The post-illumination pupil response of melanopsin expressing intrinsically photosensitive retinal ganglion cells in diabetes

Beatrix Feigl MD PhD*, Andrew J. Zele PhD*, Samantha M. Fader, Annelisa N. Howes, Catherine E. Hughes, Kris A. Jones and Rawlyn Jones

Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane 4059, Australia.

*Correspondence:
Beatrix Feigl, MD, PhD
Andrew J. Zele, PhD
Medical Retina Laboratory
Institute of Health and Biomedical Innovation
Queensland University of Technology
60 Musk Avenue
Brisbane 4059, QLD, Australia
Phone: 61 7 3138 6147. Fax: 61 7 3138 6030
e-mail: b.feigl@qut.edu.au, andrew.zele@qut.edu.au
ABSTRACT

**Purpose:** This study investigates the clinical utility of the melanopsin expressing intrinsically photosensitive retinal ganglion cell (ipRGC) controlled post-illumination pupil response (PIPR) as a novel technique for documenting inner retinal function in patients with Type II diabetes without diabetic retinopathy.

**Methods:** The post-illumination pupil response (PIPR) was measured in seven patients with Type II diabetes, normal retinal nerve fiber thickness and no diabetic retinopathy. A 488 nm and 610 nm, 7.15° diameter stimulus was presented in Maxwellian view to the right eye and the left consensual pupil light reflex was recorded.

**Results:** The group data for the blue PIPR (488 nm) identified a trend of reduced ipRGC function in patients with diabetes with no retinopathy. The transient pupil constriction was lower on average in the diabetic group. The relationship between duration of diabetes and the blue PIPR amplitude was linear, suggesting that ipRGC function decreases with increasing diabetes duration.

**Conclusion:** This is the first report to show that the ipRGC controlled post-illumination pupil response may have clinical applications as a non-invasive technique for determining progression of inner neuroretinal changes in patients with diabetes before they are ophthalmoscopically or anatomically evident. The lower transient pupil constriction amplitude indicates that outer retinal photoreceptor inputs to the pupil light reflex may also be affected in diabetes.
Introduction

Melanopsin-expressing intrinsically photosensitive Retinal Ganglion Cells (ipRGC) project to the suprachiasmatic nucleus for circadian photoentrainment and to the olivary pretectal nucleus in the midbrain to control the pupil light reflex (Dacey et al. 2005; Hattar et al. 2002). They compromise only about 0.2% of the total number of ganglion cells in the retina and have the largest dendritic tree diameters of any known primate retinal ganglion cells (Dacey et al. 2005). It has been shown in humans that ipRGCs contribute to baseline pupil diameter (Tsujimura et al. 2010), the steady-state pupil diameter (McDougal and Gamlin 2010) and the post-illumination pupil response (PIPR), a sustained constriction of >30 s after light offset (Markwell et al. 2010; Gamlin et al. 2007; Kankipati et al. 2010). In vitro ipRGC recordings in primates display a typical transient increase in firing rate at stimulus onset and a sustained firing that continues after light offset (Dacey et al. 2005). This sustained ipRGC response after light offset controls the post-illumination pupil response (Gamlin et al. 2007) that can be reliably recorded in normal participants and in patients with inner retinal disease (Kankipati et al. 2010; Kardon et al. 2010; Kardon et al. 2009; Feigl et al. 2011; Zele et al. 2011; Markwell et al. 2010).

Pupillometric studies in patients with diabetes with and without diabetic retinopathy indicate dysfunction in autonomic pupil innervation (Yang et al. 2006; Ferrari et al. 2007). While these studies indicate pupillometry is valuable for detecting autonomic neuropathy, no study has evaluated the ipRGC controlled post-illumination pupil response as a direct measure of inner neuroretinal changes in patients with diabetes without diabetic retinopathy. Indeed the clinical potential of the PIPR only now being realized (Wilhelm 2010; Markwell et al. 2010; Kardon et al. 2009; Kankipati et al. 2010; Kawasaki and Kardon 2007; Kardon et al. 2010; Feigl et al. 2011; Kankipati et al. 2011). In diabetes, neuroretinal changes can precede
microvascular changes in diabetic retinopathy by one year (Harrison et al. 2010) and a decrease in retinal nerve fiber layer thickness can occur before onset of diabetic retinopathy (Sugimoto et al. 2005; Cabrere DeBuc and Somfai 2010). In mice, ganglion cells (melanopsin expressing ganglion cells included) undergo morphological changes within three months of diabetes onset (Gastinger et al. 2008) and show a reduced PLR (Kumar and Zhuo 2011). Given that ipRGCs comprise only a small percentage of all human retinal ganglion cells (Dacey et al. 2005), their measurement may provide a novel method for the detecting inner retinal dysfunction in humans (Feigl et al. 2011; Kardon et al. 2009; Markwell et al. 2010; Kankipati et al. 2011). The aim of this exploratory case study was to evaluate the PIPR as a direct measure of inner retinal (ipRGC) function in patients with Type 2 diabetes without diabetic retinopathy.

Methods and Patients

We recorded the consensual pupil light reflex of the left eye with an infrared Pixelink camera (IEEE-1394, PL-A741 FireWire; 480 x 640 pixels; 62 frames.sec⁻¹) through a telecentric lens (Computar 2/3" 55 mm and 2X Extender C-Mount) in response to a calibrated monochromatic 14.2 log photon.cm⁻².s⁻¹, 10 sec, 7.15° stimulus (488 nm, 610 nm; 10-12 nm full-width at half maximum, Edmund Optics) presented to the right eye using a Maxwellian view optical system. The pupillometer was controlled and the data analyzed with custom software (MatLab, Mathworks). In a control study the viewing distance (1.15 m) of the 6.3° x 8.9° back-lit fixation screen (5.9 cd.m⁻²) for the consensual left eye was determined to minimize accommodation and convergence driven pupil fluctuations. The head position in the pupillometer was stabilized with temple bars, head restraint and chin rest. The pupil light reflex was measured for 50 seconds, including 10 sec pre-stimulus, 10 sec stimulus and 30 seconds post-stimulus.
The stimulus and experimental design and matching of controls to patients minimized the effect of optical changes on the pupillary light reflex. To compensate for any change in stimulus retinal irradiance due to senile miosis and/or age-related optical media changes (van de Kraats and van Norren 2007; Pokorny et al. 1987; Age-Related Eye Disease Study Research Group 2001) the stimulus was presented in Maxwellian view and corneal irradiance was set to $14.2 \log \text{photon.cm}^{-2}.\text{s}^{-1}$. The testing was conducted during the day to minimize the circadian driven reduction in ipRGC contributions to the post-illumination pupil response (Zele et al. 2011). While the short wavelength stimulus (488 nm) was chosen to maximize ipRGC contributions to the PIPR (Gamlin et al. 2007; Markwell et al. 2010), the long wavelength stimulus (610 nm) was chosen to study outer retina cone contributions to the PIPR and as a control for fatigue (Markwell et al. 2010; Gamlin et al. 2007; Kankipati et al. 2010). The 488 nm stimulus was always presented before the 610 nm wavelength stimulus at an inter-stimulus interval of ~ 5 min to account for the possibility of a bistable melanopsin and enhancement of the response after long-wavelength exposure (Mure et al. 2009; Hansen et al. 2011).

The initial pupil constriction amplitude is controlled by the outer retina because ipRGC latency is $>1.78$ s for both stimulus lights (McDougal and Gamlin 2010). Pre-stimulus baseline pupil diameter, constriction and re-dilation kinetics, and PIPR amplitudes were derived from the parameters of a best-fitting simple linear and exponential model of the PLR (Zele et al. 2011; Markwell et al. 2010; Feigl et al. 2011). The percentage PIPR amplitude was determined for each participant relative to baseline pupil diameter for the blue (blue PIPR) and red stimuli (red PIPR) (Feigl et al. 2011; Zele et al. 2011) and a net PIPR percentage change (blue PIPR-red PIPR) was calculated (Kankipati et al. 2010).
Report of cases

Seven patients with Type 2 diabetes (mean age 64 ± 6.8 years) and seven healthy age-matched participants were recruited from the Queensland University of Technology (QUT) Eye Clinic. The pupil light responses for the age-matched participants were within the range of normal values reported previously (Feigl et al 2011; Kankipati et al 2010). Table 1 defines each of the diabetic patient’s characteristics. All participants underwent an extensive ocular examination by an ophthalmologist and optometrist. Exclusion criteria included any ocular disease. Participants were phakic in both eyes and were screened for diabetic retinopathy and graded with no retinopathy based on fundus photography (Vujosevic et al. 2009). Best-corrected visual acuity was equal or greater than 6/7.5 in both eyes, except for one patient with diabetes (DM 3) who had a visual acuity equal to 6/12 due to a pterygium that caused an irregular astigmatism. However, the cornea was clear for most of its parts and the pupil response was not affected allowing equal retinal irradiance as in every other participant. Optical coherence tomography (Stratus OCT, Zeiss Meditec, California, USA) was performed in all participants and central macular thickness and nerve fiber thickness were within the normal limits (Duan et al. 2010; Seibold et al. 2010). All patients had well-controlled blood sugar (Table 1), cholesterol and blood pressure levels to exclude metabolic and systemic confounding factors (Aiello et al. 2001). Informed consent was obtained from all participants and the procedures were conducted in accordance with institutional Human Research Ethics Committee approval.

Results

Figure 1 shows the pupil light reflex for the seven patients with diabetes (DM1-DM7) for the 488 nm (blue) and 610 nm (red) stimuli. In this figure, the duration of diabetes increases from top to bottom. The kinetics of the transient pupil constriction and re-dilation for the blue
stimulus were within the normal range in diabetic participants (mean ± SD): Constriction kinetics: Diabetic, \(-1.81 \pm 0.71 \text{ mm.s}^{-1}\) (Control, \(-2.23 \pm 0.54 \text{ mm.s}^{-1}\)); Re-dilation kinetics: Diabetic, \(-0.52 \pm 0.20 \text{ mm.s}^{-1}\) (Control, \(-0.68 \pm 0.13 \text{ mm.s}^{-1}\)), indicating normal autonomic pupil innervation (Ferrari et al. 2007). The average PIPR and net PIPR of the patients with diabetes were lower compared to the healthy control group suggesting altered ipRGC function (Figure 2A, B). The blue PIPR (mean ± SD) for the diabetic group was: 93.7 ± 7.6 (Control = 88.7 ± 4.7) and the net PIPR for the diabetic group was: 4.6% ± SD 6.7 (Control = 9.4% ± 4.7) (Figure 2A, B).

In an additional analysis we evaluated the presence of dysfunction of the outer retinal photoreceptor inputs to the pupil light reflex by determining the amplitude of the transient pupil response as the maximum percent pupil change from the baseline pupil diameter (180-500 ms after stimulus onset) as defined by Kardon et al (2009). With the 488 nm stimulus, the amplitude of the 488 nm transient response is lower in the diabetic group compared to the age-matched controls (mean ± SD = 74.8% ± 7.22 vs 65.4% ± 3.34). The maximum percentage constriction amplitude is lower in the diabetics compared to the age-matched controls (mean ± SD = 58.2% ± 10.44 vs 51.3% ± 5.98) and the time to the maximum percentage constriction amplitude is longer (mean = 11.18 s ± 0.51 vs 11.65 s ± 1.08). With the 610 nm stimulus, the amplitude of the 610 nm transient response is slightly lower in the diabetic group compared to the age-matched controls (mean ± SD = 72.7% ± 5.60 vs 67.91% ± 4.37). The maximum percentage constriction amplitude is lower in the diabetics compared to the age-matched controls (mean ± SD = 60.2% ± 10.95 vs 50.2% ± 4.69) whereas the time to the maximum percentage constriction amplitude is similar (mean ± SD = 11.09 s ± 0.41 vs 11.26 s ± 0.68). All values were derived from the best fitting model to each participants PLR. The results for each diabetic patient are given in Table 1.
To demonstrate the relationship between the amplitude of the blue PIPR and diabetes duration, Figure 2C shows that the PIPR amplitude decreases with increasing diabetes duration (dashed lines show the 95% confidence interval). The slope of the regression line (R^2 = 0.61) is significantly different from zero (F_{1,5} = 7.772, p = 0.038).

Discussion

This is the first report that the post-illumination pupil response (PIPR) can be used to investigate inner retinal ipRGC function in patients with Type II diabetes. The relationship between the duration of the diabetes and blue PIPR amplitude, which was independent of age, indicates that ipRGC function may become progressively more impaired as the duration of diabetes increases. We also found a trend toward altered inner (ipRGC) and outer retinal contributions to the PLR in patients without diabetic retinopathy compared to the age-matched healthy group. These findings support the concept that inner and outer neuronal changes can occur prior to visible microvascular changes.

Studies indicate impaired pupil dynamics such as reduced baseline pupil diameter and re-dilation amplitude occur due to dysfunction in the autonomic innervation of the pupil in diabetics (Ferrari et al. 2007; Yang et al. 2006). However, none of these studies have measured the PIPR for more than a few seconds or used a defined stimulus irradiance and wavelength to isolate the ipRGC response. In one study the re-dilation pupil was smaller in patients with diabetes compared to persons without diabetes and not larger as would be expected due ipRGC dysfunction. That study (Ferrari et al. 2007) evaluated the re-dilation pupil diameter after light offset only for 3 s and at 75% of baseline pupil diameter. The PIPR however, is per definition a sustained pupil constriction longer than >30 s after light offset and therefore the past studies are not directly comparable with our report.
The transient pupil constriction in response to the 488 nm and 610 nm stimuli are controlled by the direct photoreceptor (rod and/or cone) inputs to the olivary pretectal nucleus because the dynamics of the ipRGCs are too slow (McDougal & Gamlin 2010). Given the observed lower transient pupil responses, lower and delayed maximum pupil constriction amplitudes in the diabetic patients, particularly in DM4 and DM6 (and to a lesser extent in DM2 and DM4), we infer that there may be dysfunction in the outer retinal photoreceptor inputs to the olivary pretectal nucleus in patients with diabetes.

Recent studies on ipRGC morphology in mammals and humans demonstrate injury resistance in the presence of induced chronic ocular hypertension (Li et al. 2006), toxic or hereditary neuropathy (La Morgia et al. 2010). This injury resistant characteristic of ipRGCs suggests that the measurement of the ipRGC function via the PIPR may be a useful tool for monitoring the severity and progression of neuroretinal changes in diabetes. It is worth reiterating that ipRGC dysfunction with increasing duration diabetes duration was detected even though the patients’ metabolic (e.g. HbA1c) and systemic factors (e.g. hypertension, cholesterol) were well controlled (Aiello et al. 2001). In the future, it could be of particular interest to investigate ipRGC and outer retinal function in patients with different grades of diabetic retinopathy and to correlate ipRGC function in these patients with anatomical findings using high resolution OCT.

Due to possible non-linear relationship between direct, extrinsic (rod and cone) and intrinsic ipRGCs inputs to the pupil in normal persons and those with disease, their dependence on the stimulus parameters, the possibility of ipRGC resistance to injury in disease (Li et al. 2006) and the limited number of quantitative studies exploring these relationships (Tsujimura et al. 2010; McDougal and Gamlin 2010; Zele et al. 2011; Feigl et al. 2011; Kardon et al. 2010;
Kankipati et al. 2011) further research is required to develop frameworks for differentiating, early and progressive losses inferred to represent the inner and outer retina. This could lead to the clinical application of the PLR as a rapid, non-invasive technique for monitoring the severity of the inner neuroretinal function in diabetes and in retinal disease. It is conceivable that after the measurement of a patient’s baseline PIPR, the routine measurements of the PIPR may complement conventional clinical methods such as ophthalmoscopy, fundus photography, electrophysiological techniques (Harrison et al. 2010) and OCT in the early assessment diabetic neuroretinal changes.

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References


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Figure Legends

**Figure 1.** The pupil light reflex for all seven patients (DM1-DM7) with Type 2 diabetes with no diabetic retinopathy and normal anatomical (macular and nerve fiber layer thickness) findings on OCT. The amplitude of the pupil light reflex for the blue 488 nm (blue line, model; black line, data) and red 610 nm (red line, model; grey line, data) stimuli (yellow bar in lowest panel) is plotted relative to the percentage (%) baseline pupil diameter. The first 10 s of the pupil light reflex represents the baseline pupil before stimulus onset, followed by the response delay (delay in pupil constriction latency after light onset), the transient constriction after light onset, the maintained constriction during the 10 s stimulus and an escape (re-dilation after light offset) to the post-illumination pupil response (PIPR). Patient characteristics are defined in Table 1.

**Figure 2.** Panel (A) shows the blue PIPR (mean ± SD) for the diabetic and age-matched control participants. The ipRGC function is altered in the diabetic group (less sustained, more re-dilation to baseline pupil diameter). Panel (B) shows the net PIPR percentage change (blue PIPR minus red PIPR; mean ± SD) for the two groups. The net PIPR is lower in the diabetic group, further supporting the observation for altered ipRGC function in diabetics. Panel (C) shows the blue PIPR amplitude for the diabetic patients as a function of duration of diabetes. Dashed and solid lines show the 95% confidence limits and regression line, respectively. A longer duration of diabetes is significantly correlated with a reduction in the PIPR amplitude (less sustained response).
Figure 1.
Figure 2.