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Melanopsin Gene Variations Interact With Season to Predict Sleep Onset and Chronotype

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Abstract

The human melanopsin gene has been reported to mediate risk for seasonal affective disorder (SAD), which is hypothesized to be caused by decreased photic input during winter when light levels fall below threshold, resulting in differences in circadian phase and/or sleep. However, it is unclear if melanopsin increases risk of SAD by causing differences in sleep or circadian phase, or if those differences are symptoms of the mood disorder. To determine if melanopsin sequence variations are associated with differences in sleep-wake behavior among those not suffering from a mood disorder, the authors tested associations between melanopsin gene polymorphisms and self-reported sleep timing (sleep onset and wake time) in a community sample (N = 234) of non-Hispanic Caucasian participants (age 30–54 yrs) with no history of psychological, neurological, or sleep disorders. The authors also tested the effect of melanopsin variations on differences in preferred sleep and activity timing (i.e., chronotype), which may reflect differences in circadian phase, sleep homeostasis, or both. Daylength on the day of assessment was measured and included in analyses. DNA samples were genotyped for melanopsin gene polymorphisms using fluorescence polarization. P10L genotype interacted with daylength to predict self-reported sleep onset (interaction $p < .05$). Specifically, sleep onset among those with the TT genotype was later in the day when individuals were assessed on longer days and earlier in the day on shorter days, whereas individuals in the other genotype groups (i.e., CC and CT) did not show this interaction effect. P10L genotype also interacted in an analogous way with daylength to predict self-reported morningness (interaction $p < .05$). These results suggest that the P10L TT genotype interacts with daylength to predispose individuals to vary in sleep onset and chronotype as a function of

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daylength, whereas other genotypes at P10L do not seem to have effects that vary by daylength. A better understanding of how melanopsin confers heightened responsivity to daylength may improve our understanding of a broad range of behavioral responses to light (i.e., circadian, sleep, mood) as well as the etiology of disorders with seasonal patterns of recurrence or exacerbation.

Keywords

Chronotype; Genetics; Melanopsin; Seasonal affective disorder; Sleep

INTRODUCTION

A variation in the gene for melanopsin (P10L) is associated with major depressive disorder, with a seasonal pattern (Roecklein et al., 2009), the unipolar form of mood disorders known as seasonal affective disorder (SAD). SAD is characterized by episodes of depression during the fall and winter seasons (Rosenthal et al., 1984). Etiological theories of SAD have suggested that multiple genes may contribute to multiple vulnerability factors, including biological rhythms and light sensitivity, in interaction with environmental factors, such as day-length (Levitan, 2007). Melanopsin, a photopigment expressed in the human retina, is involved in non-image-forming responses to environmental light, such as circadian photoentrainment and pupil light reflex (Hattar et al., 2003; Lucas et al., 2003; Panda et al., 2002, 2003). Although other photoreceptors contribute to these responses, variations in melanopsin functioning may explain individual differences in human light sensitivity. It is possible that SAD and some sleep and chronobiological disorders are caused by differences in entrainment of the circadian clock (Hatori & Panda, 2010), resulting from sequence variations in genes mediating nonvisual light input such as melanopsin.

Previous research suggests that melanopsin has a role in human circadian entrainment and alertness (An et al., 2009; Cajochen et al., 2005; Lockleyet al., 2003, 2006; Revell et al., 2010; Zaidi et al., 2007). Melanopsin is hypothesized to play a role in circadian influences on alertness, as well as acute, light-induced sleep and alertness responses through three mechanisms: (i) circadian entrainment of sleep propensity, (ii) homeostatic sleep regulation, and (iii) acute effects of light on alertness. In nocturnal rodents, light during the dark activity phase will acutely induce sleep (Borbely, 1976). In diurnal animals and humans, short-wavelength light has the opposite effect, leading to increased alertness as measured by α activity on waking electroencephalograph (EEG) (Zaidi, et al., 2007). Indeed, some melanopsin knockout mice do not exhibit expected acute sleep responses to light during the dark phase, whereas others exhibit a delay in acute responses to light (Altimus et al., 2008; Do & Yau, 2010; Lupi et al., 2008; Tsai et al., 2009). Many researchers have now established that melanopsin is involved in light entrainment of circadian rhythms, as recently reviewed by Benarroch (2011).

Melanopsin-expressing ganglion cells may mediate sleep and alertness effects through their projections to the central clock (suprachiasmatic nucleus; SCN) as well as other sleep and alertness regulation centers in the basal forebrain and hindbrain, including the ventral lateral preoptic nuclei (VLPO) and superior colliculus (SC; Hattar et al., 2006). In melanopsin knockout mice, acute light-induced activation of the VLPO and SC was abolished or significantly reduced (Lupi et al., 2008; Tsai et al., 2009). VLPO is of particular interest, because it receives direct input from the melanopsin-containing cells (Hannibal & Fahrenkrug, 2004) and is a region involved in non-rapid eye movement (NREM) and rapid eye movement (REM) sleep regulation (Lu et al., 2000). Likewise, the SC has been implicated in the photic regulation of sleep (Miller et al., 1998) and also receives direct input from melanopsin cells (Gooley et al., 2003). These findings suggest that a pathway from

melanopsin cells to brain regions regulating sleep underlies the circadian-independent effect of light in sleep regulation.

Regarding the homeostatic sleep drive, sleep debt accumulates during wakefulness, and is reduced during sleep. Although adenosine in the basal forebrain (BF) accumulates during prolonged wakefulness (Porkka-Hesikanen, et al., 1997; Strecker et al., 2000), the exact mechanism behind the homeostatic sleep drive is unknown. Melanopsin knockouts exhibit reduced sleep duration and reduced sleep debt as measured by electrocorticogram delta power despite sleep loss, suggesting a role for melanopsin in sleep homeostasis (Tsai et al., 2009). As recently reviewed by Dijk and Archer (2009), human sleep duration, structure, and sleep- and wakefulness-related brain electrical activity are modulated by interactions between light, circadian rhythms, and the sleep homeostat.

Others have also sought to better understand the role of melanopsin in human alertness. In one blind human, Zaidi et al. (2007) found that short-wavelength light resulted in greater increases in alertness compared with longer wavelengths, as measured by α activity on the waking electroencephalogram. This acute alerting effect in humans, in response to light, is analogous to acute induction of sleep in response to light in nocturnal rodents. Therefore, one possible human consequence to individual differences in melanopsin functioning may be decreased alertness levels during periods of low environmental light, e.g., short days in winter. Taken together, this evidence suggests that melanopsin is likely to mediate multiple influences of light on sleep and circadian timing in humans, but it remains to be determined if specific behaviors may be affected by variations in human melanopsin functioning as a result of gene sequence variations.

Individual differences in melanopsin functioning may contribute to seasonal changes in sleep and/or circadian timing, which may contribute, in turn, to SAD. Evidence for seasonal changes in sleep or circadian timing in relation to SAD is mixed. Wehr and colleagues (2001) reported later offset and longer duration of melatonin secretion in SAD patients during winter, but they saw no change in onset of melatonin secretion. Murray and colleagues (2003) reexamined this issue using self-reported chronotype, also known as diurnal preference or morningness-eveningness, which assigns individuals along the spectrum of morning types (“larks”) to evening types (“owls”) based on their preference for timing of sleep and activity. In a sample of SAD patients, a shift towards greater eveningness was associated with reduced behavioral engagement. The authors interpreted the shift towards eveningness as a delay in circadian phase, given that chronotype correlates with physiological measures of circadian phase (e.g., Baehr et al., 2000; Duffy et al., 1999). However, recent evidence indicates that individual differences in both circadian phase and homeostatic sleep drive contribute to variations in chronotype (Mongrain et al., 2006), suggesting that the seasonal shift towards eveningness in some individuals could indicate delay in phase or slowing in the accumulation of homeostatic drive, or both. Also, as Murray and colleagues (2003) suggest, shift in chronotype across seasons may be a consequence rather than cause of depressed mood. For this reason, we excluded individuals with history of mood disorder, and we control for mood using two depression rating scales in the present study.

Melanopsin sequence variations could increase risk for SAD by causing individual differences in responses to environmental light across seasons. If so, we would expect to see similar differences in at least some healthy individuals with no history of depression. Using a nondepressed sample can be an advantage, because chronobiological and sleep differences across the seasons would not be attributable to the consequences of having a mood disorder. Therefore, we hypothesize that melanopsin gene variations may be associated with individual differences in sleep timing and chronotype, i.e., early vs. late sleep-wake and

activity preferences, in this community sample. Participants completed self-report questionnaires regarding sleep, seasonal variations, and depression symptoms, as well as the Composite Scale of Morningness (CSM; Smith et al., 1989) to measure chronotype.

METHODS

Participants

Participants were all in good general health, and were part of an archival sample studied in 2001–2005 and for which the database was subsequently de-identified. All participants resided near Pittsburgh in western Pennsylvania (40°26′N) and were fluent in English. Participants were excluded if they were taking antidepressant medications or other drugs that might alter their responses to questionnaires or interview measures, if they were <30 or >54 yrs old, or reported a history of major neurological or psychological disorders, including depression, schizophrenia, or other psychotic illness. However, individuals were not excluded based on scores on the Pittsburgh Sleep Quality Inventory (PSQI; Buysse et al., 1989) in the symptomatic range, the Beck Depression Inventory–2nd Edition (BDI-II; Beck et al., 1996), or the Center for Epidemiologic Studies—Depression Scale (CES-D; Radloff, 1977). Table 2 indicates the scores on these questionnaires, and demonstrates that variation in sleep duration, mood, and other sleep and mood parameters was present. Participants were of non-Hispanic Caucasian ancestry. Participants (N = 234) were 46.6% female, and middle aged ($M \pm SD = 41.9 \pm 7.7$ yrs).

Procedures

Associations between melanopsin gene variations and data from questionnaires measuring sleep characteristics, seasonal variations, and chronotype were tested. Specifically, participants completed the Pittsburgh Sleep Quality Inventory (PSQI; Buysse et al., 1989) and two self-report depression symptom assessments. The depression symptom assessments were used as covariates to control for the effect of depression symptoms on sleep and circadian behavior. Date of assessment for each participant was recorded and used in analyses on the effect of daylength on the day of assessment. The University of Pittsburgh Institutional Review Board approved all procedures, and our study met the ethical standards established for the journal (Portaluppi et al., 2010).

Melanopsin Gene Variations

In previous analyses of melanopsin sequence variation in SAD (Roecklein et al., 2009), haplotype analysis using 12 polymorphic sites across this relatively small gene (~10.4 kb) did not reveal association between SAD and any particular haplotype, and those data indicated that the melanopsin gene spans a single haplotype block. For the present study, we included two melanopsin coding variants (rs2675703, P10L; rs1079610, T394I) and one non-coding intronic single-nucleotide polymorphism (SNP) (rs2014084) to analyze sequence variation across the melanopsin gene.

P10L SNP (rs2675703), a coding variation, results in a nonsynonymous amino acid substitution (Pro/Leu) at residue 10 of the melanopsin protein. T394I SNP (rs1079610), another coding variation, results in a nonsynonymous amino acid substitution (Thr/Ile) at residue 394 of the protein. The third polymorphism, rs2014084, is a noncoding SNP in intron 4 of the melanopsin gene (*OPN4*), and has been used in previous studies to test the role of melanopsin in psychiatric disorders, e.g., bipolar disorder (Kripke et al., 2009). The three SNPs are referred to here as P10L, T394I, and rs2014084.

Pittsburgh Sleep Quality Index

Participants completed The Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989), which measures self-reported sleep characteristics. It is a reliable and valid means to distinguish “good sleepers” from “poor sleepers” with high specificity (Buysse et al., 1989). The PSQI yields component scales of subjective sleep quality, latency, duration, efficiency, disturbances, use of sleeping medication, daytime dysfunction, and a global PSQI score as well as estimated typical times for going to sleep and waking up across the last month. Based on study hypotheses, we focused on past-month sleep and wake times in analyses.

PSQI global score has been shown to significantly differentiate between control, depressed, and sleep-disturbed individuals (Buysse et al., 1989). More so, a global PSQI cutoff score (>5) yielded diagnostic sensitivity of 89.6% and specificity of 86.5% ($\kappa = .75$, $p < .00l$) in distinguishing control subjects from individuals with a sleep disorder. The seven component scales of the PSQI had a high degree of internal consistency among a sample of healthy control subjects, patients with sleep disorders, and patients with depression (Cronbach’s $\alpha = .83$). Within each of these three groups, test-retest reliability (over an average of 28 d) was .85 for the global score and .65–.84 for the component scales (Buysse et al., 1989).

In the present study, we compared genotype groups on self-reported sleep onset, wake time, and total sleep duration across the last month. These variables were collected from the following questions: “During the past month, when have you usually gone to bed at night?” and “During the past month, when have you usually gotten up in the morning?” Sleep onset was calculated as the sum of self-reported bedtime plus self-reported average sleep latency in response to the question, “During the past month, how long (in minutes) has it usually taken you to fall asleep?” To correctly analyze those reporting going to sleep after midnight, variables were converted to number of hours since noon (sleep onset) and number of hours since midnight (wake time). Sleep duration was calculated as the difference between sleep onset and wake time. Military time is reported below as follows: 01:00 h for 1 a.m.; 12:00 h for noon; 13:00 h for 1 p.m.; and 00:00 h for midnight.

Depression Symptoms

To measure severity and frequency of depression symptoms, participants completed both the Beck Depression Inventory–2nd Edition (BDI-II; Beck et al., 1996) and the Center for Epidemiologic Studies—Depression Scale (CES-D; Radloff, 1977). CES-D is a 20-item self-report depression symptom frequency scale developed by the Center for Epidemiologic Studies (National Institutes of Health [NIH]). Participants reported how often the past week they experienced each of 20 depression symptoms. Studies using five psychiatric populations and a community sample show that the scale is valid as a screening tool to detect depression symptoms, and can be used to measure change in symptom severity over time (Weissman et al., 1977). CES-D compares well with longer self-report scales and clinician ratings of depression symptoms.

BDI-II (Beck et al., 1996) is a 21-item measure of depressive symptom severity, providing four severity options for each of the 21 items for participants to select. BDI-II has demonstrated good test-retest reliability and convergent validity (Beck et al., 1996). Both questionnaires are included, because BDI-II is the most widely used measure of the severity of depression symptoms, whereas CES-D was designed to assess current frequency of depressive symptoms. CES-D and BDI-II were included to control for depression symptoms in analyses on sleep and chronotype.

Chronotype

For the present study, we used the Composite Scale of Morningness (CSM; Smith et al., 1989) to assess chronotype. CSM is a 13-item measure based, in part, on the original Horne-Östberg Morningness-Eveningness Questionnaire (MEQ), but is easier to use with improved psychometrics. Scores on the CSM correlate highly (.95) with scores on the original MEQ. Items assess diurnal preference, sleep times, preferred times for physical and mental activities, and times of subjective alertness. Total scores range from 13 (extreme eveningness) to 55 (extreme morningness). Original cutoffs were unreliable across different age groups and new cutoffs (13–26 evening-type; 27–41 intermediate-type; 42–55 morning-type) were recommended (Natale & Alzani, 2001), although scores were analyzed as a continuous variable in the present analyses. The scale has acceptable internal consistency (Cronbach's $\alpha = .83$; Smith et al., 1989) and good test-retest reliability and predictive validity (Greenwood, 1994; Guthrie et al., 1995).

Seasonality

Seasonality is defined as the tendency to vary across the seasons in mood and behavior, and is measured using the Global Seasonality Scale (GSS) on the Seasonal Pattern Assessment Questionnaire (SPAQ; Rosenthal et al., 1987). GSS consists of six items (i.e., sleep, appetite, mood, energy level, weight, and social behavior), which are rated on a 5-point Likert scale for degree of change across seasons, ranging from 0 ("no change") to 4 ("extremely marked change"), which are then summed to derive the GSS (range: 0–24). GSS shows adequate internal consistency (Chronbach's $\alpha = .81–.85$) and test-retest reliability (Mersch et al., 2004; Young et al., 2003). Individuals are prompted to answer "To what degree do the following change *with the seasons*?" However, it is still possible that season of assessment would impact scores. However, GSS scores administered in summer and winter to an Australian sample were adequately correlated (.66; Murray, 2003). Therefore, in the present study, we tested the association between self-reported seasonality and genotype and daylength on assessment day. We hypothesized that we might find association between GSS and genotype, but not daylength, if, in fact, scores on the GSS do not vary based on season of assessment.

Genotyping

DNA was collected from participants by buccal cell sample collection swabs or EDTA-anticoagulated whole blood. The region in the melanopsin gene containing the SNPs was amplified using previously established polymerase chain reaction methods (Roecklein et al., 2009). Fluorescence polarization (FP; Chen et al., 1999) was then used to determine presence or absence of alleles. FP employs probes that hybridize to the DNA template directly adjacent to the SNP in the presence of fluorescently labeled nucleotides. The primer extends and incorporates genotype-specific labeled nucleotides, which can be observed directly using light at the appropriate wavelength. Samples were genotyped in 96-well microtiter plates and compared with sequence-verified controls run in the same plate.

Daylength

Because we hypothesize that melanopsin may mediate seasonal variations in sleep and behavior, the effect of the length of day on the day of assessment was included as an independent variable with genotype to allow for tests of interactions between genotype effects and day-length. The daylength variable was collected from U.S. Naval Observatory data for Pittsburgh, Pennsylvania (http://aa.usno.navy.mil/data/docs/Dur_OneYear.php) for the day on which assessment was completed.

Statistical Approach

Analysis of covariance (ANCOVA) was used to test main effects of genotype and daylength, as well as the interaction of genotype and daylength on dependent variables listed above, i.e., chronotype, sleep onset, wake time, sleep duration, and seasonality. Subject age, sex, and depression scores (BDI-II and CES-D total scores) were included as covariates in all analyses to control for these potentially confounding variables. Analyses based on sleep time were especially important to adjust for age due to age-related changes in sleep duration and timing (Buysse et al., 1991; Roenneberg et al., 2007). Because sleep disturbances can be symptoms of depression, we included BDI and CES-D total scores as covariates to determine if any of the associations between melanopsin polymorphisms, season, and sleep variables could be explained by depression symptom levels. We performed analyses with sleep questions from the CES-D and BDI-II removed from the total score, although results did not differ when total scores were used. Significant interactive effect between genotype and daylength was parsed by specific genotype contrasts, e.g., TT vs. CT and TT vs. CC for P10L. This involved testing for significant difference between slopes of the linear relationship between daylength and sleep onset or morningness for each specific genotype. Such specific genotype contrasts are reported in Table 4. Homogeneity of variance between the respective levels of the dependent variables across genotype groups was tested using Levene's test and is reported in Table 4. All analyses were conducted using PASW Statistics version 18.0 (Chicago, IL). Cook's distance and DFFIT were used to identify and estimate the effect of outliers on the corresponding fitted values for all and individual observations, respectively (Belsey et al., 1980; Cook & Weisberg, 1982). Analyses were repeated using a generalized linear model (GLM) with robust standard errors (Huber-White estimators for GLM), which is more robust to unequal cell sizes due to the rare TT genotype. If a large percentage of individuals with the homozygous recessive (TT) genotype at P10L were identified as outliers (see Results below), we followed the above additive model and used a separate recessive model that compared the TT genotype with the combined CT + CC genotypes (see Table 4 for results). In the additive model, we compared all three genotype groups separately. In the recessive model, we compared those with TT with the combined group of all individuals with CT or CC. Pairwise linkage disequilibrium between the three SNPs was established using Haploview 4.1 software (Barrett et al., 2005), and is reported in Table 1.

RESULTS

Genotyping

Failure rates for the sample of 234 total participants were all under 1.5% (P10L, n = 3 failed to genotype, 1.3%; T394I, n = 1 failed to genotype, .4%; rs2014084, n = 1 failed to genotype, .4%). Frequency of the genotypes at each locus did not differ significantly from that predicted by HWE, as determined using a goodness of fit chi-square test (P10L: $\chi^2_{(2,231)}$=.28, p = .60; T394I: $\chi^2_{(2,233)}$=.03, p = .85; rs2014084: $\chi^2_{(2,233)}$=.02, p = .90) and are presented in Table 3 for comparison between artificially dichotomized short and long days of questionnaire assessment. As predicted, two of the three *OPN4* SNPs assessed in this study showed strong pairwise linkage disequilibrium (LD), as indicated and defined by values of D′, LOD, and r^2 in Table 1, calculated using the present data in Haploview (Barrett et al.). Table 2 reports the mean, standard deviation, and range in the sample for each of the outcome variables and the depression scales.

Sleep Onset

The overall model including person age, sex, depression scores, P10L genotype, daylength, and P10L × daylength interaction was significant for predicting sleep onset ($F_{(9,230)}$ = 2.469,

$p < .05$). There was significant main effect of P10L, and significant interaction between P10L and daylength predicting sleep onset (see Table 4). Specific genotype contrasts are shown in Table 4 particularly for the P10L × daylength interaction, as individuals were assessed on shorter days, those with the TT genotype reported falling asleep earlier compared with the CT genotype group ($p = .014$), and there was a trend towards difference between TT and CC genotypes ($p = .065$). Levene's test was not significant, indicating that the homogeneity of variance assumption is not violated. When the analysis was repeated using GLM and Huber-White estimators, which are robust to unequal cell sizes due to the rare TT genotype, results did not change. Neither T394I nor rs2014084 was significantly associated with sleep onset. Age was associated with sleep onset as predicted ($F_{(1,230)} = 9.23$, $p < .01$).

Chronotype

The overall model including age, sex, depression scores, P10L genotype, daylength, and P10L × daylength interaction was significant for predicting greater morningness ($F_{(9,230)} = 3.315$, $p < .001$). There was significant main effect of P10L ($F_{(2,229)} = 3.328$, $p < .05$) and main effect of daylength ($F_{(1,229)} = 4.428$, $p < .05$), as well as significant interaction between P10L and daylength predicting chronotype ($F_{(2,229)} = 3.214$, $p < .05$; see Table 4, see specific contrasts between genotypes). All Levene's tests on the independently classed variables of interest were not significant, indicating that the homogeneity of variance assumption is not violated. When the analysis was repeated using GLM and Huber-White estimators, which are robust to unequal cell sizes due to the rare TT genotype, results did not change. Individuals assessed on shorter days with the TT P10L genotype reported higher CSM scores, reflecting trend toward greater morningness with decreasing daylength, a relationship that was not evident among the CC and CT genotype groups (ps = .027 and .013, respectively). Neither T394I nor rs2014084 was significantly associated with chronotype (data not shown).

Wake Time, Sleep Duration, and Seasonality

None of the genotypes was associated with either wake time or sleep duration in the study. However, BDI and CES-D scores were associated with wake time and sleep duration in the analyses with P10L (all ps < .05). A majority of the sample (n = 219) completed the SPAQ seasonality assessment. None of the genotypes was significantly associated with seasonality, contrary to hypotheses. However, in the case of T394I and rs2014084, the individual effect of daylength was significantly associated with seasonality score, as individuals who completed this questionnaire on shorter days reported greater changes in mood and behavior across the seasons compared with those completing the questionnaire on long days. For the T394I analysis, daylength had significant main effect on seasonality score ($F_{(1,217)} = 9.382$, $p < .01$), and there was similar main effect of daylength on seasonality score in the analysis with rs2014084 ($F_{(1,217)} = 9.382$, $p < .01$). Across the sample, daylength was significantly, but weakly, correlated with seasonality (Pearson correlation $r = .142$, $p < .05$). Total PSQI score was not associated with any genotypes, and as a score of >5 indicates "poor" sleepers, this is higher than our mean of 2.44 on the PSQI total score (Buysse et al., 1989; Table 2).

Outliers

For the analyses with P10L, individual genotypes identified as outliers included 4 (80%) of the individuals with the TT genotype, the genotype associated with SAD in previous reports (Roecklein et al., 2009). Samples selected as outliers were those whose Cook's distance was over .0171 and whose DFFIT absolute value was over .5264. When analyses were repeated excluding the TT genotype group, interaction between P10L and daylength on sleep onset was no longer significant. This is most likely due to overall trend similarities between the CC and CT genotype groups. Because the TT genotype group is likely to be the risk

genotype in OPN4, results are presented with the outliers included, although the TT group is very small (n = 5). In addition, given that 80% of the individuals with TT genotype were identified as outliers, we tested a recessive model comparing the TT genotype with the grouping of CC and CT genotypes, in contrast to the additive model. As with the additive models for sleep onset and chronotype, the recessive model had significant main genotype effect as well as significant P10L × daylength interaction as shown in Table 4 (*p*s = .033–.049).

DISCUSSION

Effect of P10L TT on sleep onset and chronotype varied by photoperiod on the day of assessment, but these findings only tentatively suggest possible gene by environment, i.e., daylength, interaction with TT conferring heightened responsivity to daylength. Specifically, P10L TT genotype may lead to earlier sleep onset and greater morningness when individuals are assessed on short days, and later sleep onset and greater eveningness when individuals with TT are assessed on long days. Participants with other genotypes did not vary across photoperiods. It appears this P10L × daylength interaction is a recessive gene effect, because TT was significantly different from CT for the sleep onset analysis, and TT was significantly different from both CT and CC in the chronotype analysis. However, given the small number of participants with the TT genotype, this interaction must be considered tenuous and speculative.

Findings that the P10L TT genotype may confer heightened sensitivity to daylength is surprising given sequence variations often lead to loss of function. Decreased retinal sensitivity is expected to lead to the phase delays associated with SAD, whereas here we see earlier sleep onset and greater morningness among TT genotypes. It is unknown, however, whether P10L increases or decreases sensitivity of the melanopsin protein. Some individuals with SAD have a phase advance (29%; Lewy et al., 2006), but the complement, and the majority, exhibits a phase delay (71%). On the other hand, 46% of individuals with SAD do not have a significantly phase difference from controls (Eastman et al., 1993). Although the benefits of morning light therapy for SAD appear due to phase advances caused by the timing of light (Terman & Terman, 2010), it is not clear that only phase delays are etiologically or clinically significant in SAD. The question remains, how could melanopsin variations lead to an apparent phase advance on short days, if that is what is reflected by our findings here?

To answer this question, we must consider the resultant phase on any given day is a function of advances and delays on previous days. A phase advance could result from either an increase in phase-advancing stimuli in the morning, or a decrease in phase-delaying stimuli in the evening. The first scenario is unlikely to result from melanopsin change, because short days would still be associated with lower light levels in the morning and less of a phase advance. In the second scenario, it is possible that melanopsin sequence variations lead to reduced melanopsin cell sensitivity, specifically to light in the evening. A failure to delay the clock could lead to an apparent phase advance, theoretically resulting in our observations of earlier sleep onset and greater morningness among TT genotypes on short days. In this way, P10L may cause individuals to track the timing of dusk more closely. Earlier sleep onset in winter may in turn deprive individuals of rewarding activities commonly experienced in the evening, such as social interaction, exercise, and leisure activity, increasing the risk for seasonal depression (Rohan et al., 2009). These additional risk factors for depression are likely missing from individuals in our current sample of healthy individuals, although any TT genotype effect would still be operating.

It is currently unknown how the TT genotype of the P10L mutation could be more sensitive to changes in daylength than CC or the CT genotypes. It is possible that the T allele leads to less efficient phototransduction due to changes in local structure and/or intermolecular interactions associated with proline to leucine amino acid change in the 10th residue position of the N-terminal extracellular tail. Using the PSIPRED protein structure prediction server (London, UK; Buchan et al., 2010; Jones, 1999), the secondary structure of the N-terminal extracellular tail is predicted with 90% certainty to be a coil rather than an α helix, β sheet, or a recognizable turn. Including the P10L amino acid substitution did not change this prediction. It is still possible that changes in the melanopsin extracellular tail could interfere with functions associated with this region involving molecular recognition. Given some similarity in melanopsin and rhodopsin structures and functions, function of the extracellular tail of melanopsin may be related to proper folding of the receptor, cellular processing, and chromophore regeneration (Doi et al., 1990), as well as trafficking of the protein (Borjigin & Nathans, 1994). However, this N-terminal region of melanopsin is likely to be a disordered loop, whose structure may remain unresolved because it is significantly longer (37 residues) than rhodopsin's N-terminus.

Because melanopsin may be differentially expressed as a function of acute light exposure and photoperiod in rats (Hannibal, 2006; Mathes et al., 2007), it is possible that sequence variations affect melanopsin expression levels. Yet, other explanations for our results could involve the role of melanopsin in acute alerting effects of light. As described above, Zaidi et al. (2007) found that blue light exposure resulted in increased alertness. Reduction in the acute alerting effect of light could lead some individuals to experience decreased alertness during periods of low environmental light, such as winter evening hours. This could lead individuals to retire to bed when occupational or social demands permit, perhaps more in the evening than morning.

We excluded individuals with history of depression, we controlled for depression symptoms in analyses, and we did not have significant variance in depression symptoms on the BDI-II or CES-D. Scale means were relatively low (CES-D: $M \pm SD = 7.96 \pm 7.39$; BDI-II: $M \pm SD = 4.09 \pm 4.54$), and below the cutoffs for clinically significant symptoms of depression for the CES-D (16) and BDI-II (10). Lack of significant depression symptoms in the sample, and controlling for these in the regression analyses, allows us to conclude that associations between genotype and daylength with sleep onset and chronotype are unlikely to be a consequence of mood disorder sequelae.

Genetic variation is only one of many factors affecting sleep and mood, as lighting conditions may have greater impact on sleep timing. It is possible that genotype may impact lighting conditions, if genotype influences behaviors that result in differential light exposure. Examples could be a genetic predisposition to live in more rural areas, or a genetic predisposition to withdraw socially and engage in fewer activities when light levels are low. This is an empirical question that could be tested in future studies using recent advances in actigraphy in which red, green, and blue wavelengths are measured. The value of the present study lies in identifying a possible individual difference (i.e., genotype) that interacts with daylength to influence sleep onset and chronotype. Genotype-seasonality interaction accounted for 9–12% (R^2s = .09–.12) of the variance in sleep timing and chronotype, partialing out other factors such as age, sex, and depression scores from the total variation. Given that influences on sleep and mood are likely to be polygenetic and multifactorial, 9–12% of variance attributable to the P10L by daylength interaction represents a large portion of the total variance in the timing of sleep and behavior. Gene-trait associations that have been replicated and are considered reliable in other complex traits often account for ~1% of variation (Yang et al., 2011), making our effects higher than expected for genetic studies.

It is possible that individuals with melanopsin gene variations might have decreased retinal sensitivity. Evidence indicates that individuals with SAD have lower electrooculography (EOG) ratios, indicating lower retinal sensitivity compared with controls (Lam et al., 1991; Ozaki et al., 1993), especially in winter (Ozaki et al., 1995), but that successful light therapy for SAD does not lead to corrective increases in low EOG ratios (Ozaki et al., 1993). Electroretinography (ERG) measures, however, have found that women with SAD have lower photopic ERG amplitudes compared with controls (Lam et al., 1992), and both men and women with SAD had lower scotopic ERG (Hebert et al., 2004). Photopic ERG in SAD does not differ from controls after light therapy, or in summer (Lavoie et al., 2009). SAD participants exposed to 10 000, 100, and 5 lux light for 1 h (Gagne & Hebert, 2011) showed significant decrease in ERG following 10 000 lux 1-h exposures in both summer and winter, which was not observed in controls. In a recent study using different wavelengths, ERGs were significantly lower after blue stimuli, but SAD and controls did not differ (Gagne et al., 2011). There may be other measures, such as pupillometry, that are more sensitive to melanopsin than these, since melanopsin cells are only 1–2% of the retinal ganglion cells (Provencio et al., 1998).

Limitations

Future studies should use objective rather than self-report measures of sleep and chronotype, such as actigraphy, polysomnography, or dim light melatonin onset (e. g., Ancoli-Israel et al., 2003; Pandi-Perumal et al., 2007). Importantly, PSQI is correlated with sex, psycholopathology, and self-report sleep disturbances, but not necessarily with objective measures of sleep (Buysse et al., 2008). Correlations between PSQI scores and polysomnography measures, i.e., total sleep time, sleep onset latency, etc., have been reported to be significant but low (coefficient range: −.33–.28; Backhaus et al., 2002). Buysse et al. (1989) reported no significant differences between PSQI and polysomnography measures of sleep latency, but that PSQI estimates of sleep duration and sleep efficiency were higher than estimates derived from polysomnography, suggesting that actigraphic or polysomnographic measures of sleep may clarify the relationships found in the present study.

Regarding measurement of chronotype with the CSM, this scale does not ask participants to distinguish between activity preferences on work and free days, and objective measures should be used in future studies. However, the CSM has good predictive validity, as reflected in multiple studies. Guthrie et al. (1995) found that morningness was correlated with sleep, study, and class scheduling times among students. Greenwood (1994) found adequate reliability over time in a large sample (N = 424), and Randler and Schall (2010) found that the CSM was correlated with the cortisol awakening response.

The P10L TT genotype was rare (n = 5; 2.2%), and four of the five were outliers. Outliers are defined as infrequent observations that do not follow the distribution of the sample; so, they may reflect a genuine property of the effect of TT on behavior, or may be due to measurement errors. However, Levene's test concludes that variability within the TT genotype is equivalent to that within both the CC and CT groups. This means that our test of the model that the TT group has significant effect on sleep onset or chronotype is valid. If a significant effect is present, we can be more confident that the behavioral effect is due to a genuine property of the TT genotype.

Others have included melanopsin SNPs in studies on mood and circadian behaviors. Allenbrandt et al. (2010) recently tested four melanopsin SNPs and found no association with short or long sleepers, but these authors did not include P10L (rs2675703) or rs2014084 in their analyses. Consistent with our findings, they did not find association between T394I (rs1079610) and short or long sleepers. Recently, Kripke et al. (2009) tested

four melanopsin SNPs in bipolar disorder and found no association with diagnosis. Kripke et al. (2009) included rs2014084 and T394I (rs1079610), but not P10L (rs2675703). Kripke et al. (2009) used the SNPlex genotyping system and Applied Biosystems capillary DNA analyzer (ABI, Foster City, CA), which requires there be no neighboring SNP within 15 bases. P10L SNP (rs2675703) is followed 50% of the time by rs11202106, so only certain genotyping assays will be able to genotype P10L, such as that used by Allenbrandt et al. (2010), and the FP assay used in the present study.

Conclusion

It is possible that season and/or environmental conditions may interact with genetic variation in many physiological functions, including sleep-wake cycles and mood, perhaps more than is currently recognized (Johnson et al., 2010). It is possible that melanopsin is one of multiple genes contributing to the light sensitivity vulnerability factor in SAD, and more specific information about genes for light sensitivity may allow us to optimize bright light therapy treatment for SAD (Levitan, 2007). A better understanding of the role of melanopsin in retinal light sensitivity could ultimately improve treatment of circadian, sleep, and mood disorders (Provencio, 2011).

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References

Allebrandt KV, Teder-Laving M, Akyol M, Pichler I, Muller-Myhsok B, Pramstaller P, Merrow M, Meitinger T, Metspalu A, Roenneberg T. CLOCK gene variants associate with sleep duration in two independent populations. Biol Psychiatry. 2010; 67:1040–1047. [PubMed: 20149345]

Altimus CM, Guler AD, Villa KL, McNeill DS, Legates TA, Hattar S. Rodscones and melanopsin detect light and dark to modulate sleep independent of image formation. Proc Natl Acad Sci U S A. 2008; 105:19998–20003. [PubMed: 19060203]

An M, Huang J, Shimomura Y, Katsuura T. Time-of-day-dependent effects of monochromatic light exposure on human cognitive function. J Physiol Anthropol. 2009; 28:217–223. [PubMed: 19823003]

Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. The role of actigraphy in the study of sleep and circadian rhythms. Sleep Med Rev. 2003; 26:342–392.

Backhaus J, Junghanns K, Broocks A, Riemann D, Hohagen F. Test-retest reliability and validity of the Pittsburgh Sleep Quality Index in primary insomnia. J Psychosom Res. 2002; 53:737–740. [PubMed: 12217446]

Baehr EK, Revelle W, Eastman CI. Individual differences in the phase and amplitude of the human circadian temperature rhythm: with an emphasis on morningness-eveningness. J Sleep Res. 2000; 9:117–127. [PubMed: 10849238]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Beck, AT.; Steer, RA.; Brown, GK. Beck Depression Inventory–2nd Edition manual. San Antonio, TX: The Psychological Corporation; 1996.

Belsey, DA.; Kuh, E.; Welsch, RE. Regression diagnostics: identifying influential data and sources of collinearity. Wiley series in probability and mathematical statistics. New York: Wiley & Sons; 1980. p. 85-191.

Benarroch EE. The melanopsin system: phototransduction, projections, functions, and clinical implications. Neurology. 2011; 76:1422–1427. [PubMed: 21502603]

Borbely AA. Sleep and motor activity of the rat during ultrashort light–dark cycles. Brain Res. 1976; 114:305–317. [PubMed: 963552]

Borjigin J, Nathans J. Insertional mutagenesis as a probe of rhodopsin's topography, stability, and activity. J Biol Chem. 1994; 269:14715–14722. [PubMed: 7514180]

Buchan DW, Ward SM, Lobley AE, Nugent TC, Bryson K, Jones DT. Protein annotation and modelling servers at University College London. Nucleic Acids Res. 2010; 38(Suppl):W563–W568. [PubMed: 20507913]

Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989; 28:193–213. [PubMed: 2748771]

Buysse DJ, Reynolds CF, Monk TH, Hoch CC, Yeager AL, Kupfer DJ. Quantification of subjective sleep quality in healthy elderly men and women using the Pittsburgh Sleep Quality Index. Sleep. 1991; 14:331–338. [PubMed: 1947597]

Buysse DJ, Hall ML, Strollo PJ, Kamarck TW, Owens J, Lee L, Reis SE, Matthews KA. Relationships between the Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness Scale (ESS), and clinical/polysomnographic measures in a community sample. J Clin Sleep Med. 2008; 4:563–571. [PubMed: 19110886]

Cajochen C, Munch MY, Kobialka S, Krauchi K, Steiner R, Oelhafen P, Orgül S, Wirz-Justice A. High sensitivity of human melatonin, alertness, thermoregulation, and heart rate to short wavelength light. J Clin Endocrinol Metab. 2005; 90:1311–1316. [PubMed: 15585546]

Chen X, Levin L, Kwok PY. Fluorescence polarization in homogeneous nucleic acid analysis. Genome Res. 1999; 9:492–498. [PubMed: 10330129]

Cook, RD.; Weisberg, S. Residuals and influence in regression. New York: Chapman & Hall; 1982. p. 229

Dijk DJ, Archer SN. Light, sleep, and circadian rhythms: together again. PLoS Biol. 2009; 7:e1000145. [PubMed: 19547745]

Dijk DJ, Czeisler CA. Contribution of the circadian pacemaker and the sleep homeostat to sleep propensity, sleep structure, electroencephalographic slow waves, and sleep spindle activity in humans. J Neurosci. 1995; 15:3526–3538. [PubMed: 7751928]

Dijk DJ, Duffy JF, Riel E, Shanahan TL, Czeisler CA. Ageing and the circadian and homeostatic regulation of human sleep during forced desynchrony of rest, melatonin and temperature rhythms. J Physiol. 1999; 516:611–627. [PubMed: 10087357]

Doi T, Molday RS, Khorana HG. Role of the intradiscal domain in rhodopsin assembly and function. Proc Natl Acad Sci U S A. 1990; 87:4991–4995. [PubMed: 2367520]

Duffy JF, Dijk DJ, Hall EF, Czeisler CA. Relationship of endogenous circadian melatonin and temperature rhythms to self-reported preference for morning or evening activity in younger and older people. J Invest Med. 1999; 47:141–150.

Eastman CI, Gallo LC, Lahmeyer HW, Fogg LF. The circadian rhythm of temperature during light treatment for winter depression. Biol Psychol. 1993; 34:210–220.

French J, Hannon P, Brainard GC. Effects of bright illuminance on body temperature and human performance. Annu Rev Chronopharmacol. 1990; 7:37–40.

Gagne AM, Hebert M. Atypical pattern of rod electroretinogram modulation by recent light history: a possible biomarker of seasonal affective disorder. Psychiatry Res. 2011; 187:370–374. [PubMed: 20832867]

Gagne AM, Levesque F, Gagne P, Hebert M. Impact of blue vs red light on retinal response of patients with seasonal affective disorder and healthy controls. Prog Neuropsychopharmacol Biol Psychiatry. 2011; 35:227–231. [PubMed: 21094670]

Gooley JJ, Lu J, Fischer D, Saper CB. A broad role for melanopsin in nonvisual photoreception. J Neurosci. 2003; 23:7093–7106. [PubMed: 12904470]

Greenwood KM. Long-term stability and psychometric properties of the composite scale of morningness. Ergonomics. 1994; 37:377–383. [PubMed: 8119267]

Guthrie JP, Ash RA, Bendapudi V. Additional validity evidence for a measure of morningness. J Appl Psychol. 1995; 80:186–190.

Hannibal J. Regulation of melanopsin expression. Chronobiol Int. 2006; 23:159–166. [PubMed: 16687290]

Hannibal J, Fahrenkrug J. Target areas innervated by PACAP immunoreactive retinal ganglion cells. Cell Tissue Res. 2004; 316:99–113. [PubMed: 14991397]

Hatori M, Panda S. The emerging roles of melanopsin in behavioral adaptation to light. Trends Mol Med. 2010; 16:435–446. [PubMed: 20810319]

Hattar S, Lucas RJ, Mrosovsky N, Thompson S, Douglas RH, Hankins MW. Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature. 2003; 424:76–81. [PubMed: 12808468]

Hattar S, Kumar M, Park A, Tong P, Tung J, Yau KW, Berson DM. Central projections of melanopsin-expressing retinal ganglion cells in the mouse. J Comp Neurol. 2006; 497:326–349. [PubMed: 16736474]

Hebert M, Beattie CW, Tam EM, Yatham LN, Lam RW. Electroretinography in patients with winter seasonal affective disorder. Psychiatry Res. 2004; 127:27–34. [PubMed: 15261702]

Johnson J, Wu V, Donovan M, Majumdar S, Renteria RC, Porco T, Van Gelderd RN, Copenhagena DR. Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A. 2010; 107:17374–17378. [PubMed: 20855606]

Jones DT. Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol. 1999; 292:195–202. [PubMed: 10493868]

Kripke DF, Nievergelt CM, Joo E, Shekhtman T, Kelsoe JR. Circadian polymorphisms associated with affective disorders. J Circadian Rhythms. 2009; 7(2):1–10. [PubMed: 19123938]

Lam RW, Beattie CW, Buchanan A, Remick RA, Zis AP. Low electrooculographic ratios in patients with seasonal affective disorder. Am J Psychiatry. 1991; 148:1526–1529. [PubMed: 1928467]

Lam RW, Beattie CW, Buchanan A, Mador JA. Electroretinography in seasonal affective disorder. Psychiatry Res. 1992; 43:55–63. [PubMed: 1438617]

La Morgia C, Ross-Cisneros FN, Hannibal J, Montagna P, Sadun AA, Carelli V. Melanopsin-expressing retinal ganglion cells: implications for human diseases. Vision Res. 2011; 51:296–302. [PubMed: 20691201]

Lavoie MP, Lam RW, Bouchard G, Sasseville A, Charron MC, Gagne AM. Evidence of a biological effect of light therapy on the retina of patients with seasonal affective disorder. Biol Psychiatry. 2009; 66:253–258. [PubMed: 19135188]

Levitan RD. The chronobiology and neurobiology of winter seasonal affective disorder. Dialog Clin Neurosci. 2007; 9:315–324.

Lewy AJ, Lefler BJ, Emens JS, Bauer VK. The circadian basis of winter depression. Proc Nat Acad Sci U S A. 2006; 103:7414–7419.

Lockley SW, Brainard GC, Czeisler CA. High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. J Clin Endocrinol Metabol. 2003; 88:4502–4505.

Lockley SW, Evans EE, Scheer FA, Brainard GC, Czeisler CA, Aeschbach D. Short-wavelength sensitivity for the direct effects of light on alertness, vigilance, and the waking electroencephalogram in humans. Sleep. 2006; 29:161–168. [PubMed: 16494083]

Lu J, Greco M, Shiromani P, Saper C. Effect of lesions of the entrolateral preoptic nucleus on NREM and REM sleep. J Neurosci. 2000; 20:3830–3842. [PubMed: 10804223]

Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, Yau KW. Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science. 2003; 299:245–247. [PubMed: 12522249]

Lupi D, Oster H, Thompson S, Foster RG. The acute light-induction of sleep is mediated by melanopsin-based photoreception. Nat Neurosci. 2008; 11:1068–1073. [PubMed: 19160505]

Mathes A, Engel H, Holthues T, Wolloscheck T, Spessert R. Daily profile in melanopsin transcripts depends on seasonal lighting conditions in the rat retina. J Neuroendocrinol. 2007; 19:952–957. [PubMed: 18001324]

Mersch PP, Vastenburg NC, Meesters Y, Bouhuys AL, Beersma DG, van den Hoofdakker RH, den Boer JA. The reliability and validity of the Seasonal Pattern Assessment Questionnaire: a comparison between patient groups. J Affect Disord. 2004a; 80:209–219. [PubMed: 15207934]

Miller A, Obermeyer W, Behan M, Benca R. The superior colliculus–pretectum mediates the direct effects of light on sleep. Proc Nat Acad Sci U S A. 1998; 95:8957–8962.

Mongrain V, Carrier J, Dumont M. Circadian and homeostatic sleep regulation in morningness-eveningness. J Sleep Res. 2006; 15:162–166. [PubMed: 16704571]

Murray G. The Seasonal Pattern Assessment Questionnaire as a measure of mood seasonality: a prospective validation study. Psychiatry Res. 2003; 120:53–59. [PubMed: 14500114]

Murray G, Allen NB, Trinder J. Seasonality and circadian phase delay: prospective evidence that winter lowering of mood is associated with a shift towards eveningness. J Affect Disord. 2003; 76:15–22. [PubMed: 12943929]

Natale V, Alzani A. Additional validity evidence for the composite scale of morningness. Pers Individ Dif. 2001; 30:293–301.

Ozaki N, Rosenthal NE, Moul DE, Schwartz PJ, Oren DA. Effects of phototherapy on electrooculographic ratio in winter seasonal affective disorder. Psychiatry Res. 1993; 49:99–107. [PubMed: 8153191]

Ozaki N, Rosenthal NE, Myers F, Schwartz PJ, Oren DA. Effects of season on electro-oculographic ratio in winter seasonal affective disorder. Psychiatry Res. 1995; 59:151–155. [PubMed: 8771230]

Panda S, Sato TK, Castrucci AM, Rollag MD, De Grip WJ, Hogenesch JB. Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. Science. 2002; 298:2213–2216. [PubMed: 12481141]

Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM. Melanopsin is required for non-image-forming photic responses in blind mice. Science. 2003; 301:525–527. [PubMed: 12829787]

Pandi-Perumal SR, Smits M, Spencec W, Srinivasand V, Cardinalie DP, Lowe AD, Kayumov L. Dim light melatonin onset (DLMO): a tool for the analysis of circadian phase in human sleep and chronobiological disorders. Prog Neuropsychopharmacol Biol Psychiatry. 2007; 31:1–11. [PubMed: 16884842]

Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW. Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. Science. 1997; 276:1265–1268. [PubMed: 9157887]

Portaluppi F, Smolensky MH, Touitou Y. Ethics and methods for biological rhythm research on animals and human beings. Chronobiol Int. 2010; 27:1911–1929. [PubMed: 20969531]

Provencio I. The hidden organ in your eyes. Sci Am. 2011; 304:54–59. [PubMed: 21595404]

Provencio I, Jiang G, De Grip WJ, Hayes WP, Rollag MD. Melanopsin: an opsin in melanophores, brain, and eye. Proc Natl Acad Sci U S A. 1998; 95:340–345. [PubMed: 9419377]

Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. J Neurosci. 2000; 20:600–605. [PubMed: 10632589]

Provencio I, Rollag MD, Castrucci AM. Photoreceptive net in the mammalian retina: this mesh of cells may explain how some blind mice can still tell day from night. Nature. 2002; 415:493. [PubMed: 11823848]

Radloff L. The CES-D Scale: a self-report depression scale for research in the general population. Appl Psychol Measure. 1977; 1:385–401.

Rajaratnam SM, Middleton B, Stone BM, Arendt J, Dijk DJ. Melatonin advances the circadian timing of EEG sleep and directly facilitates sleep without altering its duration in extended sleep opportunities in humans. J Physiol. 2004; 561:339–351. [PubMed: 15459246]

Randler C, Schaal S. Morningness-eveningness, habitual sleep-wake variables and cortisol level. Biol Psychol. 2010; 85:14–18. [PubMed: 20450953]

Revell VL, Barrett DC, Schlangen LJ, Skene DJ. Predicting human nocturnal nonvisual responses to monochromatic and polychromatic light with a melanopsin photosensitivity function. Chronobiol Int. 2010; 27:1762–1777. [PubMed: 20969522]

Roecklein KA, Rohan KJ, Duncan WC, Rollag MD, Rosenthal NE, Lipsky RH, Provencio I. A missense variant (P10L) of the melanopsin (OPN4) gene in seasonal affective disorder. J Affect Disord. 2009; 114:279–285. [PubMed: 18804284]

Roenneberg T, Kuehnle T, Juda M, Kantermann T, Allebrandt K, Gordijn M, Merrow M. Epidemiology of the human circadian clock. Sleep Med Rev. 2007; 11:429–438. [PubMed: 17936039]

Rohan KJ, Roecklein KA, Haaga DAF. Biological and psychological mechanisms of seasonal affective disorder: a review and integration. Curr Psychiatry Rev. 2009; 5:37–47.

Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, Mueller PS, Newsome DA, Wehr TA. Seasonal affective disorder: a description of the syndrome and preliminary findings with light therapy. Arch Gen Psychiatry. 1984; 41:72–80. [PubMed: 6581756]

Rosenthal, NE.; Bradt, GH.; Wehr, TA. Seasonal Pattern Assessment Questionnaire (SPAQ). Bethesda, MD: National Institute of Mental Health; 1987.

Smith CS, Reilly C, Midkiff K. Evalution of three circadian rhythm questionnaires with suggestions for an improved measure of morningness. J Appl Psychol. 1989; 74:728–738. [PubMed: 2793773]

Strecker RE, Morairty S, Thakkar MM, Porkka-Heiskanen T, Basheer R, Dauphin LJ, Rainnie DG, Portas CM, Greene RW, McCarley RW. Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. Behav Brain Res. 2000; 115:183–204. [PubMed: 11000420]

Terman JS, Terman M. Photopic and scotopic light detection in patients with seasonal affective disorder and control subjects. Biol Psychiatry. 1999; 46:1642–1648. [PubMed: 10624545]

Terman M, Terman JS. Circadian rhythm phase advance with dawn simulation treatment for winter depression. J Biol Rhythms. 2010; 25:297–301. [PubMed: 20679499]

Tsai JW, Hannibal J, Hagiwara G, Colas D, Ruppert E, Ruby NF, Heller HC, Franken P, Bourgin P. Melanopsin as a sleep modulator: circadian gating of the direct effects of light on sleep and altered sleep homeostasis in melanopsin(−/−) mice. PLoS Biol. 2009; 7:e1000125. [PubMed: 19513122]

Wehr TA, Duncan WC, Sher L, Aeschbach D, Schwartz PJ, Turner EH. A circadian signal of change of season in patients with seasonal affective disorder. Arch Gen Psychiatry. 2001; 58:1108–1114. [PubMed: 11735838]

Weissman MM, Sholomskas D, Pottenger M, Prusoff BA, Locke BZ. Assessing depressive symptoms in five psychiatric populations: a validation study. Am J Epidemiol. 1977; 106:203–214. [PubMed: 900119]

Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM. Genome partitioning of genetic variation for complex traits using common SNPs. Nat Genet. 2011; 43:519–525. [PubMed: 21552263]

Young MA, Blodgett C, Reardon A. Measuring seasonality: psychometric properties of the Seasonal Pattern Assessment Questionnaire and the Inventory for Seasonal Variation. Psychiatry Res. 2003; 117:75–83. [PubMed: 12581822]

Zaidi FH, Hull JT, Peirson SN, Wulff K, Aeschbach D, Gooley JJ, Brainard GC, Gregory-Evans K, Rizzo JF 3rd, Czeisler CA, Foster RG, Moseley MJ, Lockley SW. Short-wavelength light sensitivity of circadian, pupillary, and visual awareness in humans lacking an outer retina. Curr Biol. 2007; 17:2122–2128. [PubMed: 18082405]

TABLE 1

Pairwise measures of linkage disequilibrium (LD) for three OPN4 SNPs calculated with Haploview (Barrett et al., 2005)

Loci pairs	D′	LOD	r^2
rs2014084 vs. rs2675703	.251	1.92	.023
rs2014084 vs. rs1079610	.878	6.37	.080
rs2675703 vs. rs1079610	.951	85.5	.645

P10L = rs2675703 and T394I = rs1079610.

D′ = normalized value of linkage disequilibrium, a measure of the likelihood of two alleles to co-occur in a population more than would be expected by chance, given allele frequencies. This also reflects the level of recombination between two loci, and is very low for the second two comparisons, rs2014084 vs. rs1079610 and rs2675703 vs. rs1079610, but somewhat higher for the first comparison, rs2014084 vs. rs2675703.

LOD = logarithm base 10 of the odds, an alternate measure of linkage disequilibrium. As reflected by D′, the LD between the second two comparisons is higher than with the first comparison.

r^2 = coefficient of determination, or the correlation coefficient between two loci, with a maximum of 1 and minimum of zero; threshold of r^2 = .8 generally indicates high correlation.

TABLE 2

Sleep, chronotype, and mood questionnaire summary results for the total sample

	n	*M*	*SD*	Max–Min
CSM	233	39.35	7.13	17–53
PSQI Total	234	2.44	1.81	0–11
Sleep onset	234	23:03 h	01:07 h	21:00–04:00 h
Wake time	234	06:31 h	01:13 h	02:56–11:00 h
Seasonality	229	6.502	3.951	0–21
Sleep duration (h)	234	6:51 h	0:52 h	03:00–9:00 h
BDI-II	234	3.73	4.23	0–29
CES-D	232	7.34	6.81	0–39

Missing data are reflected by n's, as a few participants did not complete all measures used in this study. Specifically, one person did not complete the CSM, five did not complete the M-SPAQ, and two did not complete the CES-D. CSM is the total score on the Composite Scale of Morningness. Maximum and minimum scores obtained on each scale are listed in the "Max–Min" column. PSQI Total reflects the sum of seven component subscales, and is detailed in Buysse et al. (1989). A score >5 indicates "poor" sleepers, which is higher than our mean of 2.44 on the PSQI total score. Sleep onset, sleep duration, and wake time are taken from the PSQI. Seasonality is measured by the global seasonality score from the Modified Seasonal Pattern Assessment Questionnaire. BDI-II is the Beck Depression Inventory–2nd Edition (Beck et al., 1996) and CES-D is the Center for Epidemiologic Studies—Depression Scale (Radloff, 1977).

TABLE 3

Genotype frequencies subdivided by short and long days (n, %)

	P10L (rs2675703)			T394I (rs1079610)			rs2014084		
	CC	CT	TT	CC	CT	TT	CC	CT	TT
Short days	58 (79.5%)	13 (17.8%)	2 (2.7%)	27 (36.5%)	35 (47.3%)	12 (16.2%)	10 (13.5%)	33 (44.6%)	31 (41.9%)
Long days	128 (81.0%)	27 (17.1%)	3 (1.9%)	56 (35.3%)	79 (49.7%)	24 (15.1%)	10 (13.5%)	33 (44.6%)	61 (38.4%)
Total	186 (80.5%)	40 (17.3%)	5 (2.2%)	83 (35.6%)	114 (48.95%)	36 (15.5%)	29 (12.4%)	112 (48.1%)	92 (39.5%)

Short days were defined as occurring between the vernal and autumnal equinoxes with daylengths <12 h, and long days were defined as ≥12 h. Daylength was analyzed as a continuous variable, and is presented here dichotomized between short and long days for ease of interpretation. Failure rates for the sample of 234 total participants are not included in the totals above (P10L, n = 3 failed to genotype, 1.3%; T394I, n = 1 failed to genotype, .4%; rs2014084, n = 1 failed to genotype, .4%).

TABLE 4

Analysis of covariance of daylength and P10L genotype on sleep onset and chronotype

			F	*p*	η^2	Levene[b]
Sleep onset						
Additive model	P10L		3.337	.037*	.029	.552
	Daylength		.816	.367	.004	
	P10L × Daylength		3.345	.037*	.029	
		CC vs. TT[a]	3.430	.065		
		CT vs. TT[a]	6.090	.014*		
Recessive model	P10L (TT vs. CT&CC)		4.307	.039*	.019	.271
	Daylength		2.800	.096	.012	
	P10L × Daylength		3.894	.049*	.017	
Chronotype						
Additive model	P10L		3.328	.038*	.029	.719
	Daylength		4.428	.036*	.020	
	P10L × Daylength		3.214	.042*	.028	
		CC vs. TT[a]	4.150	.043*		
		CT vs. TT[a]	6.260	.013*		
Recessive model	P10L (TT vs. CT&CC)		5.021	.026*	.022	.468
	Daylength		6.520	.011*	.029	
	P10L × Daylength		4.621	.033*	.020	

Seasonality is the Global Seasonality Scale (GSS) from the Modified Seasonal Pattern Assessment Questionnaire. Partial eta squared (η^2) effect size for ANCOVA is estimated to be small = .01, medium = .06, and large = .14 (Cohen, 1988).

[a]In the event of significant interactions between genotype and daylength on both sleep onset and chronotype, this effect was parsed into specific genotype contrasts (e.g., TT vs. CT and TT vs. CC for P10L). This involved testing for significant difference between slopes describing the relationship between daylength and sleep onset, for example, within a specific genotype, and such specific genotype contrasts are reported above under the interaction effects.

[b]In the additive model, all three genotype groups were compared with one another. In the recessive model, the TT genotype was compared with a group including both CC and CT genotypes together. In the final column, Levene's test of equality of error variances tested the null hypothesis the error variance of dependent variables is equal across groups, and proved to be not statistically significant in any case, indicating that the variance was not significantly different between genotype groups in any analyses, despite the small number of TT genotypes. Resulting *p* value of Levene's test is not less than .05, so the null hypothesis of equal variances is retained, and there is no evidence that there is difference between the variances obtained on the dependent variables when groups are compared by genotype.

* $p < .05$.