Melanopsin expressing intrinsically photosensitive Retinal Ganglion Cells (ipRGCs) are the third photoreceptor class in the eye.[1-3] ipRGCs 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 they have roles in image forming vision.[8, 9] The non-image forming functions include the signalling 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 signalling to the olivary pretectal nucleus (OPN).[1, 2, 10-12] The most prominent ipRGC contribution to the PLR is the post-illumination 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] While 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 their function is still in its infancy. Moreover, our knowledge is predominantly derived from transgenic animal models (for comprehensive reviews see [3, 14, 16]) and 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.

**intrinsically photosensitive Retinal Ganglion Cells (ipRGGs); the "novel-old" cell**

The initial evidence of a third photoreceptor class was available as early as 1927 when Keeler et al.[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 due 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.$\mu m^{-2}$),[7] and is lower in density compared to rod and cone photopigments (~25,000 molecules.$\mu m^{-2}$), but the melanopsin signal amplification is higher. While 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 s, which is about 100-fold longer than in cones and 20-fold longer than in rods.[7] Recent measurements show that 10 h of constant light activation of ipRGCs continuously evoke action potentials, so 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 pupil light reflex 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 depolarisation in response to short wavelength (blue appearing light), high retinal irradiance (> ~11.5 log quanta.$s^{-1}$.$cm^{-2}$) lights with a $\lambda_{max}$

of about 482 nm.[1, 8, 11] The data in **Figure 1** show that the peak opn4 ipRGC spectral sensitivity derived from a criterion post-illumination pupil response (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 subserves a faster temporal response than the melanopsin elicited intrinsic response.[32] ipRGGs are thought to have unmyelinated axons consistent with the slow conduction velocities of fibres within the retinohypothalamic tract (RHT) as shown in studies of primates.[33]

**Morphological diversity of ipRGCs**

Since their initial discovery, five ipRGC subtypes (M1-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 inner plexiform layer, or stratifying in both, the outer and inner plexuses (M3).[34] Similar dendritic stratifications have been described for M1 and M2 cells in primate retinae.[31] ipRGCs have the largest identified retinal ganglion cell dendritic fields (~400 -1200 µm) but have small somata and represent only a small subset (about 3000 cells or ~0.2% - 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 behaviours (for review[16]). Given that there appears to be some

conservation of ipRGC sub-types 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), while M2 subclasses stratify in the inner IPL (ON sublamina). M3 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 due to methodological differences in their labelling. 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 to M1 subtypes,[16, 39] M1 cells display larger membrane depolarisation compared to M2 and are about 10-fold more sensitive to light.[40] Primate M1 cells also show intra-retinal 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,

colour-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 do not however, show melanopsin immunostaining but are still capable of a weak intrinsic response.[39] While 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 centre 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 pupil light reflex 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 (IGL), the centre for circadian entrainment, the ventrolateral preoptic nucleus (VLPO), the control centre of sleep, the dorsal

and ventral lateral geniculate nucleus (LGN), the lateral habenula, the medial amygdala, the supraoptic nucleus, the posterior pretectal nucleus, the intergeniculate leaflet (IGL) and superior colliculus (SC) and many more brain regions.[5, 9, 39] While 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 signalling.[9] Given that bipolar cells and conventional ganglion cells signal contrast, determining the role of melanopsin for signalling the perceptual correlate of brightness will be important for explaining human behavioural 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] though 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 re-defining of the standard model of human trichromacy,[48] 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 behaviours in animals with intact ipRGCs whereas ipRGCs 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 pupil light reflex and photoentrainment indicative of functional ipRGCs. Light has also been shown to enhance learned fear in transgenic mice, and that this requires signalling via ipRGC pathways.[50] The development of new assessment paradigms in humans, especially through use of the pupil light reflex, will provide novel techniques for assessing behaviours 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, behavioural 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 pupil light reflex (PLR) is the only measureable, non-invasive physiological response to directly reflect the behaviour of the three retinal photoreceptor classes in the human eye; rods, cones and ipRGCs. As such, non-invasive 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 favour 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 post illumination

pupil response (PIPR) amplitude and redilation time constant **(Figure 2).** (e.g. [13, 14, 18, 20-22, 24, 54-56])

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 s; the ipRGC contribution increases with longer presentation durations but the rod photoreceptor still make 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 post-illumination pupil response (PIPR) after offset of the high irradiance short wavelength light **(Figure 2)**;[11] the PIPR can be measured as percentage (or millimetre difference) to the resting baseline pupil diameter during the plateau which is typically 30 s 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 6s post-illumination [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 s light pulses, has been confirmed by spectral sensitivity of the plateau PIPR by our group (**Figure 1**) and Gamlin and colleagues.[11, 14] Additional PIPR metrics such as the 6s metric and early and late AUC have not been confirmed by spectral sensitivity but are most likely controlled

198 by the intrinsic ipRGC response as the PIPR amplitude is wavelength and irradiance
199 dependent.

200

201 Despite recent advances in understanding ipRGC function in nocturnal animal models (e.g
202 mice), there are significant knowledge gaps about how the fundamental properties and
203 functional signatures of the ipRGC light response translate to diurnal humans. There are few
204 investigations of these unique cells and their various roles in human eye diseases. Our group
205 established the role of ipRGCs in the functional differentiation of early and advanced
206 glaucoma[25] and in the measurement of the progression of diabetes using the pupil light
207 reflex.[54] The study of ipRGC function in advanced glaucoma has shown that the PIPR
208 amplitude correlates with the visual field defect.[20] A study by La Morgia et al[59] observed that
209 ipRGCs are resistant in mitochondrial optic neuropathies such as Leber hereditary optic
210 neuropathy (LHON) and dominant optic atrophy (DOA). Retinitis pigmentosa (RP) has been
211 studied in humans[14, 21, 23, 60] using the pupil light reflex to differentiate between extrinsic (rod
212 and cone) and intrinsic ipRGC contributions and show extrinsic and intrinsic losses increase
213 with disease progression.[23] Morphological studies in a rat model of RP show that ipRGC
214 density and dendritic arborization decrease in advanced stages of the disease.[34] Persons with
215 seasonal affective disorder (SAD) have a reduced post-illumination pupil response, indicative
216 of altered light signalling via ipRGCs and may have a genetic variation within the opn4 gene,
217 suggestive of a possible role of ipRGCs in its pathogenesis.[61, 62]

218

219 Under experimental conditions controlling exogenous cues of circadian activity, our
220 laboratory provided the initial evidence that ipRGCs have a circadian response synchronised
221 to melatonin onset in humans whereas outer retinal inputs to the pupil did not,[19] thereby
222 indicating the PIPR as a non-invasive marker of the circadian rhythm. Münch, Leon, Crippa

and Kawasaki[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 pupil light reflex in age-related macular degeneration (AMD)

The primary anatomical and functional changes observed in age-related macular degeneration (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 post-illumination pupil response however, has been considered in only two studies, and one showed that the PIPR was independent of age,[18] the other showed a enhanced pupil responses in healthy older persons,[24] hence further investigations are required to understand these relationships.

While 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, though 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 s pulse as shown in **Figure 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 participant's and the experiments were approved by the QUT Human Ethics Committee and conducted in accordance with the principals expressed in the Declaration of Helsinki. **Figure 3** shows the PLR a 59 year old healthy female control participant (VA 6/6) without any ocular disease. The pupil trace is the response to a large 34° diameter, 0.5 Hz sinewave stimulus [6 cycles, 11.9 s duration; 464 nm or 635 nm] centred 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 s pulse (**Figure 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 (638nm) and short (464nm) wavelength peak-to-trough phase amplitudes according to Equation 1,

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

The PAP metric (Eq. 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] (**Figure 3**), the short wavelength peak-to-trough phase amplitude is lower relative to the long wavelength amplitudes (i.e. the PAP is non-zero), possibly due to ipRGC contributions that are inhibitory in nature.[31]

In the following, we present a framework and application of the pupil light reflex as an objective behavioural measure of inner and outer retinal function in AMD. We propose that for light levels that activate melanopsin, the Phase Amplitude Percentage (PAP) and the PIPR metrics will be reduced if disease causes an alteration in ipRGC function. Given the 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 due 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 sizes will be more sensitive to inner retinal function due 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 **Figure 4** we report the pupil light reflex in two AMD stages (early and neovascular) using large stimuli and high retinal irradiances (*c.f.* **Figure 3**) to illustrate the effect of manifest AMD on ipRGC function. **Figure 4A** shows the PLR for a male patient (76 years old, VA 6/6 both eyes) with early AMD (intermediated drusen > 125 µm; AREDS classification) and **Figure 4B** shows the PLR for a female patient (74 years, VA 6/12 both eyes) with advanced neovascular AMD (AREDS 4b) who is currently undergoing anti-VEGF treatment in both eyes. **Table 1** gives the outer and inner retinal metrics and includes the confidence limits of a healthy control group for comparison (n = 5; mean = 60 years old; range 56-69 years old; 3 Female, 2 male; 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.

## Conclusion 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] retinitis pigmentosa,[14, 21, 23, 34, 60] LHON and DOA,[59] diabetic retinopathy[54] and circadian health.[19, 61, 63] It is now becoming clear that the pupil light reflex 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, ipRGC dysfunction has been associated with seasonal affective disorder (SAD), with patients showing gene variants in the opn4 photopigment having a higher risk for developing the 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 due to abnormal ipRGC inputs to the SCN, therefore these patients may be more likely to develop depression and sleep disorders. Research is currently undergoing in our laboratory to define and understand these relationships between ipRGC function and non-retinal symptoms in AMD.

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## FIGURE LEGENDS

**Figure 1.** Visual pigment nomogram of the opn4 melanopsin derived from a criterion post-illumination pupil response (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, Feigl and Zele (2010).[14]

**Figure 2.** The consensual pupil light reflex of the left eye of a healthy young participant with no retinal abnormalities (37 y/o; 6/6 VA) in response to a 10 s, 464 nm or 638 nm rectangular light pulse with a corneal irradiance of 14.5 log photon.cm$^{-2}$.s$^{-1}$ centred on the pupil of the un-dilated 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 nm 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 ~88% of the initial pre-stimulus baseline pupil diameter after 60 s (12% net PIPR; recovery rate = -.09 mm sec$^{-1}$), whereas the PIPR to the 638 nm light of the same irradiance returns to the baseline pupil diameter within 20 s post-illumination (recovery rate = -0.25 mm sec$^{-1}$). The 6s metric measures the redilation amplitude 6 seconds after light offset (60%, net PIPR = 28%). The 464 nm early area under the curve (10-20 s early AUC) PIPR is 237.1 (113.8 for the 638 nm light) and the late AUC PIPR (20-40 s) is 266.4 (3.3 for the 638 nm light).

**Figure 3**. The consensual pupil light reflex of the left eye of a healthy older participant with no retinal abnormalities (59 y/o; 6/6 VA) measured in response to a 0.5 Hz sinewave stimulus (6 cycles, 11.9 sec duration; sinewave shown on the x-axis) with a corneal irradiance of 15.1 log photon.cm$^{-2}$.s$^{-1}$ centred 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 Phase-Amplitude Percentage (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.

**Figure 4.** The data in the left column show the consensual pupil light reflex of the left eye of patients with AMD measured in response to a 0.5 Hz sinewave stimulus (6 cycles, 11.9 sec duration; sinewave shown on the x-axis) with a corneal irradiance of 15.1 log photon.cm$^{-2}$.s$^{-1}$ centred 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). **Panel A.** A patient (male, age 75 years; VA 6/7.5) with drusen due to early AMD (retinal irradiance: 464 nm = 14.45 log photon.cm$^{-2}$.s$^{-1}$; 638 nm = 14.93 log photon.cm$^{-2}$.s$^{-1}$). **Panel B.** A patient (female, age 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.

**Figure 1**

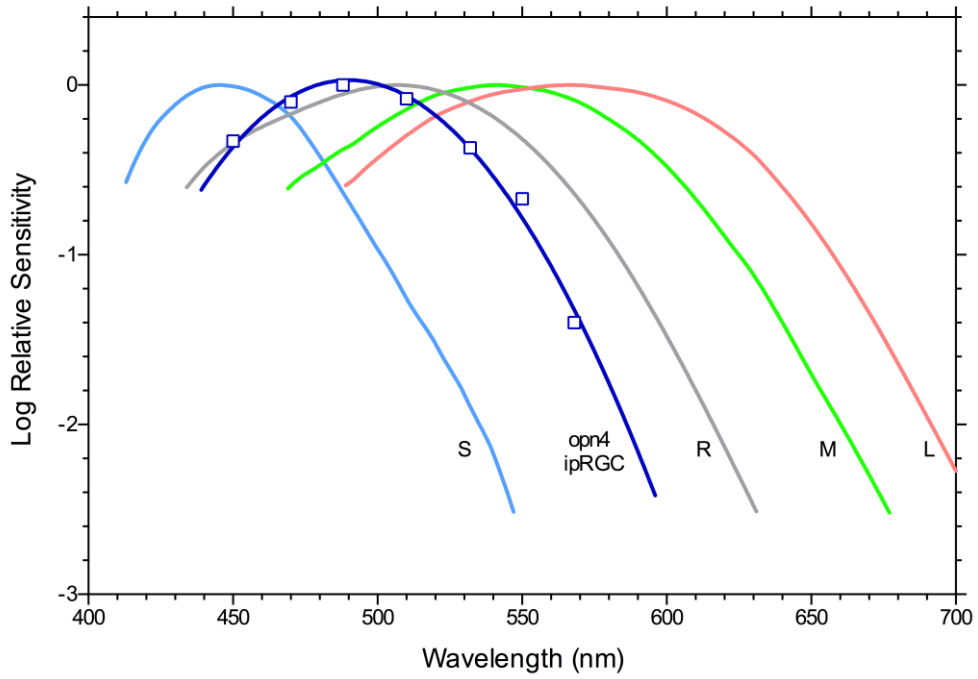


**Figure 2**

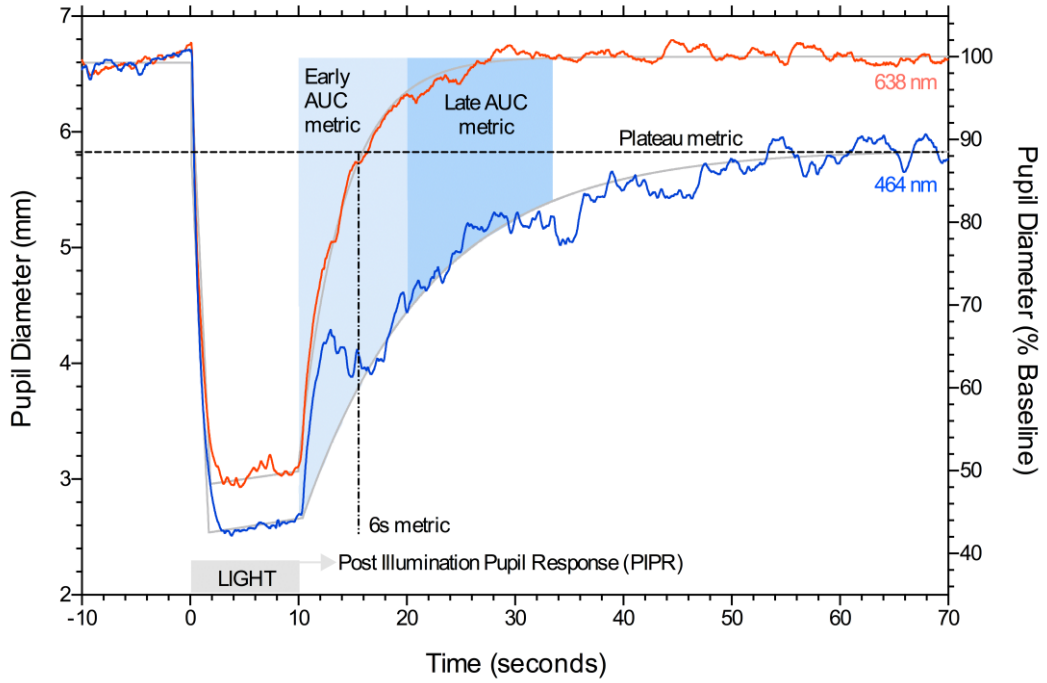

**Figure 3**

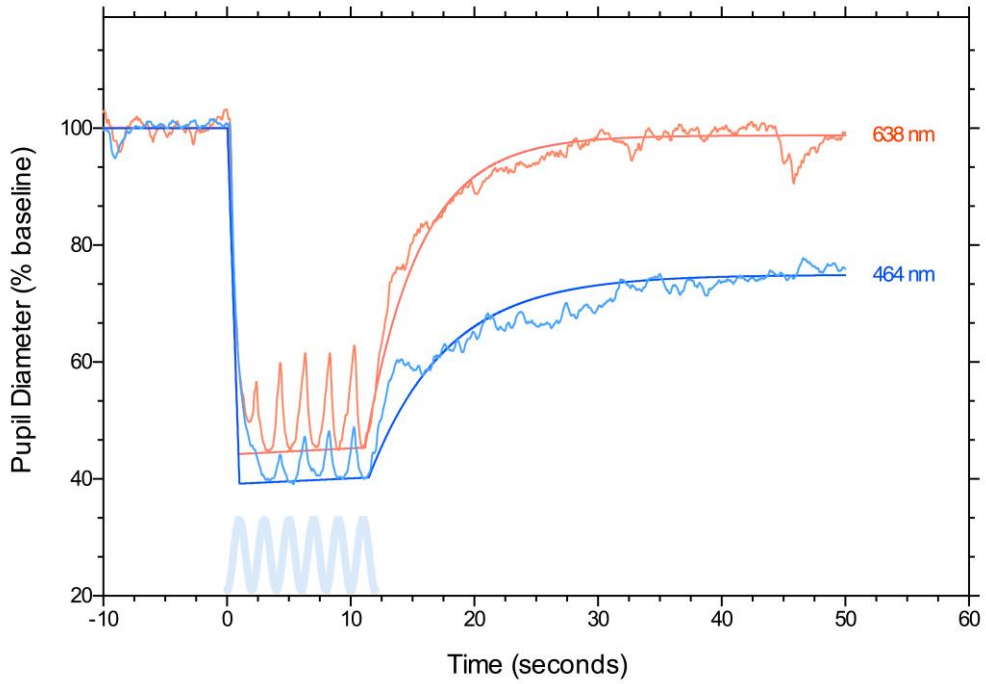


**Figure 4**