on outer retinal synaptic inputs than M1 cells that seem to respond to light using the intrinsic melanopsin pathway only.[40] Primate ipRGCs also have a spatially overlapping, color-opponent (L+M-cone)-ON and (S-cone)-OFF receptive field structure, with projections to the LGN.[8] Two additional subtypes of ipRGC cells have been discovered in mice. These are classified as M4 and M5 and stratify in the inner sublamina, with M4 being the largest of all ipRGC subtypes.[40] M4 and M5, however, do not show melanopsin immunostaining but are still capable of a weak intrinsic response.[39] Although both M1 and M2 cell subtypes are found in primate retinae,[8,35] it remains subject to further in-depth investigations if the subtypes have similar characteristics to those as shown in rodents.

ipRGC PROJECTIONS AND THEIR FUNCTIONAL CHARACTERISTICS

The axons of an ipRGC can branch out to multiple brain regions.[41] In rodents, about 80% of M1 cells project to the SCN, the master circadian clock.[37] Similarly, 80% of M1 and M2 cells project to the OPN, the control center of the pupillary light reflex, with M1 cells predominantly projecting to the OPN shell and a larger amount of non-M2 cells predominantly projecting to the OPN core.[37] The reason for differential inputs from M1 and M2 to the SCN and OPN is unknown, but it is thought that it may play a role in the overall dynamic range of the response to retinal irradiance.[37] M1 ipRGCs are considered to predominantly drive the PLR in rodents and humans.[11,42] There is evidence that Brn3b transcription factor–negative M1 cells in mice innervate the SCN, whereas Brn3b-positive M1 cells project to all other brain regions receiving ipRGC inputs, including those for the pupil control pathway, yet these cells have the same morphological and electrophysiological characteristics.[43]

ipRGC subtypes further project to the intergeniculate leaflet, the center for circadian entrainment; the ventrolateral preoptic nucleus, the control center of sleep; the dorsal and ventral lateral geniculate nucleus (LGN); the lateral habenula; the medial amygdala; the supraoptic nucleus; the posterior pretectal nucleus; the superior colliculus; and many more brain regions.[5,9,39] Although the projections of M3 cells are not known, most of the M2, M4, and M5 cells project to dorsal LGN, suggestive of a role for these subclasses in image-forming vision, in addition to projections to the core of the OPN, but for which there is presently no assigned function.[39]

Melanopsin-derived activity in the normal mouse dorsal LGN is evident as a prolonged firing of neurons when stimulated with long-duration, high-irradiance, short-wavelength stimuli, and the discrimination of high-irradiance lights from a dark background in rodless/coneless mice may reflect melanopsin signaling.[9] Given that bipolar cells and conventional ganglion cells signal contrast, determining the role of melanopsin for signaling the perceptual correlate of brightness will be important for explaining human behavioral magnitude estimation of brightness[44] and luxotonic units in the visual cortex as identified in cat[45] and macaque.[46] Melanopic metamers can produce perceptible changes in human brightness perception, but not in chromaticity,[47] although the cone-opponent receptive fields in primate ipRGCs[8] indicate that brightness changes should be accompanied by a chromaticity change. As the understanding of ipRGC contributions to image-forming vision advances, there will likely be a redefining of the standard model of human trichromacy[48] and of photometry and melanopsin photoreception.[49]

There is emerging evidence that light information mediated via ipRGCs can directly influence higher cognitive function and brain processing for emotions.[50,51] Studies demonstrate that ipRGCs can influence mood and learning through projections to the limbic areas of the brain including the lateral habenula and the medial amygdala.[2,5,51] Aberrant light cycles can cause depression-like behaviors in animals with intact ipRGCs, whereas ipRGC knock out animals do not have these symptoms.[51] A link between ipRGCs and exacerbation of migraine headache by light has been proposed based on observations that axons from ipRGCs project to dura-sensitive neurons in the posterior thalamus.[52] Photosensitive blind individuals with migraine still show a PLR and photoentrainment indicative of functional ipRGCs. Light has also been shown to enhance learned fear in transgenic mice, and that this requires signaling via ipRGC pathways.[50] The development

of new assessment paradigms in humans, especially through use of the PLR, will provide novel techniques for assessing behaviors beyond its traditional application as an objective measure of visual and pupillary pathways linking midbrain and autonomic function.

THE LIGHT REFLEX OF THE PUPIL: AN OBJECTIVE, BEHAVIORAL MEASURE OF INNER AND OUTER RETINAL FUNCTION

Since Loewenfeld's[53] seminal contribution on the pupil, the ipRGCs have been identified as a primary neural substrate of the pupillary control pathway. The PLR is the only measureable, noninvasive physiological response to directly reflect the behavior of the three retinal photoreceptor classes in the human eye: rods, cones, and ipRGCs. As such, noninvasive pupillometry techniques afford objective measurement of inner retinal (ipRGC) function and outer retinal (rod and cone) function[13,14,19,21,25,54,55] in response to high–retinal irradiance, long- and short-wavelength lights that favor outer and inner retinal responses, through analysis of the components of the light response of the pupil including the latency to constriction, the transient pupil response, the constriction amplitude,[54,55] the PIPR amplitude, and redilation time constant (Fig. 2).[13,14,18,20–22,24,54–56]

The current understanding is that the initial pupil constriction amplitude is mediated by outer retinal (rod and cone) contributions.[13,42] The outer retinal contribution to steady-state pupillary response is dominated by rods with a smaller contribution by cones for light presentations of shorter than 10 seconds; the ipRGC contribution increases with longer presentation durations, but the rod photoreceptor still makes large contributions.[13] The L- and M-cone contribution to the steady-state pupil diameter is more than a factor of three less than the ipRGC contribution.[57] At light levels above which rods are incapable of supporting image-forming vision, rods signal via the rod-cone pathway and extrinsically via the ipRGC pathway for circadian photoentrainment.[58]

The inner retinal contribution from intrinsic ipRGC activity is observed as a sustained constriction of the PIPR after offset of the high-irradiance, short-wavelength light (Fig. 2)[11]; the PIPR can be measured as a percentage (or millimeter difference) to the resting baseline pupil diameter during the plateau, which is typically 30 seconds after light offset[11,14,19]; as the net PIPR, which is the difference in the plateau amplitude of the long and short wavelength[18]; as the amplitude at 6 seconds postillumination[21]; and as the early and late area under the curve (AUC).[56] That the sustained PIPR derived from the plateau metric is controlled by the ipRGC photoresponse when assayed with high-irradiance, 10-second light pulses has been confirmed by spectral sensitivity of the plateau PIPR by our group (Fig. 1) and Gamlin and colleagues.[11,14] Additional PIPR metrics such as the 6-second metric and early and late AUC have not been confirmed by spectral sensitivity but are most likely controlled by the intrinsic ipRGC response as the PIPR amplitude is wavelength and irradiance dependent.

Despite recent advances in understanding ipRGC function in nocturnal animal models (e.g., mice), there are significant knowledge gaps about how the fundamental properties and functional

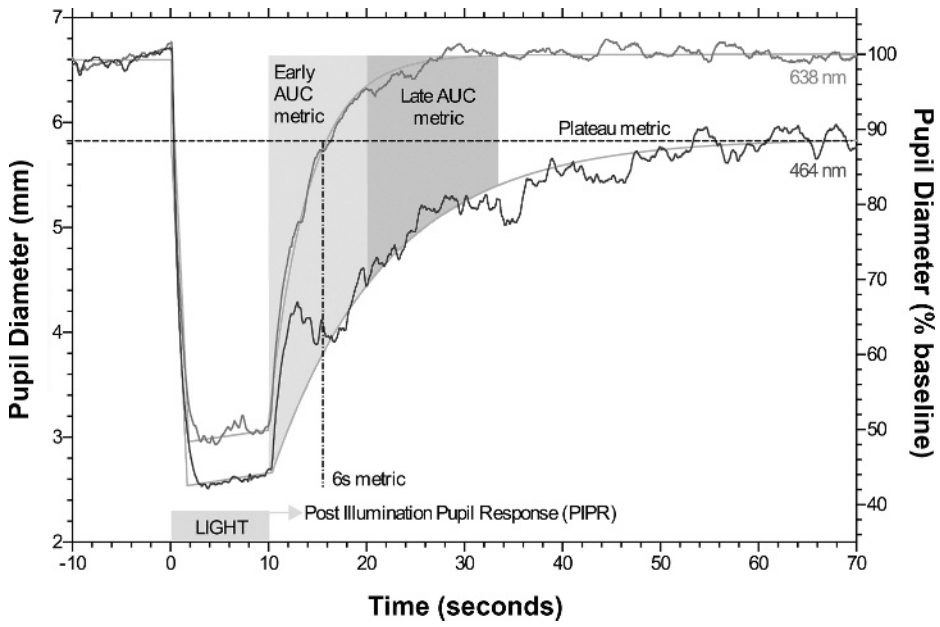

FIGURE 2.

The consensual PLR of the left eye of a healthy young participant with no retinal abnormalities (37 years old; VA, 6/6) in response to a 10-second, 464- or 638-nm rectangular light pulse with a corneal irradiance of 14.5 log photon $cm^{-2}\,s^{-1}$ centered on the pupil of the undilated fellow right eye in Maxwellian view (retinal irradiance, 464 nm = 14.15 log photon $cm^{-2}\,s^{-1}$; 638 nm = 14.35 log photon $cm^{-2}\,s^{-1}$). The PLR is indicated by the thick (638 and 464 nm) traces. Thin traces show the linear and exponential model fits. The plateau metric (horizontal dashed line) quantifies the PIPR response to the 464-nm light as approximately 88% of the initial prestimulus baseline pupil diameter after 60 seconds (12% net PIPR; recovery rate = -0.09 mm s^{-1}), whereas the PIPR to the 638-nm light of the same irradiance returns to the baseline pupil diameter within 20 seconds postillumination (recovery rate = -0.25 mm s^{-1}). The 6-second metric measures the redilation amplitude 6 seconds after light offset (60%, net PIPR = 28%). The 464-nm early AUC (10 to 20 seconds early AUC) PIPR is 237.1 (113.8 for the 638-nm light), and the late AUC PIPR (20 to 40 seconds) is 266.4 (3.3 for the 638-nm light). A color version of this figure is available online at www.optvissci.com.

signatures of the ipRGC light response translate to diurnal humans. There are few investigations of these unique cells and their various roles in human eye diseases. Our group established the role of ipRGCs in the functional differentiation of early and advanced glaucoma[25] and in the measurement of the progression of diabetes using the PLR.[54] The study of ipRGC function in advanced glaucoma has shown that the PIPR amplitude correlates with the visual field defect.[20] A study by La Morgia et al.[59] observed that ipRGCs are resistant in mitochondrial optic neuropathies such as Leber hereditary optic neuropathy and dominant optic atrophy. Retinitis pigmentosa (RP) has been studied in humans[14,21,23,60] using the PLR to differentiate between extrinsic (rod and cone) and intrinsic ipRGC contributions, showing that extrinsic and intrinsic losses increase with disease progression.[23] Morphological studies in a rat model of RP show that ipRGC density and dendritic arborization decrease in advanced stages of the disease.[34] Persons with seasonal affective disorder (SAD) have a reduced PIPR, indicative of altered light signaling via ipRGCs and may have a genetic variation within the opn4 gene, suggestive of a possible role of ipRGCs in its pathogenesis.[61,62]

Under experimental conditions controlling exogenous cues of circadian activity, our laboratory provided the initial evidence that ipRGCs have a circadian response synchronized to melatonin onset in humans whereas outer retinal inputs to the pupil did not,[19] thereby indicating the PIPR as a noninvasive marker of the circadian rhythm. Munch et al.[63] have independently confirmed this influence of the circadian clock on ipRGC inputs to the PIPR function. As the current test protocols are refined and new, rapid test methodologies emerge, the PLR assessment of inner and outer retinal dysfunction in retinal disease will find new roles in the detection and monitoring of progression of retinal and optic nerve disease and for the assessment of circadian function and dysfunction.

THE PLR IN AMD

The primary anatomical and functional changes observed in AMD occur in the paracentral retina[64]; ipRGCs spiral around the foveal pit and have their highest distribution paracentrally,[8] thus making these cells a likely target in this condition. In particular, AMD affects the outer and inner retinal layers, including retinal ganglion cells in advanced stages.[65,66] There is histological evidence of an age-related loss of ganglion cells and almost 50% loss of ganglion cells in neovascular AMD.[65] The effect of age on the ipRGC-controlled PIPR, however, has been considered in only two studies: one showed that the PIPR was independent of age[18] and the other showed enhanced pupil responses in healthy older persons[24]; hence, further investigations are required to understand these relationships.

Although established psychophysical methods such as dark adaptation,[67] mesopic vision,[68] flicker perimetry,[69] and electrophysiological techniques[70,71] can be effective and valuable for determining functional deficits in different retinal layers and different stages of AMD, they are limited to the measurement of specific retinal layers, do not assess inner and outer retina simultaneously under the same test and adaptation conditions, and can be time consuming. Pupil measurements have been recorded in AMD, although they only assessed outer retinal (rod and/or cone) contributions to the pupillary control pathway,[72,73] and the studies generally observed that pupil responses on the measured variables were dysfunctional. The measurement of ipRGC function in AMD using pupil paradigms remains to be determined.

The PLR response to high-irradiance rectangular light pulses (e.g., 10-second pulse as shown in Fig. 2) is now routinely measured in clinical studies,[18–20,23,54] and here we introduce a sinusoidal test paradigm that allows the study of inner and outer retinal contributions to the phasic pupil response. Informed consent was obtained from all participants, and the experiments were approved by the Queensland University of Technology Human Ethics Committee and conducted in accordance with the principles expressed in the Declaration of Helsinki. Fig. 3 shows the PLR of a 59-year-old healthy female control participant (visual acuity [VA], 6/6) without any ocular disease. The pupil trace is the response to a large 34-degree-diameter, 0.5-Hz sinewave stimulus (6 cycles, 11.9-second duration; 464 nm or 635 nm) centered on the pupil in Maxwellian view and with a corneal irradiance of 15.1 log photon $cm^{-2} s^{-1}$. As per the response to a 10-second pulse (Fig. 2), the sustained PIPR for the 0.5-Hz stimulus is observed after offset of the short-wavelength (464 nm) stimulus light, and the response to the control long-wavelength stimulus (638 nm) returns to baseline within about 20 seconds after light offset. As for the pulsed stimuli, metrics are available to quantify outer retinal function (e.g., maximum and transient constriction amplitudes) and inner retinal function (e.g., PIPR metrics). In addition, the phasic pupillary response to the sinewave stimulus allows analysis of the phase and peak-to-trough amplitude during the sinusoidal stimulus presentation. A "phase amplitude percentage" (PAP) parameter can then be determined from the average long-wavelength (638 nm) and short-wavelength (464 nm) peak-to-trough phase amplitudes according to equation 1:

$$\left(\frac{638\ \text{nm} - 464\ \text{nm}}{638\ \text{nm}}\right) \times 100 \qquad (1)$$

The PAP metric (equation 1) reflects inner and outer retinal interactions. For retinal irradiances below melanopsin threshold that are driven by rods and cones only,[74,75] the peak-to-trough amplitudes of the phasic response for long and short wavelengths are similar (i.e., the PAP approaches zero). However, for retinal irradiances above melanopsin threshold where the phasic response is predominantly driven by cones but with ipRGC contributions[75] (Fig. 3), the short-wavelength peak-to-trough phase amplitude is lower relative to the long-wavelength amplitudes (i.e., the PAP is nonzero), possibly due to ipRGC contributions that are inhibitory in nature.[31]

In the following, we present a framework and application of the PLR as an objective behavioral measure of inner and outer retinal function in AMD. We propose that for light levels that activate melanopsin, the PAP and the PIPR metrics will be reduced if disease causes an alteration in ipRGC function. Given that PLR further provides a measure of rod and cone function as derived from the transient pupil constriction or the amplitude of constriction, these components may be reduced owing to disease causing outer retinal deficits. The relative level of defect observed for a recording condition will depend on test parameters including the stimulus size[76] and retinal irradiance[14,21,75]; larger stimulus

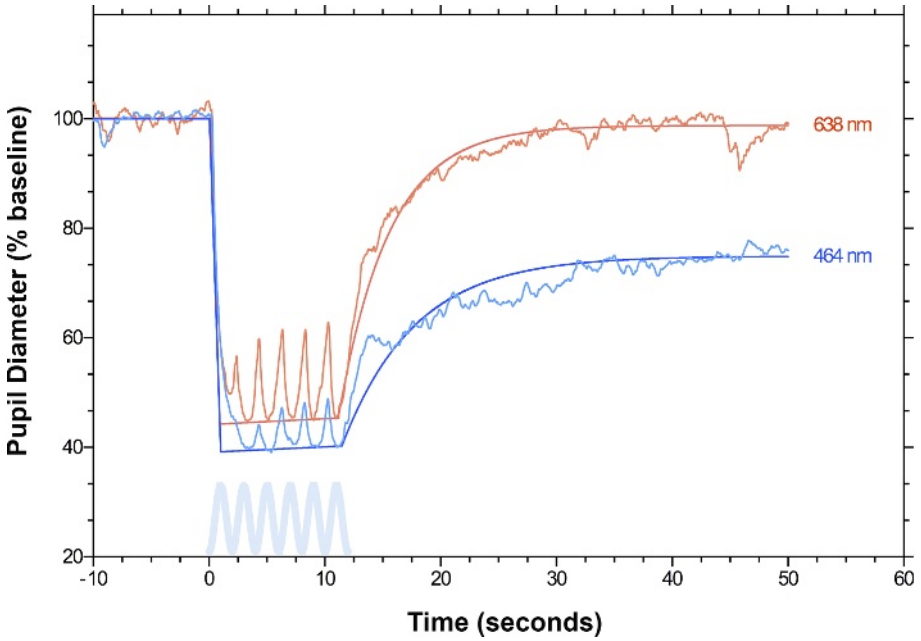

FIGURE 3.

The consensual PLR of the left eye of a healthy older participant with no retinal abnormalities (59 years old; VA, 6/6) measured in response to a 0.5-Hz sinewave stimulus (6 cycles, 11.9-second duration; sinewave shown on the *x*-axis) with a corneal irradiance of 15.1 log photon cm^{-2} s^{-1} centered on the pupil of the dilated (tropicamide 1%) fellow right eye in Maxwellian view (retinal irradiance, 464 nm = 14.6 log photon cm^{-2} s^{-1}; 638 nm = 14.90 log photon cm^{-2} s^{-1}). The PAP difference between short- and long-wavelength peak-to-trough amplitudes during the sinusoidal stimulus presentation is thought to reflect the interaction between inner and outer retinal contributions to the pupil control pathway. A color version of this figure is available online at www.optvissci.com.

sizes will be more sensitive to inner retinal function owing to the larger receptive fields of ipRGCs (conversely, smaller stimulus sizes are more sensitive to outer retinal dysfunction,[77] and retinal irradiances below melanopsin threshold provide isolation of outer retinal function).[14,21] When this framework is applied to AMD, distinct patterns of inner and outer retinal functional deficits should be apparent depending on the AMD stage, as predicted from histological,[66] psychophysical, and electrophysiological data.[78] Here, we focus on stimulus conditions designed to optimize ipRGC activation.

In the following exemplars in Fig. 4, we report the PLR in two AMD stages (early and neovascular) using large stimuli and high retinal irradiances (cf. Fig. 3) to illustrate the effect of manifest AMD on ipRGC function. Fig. 4A shows the PLR for a male patient (76 years old; VA, 6/6 both eyes) with early AMD (intermediated drusen >125 μm; AREDS [Age-Related Eye Disease Study] classification), and Fig. 4B shows the PLR for a female patient (74 years old; VA, 6/12 both eyes) with advanced neovascular AMD (AREDS 4b) who is currently undergoing anti-VEGF treatment in both eyes. Table 1 shows the outer and inner retinal metrics and includes the confidence limits of a healthy control group for comparison (n = 5; mean, 60 years; range, 56 to 69 years; three women, two men; VA, 6/6). Of note, the PIPR metrics are reduced, indicating that both the early and neovascular AMD patients have altered inner retinal ipRGC inputs to the pupillary control pathway, with the late AMD patient having predominantly a larger level of ipRGC dysfunction. There is also evidence of outer retinal dysfunction with these large, high-irradiance stimuli, in accordance with photoreceptor alterations that can occur with drusen as shown with psychophysical methods in early AMD. Future investigations are now required to comprehensively study ipRGC function in AMD and its relationship to outer retinal function with a view to developing these novel test protocols for quantifying retinal inputs to the pupillary control pathway to determine different stages of disease and possibly for monitoring progression.

CONCLUSIONS AND FUTURE DIRECTIONS

Light is required every day, with very specific irradiance, duration, and timing, to reset the circadian "body clock" and to regulate many neuronal processes. This light is received, transduced, and transmitted to the brain by ipRGCs. Research is now beginning to discover the roles of ipRGCs in human eye disease, with advances identifying the roles of ipRGCs in different stages of glaucoma,[20,25] RP,[14,21,23,34,60] Leber hereditary optic neuropathy and dominant optic atrophy,[59] diabetic retinopathy,[54] and circadian health.[19,61,63] It is now becoming clear that the PLR will have a role as a rapid clinical assessment tool to simultaneously determine inner and outer retinal function in patients with eye diseases including AMD. Novel pupil paradigms and metrics such as the sinusoidal stimuli protocol proposed here may be particularly helpful in discriminating functional impairment in AMD, in addition to other retinal/optic nerve disease, and research is ongoing to understand the sensitivity and specificity of these tests for detection and the monitoring of progression.

ipRGCs signal to brain areas linked with depression[51] and sleep,[1,11] but whether reduced ipRGC function is associated with depression and sleep disorders that are commonly found in AMD (and other ocular disease) is still to be determined. At present,

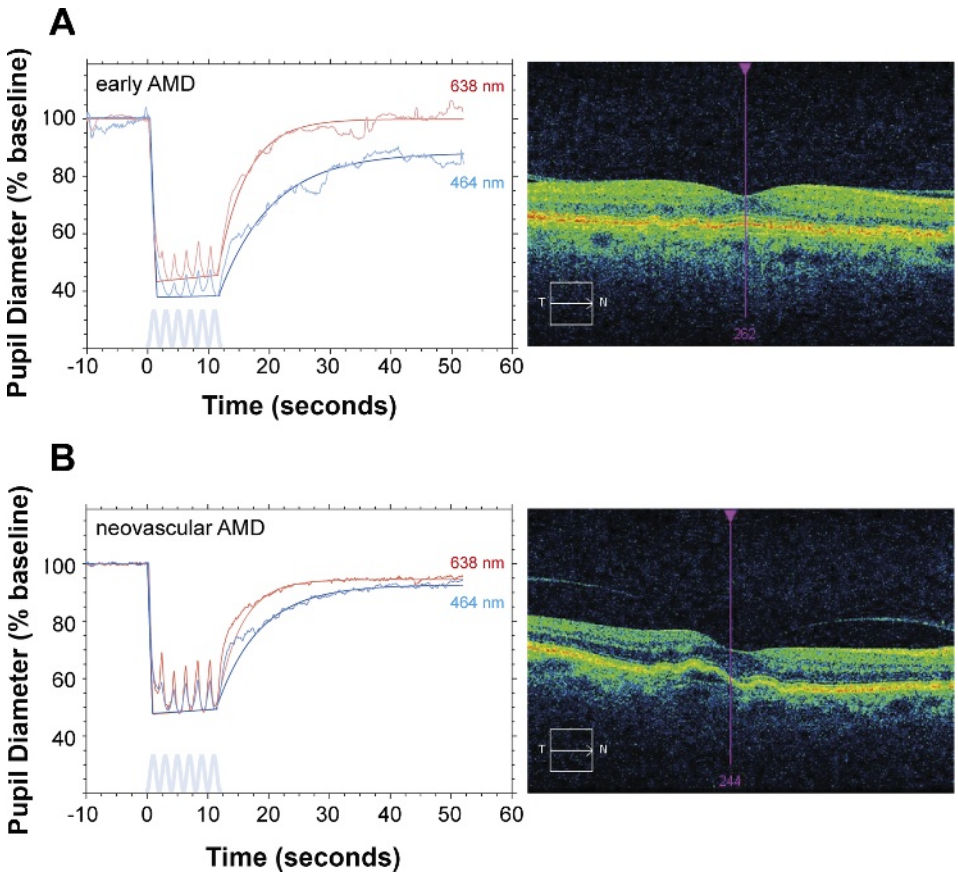

FIGURE 4.

The data in the left column show the consensual PLR of the left eye of patients with AMD measured in response to a 0.5-Hz sinewave stimulus (6 cycles, 11.9-second duration; sinewave shown on the *x*-axis) with a corneal irradiance of 15.1 log photon cm^{-2} s^{-1} centered on the pupil of the dilated (tropicamide 1%) fellow right eye in Maxwellian view. The images in the right column show the central retina as obtained with optical coherence tomography (Cirrus OCT, Zeiss, Germany). (A) A patient (male, aged 75 years; VA, 6/7.5) with drusen owing to early AMD (retinal irradiance, 464 nm = 14.45 log photon cm^{-2} s^{-1}; 638 nm = 14.93 log photon cm^{-2} s^{-1}). (B) A patient (female, aged 74 years; VA, 6/12) with neovascular AMD (retinal irradiance, 464 nm = 14.46 log photon cm^{-2} s^{-1}; 638 nm = 14.93 log photon cm^{-2} s^{-1}). The level of ipRGC dysfunction is increased in the patient with neovascular AMD. A color version of this figure is available online at www.optvissci.com.

ipRGC dysfunction has been associated with SAD, with patients showing gene variants in the opn4 photopigment having a higher risk for developing SAD.[62] Importantly, a potent treatment of SAD is short-wavelength (blue light) light therapy at irradiance levels that activate ipRGCs. Melatonin is released by the SCN to initiate the sleep phase, and melatonin secretion is suppressed by light.[79] There is evidence that AMD patients can show higher-than-normal melatonin levels.[80] Our working hypothesis is that patients with advanced AMD may have uninhibited melatonin release owing to abnormal ipRGC inputs to the SCN; therefore, these patients may be more likely to develop depression and sleep disorders. Research is currently ongoing in our laboratory to define and understand these relationships between ipRGC function and nonretinal symptoms in AMD.

TABLE 1.

Inner retina (ipRGC) and outer retina function derived from the PLR (corneal irradiance = 15.1 log photon cm^{-2} s^{-1}) in healthy control participants and AMD patients

		Outer retina (638 nm)		PIPR (inner retina; 464 nm)				
		Transient	Minimum constriction amplitude	Plateau, % model	6 s, % model	Early AUC	Late AUC	PAP
Healthy control subjects (n = 5)	Upper 95% CI	87.9	42.9	79.4	62.2	261.9	371.9	61.9
	Mean	84.0	40.2	76.6	58.7	245.2	326.0	53.5
	Lower 95% CI	80.2	37.5	73.9	55.1	228.6	280.1	45.1
Early AMD	Mean	*88.8*	40.7	*88.2*	*66.0*	*209.4*	*189.4*	*29.9*
Neovascular AMD	Mean	84.3	*49.0*	*92.6*	*75.7*	*148.9*	*127.3*	*42.8*

Values in italics indicate parameters outside the 95% confidence limits of the healthy control sample.

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