Intrinsically Photosensitive Retinal Ganglion Cell Function, Sleep Efficiency and Depression in Advanced Age-Related Macular Degeneration

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Citation: Maynard ML, Zele AJ, Kwan AS, Feigl B. Intrinsically photosensitive retinal ganglion cells (ipRGCs) are dysfunctional in various retinal and optic nerve diseases, including AMD. Non-image forming functions of these cells include signaling irradiance information for synchronizing the circadian body clock to the solar day, thus mediating photoentrainment to an altered light and dark cycle but not in ipRGC-deficient mice, demonstrating the ability of light signaling via ipRGCs to influence mood. Given that the melanopsin-mediated PIPR is significantly correlated with sleep efficiency (P = 0.008; regression, P = 0.01, R² = 0.13), but not sleep quality (P = 0.23) in the AMD group. There was no correlation between PIPR and depression scores.

RESULTS. The group with AMD showed significantly reduced pupil constrictions (P = 0.039); PIPR amplitudes (P = 0.003); global sleep scores (P = 0.01); and higher levels of depression (P < 0.001) than the control group. There was a significant correlation between the PIPR amplitude and global sleep score in the AMD group (P = 0.01). The amplitude of PIPR significantly correlated with sleep efficiency (P = 0.008; regression, P = 0.01, R² = 0.13), but not sleep quality (P = 0.23) in the AMD group. There was no correlation between PIPR and depression scores.

CONCLUSIONS. Intrinsically photosensitive RGC dysfunction in advanced AMD contributes to the observed reduction in sleep efficiency. The correlation between the melanopsin-mediated PIPR and sleep may indicate reduced photic input to the suprachiasmatic nucleus and ventrolateral preoptic area due to ipRGC dysfunction in AMD.

Keywords: intrinsically photosensitive retinal ganglion cells (ipRGCs), pupil light reflex, post-illumination pupil response, sleep, depression

PURPOSE. Melanopsin expressing intrinsically photosensitive retinal ganglion cells (ipRGC) input to multiple brain regions including those for pupil control, circadian rhythms, sleep and mood regulation. Here we measured ipRGC function and its relationship to sleep quality and depression in patients with advanced AMD.

METHODS. The melanopsin-mediated post-illumination pupil response (PIPR) was measured in 53 patients with advanced AMD (age 78.8 ± 8.8 years) and in 20 healthy controls (age 72.5 ± 3.3 years). Sleep quality and efficiency was assessed using the Pittsburgh Sleep Quality Index (PSQI). Risk of depression was determined using the Center for Epidemiologic Studies Depression questionnaire.

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dysfunction in AMD is correlated with sleep and mood disorders in patients with advanced stages of this condition. Here we measure melanopsin function using the post-illumination pupil response, and sleep and mood with validated questionnaires to assess nonvisual behavioral disorders in the AMD patients.

METHODS

Participants

Seventy three participants (41 female, 32 male) were recruited from the Queensland Eye Institute (QEI), Queensland University of Technology (QUT) eye clinic, and local optometry practices. Table 1 provides a summary of the participants’ clinical characteristics. Forty six of these participants had advanced neovascular AMD (choroidal neovascularization [CNV]; AREDS grade 4) and were under treatment with antivascular endothelial growth factor (Lucentis; Genentech, San Francisco, CA, USA, or Eylea; Regeneron Pharmaceuticals, Inc., Tarrytown, NY, USA). Seven participants had advanced geographic atrophy (GA; AREDS grade 4) and 20 participants served as healthy controls. The age range of the healthy controls is lower than that of the AMD patients (Table 1), however, this age difference was not statistically significant. All participants underwent an ophthalmic examination that included visual acuity, ophthalmoscopy, intraocular pressure measurement and optical coherence topography (OCT, Cirrus HD-OCT; Carl Zeiss Meditec, Inc., Dublin, CA, USA). Although 30 participants had intraocular lens (IOL) implants, all participants had normal iris musculature post-surgery and cataract removal surgery does not adversely affect circadian rhythm or sleep, with no difference in blue light transmission between blue-blocking and neutral IOLs. Those participants without IOLs (AMD, n = 21; control, n = 16) had crystalline lens opacities less than grade 2 (LOCS III), thus limiting the effect of blue light attenuation by the aging lens. Clinical trials show that an average of 5.6 anti-VEGF injections is administered over 12 months in patients suffering from neovascular AMD. This is in accordance with the treatment frequency in our cohort of 5.8 injections per year; therefore, the number of injections administered was grouped in increments of six for comparison of the treatment effect within a 12-month period (Table 1). Five out of 53 AMD patients were taking antidepressant medication (Zoloft; Pfizer, Inc., New York, NY, USA; Lumin; Alphapharm Pty Limited, Millers Point NSW, Australia; or Lexam; Aspen Pharma Pty Ltd, St. Leonards NSW, Australia) that could affect the pupil response.

Assessment of Sleep and Depression

Sleep was assessed using the Pittsburgh Sleep Quality Index (PSQI) questionnaire, a self-assessed screening tool that has been used to determine sleep disturbances in glaucoma and to determine sleep quality prior to measurement of the circadian response of ipRGCs. The questionnaire is primarily designed to measure sleep based on a global score. Presence and risk of depression was determined using the Center for Epidemiologic Studies Depression Scale (CES-D), a self-report test designed to measure symptoms associated with depression. The 20-item CES-D questionnaire comprises six scales reflecting the major facets of depression: depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance. These symptoms have been used in previously validated scales and the CES-D has high test-retest reliability and internal consistency. The Seasonal Pattern Assessment Questionnaire (SPAQ) was used to screen for the presence of seasonal affective disorder (SAD) and provided insight into factors such as social activity, weight, appetite, and energy that may influence mood. While the SPAQ has high specificity, it has low sensitivity and was therefore used for screening rather than as a diagnostic instrument.

Pupillometry

To assess ipRGC function, we measured the melanopsin-mediated PIPR using customized paradigms developed in our laboratory with high irradiance stimuli designed for use with a pupillographer (RAPDs; Konan Medical USA, Inc., Irvine, CA, USA). Intrinsically photosensitive RGCs produce a sustained pupil constriction after offset of short wavelength light known as the PIPR, which is absent with presentation of long-wavelength stimuli that have low melanopsin excitation. Intrinsically photosensitive RGCs also receive extrinsic signals from rods and cones which can be measured by the initial pupil constriction amplitude. Monochromatic red and blue light stimuli were presented in Newtonian view on a liquid crystal display with a 40-Hz frame rate, with a central physical barrier creating two optical channels for recording binocular pupil responses under infrared illumination. The screen was viewed at infinity through a pair of 50-mm objective lenses to produce a 25° field of view in each eye. Monochromatic stimuli included a 10-second pulse of blue light (short wavelength, 488 nm, corneal irradiance: 14.5 log quanta.cm−2.s−1, luminance: 1.3 log cd.m−2) and red light (long wavelength, 604 nm, corneal irradiance: 14.4 log quanta.cm−2.s−1, luminance: 1.0 log cd.m−2). The spectral outputs of the LED stimuli were measured with a spectroradiometer (StellarNet, Tampa, FL, USA) and irradiance and luminance was measured with a radiometer (IIT1700 Research Radiometer; International Light Technologies, Peabody, MA, USA). A target (green cross) was
used for patient fixation. A display screen mounted on the side of the pupillographer (Konan Medical USA, Inc.) allowed the examiner to monitor patient fixation during testing.

**Procedure**

Where AMD was present in both eyes, the worse eye was dilated with 1% tropicamide (Alcon Laboratories, NSW, Australia), and the preferential eye was dilated in healthy controls. The consensual PLR was measured. Participants were adapted to the dim room illumination (<1 lux) for 10 minutes prior to testing. The pupil testing started with an additional 10 seconds dark adaptation before recording. Baseline pupil diameter was then measured during 5 seconds of fixation prior to onset of the 10-second stimulus and the post-stimulus response was recorded for 40 seconds. This resulted in a 55-second interstimulus interval between the long and short wavelengths stimulus. Questionnaires were administered while the patients’ eye was dilating. All testing occurred between 8 AM and 4 PM to minimize the effect of circadian variation on the PIPR amplitude.

**Data Analysis**

The Pittsburgh Sleep Quality Index comprises seven components that are each scored on a Likert scale from 0 to 3 and then summed to provide a global score, with higher scores indicating poorer sleep quality. While global scores provide an indication of overall sleep quality, factor analysis has since shown that a multiple factor model is statistically favored over a global score, therefore, we scored and analyzed the PSQI using sleep quality and sleep efficiency components. Sleep quality included scores from the subjective sleep quality, sleep latency, and sleep disturbance components, while sleep efficiency included scores from sleep duration and calculated sleep efficiency components (time in bed versus time asleep). Sleep factor scores were calculated by multiplying the component score by the factor loading given by Magee et al. and summing them.

**Results**

There was no significant difference between CNV (n = 46) and GA (n = 7) patients for initial pupil constriction amplitude, PIPR amplitude, depression score, and global sleep score. Therefore, AMD patients with CNV and GA were combined into a single group for additional analyses. There was no significant effect of IOL surgery on all parameters. Multiple regression analysis showed no effect of age ($\beta = 0.14, P = 0.07$, $R^2 = 0.05$); IOL ($\beta = -1.8, P = 0.13, R^2 = 0.05$); and treatment frequency ($\beta = -0.2, P = 0.16, R^2 = 0.08$) on the PIPR. The mean (± standard deviation) values of all measured variables (pupil parameters, sleep and mood scores) for the AMD group and control group are given in Table 2. The long wavelength stimulus showed a low variability (±3.5%), indicative of a minimal effect of autonomic reactivity. The pupil parameters (pupil constriction and PIPR) for the AMD group were significantly reduced compared to the control group, indicating lesser pupil constriction amplitudes at stimulus onset and at post-illumination. The group with AMD had a higher global sleep score indicating poorer sleep compared to controls. Analysis of the sleep factor components demonstrated sleep efficiency scores were higher in the AMD group. However, the sleep quality score was not significantly different between groups. The group with AMD had a significantly higher level of depression compared to the control group (Table 2). All participants scored less than 4 on the SPAQ index, with the exception of one AMD patient who scored 8 out of a possible 24 and was still below the cutoff for seasonal affective disorder.

Correlation analyses identified a positive association between the PIPR amplitude and global sleep score ($r = 0.34, P = 0.01$) for the AMD group (Fig. 1A). As the amplitude of PIPR decreased, indicating a reduced sustained constriction, the
global score increased, demonstrating poorer sleep behavior \( (F_{1,51} = 6.75, \, P = 0.01, \, R^2 = 0.12) \). Correlations between the PIPR amplitude and sleep efficiency and sleep quality factors in the AMD group identified that poorer sleep efficiency was associated with a decrease in PIPR amplitude \( (r = 0.36, \, P = 0.01; \, \text{regression, } F_{1,51} = 7.55, \, P = 0.01, \, R^2 = 0.13; \, \text{Fig. 1C}) \), but no correlation was found between sleep quality and PIPR \( (r = 0.17, \, P = 0.23; \, \text{Fig. 1E}) \). Initial pupil constriction amplitude did not correlate with the global sleep score \( (r = 0.09, \, P = 0.52) \); sleep efficiency \( (r = 0.12, \, P = 0.41) \); or sleep quality \( (r = 0.05, \, P = 0.71) \) in the AMD group. Sleep scores (CNV and GA groups) did not correlate with visual acuity \( (r = -0.04, \, P = 0.77) \) or number of injections administered in the CNV group \( (r = 0.19, \, P = 0.19) \).

For the depression score analysis, 2/53 AMD patients met the criteria for major depression, 2 of the 53 AMD patients had possible major depression and 6 of the 53 AMD patients had symptoms of subthreshold depression. Five AMD patients were taking antidepressant medication; one control participant had symptoms of subthreshold depression. Five AMD patients were identified that poorer sleep efficiency was associated with a decrease in PIPR amplitude \( (r = 0.52, \, P = 0.003^*; \, \text{Fig. 2A}) \). The patients with AMD \( (n = 4) \) with major or possible major depression, according to the CES-D scores, did not have significantly reduced PIPR amplitudes compared to the mean PIPR for the AMD group. Exclusion of the patients on antidepressant medication did not affect the average pupil measurements. There was no correlation between depression scores and the initial pupil constriction amplitude \( (P > 0.05) \). Depression scores did not correlate with visual acuity in the CNV/GA group \( (r = 0.03, \, P = 0.85) \) or number of injections administered in the CNV group \( (r = 0.13, \, P = 0.38) \).

**DISCUSSION**

This is the first study to investigate the relationship between ipRGC function, sleep behavior, and depression in AMD. We hypothesized that altered ipRGC function in AMD will lead to sleep and mood disturbances due to their projections to corresponding brain areas. Based on previous studies, we proposed that aberrant light signaling through dysfunctional ipRGCs will contribute to these behavioral changes. A positive correlation was observed between reduced ipRGC function as measured by the melanopsin-mediated PIPR and poorer sleep efficiency, with ipRGC dysfunction accounting for 13% of the reduced sleep efficiency. There was no relationship between ipRGC function and risk of depression in AMD patients; however, a limitation of this study is that the number of depressed AMD patients was low, hence further studies with a larger cohort are needed.

All three photoreceptor types; rods, cones, and ipRGCs are affected in advanced AMD and contribute to the initial pupil constriction during stimulus presentation, whereas ipRGCs and rods contribute to the sustained post-illumination pupil response. At least five different ipRGC subtypes (M1-M5) project to nonimage forming centers in the rodent brain, with the M1 and M2 subtypes likely to be homologous to the outer and inner stratifying melanopsin cells found in humans and nonhuman primates. Recent mouse study using single M1 ipRGC axonal tracing and confocal microscopic analysis identified up to five different brain targets that received input from a single M1 subtype ipRGC, indicating that a single ipRGC can affect brain areas involved in numerous light-mediated behaviors. Therefore, the correlation between poor sleep efficiency and reduced PIPR may be attributed to individual dysfunctional M1 ipRGs projecting to both the SCN and OPN.

Poor sleep is a common complaint in the aged population, but there is no evidence for a significant age-related difference in sleep behavior consistent with data from our healthy control group, suggesting that the sleep problems detected in

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**TABLE 2.** Mean (± SD) Values for the Pupil Parameters, Sleep Component, and Depression Scores for AMD Patients and Healthy Control Participants

<table>
<thead>
<tr>
<th>Metrics</th>
<th>AMD Participants, ( n = 53 )</th>
<th>Control Participants, ( n = 20 )</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial constriction amplitude (% baseline)</td>
<td>65.7 ± 8.0</td>
<td>61.5 ± 6.9</td>
<td>0.059*</td>
</tr>
<tr>
<td>PIPR amplitude (% baseline)</td>
<td>91.2 ± 5.9</td>
<td>87.4 ± 4.0</td>
<td>0.003*</td>
</tr>
<tr>
<td>PSQI global Score</td>
<td>6.5 ± 3.2</td>
<td>4.5 ± 2.5</td>
<td>0.013*</td>
</tr>
<tr>
<td>Sleep quality score</td>
<td>2.6 ± 1.7</td>
<td>1.9 ± 1.2</td>
<td>0.057</td>
</tr>
<tr>
<td>Sleep efficiency score</td>
<td>1.5 ± 1.1</td>
<td>0.8 ± 0.5</td>
<td>0.001*</td>
</tr>
<tr>
<td>CES-D score</td>
<td>9.3 ± 9.1</td>
<td>2.9 ± 4.0</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

* \( P < 0.05. \)

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**FIGURE 1.** Correlation between PIPR and sleep components. The scatterplot and linear regression lines show the association between the PIPR to a blue light stimulus \( (\lambda_{\text{max}} = 448 \, \text{nm}) \) and sleep components in AMD patients \( (n = 53): \, A, \, C, \, E \) and healthy control participants \( (n = 20): \, B, \, D, \, F \). The horizontal line in the top graphs indicates a cutoff point in the global score to distinguish between “good” and “poor” sleep.
the AMD group are secondary to comorbidities rather than to aging.64 Furthermore, ipRGC function is robust to aging.62,65 with one study showing an enhanced response to high irradiance short wavelength light associated with advancing age.66 Therefore, it is unlikely that the advanced age of the participants provided the basis for the association between ipRGC dysfunction and poor sleep efficiency in this study. Other contributors to poor sleep behavior in the elderly may include changes relating to time in REM and slow-wave sleep,67 medical or psychiatric illness,64 and sleep-related disorders such as restless legs syndrome or sleep apnea.68,69 although only sleep apnea has been previously associated with AMD.70 The sleep quality component of the PSQI questionnaire considers these contributing factors as it addresses sleep disturbances and perceived sleep quality; however, these were nonsignificant in our cohort.

Depression in AMD has been attributed to functional disabilities and loss of independence due to impaired vision.24–26,70,71 Of the AMD patients in this study, 28% had either criteria for risk of depression or were taking antidepressant medication. However, no correlation between ipRGC function and depression in advanced AMD was found, hence our hypothesis for a causal relationship between the dysfunction of these cells and altered signaling to mood centers could not be evaluated in this sample. Irregular light exposure can influence cognitive and mood functions directly through ipRGCs21 as demonstrated in patients with SAD who have a reduced PIPR amplitude.22 Seasonal affective disorder may be caused by an abnormal response to seasonal light changes,72 with melanopsin gene variants increasing the risk for SAD in 5% of individuals.73 As the SAD patients had no ocular pathology, the lower PIPR amplitude may be due to irregular light exposure, although this was not measured in that study.22 Our AMD cohort did not report any symptoms of irregular light exposure, although this was not measured in ocular pathology, the lower PIPR amplitude may be due to pathologic ipRGC dysfunction that leads to aberrant inputs to the brain, rather than due to irregular light exposure. It is still unknown which ipRGC subtype(s) project to mood centers in the human brain and whether these projections are affected by AMD. Visual acuity and number of intravital injections did not correlate with the depression scores in this study. Visual impairment is a risk factor for depression, but it is not an inevitable consequence of vision loss and depression can occur regardless of the level of vision impairment.4,5,47 Further research is needed to determine the cause of the association between AMD and depression.

In conclusion, this study provides the first evidence that ipRGC dysfunction contributes to reduced sleep efficiency in patients with advanced AMD. With subtypes of melanopsin ipRGCs that stratify in different regions of the inner plexiform layer and project differentially to the SCN and OPN, this new knowledge advances insights into ipRGC contributions to AMD and its non-visual-related disorders, allowing a further understanding of this complex condition.

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