Melanopsin-driven pupil response in summer and winter in unipolar seasonal affective disorder.

Kathryn A. Roecklein[a,b,*], Peter L. Franzen[c], Delainey L. Wescott[a], Brant P.Hasler[c], Megan A. Miller[a], Shannon D. Donofry[a], Caitlin M. DuPont[a], Sarah M. Gratzmiller[c], Scott P. Drexler[d], W. Michael Wood-Vasey[e], Paul D. Gamlin[f]

[a] Department of Psychology, University of Pittsburgh, [b] The Center for the Neural Basis of Cognition, [c] Department of Psychiatry, University of Pittsburgh School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
[d] Department of Ophthalmology, University of Pittsburgh School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
[e] Department of Physics and Astronomy, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.
[f] Department of Ophthalmology and Visual Sciences, University of Alabama at Birmingham, Birmingham, Alabama, USA.

*Corresponding Author:
Kathryn A. Roecklein, Ph.D.
Associate Professor
Department of Psychology
University of Pittsburgh
210 S. Bouquet Street
Pittsburgh, PA 15206
(412) 624-4553
kroeck@pitt.edu

Abstract

A retinal subsensitivity to environmental light may trigger Seasonal Affective Disorder (SAD) under low wintertime light conditions. The main aim of this study was to assess the responses of melanopsin-containing retinal ganglion cells in participants ($N = 65$) diagnosed with unipolar SAD compared to controls with no history of depression. Participants attended a summer visit, a winter visit, or both. Retinal responses to light were measured using the post-illumination pupil response (PIPR) to assess melanopsin-driven responses in the non-visual light input pathway. Linear mixed-effects modeling was used to test a group*season interaction on the Net PIPR (red minus blue light response, percent baseline). We observed a significant group*season interaction such that the PIPR decreased from summer to winter significantly in the SAD group while not in the control group. The SAD group PIPR was significantly lower in winter compared to controls but did not differ between groups in summer. Only 60% of the participants underwent an eye health exam, although all participants reported no history of retinal pathology, and eye exam status was neither associated with outcome nor different between groups. This seasonal variation in melanopsin driven non-visual responses to light may be a risk factor for SAD, and further highlights individual differences in responses to light for direct or indirect effects of light on mood.

Keywords: depression, circadian rhythms, environment, melanopsin, ipRGC, pupillometry

1. Introduction

Ten to twenty percent of individuals receiving treatment for depression have a seasonal pattern, or seasonal affective disorder (SAD; Magnusson and Partonen, 2005). The first line of treatment, light therapy, is only effective in about half of individuals with SAD (Terman et al., 1989). To predict light therapy response, a better understanding of SAD etiology is essential. Hypotheses suggest that low winter light levels combined with retinal subsensitivity to light (Hébert et al., 2002; Lewy et al., 2007; Remé et al., 1990; Rohan et al., 2009) results in insufficient input from the retina to the brain for synchronizing the circadian clock with the solar day and direct modulation of mood and alertness (Wehr et al., 2001; Cajochen et al., 2005; Lockley et al., 2006; Schmidt et al., 2011). While both nonseasonal depression and SAD are characterized by delayed circadian rhythms, retinal subsensitivity may be particularly important in SAD due to seasonal variations in daylength and light levels.

Retinal projections to the central nervous system constitute the non-visual light input pathway and contain the photopigment melanopsin (intrinsically-photosensitive retinal ganglion cells, ipRGCs; Hattar et al., 2006; Panda et al., 2005; Berson et al., 2002). Electroretinography (ERG) is used to measure responses from rods, cones and ipRGCs en masse. Decreased ERG responses to light are a SAD biomarker (Gagné and Hébert, 2011), and a state marker of SAD episodes (Lavoie et al., 2009). However, ipRGCs are a minority of retinal photoreceptors (0.3%; Hattar et al., 2002, Berson et al., 2002). While ERG measures overall retinal signaling, it cannot readily isolate specific ipRGC responses.

In mice, depression analog studies show that ipRGCs are involved in the effects of light schedules on mood (LeGates et al., 2012), prompting the study of ipRGC functioning in human mood disorders. Decreased ipRGC responses, combined with lower winter light levels, may result in decreased input to the central circadian clock that falls below the threshold required for alignment of the circadian clock with the solar day (Roecklein et al., 2013a). Downstream implications of decreased ipRGC responsivity could explain circadian etiological hypotheses for SAD, circadian misalignment and delayed circadian phase (Lewy et al., 2006). Results of clinical interventions that realign circadian timing (Lewy, 2007), and basic studies on the links between the SCN and brain areas that regulate mood (Vadnie & McClung, 2017) suggest that circadian dysregulation is implicated in mood disorders. There may also be direct, non circadian effects of light through this pathway on mood (LeGates et al., 2014).

A recent methodological advance – the post-illumination pupil response (PIPR; Gamlin et al., 2007) allows for measurement of ipRGC responses. The PIPR captures the sustained pupil constriction after stimulus offset, isolating ipRGC activity (Gamlin et al., 2007). By taking into account the unique photic response dynamics and the sensitivity of ipRGCs to blue wavelengths, the PIPR maximizes ipRGC contributions to pupil diameter while minimizing rod and cone contributions (Kardon et al., 2009). Studies in healthy humans have established that the PIPR reflects ipRGC responsivity to light (Kawasaki and Kardon, 2007; McDougal and Gamlin, 2010). The PIPR is absent in ganglion cell disorders like anterior ischemic optic neuropathy (Kawasaki et al., 2012), and lower in other retinal ganglion cell disorders (e.g., glaucoma, diabetic retinopathy, retinitis pigmentosa; Feigl et al., 2012, 2011; Kankipati et al., 2011; Kardon et al., 2011; Kawasaki et al., 2012).

We previously reported a lower PIPR in winter in 15 SAD patients compared to 15 controls (Roecklein et al., 2013b). In unipolar depression, the PIPR was lower in 19 individuals with nonseasonal depression compared to 10 controls, but not statistically significant ($p = 0.071$; Laurenzo et al., 2016). That study found a positive association between the PIPR and daylight hours in both groups, suggesting an effect of season and/or previous light history on ipRGC responses. In individuals with traits of Bipolar Disorder, the PIPR was higher in those with mania or hypomania symptoms, but not associated with depression, possibly due to low rates of depression (Bullock et al., 2019). Both Feigl et al. (2018) and Laurenzo et al. (2016) found no statistically significant difference in nonseasonal depression and controls on the PIPR, suggesting that sample sizes larger than 29 may be needed to observe significant differences in nonseasonal depression. Berman et al. (2018) found that a combined SAD and non-seasonal depression group had a lower PIPR compared to controls, and a significant effect of daylight hours was observed across all groups. The Berman et al. (2018) study suggests a seasonal variation in the PIPR although

individuals with SAD were not tested in summer months. To date, a summer vs winter effect of season, and an interaction between diagnostic group and season has not been tested in SAD. The PIPR has also not yet been tested with chronotype, which reflects diurnal preference for activity and social interaction. Although chronotype is significantly associated with circadian phase as measured by time of dim-light melatonin onset (DLMO), DLMO predicted by chronotype score can vary by +/- 2 hours (Kantermann, Sung, & Burgess, 2015).

The aim of the present study was to examine the PIPR in both summer and winter in individuals with and without SAD. We hypothesized that the SAD group would have a lower PIPR during winter compared to nonseasonal, never-depressed controls. We included a seasonal comparison as an exploratory aim, given limited extant data on seasonal variation. Theoretically, lower retinal responsivity to light would be a vulnerability factor that leads to insufficient light input during low winter light conditions. We also tested whether recent light history measured by actigraphy was associated with PIPR magnitude. The PIPR varies across the day (Münch et al., 2012; Zele et al., 2011), so we took steps to avoid measuring individuals with SAD at a different circadian time than controls. First, we controlled for wake time and testing time. Second, we tested whether group, season, or a group*season interaction predicted testing time or wake time in addition to the PIPR. Finally, we tested whether self-reported chronotype was associated with the PIPR.

2. Methods

2.1 Participants.

Participants aged 18-65 were recruited through the Pitt+Me participant registry at the University of Pittsburgh which sent participants emails or letters if they might be eligible for our study. Participants underwent further screening if they opted to phone Pitt+Me in response to a mailing. Participants provided informed consent and the University of Pittsburgh Institutional Review Board approved all study procedures. Exclusion criteria included Psychotic Disorders, Sleep Disordered Breathing, Narcolepsy, current Substance Use Disorder or disorders requiring immediate treatment. Individuals were screened for color vision abnormalities using Ishihara's Test for Colour Deficiency 24-Plates Edition (Clark, 1924). Individuals in the Control group had no history of Mood Disorders. Individuals with Bipolar Disorder were excluded from the present study because a minority of individuals with SAD have a bipolar course, and due to a report of opposite PIPR effects in individuals with bipolar traits (Bullock et al., 2019). Participants were not excluded for antidepressant medication use.

2.2 Diagnostic evaluation.

The Structured Clinical Interview for DSM-IV Axis I disorders (SCID-I; First et al., 2002) was used to diagnose unipolar Major Depressive Disorder with a Seasonal Pattern (APA, 2000). Participants were assessed using the Structured Interview Guide for the Hamilton Depression Rating Scale, Seasonal Affective Disorder version (SIGH-SAD; Williams et al., 1992) to measure depression symptom severity, to confirm summer remission (Terman et al., 1990), and to confirm criteria for a current SAD episode in winter (Terman et al., 1990). Individuals in the SAD and Control group were recruited in summer (June 21st through September 21st) or winter (December 21st through March 21st), and then invited to return the following season.

2.3 Photoperiod.

We tested whether photoperiod on the day of testing differed between groups but did not plan to include photoperiod as a covariate in the main analyses because this variable may be redundant with season as the greatest difference in photoperiod is from summer to winter (i.e., collinear). Photoperiod was collected for each visit date for Pittsburgh from the United States Naval Observatory (https://aa.usno.navy.mil) and was calculated as the difference between the timing of dawn and dusk.

2.4 Chronotype.

Chronotype is one's self-reported propensity for sleep and activity timing to occur relatively early (i.e., morning type) or relatively late (i.e., evening type; Kantermann et al., 2015). We used the Composite Scale of Morningness (CSM; Natale and Alzani, 2001; Smith et al., 1989) as a measure of chronotype.

2.5 Eye health.

As part of the intake assessment, participants self-reported medical history including any history of retinopathy, glaucoma, cataracts, amblyopia, macular degeneration, scotoma, or night blindness. However, reports of the effect of multiple retinal pathologies on the PIPR lead us to consider whether or not participants in our study were truly free of eye health problems. We re-contacted participants to invite them to attend an eye exam at the UPMC Eye and Ear Institute (S.P.D.) including both the anterior and posterior segment of the eye, testing for cataracts, corneal pathologies, macular degeneration, glaucoma, and retinitis pigmentosa using slit lamp technology. Patients aged 50-65 or those with observed ocular changes underwent ocular coherence tomography (OCT) to test for structural retinal changes such as macular degeneration or pre-retinal membrane. The follow-up ophthalmological exam was attended by 60% of the sample.

2.6 Stimuli.

We employed a more effective light stimulus than in Roecklein et al. (2013b; 12.49 log photons/cm^2/s), using a shorter stimulus duration and increased retinal irradiance (Adhikari et al., 2015; Gamlin et al., 2007) potentially amplifying individual differences in the PIPR. Corneal irradiance was determined using a 50-micron aperture spectrophotometer (USB4000 Fiber Optic Spectrometer; Ocean Optics, Dunedin, FL). Irradiance for both red (633 nm; 15.78 nm full width half-maximum (FWHM; the span in nanometers at half maximum) and blue (468 nm; 22.68 FWHM) stimuli resulted in equivalent calculated retinal irradiance (M_{blue} = 15.332 log photons/cm^2/s, SD_{blue} = 0.101 log photons/cm^2/s; M_{red} = 15.304 log photons/cm^2/s, SD_{red} = 0.074 log photons/cm^2/s).

Estimated retinal irradiance for each participant was calculated using corneal irradiance of the stimuli, diameter of the pupil at baseline, and participant age, to account for age-related decreases in blue light transmission from the cornea to the retina. Retinal irradiance (E_r) was based on corneal irradiance (E_c), the lens transmittance for either red or blue wavelength ($T(\lambda, \text{age}) = 10^{-D}$; where $D(\lambda, \text{age})$ is the optical density, solid angular size of the light screen (Ω_{screen} = 0.378 sr; screen diameter, d = 16"; screen distance d_s = 22"), and the solid angular size of the image on the retina (Ω_{eye}, based on pupil aperture diameter a = 3--5 mm, and focal length of the human eye, f = 17.0 mm; Kankipati et al., 2010).

1. $\Omega_{screen} = 2\pi(1-\cos(\arctan(d/2)/d_s) = 0.378$ sr
2. $\Omega_{eye} = 2\pi(1-\cos(\arctan(a/2)/f) = 0.03$ -- 0.05 sr
3. $E_r = (\Omega_{eye}/\ \Omega_{screen})\ T(\lambda, \text{age})\ E_c$

Stimuli were controlled remotely using E-Prime 2 Software (Psychology Software Tools, Inc., Pittsburgh, PA). Light emitting diodes were presented at a distance of 22" from the eye, behind a 16" diameter Mylar diffuser. Although blue light can lead to photochemical injury when in the range of 400-550 nm (Sliney, 1994), the time-integrated hazard-weighted irradiance for the blue stimuli (4 seconds; ACGIH) was at least 36,000 times below hazardous levels.

2.7 Sequence of stimuli.

Participants were exposed to dim light for 60 minutes (<25 lux) to standardize the immediate light history prior to testing (Figure 1). Participants underwent 11 minutes of dark adaptation followed by four repeats of alternating 1 second blue and red stimuli. The red stimuli were followed by 90 seconds of dark, while the blue stimuli were followed by 3 minutes of dark to allow return to baseline. To minimize autonomic pupil constriction, participants were tested in isolation and only contacted through intercom if they fell asleep.

Figure 1. Stimuli and testing timeline, with periods of PIPR measurement indicated.

Duration	Light Condition
60 minutes	<25 lux
11 minutes	<1 lux
6.5 seconds	Baseline
1 second	Blue stimulus
6 seconds post-stimulus offset	PIPR 6
10-30 seconds post-stimulus offset	PIPR AUC
3 minutes post blue stimulus	Inter-stimulus interval (post Blue)
6.5 seconds	baseline
1 second	Red stimulus
6 seconds post-stimulus offset	PIPR 6
10-30 seconds post-stimulus offset	PIPR AUC
90 seconds	Inter-stimulus interval (post Red)

Notes: The Blue and Red stimuli were each repeated 4 times for a total of 8 stimuli. The total duration of darkness following each blue stimulus was 3 minutes, and following each red stimulus was 90 seconds, as return to baseline was faster following the red stimulus. Prior to each stimulus presentation, baseline pupil diameter was calculated for 6.5 seconds.

2.8 Pupillometry.

Pupil diameter was measured using near-infrared illumination and solid-state video with the EYE-TRAC (R) 6000 at 60 Hz (Applied Science Laboratories Inc., Bedford, MA). Focal length was 27 mm with an F-number of 4.5 for the EyeStart 6 camera. A fixation point and forehead and chinrests were provided. The stimulus presentation was binocularly to non-dilated pupils and the diameter of the left eye was measured. All data presented are from the left eye, while both eyes were receiving stimuli and freely reactive.

Artifacts were removed using linear interpolation and replacement of blink data points using a sliding window (MATLAB, MathWorks Inc., Natick, MA; Steinhauer et al., 2000). Baseline pupil diameter was defined as the average pupil diameter across 6.5 seconds prior to stimulus onset, and the PIPRs for both blue and red stimuli were each calculated as percent of baseline in order to control for baseline diameter (Kelbsch et al., 2019). “Non-specific” effects include elevated sympathetic activity in patients with depression causing larger mean pupil diameter (Schumann et al., 2013) and would be similar in red and blue responses, while only the blue response reflects the melanopsin influences on pupil diameter. Therefore, the Net PIPR subtracts non-specific effects from the melanopsin driven blue response.

Retinal irradiance values were regressed on the PIPR, and residual values were averaged by wavelength. PIPR at 6 seconds post stimulus offset is reported as PIPR 6. The PIPR averaged across 10--30 seconds post offset represents the area under the curve (AUC), and is abbreviated PIPR AUC (Adhikari et al., 2015). The advantages and disadvantages of these two PIPR metrics argue for the inclusion of each, although it is currently unknown whether these metrics have different implications for the functioning of ipRGCs. The PIPR 6 has been used to measure amplitude previously (Park et al., 2011), and is recommended due to greater test-retest reliability (Adhikari et al., 2015). PIPR AUC captures average diameter for 20 seconds and is therefore less susceptible to deviations from the overall trend than a single epoch like PIPR 6. However, if diameter begins to approach baseline 10-30 seconds post-illumination, PIPR AUC between subject variance will be compressed.

2.9 Light levels.

Actiwatch Spectrum ® (Phillips Respironics, Murrysville, PA) wrist-worn actigraphs recorded the amount and duration of irradiance in luminous flux (lux) from 400-700nm by minute during 4-7 days in

the same week in which PIPR testing occurred. Light data from times when the participants were awake (based on activity counts) were compared between groups and across seasons, the group*season interaction. Light levels were not included as a covariate in analyses due to missing actigraphy measures for 26 out of 86 participant visits (30%), and because light levels are redundant with season.

2.10 Statistical approach.

Mixed Linear Models including individuals attending either a winter or a summer visit or both were conducted using SPSS Statistics, V 19.0 (SPSS Inc., Chicago, IL). Main effects of group and season, and a group*season interaction were tested for PIPR 6 and PIPR AUC. Because PIPR 6 and PIPR AUC are not known to reflect different retinal responses, correction for multiple tests was not employed. Covariates included age, gender, testing time, wake time, and eye exam (i.e., passed or missing, see section 2.10 Eye health). Simple effect tests were planned to follow a significant interaction to determine whether groups differed within a single season, and whether seasonal variations were present within one group or the other. Chronotype, photoperiod, and light history were compared across group, season, and the group*season interaction using mixed linear models as well, while controlling for age, gender, and testing time.

To visually depict the group*season interaction, average PIPR waveforms were plotted. Epochs that significantly differed ($p < 0.05$) by t-test for at least 1 contiguous second are indicated with black vertical lines along the x-axis. Plots are presented for both groups within winter, both groups within summer, SAD participants between summer and winter, and Control participants between summer and winter (Figure 2).

3. Results

3.1 Pupillometry.

Groups did not differ on age, gender, or photoperiod (Table 1). Twenty-one (32%) participants attended both visits, 15 (23%) attended a winter visit only, and 29 (45%) attended a summer visit only. There was no main effect of group on photoperiod ($F_{1,79.9} = 0.01$, $p = .982$), and photoperiod differed by season as expected ($F_{1,79.9} = 6.44$, $p = .013$; Table 1). Because photoperiod was significantly correlated with season ($r = 0.833$, $p < 0.001$) and had a variance inflation factor (VIF) of 5.71 reflecting multicollinearity, it could not be included as a covariate in analyses testing the effect of season. The global percentage of data replaced due to blinks or artifacts was $M = 14.9\%$, $SD = 0.11\%$, and did not differ between groups ($F_{1,67} = 0.361$, $p = 0.55$). The average baseline pupil diameter prior to the red and blue stimuli did not differ ($F_{1,140.1} = 0.05$, $p = .824$) indicating that the inter-stimulus intervals were of sufficient duration to allow return to baseline.

Of the total sample, 35 participants (52%) completed an eye exam. The remaining 31 participants either moved out of the area, declined to participate in the eye exam, or did not respond to invitations to volunteer for the eye exam. One control participant was excluded due to decreased thickness of the ganglion cell layer in the right eye, decreased macular thickness and decreased retinal nerve fiber layer in the left eye. The remaining 65 participants were compared based on whether they passed the eye exam ($n = 34$), or the eye exam was missing ($n = 31$). The SAD and control groups did not differ in the frequency of missing exams ($X^2_{1,65} = 0.365$, $p > 0.05$). When passed vs. missing Ophthalmological exam was included as a covariate, the pattern of results did not differ, although passed vs. missing eye exam was retained as a covariate.

There were 16 participants in the SAD group taking stable doses of antidepressant medications who nevertheless met criteria for a current major depressive episode (47%). Of those 16, 6 were only taking selective serotonin reuptake inhibitors (SSRI's; Sertraline = 3; Fluoxetine = 2; Escitalopram = 1), two were taking serotonin-norepinephrine reuptake inhibitors (Duloxetine=1; Venlafaxine=1), one reported taking Trazadone, four reported taking Buproprion, two reported taking both SSRIs and Buproprion, and one participant did not specify which antidepressant. Individuals in the SAD group who were taking antidepressants or not were compared on the PIPR while controlling for wake time, testing time, and age.

No statistically significant effect of antidepressants was observed ($F_{1,32.6} = 3.43$, $p = 0.07$) and the effect of antidepressants did not vary based on season of assessment (interaction, $F_{1,32.3} = 2.76$, $p = 0.11$).

The average PIPR values, unadjusted for covariates, are reported in Table 1 as the net of the percent change from baseline for responses to blue minus red stimuli and larger values indicate a larger PIPR. The main effect of group, main effect of season, and group*season interaction were statistically significant predictors of PIPR 6 (Table 2). The main effect of group and the group*season interaction were also statistically significant predictors of PIPR AUC. To further understand the group*season interaction, simple effects were tested including covariates. Although there was a main effect of group on each PIPR metric, there was no group difference in PIPR during summer (PIPR 6, $F_{1,43} = 0.62$, $p = 0.192$, *ns*; PIPR AUC, $F_{1,50} = 1.65$, $p = 0.206$, *ns*) indicating that the main effect of group is due to the significant difference in winter in SAD compared to controls. PIPR 6 was also compared between seasons for each group separately and was not different across the seasons in the Control group ($F_{1,36} = 2.05$, $p = 0.163$, *ns*) indicating that the main effect of season on PIPR 6 is due to the significant seasonal variation in SAD.

In Figure 2, group and season comparisons were plotted and tested for significant differences per epoch (60 Hz). Groups differed in winter, but not in summer, on the Net PIPR for much of the post-stimulus interval. In addition, the control group did not typically differ across seasons, while the SAD group differed across seasons for much of the post-stimulus interval.

3.2 Diurnal variation.

Testing time ranged from 10:00 am to 7:00 pm, with 80% of participants tested prior to 4:00 pm (M = 14:03, SD = 2 hours 33 minutes). The SAD group reported waking 53.4 minutes later than controls ($F_{1,52.71} = 5.12$, $p = .028$), but no main effect of season was observed ($F_{1,27.21} = .386$, $p = .539$), nor was the group*season interaction predictive ($F_{1,25.65} = 2.43$, $p = .131$). Testing time was 2 hours 46 minutes later in winter than summer ($F_{1,77.6} = 7.17$, $p < .01$), but there was no group difference ($F_{1,78.0} = 1.25$, $p = .266$) and no group*season interaction ($F_{1,70.1} = 1.23$, $p = .291$). Having established that groups were not tested at different times, we tested whether individuals with a later chronotype might have attended PIPR sessions at later times. There was no correlation between testing time and chronotype ($R^2 = .03$, *ns*), indicating that our main analyses are unlikely to be confounded by participants in the SAD group having been tested during a different circadian time than controls. Despite no group differences in testing time, wake time and testing time were retained as covariates.

3.3 Chronotype.

The SAD group was more evening-type ($F_{1,74.8} = 4.68$, $p = .034$), but no main effect of season ($F_{1,76.0} = 2.63$, $p = .11$), and no group*season interaction ($F_{1,67.2} = .882$, $p = .419$) were observed. PIPR 6 was associated with increasing eveningness ($b = -.282$, $t(73) = -2.90$, $p = .005$), although chronotype only explained 3.6% of variation in PIPR 6 ($R^2 = .036$) and this effect was only seen in winter. PIPR AUC was not associated with chronotype ($b = -.186$, $t(83) = -1.92$, $p > .05$, $R^2 = .006$).

3.4 Average daily light levels.

Light levels were significantly lower in the winter ($F_{1,52} = 16.41$, $p < 0.001$), as expected (Table 1). No significant differences between groups ($F_{1,28} = 0.044$, $p = 0.835$), or group*season interaction ($F_{2,53} = 0.230$, $p = 0.795$) were observed. There was no significant group difference in Lux in winter ($F_{1,27} = 1.10$, $p = 0.30$) or summer ($F_{1,31} = 0.080$, $p = 0.779$). Light levels were not included as a covariate because 26 (30%) out of 86 visits did not include actigraphy due to participants declining to wear an actiwatch or watch malfunction, and also because Lux, like photoperiod, is significantly correlated with season ($R^2 = 0.61$, $p < 0.001$). Also, given that these are wrist-worn actigraphs, the light sensor was more likely to be obscured by long sleeves during winter compared to summer. When Lux was included in the mixed linear models predicting PIPR 6 and PIPR AUC, the main effect of group ($F_{1,23.5} = 8.70$, $p = .007$) and the group*season interactions were still statistically significant ($F_{2,33.2} = 4.50$, $p = .019$), but the main effect of season was no longer significant as expected ($F_{1,30.8} = 1.44$, $p = .239$), likely due to multicollinearity (VIF = 2.6).

Table 1. Post-illumination pupil response (PIPR) values, demographics, and covariates stratified by season and diagnostic group (SAD; seasonal affective disorder).

	SAD			Control			Both Groups		
	Summer	Winter	Total	Summer	Winter	Total	Summer	Winter	Total
N	16	19	34	21	22	31	37	41	65
Age	35.7±11.9	38.7±13.4	37.7±12.7	34.2±12.5	38.9±12.0	36.5±12.3	35.1±12.2	38.8±12.5	37.5±12.5
Gender			30(88%)			23(74%)			53(81%)
Testing time	15:31±2:15	14:41±2:20	15:07±2:18	12:07±1:47	12:50±2:04	12:30±1:47	13:28±2:36	13:49±2:22	13:39±2:29
Wake time	6:36±1:45	7:07±1:20	6:53±1:32	6:58±1:18	7:18±0:58	7:07±1:09	6:45±1:26	7:16±1:11	7.01±1:20
Eye exam			19(56%)			15(48%)			34(52%)
Photoperiod	13:28±0:53	10:51±0:46	12:18±1:33	13:16±0:49	11:00±0:43	12:25±1:21	13:23±0:51	10:56±0:44	12:21±1:27
Chronotype	34.3±7.5	32.4±9.5	33.2±8.4	38.9±8.3	36.9±8.1	38.2±8.2	36.7±8.2	34.1±9.1	35.6±8.6
SIGH-SAD	9.6±7.6	28.4±7.9	18.2±12.2	4.0±6.1	3.5±3.3	3.8±5.2	6.8±7.4	18.1±14.0	11.5±12.0
Lux	1,529±1,288	233±211	786±1,045	1,334±945	160±182	927±952	1,409±1,082	205±199	847±1,000
PIPR 6	23.5±7.1	19.1±10.4	21.1±9.2	23.5±7.8	26.5±6.9	25.0±7.5	23.1±7.1	23.4±9.6	23.2±8.5
PIPR AUC	14.8±5.0	10.4±8.5	12.8±7.1	14.5±8.0	16.1±6.7	15.1±7.6	14.7±6.7	12.8±8.2	13.9±7.4

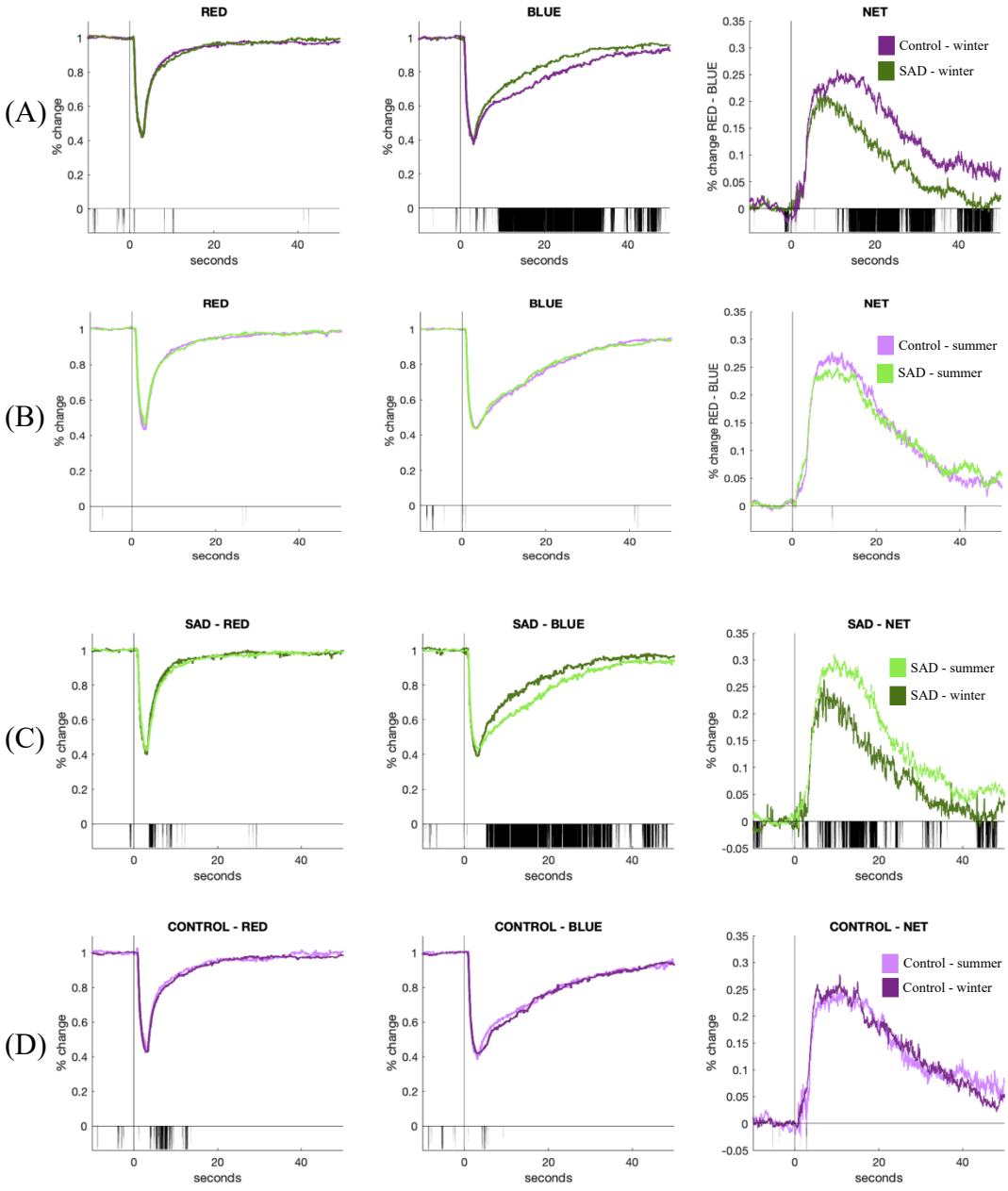
Note: Values except gender and eye exams are reported as mean plus or minus the standard deviation. Gender is reported as the number and percent of women per group. Values for PIPR6 and PIPR AUC are Net percent of baseline (e.g., 23%), which is the Red response percent of baseline (e.g., 98%) minus the blue response percent of baseline (e.g., 75%; $98\%_{red} - 75\%_{blue} = 23\%_{net}$). Net values are highest when the pupil responses to red and blue stimulus differ the most, indicating a larger PIPR. Values are unadjusted for covariates. PIPR 6 is the PIPR at 6 seconds post-stimulus, and PIPR AUC is the area under the curve, or average net pupil diameter, from 10-30 seconds post-stimulus. Rows for a given season include individuals with data from either and both seasons, leading to *N*'s reflecting total number of visits rather than number of participants. Lux is the total luminous flux of light in the visible spectrum during waking hours averaged across 4-7 days of actigraphy prior to PIPR testing. Eye exam is the number of individuals who underwent an Ophthalmological exam and were found to be free of retinal pathology. Photoperiod is the length of time, in minutes, from dawn to dusk on the day the PIPR was assessed in Pittsburgh, PA expressed as hh:mm. Because some individuals attended two visits and some attended one visit, the total number of participants per group and in the total sample are not equal to the number of visits per group in each season (i.e., 86 total visits across 65 participants). Chronotype was measured with the self-report Composite Scale of Morningness, and higher scores indicate a preference for morning activity. The Structured Interview Guide for the Hamilton Depression Rating Scale, SAD Version (SIGH-SAD) was used to measure SAD depression symptom severity.

Table 2. Effects of group, season, and the group*season interaction, as well as covariates on the post-illumination pupil response (PIPR) during different time frames post-stimulus.

	Net PIPR 6					Net PIPR AUC				
				95% CI					95% CI	
	Estimate (*SE*)	*F*	*p*	Lower Bound	Upper Bound	Estimate (*SE*)	*F*	*p*	Lower Bound	Upper Bound
Group	30.02(9.96)	9.08	**.005**	-50.34	-9.69	20.70(8.15)	6.44	**.014**	-37.01	-4.36
Season	22.02(10.07)	4.78	**.035**	-42.46	-1.59	14.91(8.50)	3.08	.084	-31.01	2.08
Group*Season		5.42	**.008**				4.93	**.010**		
Testing time	0.19(0.58)	0.12	.744	-1.34	0.97	0.50(0.55)	0.83	.366	-1.61	0.60
Wake time	-1.01(0.93)	1.18	.284	-0.86	2.89	-0.86(0.88)	0.94	.336	-0.92	2.63
Gender	2.60(3.34)	0.60	.440	-9.26	4.07	2.36(3.31)	0.51	.478	-8.97	4.23
Eye exam	3.76(2.42)	2.41	.125	-8.62	1.08	2.14(2.31)	0.86	.357	-6.77	2.48
Age	-0.46(0.10)	20.25	**<.001**	0.25	0.66	-0.53(0.10)	30.03	**<.001**	0.33	0.72

Notes: *P*-values less than 0.05 are bolded. PIPR values tested are Net (red minus blue PIPR) calculated as percent of baseline. PIPR 6 is the PIPR at 6 seconds post-stimulus, and PIPR AUC is the area under the curve, or average net pupil diameter, from 10-30 seconds post-stimulus. “Eye exam” represents whether an individual passed or was missing the eye exam. Estimates and confidence intervals are only calculated for the two decomposed levels of the group*season interaction, while the significance of the overall interaction effect is shown here.

Figure 2. Pupil diameter in response to red and blue light stimuli compared between groups in winter and summer, and between seasons in individuals with seasonal affective disorder and control participants.



Note: Average PIPR waveforms (% of baseline in pupil diameter) depicting responses to (from left to right) red, blue, and net (red minus blue) stimuli were compared by group and season in those attending both visits ($N = 22$). Top panel: (A) all participants during the winter (SAD $n = 19$; Control $n = 17$), and (B) during the summer (SAD $n = 25$; Control $n = 28$). Bottom panel: (C) Average PIPR waveforms among the SAD participants who had observations during both winter and summer ($n = 11$) and (D) Control participants with both winter and summer observations ($n = 11$). Instances of statistically significant differences transpiring at least 1 second are indicated as vertical black lines on the x-axis for the 50 second post-stimulus recording interval.

4. Discussion

This is the first report that the PIPR varies seasonally in SAD but not in controls. The PIPR was lower in winter than summer in the SAD group, and lower in SAD than controls during winter, while there was no seasonal variation in the control group. This decreased PIPR may indicate that ipRGCs are less responsive to light than is required for euthymic functioning during winter in SAD. Environmental light levels affect mood, sleep, and alertness, and light is the main signal synchronizing the circadian clock. Intrinsically photosensitive retinal ganglion cells (ipRGCs) contribute to circadian synchronization through neural connections with the suprachiasmatic nucleus (SCN), and also project to areas of the brain responsible for the acute alerting effect of light (Rupp et al., 2019). If ipRGCs are less responsive to light, input conveyed by ipRGCs to the CNS may be insufficient to maintain circadian and direct effects of light on mood, sleep, and alertness. If these findings of reduced melanopsin-driven light responses are replicated among individuals who have all been confirmed to be free of retinal health diagnoses, this would implicate the ipRGC light input pathway in the pathogenesis of SAD. Future studies will need to determine the precursor of this reduced responsivity, and the downstream processes that may lead to sleep, alertness, and depressed mood symptoms.

A retinal epiphenomenon of abnormal monoamine functioning in depression may explain a lower PIPR. Seasonal variation in monoamine levels and binding have been observed in SAD (Weng et al., 2009). While the PIPR measures melanopsin-driven responses, the PIPR might be modulated by dopamine released from dopaminergic amacrine cells (DACs; Blasic, Brown & Robinson, 2012; Liu, Spix & Zhang, 2017; Liao, Ren, Peterson, Marshak, Yau, Gamlin, Dacey, 2016). In rodents, connections between DACs and ipRGCs process information about the duration and intensity of light exposure to modulate non-image forming functions (Prigge, Yeh, Liou, Lee, You, Liu, McNeill, Chew, Hattar, Chen & Zhang, 2016; Vuong, Hardi, Barnes & Brecha, 2015; Zhang, Wong, Sollars, Berson, Pickard & McMahon, 2008; Vugler, Redgrave, Semo, Lawrence, Greenwood & Coffey, 2007). In rats, the majority of ipRGCs express the DRD1 receptor (Van Hook, Wong & Berson, 2012). Although SAD has been associated in humans with DRD4 gene variations (e.g., Levitan et al., 2004), DRD1 polymorphisms have not been reported in this group.

The present study included 34 SAD and 31 control participants, which is a larger overall cohort than in previous studies (i.e., 19 SAD participants, Berman et al., 2018; 15 SAD participants, Roecklein et al., 2015). In a sample including SAD and nonseasonal depressed participants as well as controls, Berman et al. (2018) tested a group*daylight hours interaction that is similar to our group*season interaction, but which was not significant. Berman et al. (2018) did observe a main effect of season with the PIPR being lower in winter compared to summer, and a main effect of group in that the PIPR was lower in the combined SAD and nonseasonal depression group compared to the control group regardless of season. In the present study, the group*season interaction for both PIPR 6 and PIPR AUC in this study is interpreted as a function of the large seasonal effect within the SAD group, while no seasonal variation was observed in the control group. It is possible that Berman et al. (2018) did not observe a group*daylight hours interaction because the SAD participants were not assessed in summer months (i.e., June, July, August).

PIPR 6 was associated with self-reported chronotype, while PIPR AUC was not. The association may not be important as it was only observed in winter, was in the opposite direction than predicted, and the effect size was small (R^2 = .036; Cohen, 1988). While the SAD group had a more evening chronotype than the control group, both group means fell into the intermediate chronotype group (i.e., 27-41) and the mean difference in chronotype between groups was less than 5 points. Because half of participants fell into the intermediate chronotype group, a greater range of chronotypes may be necessary for a reliable estimate. Measures of circadian phase such as dim-light melatonin onset may be superior to self-reported chronotype in estimating a relationship between the PIPR and circadian rhythm. Alternatively, direct non-circadian effects of light on mood and alertness may be a more likely mechanism by which ipRGCs influence depression than circadian photoentrainment (LeGates et al., 2014; Stephenson et al., 2012; Fernandez et al., 2018).

4.1 Strengths and Limitations

Group differences could have been confounded had groups been tested at different circadian times. However, groups did not differ in the duration of time between wake and PIPR testing or on chronotype, and groups were not tested at different times, indicating that the decreased PIPR is not merely a function of testing individuals during a time of day when the PIPR might be lower. Although testing time ranged from 10:00 am to 7:00 pm, we did not find a significant effect of testing time on PIPR, possibly because over 80% of participants were tested prior to 4:00 pm when the daily variation in PIPR is relatively stable (Zele et al., 2011; Münch et al., 2012). To determine the time at which the PIPR differs maximally between SAD and control participants multiple daily measures including evening testing times will be required.

We accounted for non-specific effects on pupil diameter due to medications or autonomic arousal by using the Net PIPR, which is the blue response minus the red response. While SSRIs have been shown to increase the sensitivity of the circadian system to light (McGlashan et al., 2018), we did not observe an effect of antidepressants on the PIPR, possibly because of sample size (SAD group = 34; antidepressants yes = 16, no = 18), because we combined antidepressants types for analysis, or because our participants taking antidepressants still met criteria for a major depressive episode during the winter PIPR assessment, so this subgroup may have a more severe illness.

Additional limitations include the rate of loss to follow-up, in that only 32% attended both visits. Future studies should include ophthalmological examinations in all participants. However, nearly 60% of the present sample passed an Ophthalmological examination, and no differences on the PIPR were observed between those who passed the exam and those who were missing exams. Finally, because the pupil will constrict before the offset of a 1-second stimulus, individuals whose pupils constricted faster would receive less overall light incident on the retina. Future studies could use shorter duration stimuli that conclude before the pupil begins to constrict.

4.2 Implications

Light therapy for SAD is effective, suggesting that light therapy compensates for subsensitivity to light (Terman et al., 1987). Although our focus is on retinal responses to light, other approaches to measuring sensitivity to light, such as melatonin suppression and fMRI, deserve mention. Multiple studies have assessed melatonin suppression as an indicator of light sensitivity in SAD. McGlashan et al. (2019) showed that individuals in a nonseasonal depressive episode had lower levels of melatonin suppression than remitted patients, which indicates reduced responsivity to light and is consistent with our findings of reduced melanopsin-driven retinal responsivity. Studies in SAD, however, have found greater melatonin suppression by light during winter (Thompson, Stinson & Smith, 1990; Nathan, Burrows & Norman, 1999) or no difference (Murphy et al., 1993). These contradictory results may be explained by current or recent antidepressant medication use which has been shown to increase responses to light (McGlashan et al., 2018).

Other studies have administered light probes during fMRI scans to assess neural responsivity to light. One study in SAD found that administering blue light heightened the hypothalamic activity in response to emotionally relevant auditory stimuli (Vandewalle, et al., 2011). In unaffected participants, McGlashan et al. (2018) measured BOLD-fMRI responses to light in the hypothalamus and found that activation in this area of the brain correlated with melatonin suppression. Melanopsin likely drives the acute suppression of nocturnal melatonin release (Prayag, Najjar, Gronifer, 2019) although even the relatively short neural pathway from the retina to the pineal has multiple potential vulnerability points. If such fMRI measures were repeated in SAD along with measures of melanopsin-driven retinal responses and circadian phase shifts, it could potentially reveal whether non-visual responses to light are impaired at the level of the retina, the SCN, the habenula, or elsewhere.

Although light treatment is effective in SAD and nonseasonal depression (Tuunainen et al., 2004), about 43-51% of individuals with SAD do not respond to light treatment (Terman et al., 1989). A reduction in melanopsin-driven retinal responses to light observed in the present study may correlate with increased risk of SAD because lower winter light levels combined with lower retinal responses could lead

input to the CNS to fall below threshold for euthymic functioning. Responses to light treatment in SAD could also vary as a function of individual differences in the PIPR. It may be possible to use the PIPR to predict response to light treatment on an individual basis, or to titrate light therapy dosage. Such individualized adjustments in light therapy could potentially reduce the time to remission and risk of relapse in this highly recurrent disorder. Although our results do not suggest one direction or another, it is hypothesized that individuals with SAD who have lower melanopsin-driven responses to light will be most likely to respond to light treatment as it may drive light levels above the necessary threshold. Individuals with SAD and less of a reduction in melanopsin-driven responses to light may not respond to light therapy because they have other risk factors for SAD.

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Figure Titles and Notes

Figure 1. Stimuli and testing timeline, with periods of PIPR measurement indicated.

Notes: The Blue and Red stimuli were each repeated 4 times for a total of 8 stimuli. The total duration of darkness following each blue stimulus was 3 minutes, and following each red stimulus was 90 seconds, as return to baseline was faster following the red stimulus. Prior to each stimulus presentation, baseline pupil diameter was calculated for 6.5 seconds.

Figure 2. Pupil diameter in response to red and blue light stimuli compared between groups in winter and summer, and between seasons in individuals with seasonal affective disorder and control participants.

Note: Average PIPR waveforms (% of baseline in pupil diameter) depicting responses to (from left to right) red, blue, and net (red minus blue) stimuli were compared by group and season in those attending both visits ($N = 22$). Top panel: (A) all participants during the winter (SAD $n = 19$; Control $n = 17$), and (B) during the summer (SAD $n = 25$; Control $n = 28$). Bottom panel: (C) Average PIPR waveforms among the SAD participants who had observations during both winter and summer ($n = 11$) and (D) Control participants with both winter and summer observations ($n = 11$). Instances of statistically significant differences transpiring at least 1 second are indicated as vertical black lines on the x-axis for the 50 second post-stimulus recording interval.