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Title: Intrinsically photoreceptive melanopsin retinal ganglion cell contributions to the pupil light reflex and circadian rhythm

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Abstract
Recently discovered intrinsically photosensitive melanopsin retinal ganglion cells contribute to the constriction, recovery and the post-illumination component of the pupil light reflex and provide the primary environmental light input to the suprachiasmatic nucleus for photoentrainment of the circadian rhythm. This review summarises recent progress in understanding intrinsically photosensitive ganglion cell histology and physiological properties in context of their contribution to the pupillary and circadian functions, and introduces a clinical framework for using the pupil light reflex to evaluate inner retina (intrinsically photosensitive melanopsin ganglion cell) and outer retina (rod and cone photoreceptor) function in the detection of retinal eye disease.

Keywords:
Circadian Rhythm;  Melanopsin;  Photoreceptors, Retinal;
Reflex, Pupillary;  Retinal Disease;  Retinal Ganglion Cells;
Introduction

The discovery of intrinsically photosensitive retinal ganglion cells (ipRGCs)\(^1\) and their unique photopigment melanopsin\(^2\) significantly altered the classical view of only four light sensitive retinal photoreceptors. Substantial progress has since been made regarding the histological distributions and functional properties of intrinsically photosensitive ganglion cells in non-primate and primate eyes. It is established that ipRGCs provide the primary environmental light input to the suprachiasmatic nucleus (SCN) for photoentrainment of the circadian rhythm.\(^3,4\) They also contribute to the constriction, recovery and the post-illumination pupilloconstriction component of the pupil light reflex,\(^5,6\) but their role in image forming vision is unclear. The temporal properties of ipRGCs are distinct from rod and cone photoreceptors; the light response of ipRGCs has a slow onset and sustained depolarization that is maintained for up to 30 seconds after light offset.\(^7\) This post-illumination sustained depolarization can be observed in the pupil reflex after light offset as an unique indicator of ipRGC function\(^5,8\) and has been termed the post-illumination pupil response (PIPR), also called the sustained pupil response.\(^8\) In the first part of this review, we consider the anatomical distribution and electrophysiological properties of intrinsically photosensitive ganglion cells and compare them with cone and rod photoreceptors. We then examine the role of ipRGC signalling in the circadian rhythm and the pupil light reflex. In the final section, a clinical framework is introduced to demonstrate how the ipRGC contribution to the pupil light reflex can be used to differentiate between inner and outer retinal processing.
Intrinsically photosensitive retinal ganglion cell (ipRGC) histology and electrophysiology

Melanopsin is the fifth human retinal photopigment, with the three cone opsins and the single rod opsin comprising the other four. It was detected in the retinal ganglion cell layer (GCL) of mice and primates\(^2\) after its first discovery in the dermal melanophores of frogs.\(^9\) Several studies have confirmed melanopsin as a retinal photopigment, in both mammals and humans.\(^7,10-12\) Retinal ganglion cells encode visual light input as a function of position, wavelength and time and project to the visual cortex via the lateral geniculate nucleus (LGN),\(^13\) as well as projecting to the olivary pretectal nucleus (OPN) and suprachiasmatic nucleus (SCN).\(^14,15\) Ganglion cells have been classified by soma, dendritic field size and density\(^13\) into an estimated 20 specialized cell sub-types.\(^16\) Of these ipRGCs comprise 0.2% of the ~1.5 million retinal ganglion cells in the human retina.\(^7\)

Intrinsically photosensitive ganglion cell dendrites branch infrequently along the inner and outermost edges of the inner plexiform layer (IPL) (Figure 1A) to create an overlapping photoreceptive mesh.\(^7,17\) Although few in number (~3000), ipRGCs have the longest dendrites and largest fields of all known ganglion cells, with diameters of 350 - 1200 µm increasing with retinal eccentricity,\(^7\) as compared to midget (~4 - 180 µm),\(^18\) small bi-stratified (~30 - 400 µm)\(^19\) and parasol (~20 - 400 µm)\(^18\) ganglion cells. Retinal ganglion cell are absent in the fovea with dendrites encircling the foveal pit.\(^7\) Like other known ganglion cell types 60 % of ipRGCs have their cell bodies in the ganglion cell layer (GCL) of the inner retina, however 40 % of ipRGC bodies are located in the inner nuclear layer.
Intrinsically photosensitive ganglion cells are classified into two subtypes according to stratification layer (Figure 1A). The inner subtype (ipRGC$_i$) has cell bodies in the GCL and stratifies in the extreme inner IPL (stratum 5), whereas the outer subtype (ipRGC$_o$) has cell bodies in both the GCL and INL and stratifies in the extreme outer IPL (stratum 1).$^7,17$ The ratio of inner to outer stratifying cells is between 1:1.1 and 1:1.5 in primates.$^7,21$ Although bi-stratifying photosensitive ganglion cells have been identified in mice, ipRGCs are primarily monostratified in the primate retina.$^{17,21,31,32}$

In addition to their intrinsic response, ipRGCs receive rod and cone input. Figure 1B shows the synapses of inner and outer stratifying ipRGCs with rod and cone pathways. Inner cells (ipRGC$_i$) contact DB6 bipolar cells in stratum 5$^{17,21}$ and transmit signals from L, M and S cones.$^{33,34}$ Rod input, which transmits to cones via gap-junctions,$^{35,36}$ may also pass via the DB6 bipolar of the cone pathway. Inner cells have also been shown to synapse with amacrine cells$^{17,37,38}$ and rod bipolar cells in rats$^{38}$. 
Outer cells (ipRGC\textsubscript{o}) co-stratify with dopaminergic amacrine cells\textsuperscript{21,37-39} and bipolar cells in stratum 1.\textsuperscript{17} In mammals ipRGC\textsubscript{o} and dopaminergic amacrine cells also synapse with bistratified ON bipolar cells via en passant ribbons.\textsuperscript{40,41} This ipRGC\textsubscript{o} ON input in the OFF IPL sub layer has not yet been confirmed in primates. These synapses suggest further unknown rod and cone pathways to both inner and outer ipRGC. The receptive fields of inner and outer stratifying cells overlap, suggesting a difference in roles that is still to be determined.

Intrinsically photosensitive ganglion cells (ipRGCs) give rise to 75 – 90% of projections to the suprachiasmatic nucleus (SCN),\textsuperscript{12,42,43} the location of the circadian biological clock.\textsuperscript{44} The majority of SCN projections in mice are from the outer stratifying cell group,\textsuperscript{45} but this difference in subgroup projections has not been demonstrated in primates. If outer and inner stratifying cells do project to different brain regions this supports a difference in roles for the cell subgroups. In addition to the SCN, ipRGCs project to the olivary pretectal nucleus (OPN) of the pretectum, the start of the pupil reflex pathway.\textsuperscript{7,11,46} Intrinsically photosensitive ganglion cells also synapse in the lateral geniculate nucleus (LGN) of the thalamus\textsuperscript{7,11,46} which relays, integrates and projects visual, auditory and somato-sensory information to the cerebral cortex and receives cortical feedback.\textsuperscript{47} The current known ipRGC projections to the SCN, the OPN and the LGN in mice are displayed in Figure 2.

Insert Figure 2 here
Rod, cone and ipRGC photopigments are isomerised on light absorption, converting 11-cis retinal to all-trans retinal. Rod and cone photopigments regenerate by binding 11-cis retinal via synthesis in the retinal pigment epithelium (RPE) to return to the active state. The retinoid processing cycle has been reviewed in detail elsewhere. Recent studies show that Müller cells also regenerate 11-cis retinal and support the rapid dark adaptation required by the cones. Regeneration of the ipRGC photopigment melanopsin is not dependant on the retinoid processing cycle and may regenerate by a different mechanism. Some invertebrate opsins and the melanopsin of primitive chordates are bistable photoisomerases that have an intrinsic light triggered regeneration where the opsin is isomerised to all-trans retinal with one photon and regenerated to 11-cis retinal with a second photon. Human melanopsin shares a common ancient origin with these bistable invertebrate opsins and early evidence suggests it may also function as a photoisomerase. The intrinsic photo-regeneration of melanopsin may be combined with further unknown extrinsic processes. Müller cells, capable of regenerating 11-cis retinal, are located adjacent to ipRGCs and may be a component of the melanopsin pigment cycling mechanism.

Intrinsically photosensitive ganglion cells display both light and dark adaption, with response amplitude and latency varying with prior adaptation level. Light adaptation produces a 0.4 log unit loss of sensitivity with a time constant of ~8 sec, measured using the human pupil light reflex. Dark adaptation increases the intrinsic sensitivity of rat ipRGCs from ~11 log photons.cm^-2.s^-1 to ~9 log photons.cm^-2.s^-1 with a time constant of
~198 minutes and rod input further increases the dynamic range to
~6 log photons.cm^{-2}.s^{-1}.7

Figure 3 shows the ipRGC spectral sensitivity derived by the authors from the measurement of a criterion post-illumination pupil response in two participants (aged 25 and 30). All reported data was collected in accordance with the requirements of the QUT Human Research Ethics Committee. The stimulus light (7.15° diameter field, 10 sec duration) was presented in Maxwellian view to the (cyclopeged) right eye at seven selected narrowband wavelength lights between 450 - 568 nm with a range of energy levels (13.4 - 14.7 log photons.cm^{-2}.s^{-1}). The consensual pupil light reflex at each wavelength and corneal irradiance was recorded for 55 seconds (10 sec pre-stimulus, 10 sec stimulus and 35 sec post-stimulus). The irradiance values were corrected for deviations from the criterion response, the data were normalised and fitted with a Vitamin A pigment nomogram.63 The peak spectral sensitivity (\lambda_{max}) was 482 nm, consistent with published reports.5,7 Additional details of the experimental pupillography set-up are given in a later section describing ipRGC contributions to the pupil light reflex.

Rods and cones show transient depolarization in response to light and display photosensitive bleaching and adaptation under continuous illumination.64 IpRGCs are also
intrinsically photosensitive but have a slow onset, sustained depolarization in response to light, even when detached from the retina.\textsuperscript{1,7,11} Figure 4A shows that the intrinsic response (no rod or cone input) of a human ipRGC to a 10 second light stimulus (470 nm, 13.3 log photons.cm\(^{-2}.s^{-1}\)) has an initial slow onset, with a latency of 3 - 10 sec, followed by sustained depolarization lasting up to 30 seconds after light offset.\textsuperscript{7} In comparison, the response of ipRGCs to rod and cone input, prior to any intrinsic response, is a rapid onset, transient depolarization with latencies of \(~150\) ms and \(~30 - 40\) ms respectively.\textsuperscript{7} Both the intrinsic response amplitude and time-to-peak of the ipRGC response increase with irradiance;\textsuperscript{1,7} the sustained depolarization (total number of spikes) is linearly proportional to the retinal irradiance in the photopic range between 11.5 and 14.7 log photons.cm\(^{-2}.s^{-1}\).\textsuperscript{7,65}

Insert Figure 4 here

The sustained, linear depolarization of the intrinsic ipRGC response to retinal irradiance, combined with a latency 100 times slower than with cone input, is consistent with its intrinsic role for mediating long term steady signalling of environmental irradiance.\textsuperscript{66} Luxotonic cells in the primate visual cortex discharge in a sustained, linear response to illumination.\textsuperscript{67} Because traditional rod and cone image forming pathways encode contrast, ipRGCs may signal this irradiance input and explain a person’s ability to quantify brightness in the absence of contrast information (eg. in a Ganzfeld).\textsuperscript{68} Brightness perception is sustained at short wavelengths near the ipRGC spectral peak compared to the faster fade-out that occurs with long wavelength light.\textsuperscript{69}
Intrinsically photosensitive ganglion cells receive input from rods and cones, via synapses with amacrine, DB6 and other bipolar cells in the inner plexiform layer (IPL)\textsuperscript{17,21} and it is not yet known if they contribute to image formation. The rod-ipRGC pathway is still to be determined but it could involve gap-junctions between rods and cones (Figure 1B).\textsuperscript{35,36}

Figure 4B shows the rod-mediated sustained \textit{ON} response of ipRGCs in response to scotopic stimulation (6 - 7.6 log photons.cm\textsuperscript{-2}.s\textsuperscript{-1}) in the dark-adapted primate retina.\textsuperscript{7} The ipRGC (L+M) cone \textit{ON} and S cone \textit{OFF} mediated responses are shown in Figure 4C. The spatially co-extensive S-\textit{OFF} and (L+M)-\textit{ON} components contribute to a colour-opponent receptive field that does not display the typical surround antagonism common to other retinal ganglion cells.\textsuperscript{7} Intrinsically photosensitive ganglion cells may subserve the S-\textit{OFF} signal, which projects to layer 4A of the primary visual cortex.\textsuperscript{70}

**Intrinsically photosensitive retinal ganglion cells and the pupil light reflex (PLR)**

The pupil light reflex (PLR) is the constriction and recovery of the pupil in response to light. In addition to attenuating the retinal illumination, a light responsive pupil can vary the depth of focus and reduce the visual effects of glare, diffraction and optical aberrations.\textsuperscript{71} A small pupil diameter also reduces photoreceptor bleaching, allowing faster dark adaptation.\textsuperscript{72}
The pupil light reflex is modulated by the autonomic nervous system which innervates two iris muscles; the sphincter pupillae (parasympathetic innervation), a smooth muscle ring located around the pupil aperture, and the dilator pupillae (sympathetic innervation), a thin muscle sheet lying between the iris stroma and the posterior pigment endothelium, radiating from the sphincter muscle to the ciliary body.\textsuperscript{71} Figure 5 overviews the parasympathetic and sympathetic pupil pathways. Retinal input to the olivary pretectal nucleus (OPN) is projected to the Edinger-Westphal nucleus (EW) where the parasympathetic pathway originates. The parasympathetic signal is transmitted via the third cranial nerve and synapses at the ciliary ganglion (CG) before the postsynaptic short ciliary nerve innervates the sphincter pupillae muscle.\textsuperscript{71} The sympathetic pathway originates in the intermediolateral columns of the cervical spinal cord (C8-T1) and synapses at the superior cervical ganglion (SCG) located at the C2-C3 vertebrae. Post synaptic fibres pass up the neck to the orbit and signals are primarily transmitted via the long posterior ciliary nerves to the dilator pupillae muscle in the iris. Other sympathetic fibres may also travel along the short ciliary nerves.\textsuperscript{71} Unlike parasympathetic fibres, sympathetic fibres do not synapse at the CG.\textsuperscript{71}

Insert Figure 5 here

Pupillary constriction to light occurs when parasympathetic cholinergic stimulation contracts the sphincter pupillae muscle.\textsuperscript{73} At light offset pupil dilation occurs via dual pathways; excitation of the $\alpha_1$ adrenergic sympathetic pathway causes dilation of the dilator
pupillae and parasympathetic inhibition of the EW relaxes the sphincter pupillae.\textsuperscript{73} The dual parasympathetic and sympathetic autonomic innervation creates a balance (tonus) in the steady state pupil. Non-photic stimuli can also induce pupil dilation. Noise, pain, surprise, pleasure and stress cause pupil dilation by increasing the sympathetic tone of the central autonomic system.\textsuperscript{72,74,75} Cognitive tasks such as number recall and mental arithmetic also cause pupil dilation by cortical inhibition of the parasympathetic pathway at the EW.\textsuperscript{76-78} This dilation increases with the level of demand\textsuperscript{77-79} and is sustained during continuous cognitive tasks.\textsuperscript{80}

Figure 6 shows the rod, cone and ipRGC contributions to the consensual pupil light reflex of a healthy, 30 year old observer. The light stimulus was a uniform 7.15° field presented in Maxwellian view to the right eye (dilated with 1 % cyclopentolate). The consensual pupil light reflex was recorded using an infrared camera (62 frames.sec\textsuperscript{-1}) and derived using custom designed Matlab (Mathworks Inc) analysis software (light and dark grey lines in Figure 6) and fitted with a simple linear and exponential model (coloured lines in Figure 6). Three light stimulus (10 sec) irradiances were used: 10.1 log photons.cm\textsuperscript{-2}.s\textsuperscript{-1} (rod only; Figure 6A), 12.2 log photons.cm\textsuperscript{-2}.s\textsuperscript{-1} (rod and cone; Figure 6B) and 14.2 log photons.cm\textsuperscript{-2}.s\textsuperscript{-1} (above the irradiance required for ipRGCs to produce a half-maximal pupil constriction at 470 nm in primates and humans;\textsuperscript{5} Figure 6C). A 488 nm stimulus was used to maximise the ipRGC contribution to the PLR and a 610 nm (control) stimulus was used to minimise it (see Figure 3).
The pupil light reflex has four major components; response latency, maximum constriction, escape and recovery. The latency of the PLR is the delay in pupil constriction after light onset. The pupil rapidly constricts to a minimum diameter which ‘escapes’ to partial constriction during a prolonged stimulus of several seconds. At light offset the pupil re-dilates returning to the initial size. The initial rapid pupil constriction is driven by rod and cone input with the latency shortening as light intensity increases up to a minimum latency of 230 ms. The minimum latency is dependent on the temporal dynamics of cones (30 - 40 ms), rods (~150 ms), iris muscle, innervation pathway and processing. Under a low photopic light level (24.6 cd.m⁻²) a pupil constriction of 1.6 - 1.9 mm takes ~0.73 sec. Intrinsically photosensitive ganglion cells have a latency of 1.78 - 10 sec and therefore do not have the temporal dynamics to contribute to the initial constriction. The maximum pupil constriction varies with stimulus intensity, duration, spectral composition, retinal size and location. The sensitivity of rods and cones change with stimulus wavelength and illumination, and the Purkinje shift occurs for both the visual system and the pupil light reflex as light levels change from photopic to scotopic. Cones are fewer in number but cover a broader spectral range compared to rods. Below cone threshold, greater initial constriction is produced by a short wavelength compared to a long wavelength stimulus of equal intensity due to rod sensitivity being higher at shorter wavelengths. Pupil escape, the re-dilation to a partially constricted state occurs for light
durations longer than 1 - 2 sec, and is produced by a combination of rod, cone and ipRGC cell input. This partial constriction may be maintained for light durations up to 100 seconds.

The ipRGC signal is responsible for the post-illumination pupil response (PIPR) of the PLR, the difference between the pupil diameter prior to stimulus onset and after light offset. The PIPR, also referred to as the sustained pupil response, is characterised by 8 - 10 seconds of re-dilation after light offset before stabilizing at ~1.5 mm less than the pre-stimulus pupil diameter, and is maintained for at least 30 seconds (stimulus: 60º, 470 nm, 13 log photons.cm⁻².s⁻¹, 10 sec). The post-illumination pupil response depends on the intensity and wavelength of the stimulus, but the effect of age, race and gender on ipRGC function was not determined in a small sample of 37 participants (26-80 yr). Further investigations are required to determine how age affects ipRGC function. Short wavelength light produces the greatest PIPR, with a half-maximal pupilloconstriction of 1.5 mm occurring for a 470 nm, 13.6 log photons.cm⁻².s⁻¹ stimulus as demonstrated in primates under pharmacological blockade of the rod and cone photoreceptors. An increased irradiance is required to produce an equivalent PIPR at longer wavelengths.

The post-illumination pupil response for a single observer is demonstrated in Figure 6C (blue line, dark grey trace) with a PIPR of 1.12 mm (81 % of pre-stimulus diameter) in response to a 7.15º, 488 nm, 14.2 log photons.cm⁻².s⁻¹ stimulus. In contrast, a long wavelength stimulus of the same irradiance (red line) demonstrates little PIPR, returning to
within 0.29 mm (~95.5 %) of the pre-stimulus diameter within 8 - 10 sec after light offset. Figure 6B shows that the PIPR is not evident at the lower irradiance (12.2 log photons.cm\(^{-2}.s^{-1}\)) for either the 488 or 610 nm stimuli. The PIPR observed in Figure 6C displays similar temporal properties to the sustained depolarization seen in vitro, in the macaque and human retina (Figure 4A).\(^1,7\) It has been confirmed in primates that the ipRGC signal is responsible for the PIPR after pharmacologically blocking rods and cones signals.\(^5\)

**Intrinsically photosensitive retinal ganglion cell contributions to circadian rhythm**

The circadian rhythm is a cycle of biochemical, physiological and behavioural processes coordinated by the suprachiasmatic nucleus (SCN) of the anterior hypothalamus.\(^92\) The SCN regulates the release of melatonin from the pineal gland to regulate the sleep/wake cycle.\(^93\) The SCN has an intrinsic rhythm of 23.81 - 24.31 hours\(^94-96\) and is synchronized to the solar day, in a process called photoentrainment, by ipRGC cell input which encodes the environmental light levels.\(^3,4\) This has been confirmed in mice lacking either rods and cones, ipRGCs or all photoreceptive cells.\(^3,4,97-102\) Intrinsically photosensitive ganglion cell input to the SCN also regulates phase shifting,\(^3,4\) where the circadian rhythm is advanced or delayed by exposure to light, with the degree of phase shifting dependent on the duration, intensity and wavelength of the light.\(^103\)
Visual function and performance shows circadian variation in sensitivity to optimize vision for photopic (cone only) and scotopic (rod only) conditions. Visual luminance sensitivity increases during the night with the greatest scotopic sensitivity occurring between midnight and 2:30 am in normally entrained participants. The cone-pathway shows faster latency (ERG b- and d-wave) in the light phase of the circadian cycle compared to the dark phase and the rod ERG b-wave amplitude decreases early in the light cycle. Thus rod and cone function varies diurnally in response to environmental light; the rhythm of ipRGC function is however unknown. The circadian expression of melanopsin mRNA peaks near dark onset while immunopositive ipRGC cell numbers peak just before light onset in mice entrained by artificial light in an animal house. The circadian rhythms of mRNA and cell numbers are lost in a continuous dark environment demonstrating that melanopsin production may be driven either directly by environmental light or involve feedback from the SCN.

Although the SCN is the master synchronizer, peripheral tissues such as the retina, heart, liver, lungs, pituitary and skeletal muscle, show self-sustained circadian oscillation of clock genes and protein expression when isolated from the SCN. The precise location of the retinal oscillator is unknown; clock gene expression has been demonstrated in rod and cone photoreceptors and the inner nuclear, inner plexiform and ganglion cells layers, in horizontal, bipolar, amacrine and ganglion cells. The retinal oscillator regulates the circadian rhythm of several retinal processes and functions independently of the SCN. For example, the retinal clock controls circadian shedding of rod outer segments, which
peak 1.5 hours into the light cycle.\textsuperscript{121-123} The retinal clock also regulates the nightly synthesis of melatonin in the outer photoreceptors, the inner nuclear layer and ganglion cell layer (reviewed by Iuvone et al., 2005 \textsuperscript{124}) which can also be photoentrained \textit{in vitro} in retinal tissue.\textsuperscript{125,126} Many other retinal processes such as dopamine synthesis and cone outer segment shedding also show a circadian rhythm but it is not yet known if these are also regulated by a local retinal clock.\textsuperscript{127-129} The effects of the central circadian rhythm and the local retinal rhythm on ipRGC function is not yet fully understood. Markwell, Feigl, Smith and Zele (2010)\textsuperscript{130} recently demonstrated circadian modulation of the intrinsically photosensitive retinal ganglion cell driven post-illumination pupil response.

\textbf{Intrinsically photosensitive retinal ganglion cell contributions to the PLR in retinal disease}

Ganglion cell dysfunction causes progressive loss of vision in ocular diseases such as glaucoma, diabetes and optic neuropathy.\textsuperscript{131} The isolation and testing of subpopulations of ganglion cells affected by disease can reveal early dysfunction. According to the \textit{reduced redundancy} hypothesis\textsuperscript{132} the impaired function of a subpopulation of cells can be detected earlier than dysfunction across ganglion cell subpopulations. By developing clinical tests which specifically target the response of subpopulations of ganglion cells, earlier detection of disease may be possible. Short wavelength automated perimetry (SWAP), flicker perimetry and frequency doubling perimetry (FDT) have been developed to bias the response to a subpopulation of ganglion cells.\textsuperscript{133-135} SWAP is designed to isolate the response of the small bistratified ganglion cells of the koniocellular pathway,\textsuperscript{133} which
comprise only 1% of the central and ~6 – 10% of the peripheral retinal ganglion cells. SWAP can improve the detection of glaucomatous ganglion cell dysfunction by 15% compared to standard automated perimetry. FDT also allows the earlier detection of diffuse ganglion cell dysfunction in glaucoma by selectively assessing cortical processes which require magnocellular input. The achromatic (white-on-white) and peripheral (>5 deg) chromatic (red-on-white) flicker perimetry can detect early losses by biasing detection to the magnocellular pathway which includes about 10% of all retinal ganglion cells, while central (<5 deg) chromatic (red-on-white) flicker perimetry biases detection to the parvocellular pathway. Flicker perimetry is more sensitive than standard automated perimetry to early foveal defects in ARM and peripheral defects in diabetes.

The effect of retinal disease on ipRGCs is currently unknown, but ipRGCs sparsely populate the retina comprising 0.2% of total retinal ganglion cells. The post-illumination pupil response (PIPR), as a direct measure of ipRGC function, may be a sensitive measure of early ganglion cell loss in the inner retina. For inner retinal diseases such as glaucoma and diabetes, it is not known if ganglion cell loss is non-selective across ganglion cell classes, selective for specific subtypes or variable between individuals with the same disease. Tests of ipRGC function may be useful in understanding the subtypes and progression of retinal disease processes.
While the PIPR may be developed as a clinical assessment technique for inner retinal disease, the pupil light reflex may also be used to assess outer retinal function. Rods and cones both contribute to the initial constriction amplitude of the PLR, but ipRGCs do not due to their slow temporal response. Figure 6 demonstrates the maximum initial pupil constriction of a healthy, young (30 yrs) observer to 10 second stimuli of 488 and 610 nm for a rod isolated stimulus (below cone threshold) (Figure 6A), a photopic cone stimulus (rod saturation) (Figure 6B), and a stimulus greater than that shown to produce half-maximal ipRGC pupilloconstriction (Figure 6C). As a clinical example of the PLR and its application in outer retinal disorders, we investigated a patient with retinitis pigmentosa. The patient was a 30 year old male with a visual acuity of 6/6 and a ring scotoma at ~15° (MD = -7.41 dB) in his right eye (central 22° field, Medmont M700). Figure 7 shows the pupil light for 10 second stimuli of 488 and 610 nm that were scotopic (Figure 7A) or photopic (Figure 7B). The patient demonstrates impairment of the isolated rod photoreceptors in Figure 7A by the reduced amplitude and duration of the initial pupil constriction for both 488 and 610 nm stimuli. Figure 7B demonstrates some additional impairment to the cone photoreceptors, with the constriction amplitude reduced for the 610 nm stimulus. Figure 7B also shows a PIPR of ~80 % for a 488 nm, 14.2 log photons.cm⁻².s⁻¹ stimulus (blue line), similar to the ~81 % PIPR of the 30 year old control observer for the same stimulus (blue line, Figure 6C) confirming normal ipRGC function in this RP patient.

Insert Figure 7 here
The use of the pupil light reflex to differentiate between outer and inner retina impairment has recently been suggested. Where a disease affects multiple retinal layers the pupil light reflex could be a useful tool in determining the inner and outer retinal contributions to the disease process. Age related maculopathy for example, causes impairment to both the inner and outer retinal layers depending on the stage of the disease and monocular PLR measurements may allow the progression of the disease through the retinal layers to be monitored over time.

**Future Directions**

The discovery of ipRGC contributions to both the pupil light reflex and circadian rhythm has applications in the study of retinal disease, chronobiology and sleep disorders. Intrinsically photosensitive ganglion cell projections to the suprachiasmatic nucleus and the olivary pretectal nucleus have been defined and open questions remain regarding the organisation of the rod- ipRGC pathway, the melanopsin pigment regeneration mechanism and the role of ipRGC contributions to the S-cone OFF pathway for colour vision. The pupil light reflex is currently used in clinical practice to test the optic nerve pathway. Clinical protocols which use the PIPR to detect and monitor early retinal disease are being developed. If the PIPR is to assess inner retinal function, the normal ageing effects on the response also need to be quantified. The aetiology of many sleep disorders, such as advanced sleep phase disorder and delayed sleep phase disorder, are currently unknown and the assessment of ipRGC function using the PIPR may also contribute to understanding
the pathogenesis of circadian disorders. A clinical pupil test however, must control factors that influence the autonomic nervous system, such as noise, stress, cognitive tasks and autonomic drugs.\textsuperscript{72,74-78,144} Previously, the use of pupillometry has been limited by large inter-individual differences in pupil response\textsuperscript{145} and this needs to be overcome for a clinical PLR test to be successful. Research in our laboratory is currently under way to address some of these questions and to optimise the clinical parameters to evaluate the ipRGC response. In the future, a new clinical protocol may extend the current pupil measurement techniques to identify and monitor the progression of retinal eye disease and in determining the inner and outer retinal contributions to the progression of pathology.
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Table 1

Caption

Table 1. Location, distribution and anatomy of primate intrinsically photosensitive retinal ganglion cells compared with rod and cone photoreceptors.

<table>
<thead>
<tr>
<th></th>
<th>ipRGCs</th>
<th>Rods</th>
<th>L, M &amp; S Cones</th>
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</thead>
<tbody>
<tr>
<td><strong>Location</strong></td>
<td>Inner Retina</td>
<td>Outer Retina</td>
<td>Outer Retina</td>
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<tr>
<td><strong>Number in Retina</strong></td>
<td>~3000 †</td>
<td>92 million †</td>
<td>4.6 million †</td>
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<tr>
<td><strong>Peak Cell Density</strong></td>
<td>20 - 25 cells.mm$^{-2}$ at 2° eccentricity †</td>
<td>176 200 cells.mm$^{-2}$ at ~21° eccentricity †</td>
<td>199 000 cells.mm$^{-2}$ at fovea † (L &amp; M cones)</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>2600 cells.mm$^{-2}$ at 0.6° eccentricity † †††† (S cones)</td>
</tr>
<tr>
<td><strong>Cells Bodies</strong></td>
<td>40 % INL, 60 % GCL †</td>
<td>ONL</td>
<td>ONL</td>
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<tr>
<td><strong>Dendrite Stratification</strong></td>
<td>Extreme outer and inner IPL †</td>
<td>OPL</td>
<td>OPL</td>
</tr>
<tr>
<td><strong>Input</strong></td>
<td>Intrinsically photosensitive †</td>
<td>Intrinsically photosensitive</td>
<td>Intrinsically photosensitive</td>
</tr>
<tr>
<td></td>
<td>Rod &amp; Cone input †</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Peak λ Sensitivity</strong></td>
<td>482 nm †</td>
<td>507 nm ‡</td>
<td>440, 543 and 566 nm † †</td>
</tr>
<tr>
<td><strong>Photopigment</strong></td>
<td>Melanopsin §§§</td>
<td>Rhodopsin †††</td>
<td>Cyanolabe ‡‡‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chlorolabe &amp; Erythrolabe †††</td>
</tr>
<tr>
<td><strong>Synapses</strong></td>
<td>DB6 Bipolar Cells †</td>
<td>Rod-Cone gap junctions †††</td>
<td>Cone midget, parasol &amp; bistratified bipolar cells</td>
</tr>
<tr>
<td></td>
<td>Amacrine Cells †</td>
<td>Rod ON Bipolar cells †</td>
<td>Horizontal cells ††</td>
</tr>
</tbody>
</table>

Footnote
† Dacey et al. (2005)‡
‡ Curcio et al. (1990)²⁰
§ Dacey et al. (2006)²¹
¶ Crawford (1949)²²
†† Schneeweis & Schnapf (1995)²³
‡‡ Dacey et al. (1996)²⁴
 §§ Smith & Pokorny (1975)²⁵
¶¶ Daw et al. (1990)²⁶
††† Boll (1877)²⁷
‡‡‡ Rushton (1959)²⁸
 §§§ Provencio et al. (2000)²
¶¶¶ Marks et al. (1964)²⁹
†††† Calkins (2001)³⁰
Figure 1. Schematic of the primate retinal layers showing location and synapses (chemical synapses, filled circles) of inner and outer stratifying ipRGCs in primates. (A) Inner stratifying photoreceptive ganglion cell bodies (ipRGC$_{i}$) are located in the ganglion cell layer (GCL) and their dendrites stratify along the extreme inner strata (S5) of the inner plexiform layer (IPL$_{i}$). Outer stratifying photoreceptive ganglion cell bodies (ipRGC$_{o}$) are
located in both the ganglion cell layer (GCL) and the inner nuclear layer (INL) and their
dendrites stratify along the extreme outer strata (S1) of the inner plexiform layer (IPLo).

(B) Cone input is transmitted to ipRGCi via DB6 cone bipolar cells (DB6).17,21 Rod input to
ipRGCi may be transmitted via rod-cone gap-junctions (GJ) and the DB6 bipolar cells of
the cone pathway; rod input along the rod pathway, via ON rod bipolar (RB), AII amacrine
cells (AII) and ON cone (B_on) and OFF cone (B_off) bipolars, is yet to be determined in
primates although synaptic contact has been shown between rod bipolars and ipRGCi in
rats.38 Synaptic contact also occurs between ipRGCo and dopaminergic amacrine cells
(A_d)21,37,38 and bipolar cells (B)17; ipRGCi synapse with unspecified amacrine cells (A).

Abbreviations: outer segment (OS), outer nuclear layer (ONL), outer plexiform layer
(OPL) and nerve fibre layer (NFL).
Figure 2. Intrinsically photosensitive ganglion cell (ipRGC) projections to brain locations and the associated circuits. The ipRGCs and their axons are shown in dark blue and their principal targets in red. Intrinsically photosensitive ganglion cells project to the suprachiasmatic nucleus (SCN) for entrainment of the biological circadian rhythm. The SCN regulates the expression of melatonin from the pineal gland (P), with a sympathetic pathway (orange) synapsing at the intermediolateral nucleus (IML) and superior cervical ganglion (SCG). Intrinsically photosensitive ganglion cells also project to the olivary pretectal nucleus (OPN) contributing to both the sympathetic (not shown) and parasympathetic pupil reflex pathways. The parasympathetic pupil pathway (light blue) synapses at the Edinger–Westphal nucleus (EW) and the ciliary ganglion (CG) before reaching the iris muscles (I). The final target of ipRGC projections are two regions of the lateral geniculate nucleus in the thalamus: the ventral division (LGNv) and the intergeniculate leaflet (IGL). The LGN processes, integrates and projects to the visual cortex for image formation (pathway not shown). Reproduced with permission from Berson.48
Figure 3. Spectral sensitivity of the human retinal photopigments: S-cone ($\lambda_{\text{max}} = 440$ nm), ipRGC ($\lambda_{\text{max}} = 482$ nm), Rod ($\lambda_{\text{max}} = 507$ nm), M-cone ($\lambda_{\text{max}} = 543$ nm) and L-cone ($\lambda_{\text{max}} = 566$ nm). Cone spectral data from Smith & Pokorny\textsuperscript{25}; Rod spectral data from Crawford\textsuperscript{22}; ipRGC spectral data collected in the Visual Science and Medical Retina Laboratory, QUT. See text for details.
Figure 4. Intracellular voltage recordings of a human ipRGC in vivo. (A) The slow, sustained intrinsic photoresponse of the ipRGC in response to a 10 sec, 550 nm, 13.5 log photons.cm$^{-2}$.s$^{-1}$ light pulse under pharmacological blockade of the rod and cone photoreceptors. (B) The rod-mediated response of the ipRGC to a 10 sec, 550 nm, low scotopic light pulse of 7.6 log photons.cm$^{-2}$.s$^{-1}$. (C) The (L+M) cone ON and S cone OFF isolated responses of the ipRGC. Reproduced with permission from Dacey and colleagues.$^7$
Figure 5. Anatomical drawing showing the direct and consensual pupillary light reflex pathways and the parasympathetic and sympathetic innervation of the iris in primates. See text for details. Reproduced with permission from McDougal & Gamlin.71
Figure 6. The consensual pupil light reflex (PLR) of a 30 year old female with 6/5 acuity and normal ocular health. The average initial pupil diameter is indicated by the horizontal black dashed line. Light onset is indicated by the vertical dashed line, duration by the grey box. Pupil data represented by the light and grey lines and pupil model data by blue and red.
lines. Pupil light reflex components shown: Pre-stimulus pupil diameter (-10 - 0 sec), response latency (0 - 0.3 sec), maximum constriction, escape (1 - 10 sec) and recovery (10 - 45 sec). (A) Pupil Light Reflex for scotopic 10.1 log photons.cm^{-2}.s^{-1}, 488 nm (blue line) and 610 nm (red line) 10 sec stimuli. (B) Pupil Light Reflex for photopic (above cone threshold) 12.2 log photons.cm^{-2}.s^{-1}, 488 nm (blue line) and 610 nm (red line) 10 sec stimuli. (C) Pupil Light Reflex for photopic (above cone and ipRGC threshold) 14.2 log photons.cm^{-2}.s^{-1}, 488 nm (blue line) and 610 nm (red line) 10 sec stimuli. The post-illumination pupillary reflex of 81 % is shown by the blue dashed line. Data collected in the Visual Science and Medical Retina Laboratory, QUT. See text for details.
Figure 7. The consensual pupil light reflex (PLR) of a 30 year old male patient with retinitis pigmentosa with 6/6² acuity and a visual field ring scotoma defect of ~15° (~7.41 dB) measured with a central 22° (30° nasal) visual field test. Pupil data is represented by the light and grey lines and pupil model data by blue and red lines. (A) Pupil Light Reflex for scotopic (below cone threshold) 10.1 log photons.cm⁻².s⁻¹, 10 sec stimuli (488 nm, blue line; 610 nm, red line). (B) Pupil Light Reflex for photopic (above cone and ipRGC threshold) 14.2 log photons.cm⁻².s⁻¹, 10 sec stimuli (488 nm, blue line; 610 nm, red line). The unimpaired post-illumination pupillary reflex demonstrates normal ipRGC cell and inner retinal function. Data collected in the Visual Science and Medical Retina Laboratory, QUT. See text for details.