Contributions of the Melanopsin-Expressing Ganglion Cells, Cones, and Rods to the Pupillary Light Response in Obstructive Sleep Apnea

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PURPOSE. To investigate the impact of obstructive sleep apnea (OSA) on the contribution of inner and outer retinal photoreceptors to the pupillary light response (PLR).

METHODS. Ninety-three eyes from 27 patients with OSA and 25 healthy controls were tested. OSA severity was graded according to the apnea-hypopnea index. PLR was measured monocularly with an eye tracker in a Ganzfeld in response to 1-second blue (470 nm) and red (640 nm) flashes at −3, −2, −1, 0, 1, 2, and 2.4 log cd/m². Peak pupil constriction amplitude, peak latency, and the postillumination pupil response were measured. The Cambridge Colour Test, standard automatic perimetry, spectral domain optical coherence tomography, polysomnography, and the Pittsburgh Sleep Quality Index were used.

RESULTS. OSA patients have a significantly decreased peak pupil constriction amplitude for blue stimuli at −3, −2, −1, 1 log cd/m² and at all red flash luminances (P < 0.050), revealing reduction of outer retina contributions to PLR. OSA patients showed reduced peak latency for blue (−2, 0, 2, 2.4 log cd/m²) and red stimuli (−2, 0 log cd/m²; P < 0.040). No significant difference was found in the melanopsin-mediated PLR.

CONCLUSIONS. This study is the first to evaluate the inner and outer retinal contributions to PLR in OSA patients. The results showed that the outer retinal photoreceptor contributions to PLR were affected in moderate and severe OSA patients. In contrast, the inner retina contributions to PLR are preserved.

Keywords: obstructive sleep apnea, pupillary light responses, classical photoreceptors, intrinsically photosensitive retinal ganglion cells, circadian rhythm, retinal nerve fiber layer thickness, visual field defect

Obstructive sleep apnea (OSA) is a highly prevalent chronic sleep disorder. According to some epidemiologic studies, prevalence of moderate-to-severe OSA varies from 6% to 17% in the general adult population1,2 and reaches a maximum between the fifth and seventh decades.2 OSA involves repeated episodes of partial or complete obstruction of the upper airway during sleep,3 associated with a decreased oxyhemoglobin saturation and a consequent arterial hypoxemia and hypercapnia, repetitive arousals, intrathoracic pressure changes, hypoxia, endothelial dysfunction, oxidative stress, and activation of systemic inflammation.4–7 OSA patients present an autonomic nervous system (ANS) imbalance due to sleep fragmentation, which involves recurrent arousals that can precipitate post- apneic surges in blood pressure and heart rate, which may occur even during wakefulness.8,9 These pathophysiological changes have been associated with increased risk for several systemic and neurodegenerative diseases involving cardiovascular, metabolic, neurologic,10 Parkinson11 and Alzheimer disease,12 as well as with some ophthalmic disorders, such as glaucoma,13 floppy eyelid syndrome,14,15 nonarteritic ischemic optic neuropathy,16 and retinal vein occlusion.17

The visual system requires large amounts of metabolic energy and the photoreceptor cells demand high oxygen consumption.18 The retina is particularly susceptible to oxidative stress. As a consequence, the deoxygenation and re- oxygenation phenomena associated with apnea may contribute to endothelial injury and compromise the optic nerve integrity and its function.7,10 In fact, many studies have reported a strong link between OSA and optic nerve disorders, associated with a decrease of nerve fiber layer (RNFL) thickness.7,19–21 This thinning of the RNFL in OSA may lead to a dysfunction of the retinal ganglion cells (RGC). In fact, a disrupted circadian rhythm and abnormal melatonin secretion pattern in OSA patients have been reported,22–24 and it is known that the RGC subpopulation of intrinsically photosensitive ganglion cells (ipRGCs) is involved in circadian photoentrainment and in the
Pupil Light Response in Obstructive Sleep Apnea

regulation of melatonin secretion. These RGCs, also known as the third class of photoreceptor, contain the melanopsin photopigment and are known to mediate nonimage-forming visual functions, which include a wide range of responses to light—circadian photorefractometry, sleep regulation, pupillary light response (PLR), and modulation of mood and learning. The possible effects of OSA upon the ipRGCs has not been investigated so far.

The PLR can be used as a noninvasive tool and as an alternative to electroretinography to evaluate retinal and optic nerve functions. It is possible to assess the contribution of outer (rod and cone) and inner (ipRGC) retinal photoreceptors by the characteristics of the response to light stimuli of appropriate wavelengths and intensities for each photosensitive cell group. The outer retinal contributions to the PLR are responsible for the transient constriction, while the inner retinal contribution mediates the sustained constriction during the return to the baseline pupil amplitude.

Therefore, the goal of this study was to evaluate the contribution of inner and outer retinal photoreceptors to the PLR in patients with moderate and severe OSA and to correlate these findings with parameters of visual function (visual field, visual acuity measurement, optical coherence tomography [OCT]) and polysomnography (PSG)).

METHODS

Study Design

This cross-sectional study was designed to investigate the contributions of the inner and outer retina to the PLR in OSA patients. This study was approved by the Brazilian National System for Ethics in Research – SISNEP, Brazil (CAAE: 15172113.0.0000.5561) and conducted in strict adherence to the tenets of the Declaration of Helsinki. Informed written consent was obtained from all study participants.

Subjects

Ninety-three eyes from 27 patients with OSA aging from 37 to 73 years (mean = 54.28 ± 8.89 years, 13 females) and 25 healthy control participants aging from 38 to 74 years (mean = 52.60 ± 9.13 years, 17 females) were recruited from the Department of Otolaryngology of the University of Sao Paulo in Brazil. Participants were all those who met the inclusion criteria and did not fit in the exclusion criteria described below. All subjects underwent a complete ophthalmologic evaluation, including a medical history review, best-corrected visual acuity measurement, gonioscopy, slit-lamp biomicroscopy, dilated funduscopy examination using a 78 diopter (D) lens, refraction, intraocular pressure (IOP) measurement, visual field (VF) test mean deviation (MD) by spectral-domain OCT) and polysomnography (PSG).

Polysomnography (PSG)

The diagnosis of OSA was based on results of a full night assisted PSG diagnosis at the Sleep Laboratory of the Department of Otolaryngology of the University Hospital “Hospital das Clínicas” of Sao Paulo University. PSG assessment was carried out using the polysomnograph Embla (Embla s4000: Embla Systems, Inc., Broomfield, CO, USA) and data were analyzed using Remlogic 1.5 software (Embla Systems, Inc.). PSG data recording followed the American Academy of Sleep Medicine recommendations and included electroencephalogram (F4/M1, C4/M1, O2/M1), submental electromyogram, tibialis anterior muscle electromyogram, electrooculogram (E1/M2, E2/M2), electrocardiogram, airflow (nasal cannula and oronasal thermistor), respiratory effort with thoracic and abdominal inductance plethysmographic belts, pulse oximetry measurements, body position, and snoring intensity. All the sleep tests were manually scored by a certified sleep physician.

The Pittsburgh Sleep Quality Index

Subjective assessment of sleep quality was performed using the Pittsburgh Sleep Quality Index (PSQI). Participants were required to complete this questionnaire, which consists of seven subscales with 19 questions that represent the total sleep quality score ranging from 0 to 21, where higher scores indicate worse sleep quality. Patients with a global score equal to 5 or higher were considered poor sleepers.

Rod, Cone, and ipRGC Contributions to the PLR

Stimuli consisted of a 1-second blue (470 nm) and red (640 nm) light flashes, generated in a Ganzfeld (Q450; Roland Consult, Brandenburg, Germany), controlled by the A-pattern simulation system (RETI–port; Roland Consult). We use photopic luminance values. The units are given as log cd/m². Flashes were presented at the following luminances: −3, −2, −1, 0, 1, 2, 2.4 log cd/m². Photopic and melanopic weighted “alpha-opic” luminance calculated according to Lucas et al. are shown in Supplementary Table S1. The Ganzfeld was factory calibrated; we verified this with a Konica Minolta CS-100A luminance and color meter for the luminance levels (0, 1, 2, 2.4 log cd/m²). For the red and blue flashes, we used specific light-emitting diodes (LEDs) of the Ganzfeld with peak wavelengths at 470 (blue) and 640 nm (red) and full width at half maximum of 26 and 17 nm, respectively. We chose stimulus duration, wavelengths, and luminance based on previous studies. To evaluate the activity of the ipRGCs, flashes at 470 nm were used with luminance from 1 to 2.4 log plot cd/m², alternating with flashes at 640 nm (which falls away from the peak of the melanopsin light absorption spectrum). We used...
flashes at 470 nm below 0 log cd/m² to isolate mainly the rod contributions to the PLR. The rods mediate the transient PLR phase, particularly at low luminance. However, rods have been recently revealed to respond at low and medium intensity red stimuli, and it seems that they contribute at very bright light intensities to circadian photoentrainment as well. It is also known that cones have a contribution to the transient PLR phase, especially in response to long wavelength stimuli (640 nm), and high luminance.

Procedure

Measurements of the PLR were done with an infrared eyetracking camera system (View Point, Arrington Research, Scottsdale, AZ, USA) at 60 Hz sample rate of real-time pupil recording. Subjects were dark adapted for 10 minutes. Both eyes were tested monocularly by covering one eye with an eye patch, in a random order. After dark adaptation, for each luminance level a red flash was presented followed by a blue flash after the offset recording of the red flash. All flashes were 1 second in duration. Responses to flashes below 0 log cd/m² were separated by at least 30 seconds of dark adaptation. For the photopic range of luminances, 1, 2, 2.4 log photopic cd/m² we used an interstimulus interval of 90 seconds of dark adaptation.

This cycle is repeated for each luminance

PLR Analysis

Pupillometric recordings were analyzed using the average pupil diameter during 3 seconds before the flash onset as a baseline value. Noise and blinking artifacts were removed from recordings with median filtering using a 200-ms window and derivative filtering. Responses irreparably contaminated (eye blinks or slow drifts >200 ms) were excluded. The filtered response values were divided by the baseline pupil diameter to normalize the PLR parameters and compare the results among different subjects. Normalized peak pupil constriction amplitude was estimated as the maximum pupil constriction and expressed relative to the baseline value. The peak latency (PL) was defined as the time from stimulus onset to the peak pupil constriction amplitude (PA). The PIPR was expressed as the median pupil diameter between 6 and 8 seconds after the flash offset, relative to the baseline. The percentage of rejected peak pupil constriction amplitude responses at all luminance was 3.16% and for the PIPR it was 1.65%.

Cambridge Colour Test (CCT)

Measurements of the color discrimination thresholds were done using the CCT v2.0 in its Tri-vector and Ellipses protocols with stimuli generated by a ViSaGe 2/S graphics card (Cambridge Research Systems, Rochester, England, UK) and a gamma corrected Sony FD Trinitron color monitor (Model GDM-F500T9; Sony Corporation, Tokyo, Japan). The procedure of calibration was performed using the OptiCAL 200-E photometer and the standard calibration routine of the VSG Desktop library (version 8.0) provided by Cambridge Research Systems.

The design of the CCT is based on the same principle as the Ishihara pseudoisochromatic plates. The adoption of spatial noise and luminance noise in the stimuli is intended to eliminate the influence of spatial contour or luminance clues in the color discrimination response. In the CCT, a mosaic composed of small circles of several sizes and luminance constitutes both the target and the background. A subset of these circles presented at a different chromaticity from the background forms a Landolt “C” stimulus target. The diameter of the circles varied from 5.7 to 22.8 arcmin and their luminance ranged from 7 to 15 phot cd/m². The background chromaticity was set at u’ = 0.1977 and v’ = 0.4689, in CIE 1976 Color Space. Subjects were tested in a darkened room and seated at 3 m from the monitor, the outer diameter of the Landolt’s “C” subtended 4.4° visual angle, the inner diameter 2.3°, and the gap subtended 0.8°.

Monocular tests were performed in both eyes in a randomly selected order. The target was randomly presented with the gap at one of the following four positions: up, down, right, or left (4-alternative forced choice). Subjects were instructed to identify the position of the gap in the “C” using a remote response box control (CT6, CRS). The CCT was performed in its two testing procedures, the Trivector test used to measure thresholds along three confusion axes, proton, deutan, and tritan, and the Ellipses test used to estimate a MacAdam ellipse interpolated along the thresholds obtained at each of eight vectors.

Optical Coherence Tomography (OCT)

Analysis of the peripapillary RNFL thickness was performed using a Cirrus HD-OCT (software v. 5.2, model 4000; Carl Zeiss Meditec, Inc.), which uses a super-luminescent diode scan with a center wavelength of 840 nm and an acquisition rate of 27000 A-scans per second at an axial resolution of 5 µm. Peripapillary RNFL measurements were obtained using a
The Shapiro-Wilk test was performed to assess the normal distribution and graphic inspection of the data. Data from both eyes for each participant were collected to increase potentially the power of the study. In order to compare data across groups, we used a general estimating equations (GEE) method to adjust within subject intereye correlations.61,62 Furthermore, single-eye analyses were made using the Mann-Whitney U test, left eyes were chosen for this specific analysis (see Supplementary Table S2). For variables with parametric distributions, a t-test was performed and for variables with nonparametric distributions, Mann-Whitney U test was used. To analyze sex and age differences between groups, \( \chi^2 \) test was conducted for sex, and an ANOVA for age. ANOVA was performed with Brown-Forsythe correction. Pearson and Spearman correlation coefficients were used to measure the degree of association between some variables that showed losses in the OSA group. For the linear regression analysis, we used only the variables that had a moderate or strong correlation. Whenever both eyes were eligible, the right eye was arbitrarily chosen for this specific analysis.

For all statistical tests, the level of significance was set at \( \alpha = 0.05 \). We used SPSS (version 24.0, SPSS Statistics; IBM, Corp, Armonk, NY, USA) and Stata (version 12.0, StataCorp, College Station, TX, USA) as the software for conducting analyses.

The datasets supporting the conclusions of this manuscript are available on open science framework: https://osf.io/7qwhn.

### Table 1. Demographic, Clinical, and Polysomnographic Data of all Participants

<table>
<thead>
<tr>
<th></th>
<th>Control Group Mean ± SD</th>
<th>95% CI</th>
<th>OSA Group, Moderate to Severe OSA Mean ± SD</th>
<th>95% CI</th>
<th>( P ) Value</th>
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<tbody>
<tr>
<td><strong>Demographic</strong></td>
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<tr>
<td>Gender</td>
<td></td>
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</tr>
<tr>
<td>Female</td>
<td>17 (68%)</td>
<td>31</td>
<td>13 (48.1%)</td>
<td>24</td>
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</tr>
<tr>
<td>Male</td>
<td>8 (32%)</td>
<td>15</td>
<td>14 (51.9%)</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>46</td>
<td>27</td>
<td>47</td>
<td>0.109*</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.28 ± 8.89</td>
<td>50.61–57.95</td>
<td>52.60 ± 9.13</td>
<td>48.98–56.21</td>
<td>0.505†</td>
</tr>
<tr>
<td><strong>Ophthalmologic characteristics</strong></td>
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<tr>
<td>BCVA, logMAR</td>
<td>0.05 ± 0.07</td>
<td>0.03–0.07</td>
<td>0.18 ± 0.61</td>
<td>–0.04 to 0.40</td>
<td>0.506\†</td>
</tr>
<tr>
<td>SE, D</td>
<td>1.21 ± 1.42</td>
<td>0.69–1.71</td>
<td>0.15 ± 1.35</td>
<td>–0.36 to 0.66</td>
<td>0.005‡</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>13.65 ± 1.84</td>
<td>13.00–14.29</td>
<td>14.85 ± 2.18</td>
<td>14.08–15.62</td>
<td>0.008‡</td>
</tr>
<tr>
<td>Cup-to-disc ratio</td>
<td>0.40 ± 0.15</td>
<td>0.35–0.45</td>
<td>0.53 ± 0.58</td>
<td>0.28–0.78</td>
<td>0.584‡</td>
</tr>
<tr>
<td>MD SAP, dB</td>
<td>–1.08 ± 2.02</td>
<td>–1.79 to –3.76</td>
<td>–1.08 ± 1.47</td>
<td>–1.60 to –5.59</td>
<td>0.997\‡</td>
</tr>
<tr>
<td>PSD SAP</td>
<td>2.86 ± 1.92</td>
<td>2.19–3.55</td>
<td>2.34 ± 1.00</td>
<td>1.99–2.69</td>
<td>0.581‡</td>
</tr>
<tr>
<td>VFI SAP</td>
<td>97.26 ± 4.19</td>
<td>95.80–98.72</td>
<td>97.48 ± 1.69</td>
<td>96.78–98.18</td>
<td>0.071\‡</td>
</tr>
<tr>
<td>OCT RNFL thickness, ( \mu )m</td>
<td>96.53 ± 8.26</td>
<td>93.81–99.24</td>
<td>99.41 ± 13.94</td>
<td>94.55–104.28</td>
<td>0.297\‡</td>
</tr>
<tr>
<td>Superior</td>
<td>120.71 ± 17.10</td>
<td>115.09–126.33</td>
<td>120.38 ± 16.88</td>
<td>114.49–126.27</td>
<td>0.935\‡</td>
</tr>
<tr>
<td>Nasal</td>
<td>71.61 ± 9.65</td>
<td>68.43–74.78</td>
<td>75.38 ± 15.09</td>
<td>70.12–80.65</td>
<td>0.217\‡</td>
</tr>
<tr>
<td>Inferior</td>
<td>129.18 ± 14.72</td>
<td>124.55–134.02</td>
<td>151.03 ± 25.62</td>
<td>122.09–159.97</td>
<td>0.693‡</td>
</tr>
<tr>
<td>Temporal</td>
<td>65.42 ± 10.44</td>
<td>59.99–66.85</td>
<td>70.29 ± 15.09</td>
<td>65.03–75.56</td>
<td>0.033‡</td>
</tr>
<tr>
<td><strong>Sleep characteristics</strong></td>
<td></td>
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<td>PSG</td>
<td></td>
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<tr>
<td>AHI (events/h)</td>
<td>5.25 ± 4.38</td>
<td>2.91–7.59</td>
<td>40.87 ± 26.13</td>
<td>33.20–48.54</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>ODI (events/h)</td>
<td>5.26 ± 7.20</td>
<td>1.42–9.09</td>
<td>19.39 ± 16.81</td>
<td>13.87–24.92</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Total sleep time (min)</td>
<td>391.19 ± 35.83</td>
<td>372.09–410.28</td>
<td>351.40 ± 60.58</td>
<td>333.62–369.19</td>
<td>0.010‡</td>
</tr>
<tr>
<td>Total arousal index (events/h)</td>
<td>12.83 ± 6.79</td>
<td>9.21–16.44</td>
<td>41.33 ± 25.17</td>
<td>33.77–48.90</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>SaO(_2) minimum</td>
<td>88.25 ± 5.33</td>
<td>86.47–90.03</td>
<td>78.30 ± 7.52</td>
<td>76.09–80.51</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Wake after sleep onset (min)</td>
<td>57.46 ± 22.35</td>
<td>45.55–69.57</td>
<td>95.66 ± 49.84</td>
<td>81.03–110.29</td>
<td>0.006‡</td>
</tr>
<tr>
<td>REM sleep (%)</td>
<td>21.03 ± 3.91</td>
<td>18.94–25.11</td>
<td>17.83 ± 15.15</td>
<td>13.38–22.28</td>
<td>0.014‡</td>
</tr>
<tr>
<td>PSQI</td>
<td></td>
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<tr>
<td>Sleep efficiency</td>
<td>0.24 ± 0.57</td>
<td>0.07–0.41</td>
<td>0.84 ± 1.14</td>
<td>0.49–1.19</td>
<td>0.006‡</td>
</tr>
<tr>
<td>Sleep latency</td>
<td>1.17 ± 0.85</td>
<td>0.92–1.45</td>
<td>1.23 ± 1.03</td>
<td>0.91–1.54</td>
<td>0.952‡</td>
</tr>
<tr>
<td>Daytime dysfunction</td>
<td>0.65 ± 0.74</td>
<td>0.41–0.85</td>
<td>1.39 ± 1.06</td>
<td>1.06–1.71</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Sleep duration</td>
<td>0.61 ± 0.77</td>
<td>0.38–0.84</td>
<td>1.43 ± 8.46</td>
<td>1.17–16.97</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Sleep disturbance</td>
<td>1.43 ± 0.58</td>
<td>1.26–1.61</td>
<td>2.11 ± 7.84</td>
<td>1.88–2.35</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>Sleep medication</td>
<td>0.26 ± 0.85</td>
<td>0.01–0.51</td>
<td>0.39 ± 0.89</td>
<td>0.11–0.66</td>
<td>0.150‡</td>
</tr>
<tr>
<td>Sleep quality</td>
<td>0.89 ± 0.71</td>
<td>0.68–1.10</td>
<td>1.93 ± 0.82</td>
<td>1.68–2.68</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>PSQI global score</td>
<td>5.24 ± 3.18</td>
<td>4.29–6.18</td>
<td>9.32 ± 4.27</td>
<td>8.02–10.62</td>
<td>&lt;0.001‡</td>
</tr>
</tbody>
</table>

BCVA, best-corrected visual acuity; PSD, pattern standard deviation; VFI, visual field index; REM, rapid eye movement; SaO\(_2\), oxyhemoglobin saturation; SE, spherical equivalent.

* Pearson’s \( \chi^2 \) test.
† Brown-Forsythe ANOVA.
‡ Mann-Whitney U test.
§ t-test.
RESULTS

This study included 47 eyes from 27 patients with OSA aging from 37 to 72 years (mean ± SD = 52.60 ± 9.13 years, 13 females) and 46 eyes from 25 healthy control participants aging from 38 to 74 years (54.28 ± 6 years, 17 females). Eleven eyes (7 from OSA patients and 4 from controls) were excluded according to exclusion criteria. OSA severity was categorized based on the AHI, into moderate (22 eyes) and severe (25 eyes). For statistical analyses, moderate and severe OSA patients were combined into a single group. There was no difference in terms of age, sex, or visual acuity (P > 0.050). Spherical refractive error and IOP showed no significant differences between all participants met the inclusion criteria (IOP < 21 mm Hg and spherical refraction within ± 5.00 D and cylinder refraction within ± 3.00 D, to avoid that high refraction errors could interfere in retinal anatomy and consequently affect the PLR results). Neither the mean VF test by SAP nor the mean RNFL thickness measured with OCT for all quadrants differed significantly between groups (P > 0.050) with exception of the temporal quadrant (P = 0.033). Table 1 shows relevant data on demographic, ophthalmologic, and sleep parameters of the groups.

Rod, Cone, and ipRGC Contributions to the PLR

Table 2 presents the mean PLR measurements for all participants. Figure 2 presents the normalized mean PLR to different flash luminances for control and OSA groups. The normalized peak pupil constriction amplitude of the transient phase as a function of blue and red flash luminance for the groups is shown in Figure 3. Comparison between OSA and control groups for peak pupil constriction amplitude in response to blue stimuli showed significant differences at −3 log cd/m² (P = 0.015), −2 log cd/m² (P = 0.041), −1 log cd/m² (P = 0.008), and 1 log cd/m² (P = 0.038). Significant differences were found at all luminance conditions of the red flash (P < 0.050).

Figure 4 shows the PIPR amplitude of the PLR between 6 and 8 seconds after light offset for the blue stimuli. Statistical comparisons between groups showed no significant differences in the contributions of ipRGCs to the PLR (P > 0.050).

Figure 5 shows the average of the PL of the PLR to blue and red stimuli. PL comparisons between OSA and control groups showed no significant differences between each group in terms of blue and red stimuli. PL comparisons between groups showed no significant differences in the contributions of ipRGCs to the PLR (P > 0.050).

Cambridge Colour Test (CCT)

Results of the Trivector and Ellipse protocols (Figs. 6, 7) did not show significant differences between OSA and control groups (P > 0.050).
Sleep Parameters

OSA patients had significant differences in the PSG parameters and higher PSQI components (P < 0.015) than controls as it is expected in OSA, except for sleep latency and sleep medication questions of the PSQI (P > 0.150; Table 1).

Relations Between PLR and Clinical Measures in OSA

Analysis of the transient PLR amplitude indicated significant correlations among some visual functions, retinal, morphologic parameters, and sleep parameters. There was a negative correlation between ODI and the PLR peak pupil constriction amplitude for the blue flash luminance at −1 log cd/m² (R = −0.516 R² = 0.266; P = 0.003; Supplementary Fig. S1). A significant positive correlation was observed between the mean OCT T with ODI (R = 0.516 R² = 0.266; P = 0.008; Supplementary Fig. S2). There was a positive correlation between the PLR peak pupil constriction amplitude for the blue flash luminance at −2 log cd/m² and the total sleep time (TST; R = 0.490 R² = 0.240; P = 0.003; Supplementary Fig. S3).

DISCUSSION

To our knowledge, this is the first study to investigate the OSA impact on the rod/cone and ipRGC contributions to PLR. We assessed the PLR under conditions designed to preferentially elicit the contribution of the inner and outer retinal photoreceptors and we endeavored to ensure that our study included only patients diagnosed with OSA without comorbidities that might have affected the visual system. Our data showed at low luminance levels (−3, −2, and −1 log cd/m²) for the blue flash and at all luminance levels for the red flash the OSA group had a significantly lower peak pupil constriction amplitude relative to the control group. Therefore, at low luminance levels, the contributions of the rods to the PLR were significantly lower in comparison to the control group. A decrease of the peak pupil constriction amplitude responses for long-wavelength stimuli at high-luminance points out to a reduced rod/cone contribution in moderate and severe OSA patients. Additionally, the OSA group exhibited longer constriction latency. It has been previously shown that pupil latency can be delayed in some diseases due to an afferent-based pathway dysfunction.63,64
In the avascular outer retina, rod photoreceptors are among the most metabolically active cells of the central nervous system and during darkness, the metabolic process requires the highest oxygen consumption of any tissue of the body.\textsuperscript{65} The oxygen consumption of rods and cones increases as light levels fall.\textsuperscript{65,66} OSA-induced hypoxia leads to inflammatory responses, vascular dysregulation, endothelial dysfunction, and oxidative damage, and it is thought to be one of the most critical pathogenesis factors for ischemic retinal diseases, such as proliferative diabetic retinopathy, retinal vessel occlusion, and oxygen-induced retinopathy.\textsuperscript{5,6,15} Here, correlations between the PLR and sleep parameters indicated that a reduced peak pupil response to blue flash luminance at $-2$ and $-1$ log cd/m$^2$ (rod condition) was associated with reduced TST and increased levels of the ODI, respectively. Thus, dysfunctional contributions of the outer retina to PLR found in this study may be associated with OSA’s chronic and intermittent hypoxia.

Even though the RGCs are sensitive to hypoxic conditions, which may cause damage or loss of these cells, the ipRGCs seem to have an injury-resistant character compared with other retinal cells.\textsuperscript{67–71} Our findings agree with this observation as the ipRGC contributions to the PLR assessed by the amplitude of the PIPR between 6 and 8 seconds were preserved in OSA patients.

The ipRGC cells, especially the M1 ipRGC subtype, send their axons to the suprachiasmatic nucleus (SCN) of the hypothalamus to mediate circadian regulation and to other brain nuclei, including the olivary pretectal nucleus involved in the PLR.\textsuperscript{72,73} Melatonin secretion is driven by the master circadian clock SCN and it has been considered a stable biomarker, the gold standard of circadian phase entrainment.\textsuperscript{74,75} Some studies have reported that in OSA patients there is an alteration in circadian rhythms and melatonin...
secretion but our results did not find reduced melanopsin-driven responses in OSA patients. Moreover, in this study, the OSA group presented an enhanced but not statistically significant ipRGC response to PLR compared with controls (Fig. 4). Leon et al. have reported a delayed pupillary re-dilation response after a bright blue light offset significantly greater in patients with outer retinal disease compared with controls. They proposed that the loss of rod and cone contributions to the PLR might be pathologically altered in favor of the melanopsin pupillary response. Further, a previous work of Hannibal et al. reported a higher density in the temporal retina of M1 ipRGC cells, which project mainly to the olivary pretectal nucleus and SCN. Therefore, the enhanced PIPR of the OSA group may be associated to reduced outer retinal photoreceptor contributions to PLR in favor of melanopsin and/or to the only significant difference found in the temporal quadrant of the RNFL thickness, but it is important to note that the mean RNFL thickness values for both groups were within the normal range.

Previous papers have reported a strong link between OSA and optic nerve disorders. Studies of electrophysiology and RNFL thickness have reported conflicting findings in OSA patients, some showed significant reduction of the RNFL thickness, pattern electroretinogram (PERG), and visual evoked potential (VEP) while others found no decrease or alterations of ERG. Our results did not show any reduction of RNFL thickness in OSA patients in agreement with previous studies by Salzgeber et al., Ferrandez et al., and Yu et al., which did not find significant thinning of the RGC layer or macular thickness in OSA patients. In contrast, the contribution of the inner retina photoreceptors, rod and cones, to the PLR were affected in moderate and severe OSA patients. In contrast, the contribution of the inner retina photoreceptors to the PLR was preserved.

In summary, this is a pioneer study that evaluated the impact of OSA on photoreceptor contributions to PLR. As noted above, we did not find color vision defects, reduction of RNFL thickness or VF in OSA patients, but we found significant differences only through the pupillometry protocol. The contributions of the outer retina photoreceptors, rod and cones, to the PLR were affected in moderate and severe OSA patients. In contrast, the contribution of the inner retina photoreceptors to the PLR was preserved. In this context, results indicate that pupillometry can be used as a sensitive noninvasive and promising method for early detection of retinal dysfunction even before major symptoms or ocular diseases appear.

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