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 population and reaches a maximum between the fifth and seventh decades. OSA involves repeated episodes of partial or complete obstruction of the upper airway during sleep, 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. 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. These pathophysiological changes have been associated with increased risk for several systemic and neurodegenerative diseases involving cardiovascular, metabolic, neurologic, Parkinson and Alzheimer disease, as well as with some ophthalmic disorders, such as glaucoma, floppy eyelid syndrome, nonarteritic ischemic optic neuropathy, and retinal vein occlusion.

The visual system requires large amounts of metabolic energy and the photoreceptor cells demand high oxygen consumption. 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. 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. 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, and it is known that the RGC subpopulation of intrinsically photosensitive ganglion cells (ipRGCs) is involved in circadian photoentrainment and in the...
regulation of melatonin secretion. These RGCs, also known as the third class of photoreceptor,\textsuperscript{24,25} contain the melanopsin photopigment and are known to mediate nonimage-forming visual functions, which include a wide range of responses to light—circadian photoentrainment, sleep regulation, pupillary light response (PLR), and modulation of mood and learning.\textsuperscript{27–31} 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.\textsuperscript{32–40} 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.\textsuperscript{27,33,41–45} 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.\textsuperscript{27–31}

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 perimeter, color vision), retinal morphology (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 \(\pm\) 54.28 \(\pm\) 8.89 years, 13 females) and 25 healthy control participants aging from 38 to 74 years (mean \(\pm\) 52.60 \(\pm\) 9.13 years, 17 females) were recruited from the Department of Otolaryngology of Medical School 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 standard automated perimeter (SAP) using the Swedish interactive threshold algorithm (STTA Standard 24–2; Carl Zeiss Meditec, Inc., Dublin, CA, USA), and RNFL thickness evaluated by spectral-domain OCT. All tests were performed within 4-month intervals.

The inclusion criteria were age between 35 and 75 years, best-corrected visual acuity equal to or better than 0.3 logMAR in the study eye, spherical refraction within \(\pm\) 5.00 D and cylinder refraction within \(\pm\) 3.00 D, transparent ocular media (lens opacity \(<\)1) based on the Lens Opacities Classification System III system,\textsuperscript{46} a normal VF (defined as a Glaucoma Hemifield Test [GHT] within normal limits and a MD \(\geq\) -2.0 dB), and an IOP less than 21 mm Hg. On the other hand, the exclusion criteria were concomitant ocular disease, history of ophthalmic surgery, neurologic or psychiatric illness, diabetes, smoking or alcohol abuse history, use of topical or systemic medication, which could potentially affect the pupillary response.

Only patients diagnosed with moderate or severe OSA were enrolled in the study. OSA severity was graded according to the apnea-hypopnea index (AHI) calculated as the number of events of desaturation and apnea or hypopnea (cessation or decrease in respiratory airflow, respectively) per hour of sleep. Subjects with an AHI of 15 or more and less than 30 were regarded as having moderate OSA or severe OSA if the AHI 30 or more. AHI and the oxygen desaturation index (ODI), which is the number of times per hour of sleep that the blood’s oxygen level drops by a certain degree from baseline, were used for correlation.

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 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\textsuperscript{4} and included electroencephalogram (F4/M1, C4/M1, O2/M1), submentum 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).\textsuperscript{47} 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/m\(^2\). Flashes were presented at the following luminances: \(-3, -2, -1, 0, 1, 2, 2.4\) log cd/m\(^2\). For the red and blue flashes, we used specific wavelengths and intensities for each photosensitive cell group.27–31 The possible effects of OSA upon the ipRGCs has not been investigated so far.

To evaluate the activity of the ipRGCs, flashes at 470 nm were used with luminance from 1 to 2.4 log phot cd/m\(^2\), alternating with flashes at 640 nm (which falls away from the peak of the melanopsin light absorption spectrum). We used...
Response values were divided by the baseline pupil diameter to express relative to the baseline value. The peak latency (PL) was defined as the time from stimulus onset to the peak pupil constriction amplitude, and the sustained reponse involves 10 subjects was conducted and the statistical analysis processing. 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, protan, 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
TABLE 1. Demographic, Clinical, and Polysomnographic Data of all Participants

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th></th>
<th></th>
<th>OSA Group, Moderate to Severe OSA</th>
<th></th>
<th></th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean ± SD</td>
<td>Eyes</td>
<td>95% CI</td>
<td>n</td>
<td>Mean ± SD</td>
<td>95% CI</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17 (68%)</td>
<td></td>
<td>31</td>
<td></td>
<td>13 (48.1%)</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Male</td>
<td>8 (32%)</td>
<td></td>
<td>15</td>
<td></td>
<td>14 (51.9%)</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td></td>
<td>46</td>
<td></td>
<td>27</td>
<td></td>
<td>47</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.28 ± 8.89</td>
<td>50.61–57.95</td>
<td></td>
<td></td>
<td>52.60 ± 9.13</td>
<td>48.98–56.21</td>
<td>0.505†</td>
</tr>
<tr>
<td>Ophthalmologic characteristics</td>
<td></td>
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</tr>
<tr>
<td>BCVA, logMAR</td>
<td>0.05 ± 0.07</td>
<td>0.03–0.07</td>
<td></td>
<td></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></td>
<td></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></td>
<td></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></td>
<td></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></td>
<td></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>2.19–5.55</td>
<td></td>
<td></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></td>
<td></td>
<td>97.48 ± 1.69</td>
<td>96.78–98.18</td>
<td>0.071‡</td>
</tr>
<tr>
<td>OCT RNFL thickness, μm</td>
<td>96.53 ± 8.26</td>
<td>93.81–99.24</td>
<td></td>
<td></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></td>
<td></td>
<td>120.38 ± 16.88</td>
<td>114.49–126.27</td>
<td>0.955§</td>
</tr>
<tr>
<td>Nasal</td>
<td>71.61 ± 9.65</td>
<td>68.43–74.78</td>
<td></td>
<td></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></td>
<td></td>
<td>131.03 ± 25.62</td>
<td>122.09–139.97</td>
<td>0.694‡</td>
</tr>
<tr>
<td>Temporal</td>
<td>65.42 ± 10.44</td>
<td>59.99–66.85</td>
<td></td>
<td></td>
<td>70.29 ± 15.09</td>
<td>65.03–75.56</td>
<td>0.033‡</td>
</tr>
<tr>
<td>Sleep characteristics</td>
<td></td>
<td></td>
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<tr>
<td>PSG</td>
<td></td>
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</tr>
<tr>
<td>AH1 (events/h)</td>
<td>5.25 ± 4.38</td>
<td>2.91–7.59</td>
<td></td>
<td></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></td>
<td></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></td>
<td></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></td>
<td></td>
<td>41.33 ± 25.17</td>
<td>33.77–48.90</td>
<td>&lt;0.001‡</td>
</tr>
<tr>
<td>SaO2 minimum</td>
<td>88.25 ± 3.33</td>
<td>86.47–90.03</td>
<td></td>
<td></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></td>
<td></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>18.94–23.11</td>
<td></td>
<td></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>
<tr>
<td>Sleep efficiency</td>
<td>0.24 ± 0.57</td>
<td>0.07–0.41</td>
<td></td>
<td></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></td>
<td></td>
<td>1.23 ± 1.03</td>
<td>0.91–1.54</td>
<td>0.952‡</td>
</tr>
<tr>
<td>Daytime dysfunction</td>
<td>0.63 ± 0.74</td>
<td>0.41–0.85</td>
<td></td>
<td></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></td>
<td></td>
<td>1.43 ± 8.46</td>
<td>1.17–16.09</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></td>
<td></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></td>
<td></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></td>
<td></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></td>
<td></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; SaO2, oxyhemoglobin saturation; SE, spherical equivalent.

* Pearson’s X² test.
† Brown-Forsythe ANOVA.
‡ Mann-Whitney U test.
§ t-test.

The circular sweep with a fixed diameter of 3.45 mm around the optic disc. All information was reviewed and confirmed for the absence of movement artifacts, good centering on the optic disc, and a signal strength above 7. Scans were also evaluated in terms of the adequacy of the algorithm for detecting the RNFL. Only scans without overt algorithm failure in detecting the retinal borders were included.

Data Analysis
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, 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 ɑ = 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.
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 ± 8.89 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 statistically significant difference between each group in terms of age, sex, or visual acuity (P > 0.050). Spherical refractive error and IOP showed a difference between groups (P < 0.050). Spherical refractive error and IOP showed a difference between groups (P < 0.050). Spherical refractive error and IOP showed a difference between groups (P < 0.050).

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 < 0.013), −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 for the blue and red stimuli showed significant differences (P < 0.050) at −2, 0, 2, 2.4 log cd/m², and at −2 and 0 log cd/m², respectively.

It is worth noting that the above PLR significant differences are based on the statistical analysis from both eyes for each participant. However, most of the significant differences remain with the analysis of one eye for each participant (see Supplementary Table S2).

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 $–1 \log \text{cd/m}^2 (R = –0.516 \ R^2 = 0.266; P = 0.003$; Supplementary Fig. S1). A significant positive correlation was observed between the mean OCT T with ODI ($R = 0.516 \ R^2 = 0.266; P = 0.008$; Supplementary Fig. S2). There was a positive correlation between the PLR peak pupil constriction amplitude for the blue flash luminance at $–2 \log \text{cd/m}^2$ and the total sleep time (TST; $R = 0.490 \ R^2 = 0.240; P = 0.003$; 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 co-morbidities that might have affected the visual system. Our data showed at low luminance levels ($–3, –2,$ and $–1 \log \text{cd/m}^2$) 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.$^{55,64}$
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. The oxygen consumption of rods and cones increases as light levels fall. 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. 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/m² (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. 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. 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. Some studies have reported that in OSA patients there is an alteration in circadian rhythms and melatonin...
al.88 suggest that the vascular changes might occur before dysfunction but not the death of the RGC. Findings by Yu et al. reported an impairment in the VF evaluated by SAP, suggesting that the decrease or alterations of ERG.83 Our results did not show a decrease 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.79 The fact that several studies found no differences in the association with retinal diseases.20,92,93 In this study, the onsets of the disease remains uncertain because it was based on the patients' reports, which in most cases did not exceed 4 years. Therefore, OSA patients were evaluated in a baseline condition. These facts show a limitation in the design of this cross-sectional study; longitudinal studies with larger cohorts are needed to assess the progressive effect of OSA on the photoreceptors and its relation to a short- or long-term treatment.

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 appears.

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10. Ferrandez et al.87 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 PLR might be pathologically altered in favor of the 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.78 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 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).

**FIGURE 7.** MacAdam Ellipses presented in the CIE chromaticity diagram (1976, u’v’ units) for control and OSA groups. The colors within the ellipse are those not discriminated from the reference background. The larger the area of the ellipse the worse is the discrimination.


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