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

Michelle L. Maynard, Andrew J. Zele, Anthony S. Kwan and Beatrix Feigl

1Medical Retina and Visual Science Laboratories, Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Australia
2School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia
3School of Optometry and Vision Science, Queensland University of Technology, Brisbane, Australia
4Queensland Eye Institute, South Brisbane, Australia
5Faculty of Health and Behavioural Sciences, University of Queensland, Australia

Correspondence: Beatrix Feigl, Queensland University of Technology, 60 Mulk Avenue, Brisbane, Queensland 4059, Australia; b.feigl@qut.edu.au.
Submitted: August 31, 2016
Accepted: January 8, 2017
Citation: Maynard ML, Zele AJ, Kwan AS, Feigl B. Intrinsically photosensitive retinal ganglion cells (ipRGC) dysfunction in advanced age-related macular degeneration. Invest Ophthalmol Vis Sci. 2017;58:990–996. DOI:10.1167/iovs.16-20659

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.

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

Melanopsin-expressing 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 phototransmission through projections to the suprachiasmatic nucleus (SCN) and sleep induction through activation of the ventrolateral preoptic area. Intrinsically photosensitive RGC dysfunction could therefore alter sleep-wake cycles and affect sleep quality. Intrinsically photosensitive RGCs also signal to the olivary pretectal nucleus (OPN) for mediation of the pupil light reflex (PLR), which is an emerging method for the direct assessment of ipRGC function in healthy and diseased eyes and in persons with chronobiological disorders. Two recent studies observed that sleep duration was altered in advanced AMD patients; sleep duration decreased in wet AMD and increased in dry AMD, but the effect of aberrant ipRGC signaling in AMD as a contributing factor was not evaluated. Supporting evidence for an association between ipRGC dysfunction and sleep disorders is provided in primary open angle glaucoma patients, where there is also decreased sleep quality.

Intrinsically photosensitive RGCs project to brain areas implicated in mood regulation and patients with seasonal affective disorder (SAD) have a dysfunctional melanopsin-mediated PIPR, indicative of a role of aberrant ipRGC signaling in the presence of SAD. In mouse models, depression-related behavior in wild-type mice is observed in response 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 affected in early and late AMD and the prevalence of depression in AMD patients is as high as 39%, with its origin likely to be multifactorial, we determined the role that ipRGC dysfunction in AMD patients has in depression. Based on this evidence, the study aim is to investigate whether ipRGC...
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). Although 30 participants had intraocular lens (IOL) implants, all participants had normal iris musculature postsurgery and cataract removal surgery does not adversely affect circadian response; this was considered in the statistical analysis. There was no ocular disease other than AMD. Written informed consent was obtained from all participants and the study was conducted in accordance with the requirements of the Queensland University of Technology Human Research Ethics Committee and the tenets of the Declaration of Helsinki.

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, \( \lambda_{\text{max}} = 488 \text{ nm} \), corneal irradiance: 14.5 log quanta.cm\(^{-2}.s^{-1} \), luminance: 1.3 log cd.m\(^{-2} \)) and red light (long wavelength, \( \lambda_{\text{max}} = 604 \text{ nm} \), 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.

The Center for Epidemiologic Studies Depression questionnaire is also scored on a Likert scale from 0 to 3, with higher scores indicating more depression symptoms, weighted by frequency of occurrence during the past week. A cutoff score of 16 helps in identifying people at risk for clinical depression. Using continuous scoring criteria as described by Radloff, patients were identified as having no clinically significant depression symptoms, subthreshold depression symptoms, possible or probable major depressive symptoms, or having met major depressive episode criteria. Where depressive symptoms were a concern, the treating ophthalmologist for the AMD patients was informed, and control participants were advised to see a general practitioner. The Seasonal Pattern Assessment Questionnaire measures seasonal variations in six items (mood, appetite, weight, sleep, energy, and socializing) by scoring each item on a 5-point scale ranging from 0 (no change) to 4 (marked change). The global score (range 0–24) was calculated by adding the individual item scores and a score ≥11 identified the presence of SAD. Pupil analysis included identification and extraction of blink artefacts during analysis of pupil recordings by a customized software algorithm with linear interpolation. The data were fitted with linear and exponential models and analyzed according to our established protocols. To control for individual differences in resting pupil diameter, all data are reported as a percentage of the resting baseline pupil diameter (average pupil diameter during a 5-second prestimulus period), a larger percentage value indicates smaller constriction amplitude. The PIPR was quantified at 6 seconds after light stimulus offset. Initial pupil constriction was defined as the minimum pupil diameter within 3 seconds after stimulus onset. The short wavelength stimulus with high melanopsin excitation (609.8 μ-opic lux) served to measure the intrinsic ipRGC response. The long wavelength stimulus with low melanopsin excitation (9.7 μ-opic lux) was presented as the control and to account for autonomic pupillary reactivity and is not reported.

Statistical Analysis
Statistical analyses were performed using commercially available statistical software (IBM SPSS, version 21; IBM Corporation, Armonk, NY, USA). Normality was assessed using the Shapiro-Wilk test. Independent sample t-tests evaluated each variable (presence of IOL, initial constriction, PIPR, sleep quality, sleep efficiency, and level of depression) between the CNV and geographic atrophy patients, as well as between the control group and AMD group. Spearman's correlation coefficient was used to determine the association between the pupil metrics (initial constriction and PIPR) and sleep (global score, sleep quality, and sleep efficiency), as well as between the pupil metrics and depression scores within the AMD group. These analyses were repeated after excluding the five patients who were taking antidepressant medication. The association between visual acuity and number of injections administered with depression and sleep scores was determined using correlation analysis. Where a significant correlation was found, linear regression analyzed the relationship between sleep and depression scores and the pupil metrics. Multiple regression analysis was completed to account for the effect of age, presence of IOL, and treatment frequency. A value of $P < 0.05$ was considered statistically significant.

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 ($β = 0.14, P = 0.07, R^2 = 0.05$); IOL ($β = -1.8, P = 0.13, R^2 = 0.03$); and treatment frequency ($β = -0.2, P = 0.16, R^2 = 0.08$) 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 (±3.3%), 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
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.

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 \( (r = 0.36, P = 0.01; \) regression, \( F_{1,51} = 7.55, P = 0.01, \ R^2 = 0.13; \) Fig. 1C), but no correlation was found between sleep quality and PIPR \( (r = 0.17, P = 0.23; \) 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 taking antidepressant medication; one control participant had symptoms of subthreshold depression. Five AMD patients were taking antidepressant medication; one control participant had symptoms of subthreshold depression.

**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,17,21 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 AMD55 and contribute to the initial pupil constriction during stimulus presentation,7 whereas ipRGCs and rods contribute to the sustained post-illumination pupil response.12,15 At least five different ipRGC subtypes (M1–M5)56,57 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.58–60 A 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.61 Therefore, the correlation between poor sleep efficiency and reduced PIPR may be attributed to individual dysfunctional M1 ipRGCs projecting to both the SCN and OPN.

Poor sleep is a common complaint in the aged population,62 but there is no evidence for a significant age-related difference in sleep behavior63,64 consistent with data from our healthy control group, suggesting that the sleep problems detected in...
the AMD group are secondary to comorbidities rather than to aging.\textsuperscript{64} Furthermore, ipRGC function is robust to aging.\textsuperscript{62,65} with one study showing an enhanced response to high irradiance short wavelength light associated with advancing age.\textsuperscript{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,\textsuperscript{67} medical or psychiatric illness,\textsuperscript{64} and sleep-related disorders such as restless legs syndrome or sleep apnea.\textsuperscript{68,69} although only sleep apnea has been previously associated with AMD.\textsuperscript{69} 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.\textsuperscript{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 ipRGC function and depression in advanced AMD was found, and its nonvision-related disorders, allowing a further understanding of this complex condition.

Acknowledgments

Supported by Australian Research Council Discovery Projects (ARC-DP140100333: AJZ, BF), and an IHBI Vision and Eye Program Grant (BE AJZ). The authors alone are responsible for the content and writing of the paper.

Disclosure: M.L. Maynard, None; A.J. Zele, None; A.S. Kwan, None; B. Feigl, None

References


50. Münch M, Léon L, Crippa SV, Kawasaki A. Circadian and wake-dependent effects on the pupillary light reflex in response to


