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Pupillary response abnormalities in depressive disorders

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Abstract

Depressive disorders lack objective physiological measurements to characterize the affected population and facilitate study of relevant mechanisms. The melanopsin-mediated light signaling pathway may contribute to seasonal variation and can be measured non-invasively by pupillometry. We prospectively studied changes in melanopsin-mediated pupillary constriction in 19 participants with major depressive disorder (MDD) and 10 control across the summer and winter solstices. The melanopsin-mediated response, as measured by the pupil's sustained constriction six s after a high intensity blue light stimulus, was marginally attenuated in those with MDD relative to controls ($p=0.071$). The participants with MDD unexpectedly showed a significantly reduced transient pupillary response to low intensity red ($p=0.011$) and blue light ($p=0.013$), but not high intensity red and blue light. Sustained pupillary constriction in response to high intensity blue light was more pronounced with increasing daylight hours ($p=0.037$) and was more strongly related to objectively measured versus estimated light exposure. Melanopsin-mediated impairments in pupil response may serve as a biological marker for vulnerability to depression in low light conditions. Assessment of these and other responses to light stimuli, such as response to low intensity light, may be useful for the study of the neurobiology of MDD and related mood disorders.

Keywords

Major depressive disorder; Pupillometry; Seasonality; Bipolar disorder

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1. Introduction

The Global Burden of Disease Study found MDD to be the fourth leading cause of disability worldwide, following lower respiratory tract infections, diarrheal diseases, and perinatal disorders (Ustün et al., 2004). Depressive disorders have a 16% lifetime prevalence in the United States (Kessler et al., 2003) and are associated with occupational and psychological disability (Judd et al., 2000, 2008). The heritability, calculated as 0.37, for unipolar depressive disorders is moderate (Sullivan et al., 2000), but genome-wide association studies have been limited in their ability to link specific genes with increased risk for major depression (Ripke et al., 2013). This indicates there are no single causal genes that by themselves lead to depression, and thus, the development of major depressive disorder is multifactorial. Therefore, research aimed at identifying intermediate phenotypes or endophenotypes is valuable (Glahn et al., 2012), especially considering major depressive disorder currently lacks any reliable, objective, or quantifiable biomarker.

SAD is a current diagnostic subtype of major depressive disorder, defined in the DSM-V as recurrent major depressive disorder with the extra diagnostic criterion “with seasonal pattern,” (Association, 2013) and has a lifetime prevalence of 2.6% (Levitt and Boyle, 2002). Some studies found higher prevalence of SAD at higher latitudes, suggesting at least some individuals are vulnerable to developing a disordered mood in response to the environmental stress of changing light (Imai et al., 2003; Kegel et al., 2009), but prospective studies show a lack of diagnostic stability for SAD (Leonhardt et al., 1994; Sakamoto et al., 1995; Schwartz et al., 1996; Thompson et al., 1995). In the longest follow-up study to date, seasonality was not stable from one decade to the next, yet a winter predominance for depression was confirmed (Cobb et al., 2014). The episodic and multifactorial nature of mood disorders renders such studies based on clinical features challenging. A quantifiable biological measure to assess vulnerability to seasonal exacerbation in mood disorders would provide advantages compared to clinical interviews that rely on the timing of episodes. The pupillary light reflex, measured with pupillometry, has the potential to be one such measure, as it can assess melanopsin-mediated physiologic changes in response to a bright blue light at the peak wavelength sensitivity of melanopsin pigment.

Studies of melanopsin gene variation and seasonal affective disorder suggest a potential relationship between melanopsin-mediated retinohypothalamic tract pathways and SAD (Roeklein et al., 2012, 2013b). The retinal circuitry for transmitting environmental light signals used in chronobiological functions has recently been found to involve melanopsin, a photopigment found in intrinsically photosensitive retinal ganglion cells (ipRGCs) (Guler et al., 2008; Kawasaki and Kardon, 2007). These cells may be indirectly activated by rod and cone photoreceptors or directly activated by intrinsic melanopsin pigment. LeGates and colleagues have provided compelling evidence for the induction of depression-like symptoms in mice by shortening the photoperiod (7 h light/dark cycle) mediated through ipRGCs (LeGates et al., 2012). In healthy humans, the pupillary light reflex has also shown varying responsiveness in relation to changes in seasonal light (Münch et al., 2016).

The melanopsin-mediated pathway is most responsive to bright blue light with a peak sensitivity at a 485 nm wavelength, which causes a sustained contraction of the pupil even

after the termination of the blue-light stimulus. This sustained contraction is called the post-illumination pupil response (PIPR), and at 6-s post-illumination, it is due to the activation of melanopsin causing sustained ipRGC signaling (Adhikari et al., 2015; Park et al., 2011). A red light stimulus will activate the melanopsin-containing retinal ganglion cells via photoreceptors without activating the melanopsin itself. Red light will cause a similar initial, transient pupillary contraction, but with a much faster subsequent dilation back to baseline size, as compared to the sustained reaction to a photopically matched intensity blue light stimulus due to the lack of melanopsin activation in ipRGCs by red light (Park et al., 2011).

The purpose of the current study is to use an innovative approach to understand the influences of light on MDD. We utilized pupillometry to characterize retinal function in individuals with mood disorders as a function of seasonal changes in daylight exposure. When the retina is exposed to 485 nm wavelength light, the melanopsin-mediated pathway is activated causing signals to travel to the suprachiasmatic nucleus via the retinohypothalamic tract and also to the midbrain resulting in the PIPR. Due to the proposed relationship between the melanopsin-mediated retinohypothalamic tract pathway and SAD (Roecklein et al., 2012, 2013b), we were interested in how the melanopsin-mediated PIPR may vary based on a person's diagnosis with seasonal depression and hours of daylight at different seasons. We suspected that those vulnerable to seasonal depressive episodes would show decreased ability to transmit light signals to the brain, which could be quantifiable by comparing pupillary light reflexes. Specifically, we hypothesized that the participants with MDD, particularly those prone to seasonal depressions, would have reduced melanopsin-mediated pupillary light reflexes (PIPR) to high intensity blue light relative to controls.

2. Methods

2.1. Sample

We recruited 19 participants between the ages of 18 and 50 with prior diagnoses of MDD based on structured diagnostic interview through prior participation at the Iowa site of the Genetics of Recurrent Early-Onset Depression II (GenRED II) study (Shi et al., 2011). Participants were recruited from the GenRED II study's participant list as they had already been well phenotyped, and assigned a research diagnosis of MDD. Participants with MDD were evaluated during two different periods a year, each within one month of either the summer or winter solstice. Within each period, they had two visits scheduled approximately two weeks apart. Study visits occurred between 1/2014 and 1/2016. Another 10 participants without major depression served as controls and underwent pupillometry assessments up to every month over the course of an entire year. Assessments were confined to the period surrounding the solstice for those with MDD to reduce participant burden.

2.2. Psychometrics

After consenting at the first scheduled visit, the MDD participants completed a structured diagnostic interview using the Depression and Seasonality Interview – Ontario (DSI-O) to confirm the mood disorder diagnosis (Schaffer et al., 2003). This same interview also included the Seasonal Physical Activity Questionnaire (SPAQ) (Magnusson, 1996), and a chronotype questionnaire, the Composite Scale of Morningness (Folkard et al., 1979). At

both the summer and winter solstice appointments the participants also completed surveys to assess mood including the clinician-administered Hamilton Depression Rating Scale-Seasonal Affective Disorders Version (Williams et al., 1988). Self-reported scales included the Major Depression Inventory (Olsen et al., 2003), Patient Health Questionnaire-9 (Kroenke et al., 2001), and the Generalized Anxiety Disorder-7 (Spitzer et al., 2006). The duration and quality of sleep during both seasons were assessed using the Pittsburgh Sleep Quality Index (Buysse et al., 1989). Sun exposure during each season was examined using the Sun Exposure Questionnaire (Hanwell et al., 2010). Physical activity and light exposure were also measured utilizing the Actiwatch-L which was worn in the two weeks between visits in each seasonal period.

2.3. Pupillometry

Pupillary light reflexes were tested using a DP2000 Neurooptics binocular pupillometer (Neurooptics, Irvine, CA), which consists of an articulating arm attached to an optical head. The optical head contains two miniature video cameras and a bank of light emitting diodes, which can transmit red, green, blue, and white lights over a 5 log unit intensity range. Software allowed photopically matched light intensities to be selected for red and blue light intensities. This allowed for the comparison of rod, cone, and intrinsic melanopsin-mediated pupil response variation under conditions of low mesopic levels of adaptation. Rod responses were tested with three low intensity 1-s blue light (463 nm) stimuli given at 1 cd/m². Cone weighted pupil responses were tested with low intensity, 1 cd/m² (0 log cd/m²), 1-s red light and high intensity red light (2.6 log cd/m²). The intrinsic melanopsin-mediated post-illumination pupil responses at 6 s following termination of the light stimulus were tested with a 1-s bright blue light stimuli (2.6 log cd/m²) compared to 1-s bright red light (622 nm) stimuli (2.6 log cd/m²). The red light activated the melanopsin-containing retinal ganglion cells via the rods and cones without direct activation of the melanopsin and served as an internal control compared to the post-illumination sustained pupil response to the photopically matched bright blue light stimulus. Recordings of pupillometry data from one subject are illustrated in Fig. 1.

The recorded pupil responses were analyzed by automated software developed at the University of Iowa (Randy Kardon M.D., Ph.D. and Pieter Poolman, Ph.D.), which averaged the right and left pupil responses that are simultaneously elicited for each light stimulus. Pupil tracing during intermittent blinks were interpolated using a validated software program. The transient and sustained pupil contractions were reported as percent pupil contraction (contraction amplitude in mm divided by baseline pupil size in mm).

2.4. Statistical analysis

Analyses were conducted using SAS 9.4 (SAS Institute, Inc., Cary, NC). Linear mixed models were used to analyze the between groups variation in pupillary constriction in response to the different light stimuli. Appendix A contains a detailed description of the models that were fitted to the data. The primary outcome of interest was the delayed (6 s post-illumination pupil sustained contraction) response to high intensity blue light. More immediate, transient contractile responses to high and low intensity red and blue light were also analyzed. Model 1 ignored a potential relationship between pupillary constriction and

the number of daylight hours on the day of testing. Model 2 included a linear component for the potential effect of daylight hours. The modeling of a linear relationship in linear mixed models allowed for estimation with as few as two time points in the MDD group while maximizing the data from control participants with repeated correlated observations. The estimated variances of the random effects were used to obtain the standard errors of the fixed effects and to test their significance (Searle et al., 1992; Searle, 1971).

3. Results

A total of 122 pupillometry measurements were taken on 29 subjects in the sample. The MDD group included 19 participants with 58 observations, and the control group included 10 participants with 64 observations. Table 1 displays the sample characteristics. The sample ($N=29$) had a mean age of 47.1 years old on study intake and 72% were female. The MDD group, previously diagnosed by structured interview during the GenRED II study, underwent repeat structured diagnostic interview with one participant updated to bipolar I disorder. Seven (37%) participants in the MDD group met criteria for SAD based on the DSI-O. The measured pupillary responses in the SAD subset did not clinically or statistically differ from the pupillary responses in the remainder of the MDD group and the groups were subsequently merged for analyses. The group with MDD had a Hamilton Depression Rating Scale mean score of 7 ($SD=7$) during the summer evaluation and 6 ($SD=5$) during the winter evaluation.

Fig. 2 illustrates the stimuli responses by number of daylight hours for each group without the statistical interpretation of Models 1 or 2. These response values represent the proportion of the pupil's size following a light stimulus compared to the pupil's baseline size. In Fig. 1, "Red1" and "Blue1" indicates the transient % contraction response to low intensity red-light and blue-light stimulus respectively. "Red400" and "Blue400" indicates the transient % contraction response to the high intensity red-light and blue-light stimulus respectively. "Blue400S7" indicates the sustained % contraction response 6 s after the termination of the 1-s high intensity blue-light stimulus. In Model 1, which isolated group differences, the measurement of melanopsin-mediated sustained contraction 6 s after high intensity blue light was attenuated amongst those with MDD relative to controls with a pupil response difference between groups of 0.074 or 7.4% pupil contraction amplitude difference ($p=0.071$). Those with MDD also demonstrated significantly attenuated transient responses to low intensity red light (response difference=0.087 or 8.7% pupil contraction amplitude difference, $p=0.011$), and low intensity blue light (response difference=0.063 or 6.3% pupil contraction amplitude difference, $p=0.013$). Transient pupil contractions to high intensity red light (response difference=0.013 or 1.3% pupil contraction amplitude difference, $p=0.39$) and high intensity blue light (response difference=0.028 or 2.8% contraction amplitude difference, $p=0.21$) did not significantly differ between groups.

In the corresponding linear mixed model (Model 2) that included the effect of varying daylight hours (depending on the season at which testing occurred), the measurement of sustained contraction 6 s after high intensity blue light remained attenuated in those with MDD with a pupil response difference between groups of 0.072 or 7.2% contraction amplitude difference ($p=0.071$). The difference between groups was significant for low

intensity red light (response difference=0.087 or 8.7% pupil contraction amplitude difference, $p=0.011$), and low intensity blue light (response difference=0.064 or 6.4% contraction amplitude difference, $p=0.013$), while the differences between groups in response to high intensity red light (0.013) and high intensity blue light (0.027) remained similarly insignificant. Daylight hours significantly augmented sustained pupillary constriction following high intensity blue light at a rate of 0.005 or 0.5% pupil contraction increase per hour of daylight ($p=0.037$) and there was no significant difference in this augmentation rate between the two groups ($p=0.45$). The effect of daylight hours on the pupil response was not observed for low intensity red light (hourly reduction=0.001, $p=0.64$), low intensity blue light (hourly reduction=0.001, $p=0.67$), high intensity red light (hourly reduction=0.000, $p=0.83$), or high intensity blue light (hourly reduction=0.001, $p=0.40$).

Considerable individual variability in sustained response to high intensity blue light was observed among participants in the MDD group relative to the control group as illustrated in Fig. 3. Within the MDD group, which had objective measurements of light exposure, the relationship between daylight hours and measured light exposure varied most greatly around the summer solstice as illustrated in Fig. 4. Within the MDD group, sustained pupillary constriction following high intensity blue light was more strongly related to measured light lux exposure than it was to estimated light exposure based on daylight hours (Fig. 5). The estimation results for the linear mixed Model 2, was used to compare the effect of daylight hours exposure to light exposure measured from the light sensor on the Actiwatch-L as it affects pupil response. This comparison was analyzed using only the data from the MDD group as controls had no recorded data on light exposure with the Actiwatch-L. In this analysis, a significant relationship between sustained post-illumination pupil response to high intensity blue light and daylight hours was not found (slope=-0.008, $p=0.196$). However, there was a significant positive effect of measured light exposure, in units of lux, measured with the Actiwatch-L and sustained pupil constriction (slope=-0.006, $p=0.025$), as well as measured light exposure on the initial transient pupil contractile response to high intensity blue light (slope=-0.002, $p=0.023$). Since hours of daylight exposure is in different units compared to the lux units measured from the Actiwatch-L, the slope of the relationships cannot be directly compared.

The findings of Model 1 and 2 were not substantively altered when a covariate for use of anticholinergic medication was added to the existing model ($p=0.4690$, and $p=0.469$ respectively). During the evaluation periods, 8 MDD participants and 0 control participants were using medications with anticholinergic effects including diphenhydramine, oxybutynin, tiotropium, and promethazine. Effects of age and gender were also found to be statistically insignificant at the $p=0.10$ level and also did not substantively alter our findings.

4. Discussion

As hypothesized, participants with MDD demonstrated reductions in the post-illumination pupil response to high-intensity blue light, suggestive of impaired melanopsin-mediated light response and inversely related to the number of daylight hours (i.e., less pronounced in winter months). These findings are similar to a recent study showing a less sensitive post-

illumination pupil response in patients diagnosed with SAD (Roecklein et al., 2013a). The reduction in post-illuminated pupil response in the MDD group did not reach statistical significance at the $p < 0.05$ level, and the direct relationship between the retinohypothalamic tract and MDD cannot be confirmed at this time. Therefore, future studies with adequate power to assess moderation by season are warranted. Contrary to our expectations, there were statistically significant attenuated transient pupillary responses to low intensity red light and low intensity blue light stimuli in those with MDD relative to controls. These light responses were not affected by the seasonal change in daylight hours. The mean difference in the transient, initial pupillary responses was not significant with high intensity blue and high intensity red light. This indicates that depressive symptoms might be associated with a decreased response to lower intensity light stimuli, which identifies a previously unrecognized physiological difference in this group compared to controls. This difference is likely to be mediated by rods and cones, although the low intensity effect was not significantly affected by seasonal differences in light exposure in daylight hours.

For the purpose of our analyses, we collapsed seasonal and non-seasonal MDD into one experimental group after these groups did not show significant differences in pupillary responses. This allowed for an experimental group containing all participants with MDD regardless of subtype that could be compared to the control group. Further, new data from our group supports prior findings that seasonality in MDD as assessed by a standardized questionnaire, is not stable over long-term follow-up, in spite of seasonal effects on mood with winter as the highest risk period for depression (Cobb et al., 2014; Leonhardt et al., 1994; Sakamoto et al., 1995; Schwartz et al., 1996; Thompson et al., 1995). The melanopsin-mediated impairments in pupil response that we observe in those with MDD may serve as a biological marker of light-related vulnerability to depression. Interestingly, greater variability was seen in this measure amongst those with MDD, relative to controls. Future studies could assess whether this measure might allow for the discernment of more biologically homogeneous subgroups of individuals with MDD without relying on a clinical determination that does not remain stable and consistent over long-term follow-up and may be influenced by other factors. A small study found evidence that a variant of cryptochrome 2 (*CRY2*), which establishes day-night cycles, was associated with a more chronic course for mood disorder (Fiedorowicz et al., 2012) and future work could assess whether this or other genetic abnormalities influence melanopsin-mediated pupillary response in MDD.

The key limitation of this study is the small sample size, which was constrained by restricting recruitment to participants in the Iowa GenREDII database, established approximately ten years prior to the current study. Our participants may not have been representative of the general population with MDD, and, in fact, a greater proportion of our sample was treated than has been observed in representative samples (Kessler et al., 2003). Many of the participants in the MDD group were using medications with anticholinergic effects to treat symptoms of other diseases, which did not seem to impact our results on adjustment. Future studies are needed to better discern medication effects. Participants also varied with regard to severity of symptoms and future longitudinal studies could be designed to assess changes from depressive episodes to remission. Further, not all patients appear to get the same light exposure even in the same season as evidenced by luxometer data from the Actiwatch in Fig. 3, indicating that some participants may have spent more time inside,

away from the increased sunlight. Wide variations in individual light exposure have also been documented in previous studies (Aggio et al., 2015; Schmalwieser et al., 2010; Thieden, 2008). Future studies in larger and more generalizable samples in conjunction with light exposure measurements would strengthen the external validity of our findings. Strengths of this study included the prospective design, the use of the Neuroptics binocular pupillometer to differentiate transient rod and cone mediated pupil light reflexes from melanopsin-mediated post-illumination sustained pupil responses, and the gathering of data across the year, with focused recruitment surrounding the extreme periods of daylight exposure in summer and winter.

4.1. Conclusions

There is a quantifiable difference in pupillary light reflexes between healthy people and those with MDD. Pupillary constriction is attenuated in low light rod-cone mediated pathways and in the melanopsin-mediated pathway in those with a history of MDD. Further research may help to standardize the pupil response differences, which would create a more objective means to assess depressive diseases compared to using standardized interviews. This may provide useful pilot data to support further study of the genetics, neurobiology, and treatment of recurrent mood disorders and their seasonal determinants.

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Appendix A. Description of the models used for statistical analysis

Our data includes repeated measures on normal and MDD subjects. Random subject effects are included in all statistical models as the levels of measurement differ across subjects. Our models incorporate a linear effect of the number of daylight hours. A linear effect can be estimated reliably as observations are made over a large range of number of daylight hours. Nonlinear effects of daylight hours, however, could not be estimated as the data for MDD subjects were limited to high and low values.

The following models were considered

$$Y_{gjk} = \mu + \delta_g + S_{j(g)} + \varepsilon_{k(gj)} \quad \text{Model 1}$$

$$Y_{gjk} = \mu + \delta_g + S_{j(g)} + \beta(DH_{gjk}) + \varepsilon_{k(gj)} \quad \text{Model 2}$$

$$Y_{gjk} = \mu + \delta_g + S_{j(g)} + \beta(DH_{gjk}) + \beta_g(\text{Group} \times DH) + \varepsilon_{k(gj)}. \quad \text{Model 3}$$

The subscript g refers to the group ($g=1$ and 2 , for normal and MDD subjects). The parameters δ_g represent the fixed group effects and the difference $\delta_2 - \delta_1$ expresses whether the average measurements differ across groups. The subscript j refers to the subject. Subjects are nested within groups and $S_{j(g)}$ represents a random subject effect with variance $\sigma_{\text{Subject}}^2$. The subscript k refers to the replication taken on the j th subject within the g th group, and $\varepsilon_{k(gj)}$ is the random within-subject effect with variance σ_{Error}^2 . The two variances determine

$$\text{the correlation between measurements on the same subject, } \lambda = \frac{\sigma_{\text{Subject}}^2}{\sigma_{\text{Subject}}^2 + \sigma_{\text{Error}}^2}.$$

Model 1 does not incorporate the number of daylight hours, and merely tests whether there is a difference among the two group means (normal and MDD subjects). Models 2 and 3 incorporate a linear effect of the number of daylight hours, DH_{gjk} . The parameter β in Model 2 reflects the average change in the measurement for each additional hour of daylight and assumes that this change is the same for the normal and the MDD groups. Model 3 includes an interaction component between group and the number of daylight hours. The coefficient β in Model 3 represents the average regression slope and the two interaction effects β_{Normal} and β_{MDD} (with $\beta_{\text{Normal}} + \beta_{\text{MDD}} = 0$) express the deviations of the group-specific slopes from the average slope. Model 3 allows us to investigate whether the relationship between the reduction and the number of daylight hours changes with group.

Models 1 through 3 are special cases of mixed linear regression models, also referred to as repeated measurements models. Mixed linear regression models are studied in detail by Searle (1971) and Searle et al. (1992) and were carried out with the SAS PROC MIXED procedure of the SAS 9.4 statistical software (SAS Institute, Inc., Cary, NC). Parameter estimates for fixed effects and estimates of the two variances (for random subject effect and the noise component) were obtained through restricted maximum likelihood (REML). With repeated measures it is important to incorporate random subject effects as the estimated variances determine the correlation between measurements on the same subject which, in turn, affects the standard errors of the fixed-effects estimates (Searle et al., 1992; Searle, 1971). A large correlation implies that the significance and standard errors of the fixed effects is influenced mostly by the number of subjects, and not the number of observations that are taken on each subject.

Model simplification occurs when considering only MDD subjects, as then there is no group effect. For MDD patients we consider two alternative explanatory variables: the number of daylight hours DH and the luxometer reading LUX (unfortunately, LUX readings were not available for normal subjects). This leads to the mixed linear regression models

$$Y_{jk} = \mu + s_j + \beta_{\text{DH}}(DH_{jk}) + \varepsilon_{k(j)} \quad \text{Model 4}$$

$$Y_{jk} = \mu + s_j + \beta_{LUX}(LUX_{jk}) + \varepsilon_{k(j)} \quad \text{Model 5}$$

Fixed effects β_{DH} and β_{LUX} and variance components $\sigma_{\text{Subject}}^2$ and σ_{Error}^2 are again estimated with REML. Estimates of the variances are used to determine the standard errors of the estimated fixed effects.

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Fig. 1. Example pupillometry recordings

Fig. 1 shows an example of pupil light reflexes from a patient with depression recorded during the summer (top) and winter (bottom). The right and left pupil movements were averaged together in response to binocular stimuli (both eyes open and stimulated simultaneously). Four pupil light reflexes in response to varying intensity and wavelength are shown for summer and winter; low intensity red and blue light stimuli and high intensity red and blue light stimuli. A one s duration stimulus was used (square wave stimulus tracing shown at the top in black, ending at 0 s). The pupil responses were normalized to the baseline pupil size recorded during the one s before the light stimulus to give the percent of baseline size at each time point, sampled at 30 Hz. The time of year when this patient's testing occurred in the summer corresponded to 15 h of daylight and in winter to 10 h of daylight. The subject also wore a wristwatch activity monitor with light meter which recorded a light level of 2255 lx during the summer and 207 lx in the winter. Note that the transient pupil responses mediated by rods and cones were greater during the summer. The sustained post-illumination pupil response (PIPR) to bright blue light provides a measure of melanopsin-mediated pupil contraction, which was also prolonged during the summer compared to the winter in this patient. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 2. Scatterplots of stimuli responses against the number of daylight hours by group (depressed versus normal)

The panels show the responses by group to low intensity red light (Red1), low intensity blue light (Blue1), high intensity red light (Red400), high intensity blue light (Blue400), and the primary outcome of interest, sustained post-illumination pupil constriction to high intensity blue light (Blue400S7). The response, on the y-axis, can be understood as the proportion of the pupil's size following a light stimulus compared to the pupil's baseline size (e.g. -0.2 equals a 20% sustained pupil constriction measured 6 s following termination of a one s light stimulus). Regression lines (solid for control group, dashed for MDD group) model the impact of daylight hours by group, and unlike the mixed models presented, assume independent observations. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Scatterplots of the sustained response to high intensity blue light (Blue400S7) against the number of daylight hours for the two groups of participants

Observations from the same subject are connected to show how response changes with daylight hours. The y-axis represents the percent pupil contraction of the post-illumination pupil response to a one s duration bright blue light (e.g. -0.2 equals a 20% sustained pupil contraction measured 6 s following termination of a one s light stimulus). A negative slope indicates a greater sustained post-illumination pupil contraction with increasing daylight hours of light exposure during the summer compared to the winter. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Fig. 4. Scatterplot of the participants with MDD luxometer response against the number of daylight hours, with observations from the same subject connected

The figure shows that in the weeks surrounding the winter solstice, the variations in the luxometer reading (light intensity sensor on the actiwatch, reported on the y-axis as “LightLuxAve”) were quite small. In the weeks, surrounding the summer solstice, we observed a large variation in luxometer readings, presumably due to greater variability in outdoor activities. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

Fig. 5. Scatterplots of responses to sustained pupillary constriction (post-illumination pupil response) following high intensity blue light (Blue400S7) against the number of daylight hours and against the average light lux for depressed subjects

The y-axis represents the percent pupil contraction of the post-illumination pupil response to a one s duration bright blue light (e.g. -0.2 equals a 20% sustained pupil contraction measured 6 ss following termination of a one s light stimulus). Attenuated sustained responses to high intensity blue light are seen with fewer daylight hours (left panel) and lower light exposure (right panel). Unlike the mixed models presented in the primary models, the regression lines shown do not take into account subject effects and treat all observations as independent. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Clinical and sociodemographic characteristics of the sample.

Variable	Total sample	Mood disorder	Controls
	N=29	N=19	N=10
Mean (SD)			
Age	47.1 (11.6)	47.8 (11.3)	46.0 (12.6)
N (%)			
Female gender	21 (72%)	15 (79%)	6 (60%)
Major Depressive Disorder	18 (62%)	18 (95%)	0 (0%)
Married		11 (58%)	
Unemployed/Disabled	3 (10%)	3 (16%)	0 (0%)
Education			
Less than High School	1 (3%)	1 (5%)	0 (0%)
High School Graduate	11 (38%)	6 (32%)	5 (50%)
College Graduate	17 (59%)	12 (63%)	5 (50%)
Medication			
Anticholinergic	8 (28%)	8 (42%)	0 (0%)
Antidepressant	16 (55%)	16 (84%)	0 (0%)
NDRI	8 (28%)	8 (42%)	0 (0%)
SSRI	7 (24%)	7 (37%)	0 (0%)
SNRI	3 (10%)	3 (16%)	0 (0%)
MAOI	0 (0%)	0 (0%)	0 (0%)
TCA	0 (0%)	0 (0%)	0 (0%)