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PER2 rs2304672 Polymorphism Moderates Circadian-Relevant Reward Circuitry Activity in Adolescents

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Abstract

Background—Reward behavior in animals is influenced by circadian genes, including clock-pathway genes such as Period2 (*PER2*). Several forms of psychiatric illness are associated with both altered reward function and disturbances in circadian function. The *PER2* single nucleotide polymorphism (SNP) *rs2304672* has been associated with psychiatric illnesses involving reward dysfunction. Associations among circadian genes, function in neural reward circuits, and circadian-influenced behavior have not yet been studied in humans, however.

Methods—90 healthy adolescents underwent functional magnetic resonance imaging during a guessing task with monetary reward, genotyping for two *PER2* SNPs (*rs2304672*, *rs2304674*), and actigraphy to measure sleep in their home environments. Weekend sleep midpoint, a behavioral index of circadian function, was derived from actigraphy. Puberty was measured by physical exam.

Results—The *rs2304672* SNP predicted blood oxygenation level-dependent response to monetary reward as constrained by sleep midpoint. Later sleep midpoint was associated with reduced activity in a key component of reward circuitry, medial prefrontal cortex (mPFC; Brodmann area 9/10/32), to reward outcome ($p_{\text{corrected}} < .05$). G allele carriers showed reduced activity in mPFC relative to CC homozygotes.

Conclusions—Our findings are the first to indicate that circadian genes have a significant impact upon circadian-relevant reward circuitry in humans. These findings have the potential to elucidate gene-brain-behavior relationships underlying reward processing and psychopathology.

Keywords

Brain function; circadian function; clock-pathway genes; development; reward; *PER2*

Altered reward function is increasingly documented as an important feature of psychiatric illnesses in humans, including major depressive disorder and bipolar disorder (1,2),

substance use disorders (3,4), and schizophrenia (5). These illnesses are also characterized by disturbances in circadian function (6,7). Bipolar disorder, for example, involves a tendency toward eveningness, such that alertness and activity level peak later, rather than earlier, in the day (8). Major depressive disorder is also associated with greater eveningness, with the association postulated to occur through mechanisms involving reward responsiveness (9). Furthermore, among healthy adults, eveningness in men is associated with two reward-related traits: low harm avoidance and high novelty seeking (10).

It is now well established in preclinical research that reward function is influenced by circadian genes. Animal studies, for example, report that circadian genes from the clock pathway influence behavioral indices of reward function, including cocaine sensitization (11,12). Mice carrying a mutation of the *CLOCK* gene exhibit hyperactivity, greater valuing of cocaine, a behavioral constellation that is considered a model for bipolar disorder in humans, and altered dopamine function (13). *Period2* (*PER2*), a circadian clock gene, is one particular gene that has been proposed as critical to functional regulation of reward-related neural circuitry because expression of its product protein occurs at high levels in subcortical limbic regions supporting reward processing (14) and varies with circadian-rhythmic fluctuations (14). *PER2* mutant mice exhibit disrupted behavioral rhythms (15), hypersensitization to cocaine (16), and high alcohol intake (17).

Despite the foregoing findings indicating the likely important influence of circadian genes on reward dysfunction, little is known regarding the association of circadian genes with activity in reward-related neural circuitry in humans. Reward circuitry is conceptualized as critical to the pathophysiology of depression (18), and neuroimaging studies indicate functional abnormalities in reward-related neural circuitry in major depression (19,20) and bipolar disorder (R. Nusslock, unpublished data). In parallel, association studies suggest a relation between *PER2* functional polymorphisms, including the single nucleotide polymorphisms (SNPs) *rs2304672* and *rs2304674*, and psychosis (for both SNPs, G was the risk allele) (21). Studies directly examining gene–brain–behavior associations are, however, essential for elucidating the mechanisms through which genetic polymorphisms influence complex behavioral phenotypes.

We used functional neuroimaging of monetary reward response and actigraphy measures of circadian-influenced behavior to examine associations between two *PER2* SNPs, *rs2304672* and *rs2304674*, and circadian behavior–relevant reward neural circuitry function in humans. We investigated neural response to reward as constrained by association with sleep midpoint, a construct reflecting the influence of circadian function on sleep timing and likely to represent altered circadian patterning relevant to psychopathology. We examined healthy adolescents who were younger than early adulthood/late adolescence, the most common age of first onset of major psychiatric illnesses (22), to avoid both the potential confounds of current psychopathology and resilience. The focus on healthy adolescents also allowed examination of the extent to which any influence of *PER2* polymorphisms on reward circuitry function was associated with pubertal status, given the dramatic changes in circadian and reward function during adolescence (23,24).

Methods and Materials

Participants

The final sample included 90 healthy adolescents (Table 1). From the original 127 adolescents, participants were excluded for claustrophobia ($n = 2$), neurological or learning disorder ($n = 2$), excessive movement ($n = 20$), and genotyping difficulties ($n = 13$). Of the 90 participants, 28 declined to participate in the actigraphy assessment ($n = 28$) and were therefore excluded from analyses of genotype effects. Consistent with our approach to

affective development (23), we classified adolescents as pre/early pubertal if they were Tanner breast/genital Stage 1 or 2 ($n = 27$) and as mid/late pubertal if they were Stage 3, 4, or 5 ($n = 63$).

Adolescents were recruited from the community through advertisements, flyers, and demographically targeted phone lists. The sample was intended to have a narrow age range—11 to 13 years—but to include variability in pubertal development. Girls were 11 to 12 years old ($M = 11.43$, $SD = .54$), and boys were 12 to 13 years old ($M = 12.48$, $SD = .50$), based on findings that girls in the United States undergo puberty earlier than boys (25,26). Adolescents were free of current and lifetime psychiatric disorders by parent report; history of head injury, serious medical illness, psychotropic medication, alcohol use, or illicit drug use; and did not have braces. Adolescents underwent physical examination by a trained nurse to determine sexual maturation stage for breast/genital development using criteria specified by Marshall and Tanner (27). Depressive symptoms were measured by self-report on the Mood and Feelings Questionnaire (28). Procedures were approved by the University of Pittsburgh Institutional Review Board. Parents or legal guardians provided written informed consent, and participants provided verbal assent.

Genotyping

High-molecular-weight DNA was isolated from ethylenediamine tetraacetate anticoagulated whole-blood samples obtained from all participants using the Puregene kit (Gentra Systems, Minneapolis, Minnesota). Each sample was genotyped for two *PER2* SNPs—*rs2304672* and *rs2304674*—using the Sequenom iPLEX assay in the Genomics and Proteomics Core Laboratory, University of Pittsburgh (<http://www.genetics.pitt.edu>). Genotypes were scored by comparison to sequence-verified standards. Participants were classified by genotype as follows (Table 1). Two genotype groups were created for each SNP, based on frequency. For *rs2304672*, participants were classified as CC allele homozygotes ($n = 78$) or G carriers ($n = 12$). For *rs2304674*, participants were classified as TT homozygotes ($n = 41$) or C carriers ($n = 48$). Consistent with crosspopulation findings of allele frequencies (29), only one participant was a GG homozygote for *rs2304672*, and eight were CC homozygotes for *rs2304674*. For analyses, the *rs2304672* GC heterozygotes and the rare *rs2304672* GG homozygotes were combined, as were the *rs2304674* CT heterozygotes and the rare *rs2304674* CC homozygotes.

Reward Circuitry Activity

Reward Paradigm—The functional magnetic resonance imaging (fMRI) paradigm was a slow event-related card-guessing game (20) that allows examination of response to anticipation and receipt of monetary reward. Participants received win, loss, or no-change feedback for each trial. Participants were told that their performance would determine a postscan monetary reward, with \$1 for each win and 50 cents deducted for each loss. Trials were presented in fixed, pseudorandom order with predetermined outcomes that were identical across participants. Trials were presented in 4 runs, with 12 trials per run and a balanced number of trial types within runs (e.g., 6 possible-win trials in each run). During each trial, participants guessed via button press whether the value of a visually presented card was high or low (3 sec); learned the trial type (possible-win or possible-loss) and anticipated feedback (12 sec); and received outcome feedback (1 sec plus 11-sec intertrial interval). Feedback consisted of a computer-generated number and then a green upward-facing arrow for a win outcome, a red downward-facing arrow for a loss outcome, or a yellow circle for a neutral outcome. Participants were unaware of fixed outcome probabilities, and their engagement and motivation were maintained by verbal encouragement between runs. Participants practiced the task before the scan and did not exhibit a change in reaction time across task runs. As in our previous studies with other

samples (30), genotype groups did not differ in reaction time. During debriefing, all participants stated that they understood the task, thought that outcomes were due to chance, and found the task engaging.

fMRI Acquisition, Processing, and Analysis—Each participant was scanned using a Siemens 3T Allegra scanner (Siemens, Malvern, Pennsylvania). Blood oxygen level–dependent functional images were acquired with a gradient echo planar imaging sequence and covered 34 axial slices (3 mm thick) beginning at the cerebral vertex and encompassing the entire cerebrum and the majority of the cerebellum (repetition time/echo time = 2000/25 msec, field of view = 20 cm, matrix = 64 × 64). All scanning parameters were selected to optimize the quality of the blood oxygen level–dependent signal while maintaining a sufficient number of slices to acquire whole-brain data. Before the collection of fMRI data for each participant, we acquired a reference echo planar imaging scan that we visually inspected for artifacts (e.g., ghosting) and for good signal across the entire volume of acquisition. The fMRI data from all included participants were cleared of such problems.

Whole-brain image analysis was completed using SPM5 (<http://www.fil.ion.ucl.ac.uk/spm>). For each scan, images for each participant were realigned to the first volume in the time series to correct for head motion. Data sets were then selected for quality based on our standard small-motion correction (<4 mm for adolescents) (20). Realigned images were spatially normalized into standard stereotactic space (Montreal Neurological Institute template) using a 12-parameter affine model. Normalized images were smoothed with a 6-mm full-width at half-maximum Gaussian filter. Voxelwise signal intensities were ratio normalized to the whole-brain global mean.

Preprocessed data sets were then analyzed using first-level random effects models that account for scan-to-scan variability and second-level random effects models that account for participant-to-participant variability to determine task-specific regional responses. For each participant and scan, condition effects (i.e., main effects of task) at each voxel were calculated using a *t* statistic, producing a statistical image for the two contrasts of interest: 1) reward anticipation greater than baseline and 2) reward outcome (i.e., win) greater than baseline. Baseline was defined as the last 3 sec of each intertrial interval. Because the study focused on reward-related brain function, analyses included trials involving reward anticipation and reward (win) outcome. On the basis of our hypotheses and previous strategy, analyses focused on the first run (i.e., six reward trials), because these data are less likely to reflect fatigue, boredom, and habituation, which is a particular concern because striatal response tends to diminish with repeated experience of a reward (3). To confirm this, we computed correlations between neural response in our regions of interest during run 1 only and response in these areas across all four runs. As predicted, and consistent with our findings in other samples (20), correlations were weak and nonsignificant (e.g., $r = .21$, $p = .17$ for mPFC during reward outcome).

Circadian Characteristics

Actigraphy was conducted after the fMRI assessment, over two weekday nights and two weekend nights in participants' home environments, using Octagonal Basic Motionlogger Actigraphs (Ambulatory Monitoring, Ardsley, New York). Participants wore the actigraph on their nondominant wrist from Friday at 4 PM until awakening Tuesday morning, removing it only for contact sports, swimming, or bathing. Actigraphs recorded continuously. Participants pressed a button to indicate when they were trying to go to sleep and when they woke up.

Actigraphy data were preprocessed and scored by trained coders in 60-sec epochs using Action W 2.5. Sleep onset and offset times were determined using the actigraph record,

supported by the button-press marker and sleep diary. Data were processed using the Cole-Kripke procedure (31).

The actigraphy variable of interest was weekend *sleep midpoint*, which indicates individual differences in bedtime/waketime and reflects circadian alignment (32). We focused on the weekend because it allowed participants to be assessed during more natural, self-selected schedules. Genotype groups did not differ in sleep midpoint [$F(1,59) = 2.58, p = .11$ for *rs2304672*; $F(1,59) = .27, p = .61$ for *rs2304674*] or sex [$F = .36, p = .55$]. Sleep midpoint varied by race (European American participants had earlier sleep midpoint than non-European American; $F = 6.47, p = .01$; $M = 236.9$ min past midnight, $SD = 61.1$ and $M = 281.2, SD = 61.8$, respectively), and age (consistent with developmental findings, sleep midpoint increased with age: $r = .29, p = .02$). Sleep midpoint was negatively, moderately correlated with total sleep time ($r = -.36, p < .01$) but was uncorrelated with sleep quality ($r = .13, p = .46$).

Statistical Analysis

We first examined neural activity associated with reward outcome and reward anticipation using within-sample analyses in a priori neural regions of interest, defined anatomically using the WFU PickAtlas Tool (v1.04), that are implicated in reward and emotion processing. These regions were ventral and dorsal striatum (30); medial prefrontal cortex (mPFC), including medial Brodmann area (BA) 9, medial BA 10, and BA 32 (23); orbitomedial prefrontal cortex; and the amygdala (see Table S1 in Supplement 1 for whole-brain findings).

Next, the association of reward-related neural activity with circadian function was tested using regressions of neural activity against sleep midpoint, with a voxelwise threshold of $p < .05$ and minimum extent of 10 contiguous voxels. These analyses included the 63 participants who had actigraphy data. Simulations in the AlphaSim program in AFNI were employed to estimate the minimum number of contiguous voxels in each cluster required to avoid Type I error (cluster-level threshold $p < .05$) in each region of interest. The results of these analyses were then used as a functional mask for the subsequent analyses, so that later analyses examining the effect of genotype (two groups for each *PER2* SNP) on reward-related neural activity could be constrained to activity in neural regions 1) activated by the task and 2) relevant to circadian behavior.

We then used second-level factorial models to examine the effect of genotype on reward-related neural activity in the circadian-relevant reward-related functional mask from the regressions described earlier. To maximize the use of available data, these analyses included all 90 participants with genotype and fMRI data. For these analyses, race and sex were included as covariates. Age was also included as a covariate for models with *rs2304674* because TT homozygotes were slightly older than C carriers [$M = 12.19, SD = .74$ and $M = 11.60, SD = .65$, respectively; $F(1,60) = 11.00, p < .005$]. Results were corrected for multiple comparisons using AlphaSim program as described earlier.

Finally, we examined the development $\times$ genotype interaction on activity in circadian-relevant, reward-related clusters using second-level factorial models with genotype and puberty (pre/early, mid/late groups) as independent factors, sex, age, and race as covariates, and the AlphaSim program to correct for multiple tests.

Results

Allele and Genotype Frequencies

The allele frequencies for both candidate genotypes in the participant cohort were comparable to those reported for adults in larger samples and did not deviate from Hardy–Weinberg equilibrium ($p > .10$). Genotype groups for *rs2304672* (78 CC homozygotes; 12 G carriers) and *rs2304674* (41 TT homozygotes; 48 C carriers) did not differ with regard to sex ($\chi^2 = .007$, $p = .93$; $\chi^2 = .096$, $p = .76$, respectively), ethnicity/race ($\chi^2 = .34$, $p = .56$; $\chi^2 = 1.27$, $p = .26$, respectively), age ($F = .31$, $p = .58$; $F = 3.33$, $p = .07$, respectively), or likelihood of providing actigraphy data ($\chi^2 = .59$, $p = .44$; $\chi^2 = .85$, $p = .36$, respectively).

Reward Neural Circuitry Activity

As in previous studies using the paradigm we used in this study (20,23), participants activated reward neural circuitry, including the striatum, mPFC, and amygdala, during reward outcome and reward anticipation (Table 2; Figure 1). These findings were generally confirmed in whole-brain analyses (Table S1 in Supplement 1).

Circadian Characteristics and Reward Circuitry Activity

Sleep midpoint in the 63 participants who had complete neuroimaging, genetic, and actigraphy data was negatively correlated with activity in a large cluster in mPFC centered on BA 9, and extending into BAs 10/32, during reward outcome versus baseline (338 voxels, $t = 3.14$, $p_{corrected} < .05$, max voxel: $[-2, 46, 27]$; Figure 2). Here, later sleep midpoint was associated with lower mPFC activity. To confirm that results were not due to the association of later sleep midpoint with sleep restriction or deprivation, the regression for sleep midpoint was also conducted with total sleep time as a covariate. Results for mPFC remained significant in this model. Sleep midpoint was not correlated significantly with activity in any reward-related regions during reward anticipation. Because results were limited to reward outcome, the following analyses focused on neural response to reward outcome.

Genotype Modulation of Circadian-Relevant, Reward Circuitry Activity

In the 90 participants who had complete genetic and neuroimaging data, circadian-relevant neural activity during reward outcome differed for *rs2304672* genotype groups. Specifically, in a large dorsal mPFC cluster encompassing medial BA 9 and extending into BA 32, carriers of the G allele, a risk allele for schizophrenia (22) showed less activity than CC homozygotes (87 voxels, $t = 2.62$, $p_{corrected} < .05$, max voxel: $[-3, 37, 29]$; Figure 3). Genotype groups for *rs2304674* did not differ significantly on circadian-relevant neural activity.

In light of possible differences in allele frequency across racial groups, we tested the association of *rs2304672* genotype and circadian-relevant activity during reward outcome in the mPFC cluster defined earlier within the European American subsample (based on self-reported race, given its strong association with genetic markers of ancestry) (33). Results were consistent with those for the entire sample, although they did not meet the AlphaSim correction criterion (17 voxels, $t = 2.13$, $p_{uncorrected} < .05$, max voxel: $[-2, 44, 20]$; Figure S1 in Supplement 1). This is likely related to the reduced statistical power for this smaller sample (38 CC homozygotes, and 7 G carriers).

We also tested whether findings were confounded by depressive symptoms. G carriers did not differ from CC homozygotes in level of depressive symptoms [$F(1,89) = .56$, $p = .46$], and depressive symptoms were uncorrelated with mPFC response ($r = .09$, $p = .42$), indicating that our findings were not due to a confound by depression.

To examine whether less mPFC response in the G carrier group could indicate individual differences in regulation of reward responding, we tested correlations between the mean mPFC response in the cluster for which the *rs2304672* genotype groups differed and the mean striatal response to reward outcome. Whereas G carriers exhibited a strong, negative association between mPFC response and ventral striatal response to reward outcome ($r = -.72, p = .008$), CC homozygotes exhibited a weak, nonsignificant association between these regions ($r = .16, p = .16$).

Finally, to examine whether findings were specific to reward response, we conducted analyses for the association of *rs2304672* genotype with neural response to loss. In a whole-brain analysis, there were no clusters in which CC and G carrier groups differed in response to loss anticipation or loss outcome. This suggests that *PER2* genotype was associated with neural response to reward specifically, rather than to outcome or task engagement generally.

Puberty Associations with *PER2* Modulation of mPFC Function

Developmental group did not moderate the effect of *rs2304672* genotype on mPFC activity during reward outcome.

Discussion

This study is the first to report in humans that a polymorphism in a clock-pathway gene influences circadian-related activity within reward circuitry during reward processing. By examining *PER2* genotype, reward circuitry function, and actigraphy measures of circadian function, our findings make a significant contribution to the growing literature on circadian genes and reward processing, suggesting a mechanism through which eveningness, reward processing, and psychiatric disorders may be associated.

The association between decreased mPFC activity to reward outcome and sleep behavior—that, in turn, appears to be moderated by genetic variation in *PER2 rs2304672*—suggests a key role for clock-pathway genes in reward function in humans. This SNP has also been implicated in abdominal obesity and difficulty complying with behavioral weight-reduction treatment (34), suggesting another form of association with reward function. (Although considered an informative SNP in a study of alcohol consumption in humans (17), *rs2304672* was not ultimately included in that study's haplotype analysis.) Aside from an extant study reporting influence of a *CLOCK*-gene SNP on anterior cingulate response to affective words in inpatients with depression (35), examination of relationships between reward circuitry and circadian genes has largely been restricted to animal studies. In the current study, we were able to consider the potential roles of sleep deprivation and depressive symptoms in our findings. Importantly, our findings appear to be associated with circadian function rather than simply sleep, and our genotype findings do not appear to be driven by higher depressive symptoms in the G carrier group. Because, as in previous studies, our study design limits our interpretations about direction of effects, future studies could also address the direction of influence of these variables. Nonetheless, given emerging models highlighting the role of mPFC in reward (37), our findings therefore suggest that polymorphisms in clock-pathway genes such as *PER2* may moderate normal variability in circadian function and individual differences in reward function with implications for mental and physical health.

In this study, the *PER2 rs2304672* G allele was associated with less activity in a mPFC region postulated to regulate striatal response to emotionally salient stimuli (36) and implicated in self-related cognition and action monitoring (37). Reduced activity in this mPFC region to reward in individuals with behavioral tendencies toward late sleep may therefore indicate suboptimal regulation of striatal activity, together with reduced self-

awareness and self-monitoring of behavior in response to reward. Our findings of a strong, negative correlation between mPFC response and striatal response in G carriers provides some support to this regulatory hypothesis because they may indicate weaker inhibition of striatal response. We can only speculate about possible causal associations, but perhaps tendencies toward low self-regulation in response to reward lead to greater activity levels in the evening—when the potential to engage in reward-related behaviors is high—and thus later sleep. Alternatively, a circadian-based tendency toward reward-seeking behavior and later sleep times could serve to inhibit the function of the mPFC. Future experimental studies have the potential to test these intriguing possibilities.

Although we did not find a direct association between genotype and sleep midpoint, our results underscore the importance of an imaging genetics approach to individual differences. Together with extant findings of abnormal reward-related circuitry function in depression and schizophrenia (5,19–20), disrupted circadian rhythms in bipolar disorder (6), and an overrepresentation of the G allele of the *PER2* rs2304672 SNP in schizophrenia (22), obesity (34), and alcohol consumption (17), our findings suggest that circadian gene-moderated function in reward circuitry may be a pathway for future risk for major psychiatric and physical illnesses.

Notably, the differences we found based on *PER2* genotype were evident during reward outcome. This suggests that the enjoyment of reward could be particularly influenced by this circadian gene. However, because we constrained our genotype analyses to circadian-relevant regions, and reward anticipation was not associated with sleep midpoint, we did not conduct gene $\times$ condition analyses to directly test for the association of rs2304672 with appetitive versus consummatory reward processes. Additionally, the *PER2* SNP rs2304674 was not associated with circadian-relevant reward activity in any neural regions. This suggests that the two SNPs, although located near each other in *PER2* and not in high linkage disequilibrium, might have differing specificity for reward function, at least as it is relevant to circadian-driven sleep behavior.

Our previous findings from this study (with some individuals within the current sample) indicated that puberty is associated with reduced activity in neural reward circuitry to rewarding outcomes (23) and that sleep patterns typical of adolescent development are also associated with reduced activity in neural reward circuitry (38). There were no puberty-moderated genotype effects on reward-related neural activity in this study, however, which suggests that genetic variation in the circadian system exerts similar influence on reward function across pubertal development. In addition, we note that because we based our functional masks of genotype effects on the results of sleep midpoint analyses, and our genotype groups did not differ in their participation in the actigraphy assessment, our findings for rs2304672 are not likely to be biased by either genotype group's pattern of behavior.

A limitation of this study is the unknown function of the *PER2* SNPs. Our findings, however, provide insight into mechanisms by which rs2304672 may influence activity in reward circuitry in individuals prone to late sleep phase based on circadian function. In addition, although the focus on a healthy sample was appropriate for testing hypotheses about normal individual differences in circadian function and reward function, it will be valuable in future studies to examine the relation between clock-pathway gene functional polymorphisms and reward circuitry in individuals with psychiatric illnesses such as bipolar disorder, schizophrenia, and addiction. A methodological issue is that, given concerns about the attenuation of neural response to reward over time, we focused on the first run of our fMRI task, which limited the number of trials included in analyses. Additional analyses conducted with the entire task yielded findings that were similar to but weaker than those

reported here, which suggests the value of focusing on early trials. Future studies should balance considerations of habituation and fatigue with psychometric considerations of task reliability. Finally, we consider our findings preliminary because the small number of G carriers in the sample limited our statistical power to detect both associations in the European-ancestry subsample (in which, interestingly, both genotype groups exhibited decreased mPFC response to reward outcome) and possible interactions between genotype and development in predicting reward-related neural activity. Future work could examine larger European-ancestry samples as defined by genetic ancestry markers, as well as possible developmental changes in *PER2* genotype influence on reward-related neural function.

This study is the first to report gene– brain– behavior associations involving a clock-pathway gene functional polymorphism and reward function in humans. We show that genetic variation in the circadian *PER2* gene SNP *rs2304672*, previously associated with psychiatric illness, moderates circadian-relevant mPFC activity to reward. Our findings provide the foundation for understanding the influence of circadian genes on neural circuitry mediating individual differences— both typical and pathological—in reward-related behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Neural response to the anticipation (upper panel) and outcome (lower panel) of monetary reward. Results are masked for the following anatomic regions of interest: striatum, medial prefrontal cortex, orbitofrontal cortex, and amygdala.

Figure 2.

Association between medial prefrontal cortical (PFC) response to reward outcome and sleep midpoint. Scatterplot values reflect mean activation across the entire cluster depicted in the image. Results are masked by the findings for response to reward outcome in the region. Results remained significant when the outlier with sleep midpoint close to 8 AM was excluded.

Figure 3. Differences between *PER2* rs2304672 genotype groups in neural response to reward outcome. Results are masked by medial prefrontal cortex findings for the association between sleep midpoint and response to reward outcome. Boxplots depict neural response by group.

Table 1

Descriptive Statistics for Demographic Variables, by Genotype Group

	Total Sample	PER2 rs2304672		PER2 rs2304674	
		CC	G Carrier	TT	C Carrier
<i>N</i>	90	78	12	41	48
Age	11.9 (.7)	11.9 (.7)	11.8 (.8)	12.1 (.7)	11.8 (.8)
Sex (% Female)	51.1	51.3	50.0	48.8	52.1
Race (%)					
European American	76.7	75.6	75.0	82.9	72.9
Non-European American	23.3	24.4	25.0	17.1	27.1
Pubertal Developmental Group (%)					
Pre/early	30.2	29.5	33.3	26.8	33.3
Mid/late	69.8	70.5	66.7	73.2	66.7
Depressive Symptoms	7.15 (8.06)	6.96 (8.09)	9.11 (8.48)	7.10 (8.98)	7.34 (7.39)
Sleep Midpoint	4:08 AM (1:04)	4:13 AM (1:06)	3:39 AM (00:47)	4:14 AM (1:16)	4:05 (00:54)
Total Sleep (Hours)	8.51 (1.01)	8.48 (0.95)	8.60 (1.48)	8.35 (.95)	8.63 (1.08)

Age, depressive symptoms, and sleep variables are presented as mean (SD). Depressive symptoms are based on total score on the Mood and Feelings Questionnaire (28). Sleep data are based on the subsample of 63 participants with actigraphy data.

Table 2
Reward-Related Neural Response in A Priori Regions of Interest, by Task Condition

Neural Region	Talairach Coordinates of Maximum Voxel in Cluster			Cluster Size	t
	x	y	z		
Reward Outcome					
Striatum (caudate)	-4	-1	13	901	8.22
mPFC, BA9 (BA 6/8/10/24/32)	0	37	35	1025	4.70
Amygdala	-16	-3	-18	43	3.58
	22	1	-15	23	2.26
Reward Anticipation					
Striatum (caudate)	6	1	17	697	6.01
mPFC, BA 10 (BA 8/9)	4	50	38	584	4.71
Amygdala	-16	-5	-18	48	4.85

$p_{\text{corrected}} < .05$ for all. $df=62$. The first region listed contains the maximum-significance voxel.

BA, Brodmann area; mPFC, medial prefrontal cortex.