SLEEPJ, 2019, 1–10

doi: 10.1093/sleep/zsz034
Advance Access Publication Date: 5 February 2019
Original Article

ORIGINAL ARTICLE

Nocturnal heart rate variability moderates the association between sleep–wake regularity and mood in young adults

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Abstract

Study Objectives: Sleep–wake regularity (SWR) is often disrupted in college students and mood disorders are rife at this age. Disrupted SWR can cause repetitive and long-term misalignment between environmental and behavioral cycles and the circadian system which may then have psychological and physical health consequences. We tested whether SWR was independently associated with mood and autonomic function in a healthy adult cohort.

Methods: We studied 42 college students over a 3 week period using daily sleep–wake diaries and continuous electrocardiogram recordings. Weekly SWR was quantified by the interdaily stability of sleep–wake times (IS_{SW}) and mood was assessed weekly using the Beck Depression Inventory-II. To assess autonomic function, we quantified the high-frequency (HF) power of heart rate variability (HRV). Linear mixed effects models were used to assess the relationship between repeated weekly measures of mood, SWR, and HF.

Results: Low weekly IS_{SW} predicted subsequent poor mood and worsening mood independently of age, sex, race, sleep duration, and physical activity. Although no association was found between IS_{SW} and HF, the IS_{SW}-mood association was significantly moderated by nocturnal HF, i.e. reported mood was lowest after a week with low IS_{SW} and high HF. Prior week mood scores did not significantly predict the subsequent week's IS_{SW}.

Conclusions: Irregular sleep–wake timing appears to precede poor mood in young adults. Further work is needed to understand the implications of high nocturnal HRV in those with low mood and irregular sleep–wake cycles.

Statement of Significance

Modern societal constraints can lead to irregular sleep and wake timing in early adulthood. Detrimental effects on psychological wellbeing and physical health are just beginning to emerge. This study shows that irregular sleep–wake cycles independently predict poor mood and change in mood during weeks with high nocturnal heart rate variability in college students. Three-quarters of chronic mental health burden begin before the age of 24; future studies are warranted to test whether increased sleep–wake regularity improves mood and wellbeing at an early stage in adulthood. The observed nocturnal heart rate variability pattern is surprising and may represent maladaptive changes in vagal modulation with unclear long-term health consequences.

Key words: sleep wake regularity; mood; heart rate variability; autonomic function; circadian misalignment

Submitted: 14 October, 2018; **Revised:** 3 January, 2019

Introduction

The sleep–wake cycle displays a ~24 hr rhythm that is regulated in part by the circadian system, but is also influenced by environmental factors such as work and school schedules [1]. Measuring the fluctuations in lifestyle and daily routine, in particular, sleep–wake regularity (SWR), is an emerging field in sleep research [2–5]. We know that the intrinsic circadian system is slow to accommodate sleep schedule changes [6], and misalignments with individuals' actual sleep–wake cycle, as occasioned by shift work and irregular sleepwake times, have been associated with many adverse health consequences, including poor mood and perceived wellbeing [2, 3, 7–11].

A popular marker of sleep schedule fluctuation has been the concept of social jetlag (SJL), the difference in relative sleep–wake times between weekday and weekend nights [6, 12]. However, SJL uses an arbitrary dichotomization based on the assumption that work-life constraints are greatest during the traditional Monday–Friday paradigm; it is unclear whether these findings generalize to younger adults, such as college students, who are yet to enter the workforce. They may also exhibit rapidly changing daily sleep–wake times, which is believed to have the largest impact on the regulation of the circadian system [2]. One of the most commonly used measures of regularity that can account for day-to-day fluctuations, regardless of the day of the week, is the interdaily stability (IS) [13–15]. Given that occurrences of poor mood alone in earlier life have shown clear associations with later life chronic mental health consequences [16–18], the primary aim of this study was to examine the relationship between environmental-imposed SWR (as measured by the IS of sleep–wake times, IS_{sw}), and mood in healthy, young college students.

In addition, altered functioning of the circadian system may underlie the adverse consequences of irregular sleep–wake and daily activity cycles via circadian influences on the autonomic nervous system (ANS) [19–22]. The ANS system maintains homeostatic control via the sympathetic and parasympathetic systems responding as appropriate during times of stress. Altered autonomic function, as characterized by heart rate variability (HRV) [23], has been linked to premature aging and a host of pathological states, including cardiovascular disease [24–26] and chronic mental health disorders [27–29]. It has been suggested that chronic imbalance between the sympathetic and parasympathetic systems leads to excessive energy demands and partially accounts for the various disease states associated with HRV [23].

However, there have been few studies to date examining the relationship between SWR and autonomic function. Studies involving HRV and circadian/sleep regulation have mainly been in disorders such as insomnia [30], tested sleep deprivation [31], or classified sleep stages [32]. Therefore, the second aim of our study was to examine the effect of SWR on autonomic function. Finally, we also explored the relationship between other potential contributors and circadian misalignment (social jetlag, chronotype, and delayed bedtime) on SWR, mood, and autonomic function.

Methods

Participants

Forty-two students at local colleges completed the study. All participants were between 19 and 29 years old (Mean [SD]: 22.7 [2.7] years old; 22 females and 20 males). Racial make-up included 18 (42%) Caucasians, 18 (45%) Asians, and 5 (12%) African Americans. All participants were healthy, nonsmoking, and drug- and medication free (except oral contraceptives), and denied sleep or psychiatric disorders. Specifically, participants were asked about their habitual sleep–wake times, duration of sleep, problems falling asleep (insomnia), falling asleep at inappropriate times (narcolepsy), and daytime fatigue or sleep apnea diagnosis. Any history of psychiatric care or diagnoses, medications (particularly antidepressants, antianxiety, antipsychotics, substance abuse, or sleeping pills) led to exclusion. The study was approved by the local Institutional Review Board. Participants provided informed consent prior to participation.

Protocol and data collection

The study consisted of an initial in-lab visit and a 3 week ambulatory phase. Participants were screened by phone, seen at an initial visit to the laboratory to determine eligibility, and again at the end of the study period. During the initial visit, body weight, height, body-mass index (BMI), blood pressure (BP), heart rate (HR), and respiratory rate (RR) were recorded. Participants also completed the Morning–Evening Questionnaire (MEQ score) [33] for chronotype assessment, and the Beck Depression Inventory-II (BDI) questionnaire for assessment of mood [34]. During the ambulatory phase, participants wore a Holter monitor (DF-010, DELBio, INC.) for continuous electrocardiogram (ECG) recordings and were asked to complete the sleep–wake diary and physical activity diary each day.

Sixty-two candidates were invited to the lab visit after initial phone screening. Fifty-eight completed the initial visit and were eligible for the study. Sixteen withdrew within the first week (the main issue was the continuous ECG monitor strap on the chest, some moved away from the city, reported that daily diaries were too tedious, or no reason given). Thus, 42 students completed the study and their data entered our analysis.

Sleep–wake regularity

Sleep–wake diaries were reviewed with participants at the final visit to confirm sleep–wake patterns. The sleep–wake cycles were then plotted alongside the ECG-derived R–R intervals (RRI; see Figure 1 for representative participant data for 1 week). We found the majority of sleep schedules matched HR (inverse of RRI) responses consistent with sleep (increased RRI; decreased HR).

To quantify the regularity of the sleep–wake cycles in each week, we used the interdaily stability (IS_{sw}) analysis. This provides a measure of the extent to which a periodic time series is similar in shape from cycle to cycle, and has been widely used to quantify the stability of daily motor activity rhythms [13, 15, 35]. In this study, we applied the analysis to the time series of sleep–wake cycles and obtained IS_{sw} as a measure of SWR [36]. Ranging from 0% to 100%, larger IS_{sw} values indicate more similar sleep–wake schedules from day to day (e.g. 100% indicates the exactly same sleep–wake timings everyday) (see Supplementary Figure S1).

Finally, we also used the sleep–wake diaries to calculate delayed bedtime as the total time spent (at least 2 hr) past their

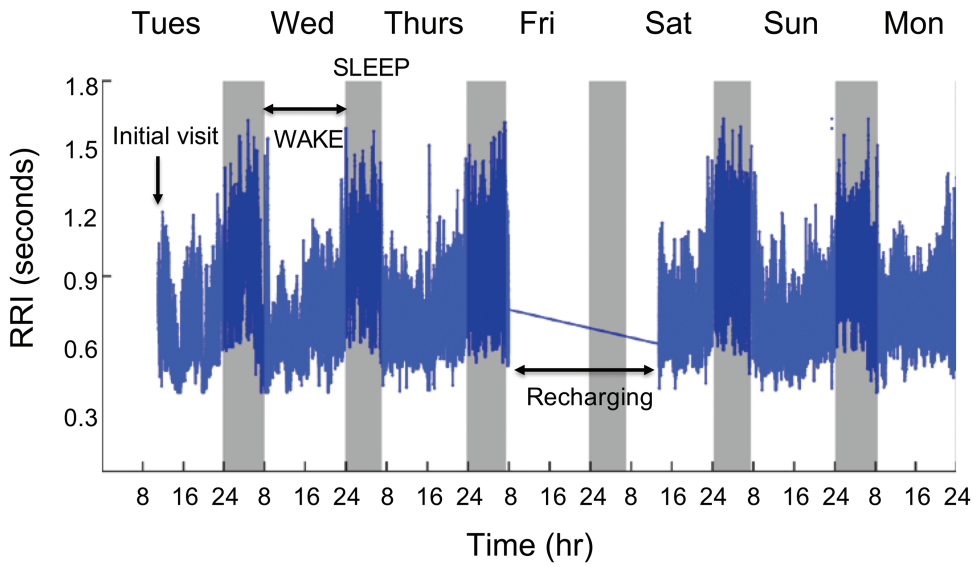

Figure 1. Subject sleep–wake diary and electrocardiogram raw data. Representative subject week 1 data (7 days, 6 nights) for ECG-derived R–R interval values (RRI). Shaded regions represent reported sleep periods from diaries. Note the rise in RRI (fall in heart rate) during sleep periods. Gap in data due to scheduled recharging on the 4th day.

habitual sleep times per week. Social jetlag was calculated as the mean difference between the midpoint of sleep during weekdays and weekends across the 3 week period [6, 12]. Physical activity (PA) questionnaires were used to generate weekly Metabolic Equivalent of Task (MET)-minutes products as a quantitative marker of physical activity levels [37].

Mood assessment

To assess mood, the BDI questionnaire was administered for all participants at baseline during the initial visit, and at the end of week 3 during the final visit. After an interim analysis of the first 27 participants, we modified the protocol by adding two additional BDI questionnaires to be completed by the final 15 participants at home, immediately after weeks 1 and 2. This enabled us to better examine the temporal relationship between IS_{sw} and BDI without any reported increase in participant task fatigue. In addition to weekly BDI scores, we calculated a change in BDI (ΔBDI) using the participant's initial visit BDI score as a baseline. In total, 72 end-of-week BDI questionnaires (42 week 3 BDI scores, plus 30 additional BDI scores from the final 15 participants for weeks 1 and 2) were analyzed in this study.

Heart rate and heart rate variability

During the initial in-lab visit, all participants were instructed to wear a Holter monitor continuously for 3 days before taking off and charging the device for 1 day (see Figure 1 for representative participant data for 1 week). The same 4 day cycle was repeated over 21 days before the second in-lab visit. Thus, for each participant, there were up to 16 days of ECG data. The average duration of high-quality, continuous ECG recordings that entered analysis for each participant was ~250 hr (10.5 days), evenly represented across 3 weeks. Heart rate was calculated as the inverse of the RRI and qualitatively used where available to corroborate sleep–wake times. A third of the ECG data were obtained from sleep episodes.

For the analysis of HRV, cardiac interbeat intervals were first obtained using a QRS wave detector based on the amplitude and first derivative of the ECG waveform and were verified by a trained technician to ensure that only "normal-to-normal" *R*-wave times were included. Power spectra were calculated by fast Fourier transform of 3.41 Hz cubic spline resampled data across nonoverlapped 10 min time windows. The high-frequency (HF) power at 0.15–0.40 Hz was used as the primary measure of autonomic activity. The other frequency- and time-domain HRV indices were also explored: low-frequency power (LF: 0.04–0.15 Hz), ratio of LF and HF powers (LF/HF), the standard deviation (SDNN), square root of the mean of squares (RMSSD), and percentage > 50 ms (pNN50) of differences between adjacent normal-to-normal intervals. Using participants' sleep–wake diaries, 30 min either side of each sleep–wake transition was excluded. HRV data were obtained in 10 min bins for the entire study period and then separated into sleep (HF sleep) and wake (HF wake) episodes for the 3 week study period, according to participants' sleep–wake diaries.

Statistical analysis

To estimate the effect of a student's weekly IS_{sw} on his/her subsequent end-of-week BDI and change in BDI, we used a linear mixed effects model (LMM) with subject as a random factor for intercept, and restricted maximum likelihood to account for the repeated weekly measures across the 3 week study. We constructed models accounting for potential covariates: demographics (age, sex, and race), physical activity, and sleep duration. Similar models were used to explore the effects of chronotype, delayed bedtime and social jetlag, separately, while controlling the effect of IS_{sw}. For the purposes of data presentation, the mean participant IS_{sw} was calculated and grouped into lower and upper half of participant IS_{sw}. Weekly IS_{sw} were also binned into quartiles for data visualization. Partial correlations were used for nonrepeated measures. All analyses were performed using JMP Pro 13 (SAS Institute Inc., Cary, NC).

Variables were log-transformed if found not to be normally distributed (e.g. HF and LF). Data are presented as mean (±standard deviation), unless otherwise indicated. Statistical significance was accepted as $p < 0.05$.

Results

Sleep–wake regularity is independent of sleep duration and physical activity

Table 1 summarizes student demographics (age, sex, and race), baseline vital signs, diary-derived results, and HRV results dichotomized by lower and upper half of mean participant IS_{SW}, which was 71.6 (13.4), and ranged from 61.9 (lower half) to 81.4 (upper half). The students slept 7.7 (0.7) hr per night on average. We did not find any significant relationship between weekly IS_{SW} and sleep duration ($p = 0.59$) or physical activity ($p = 0.23$) in linear mixed model (LMM) analysis (Figure 2, A and B). Moreover, no significant relationship was observed between demographics, baseline HR, RR, BP, BMI, or number of nights completed and IS_{SW} (all $p > 0.05$).

Sleep–wake regularity is an independent predictor of mood and change in mood

The mean participant Beck Depression Inventory-II score (BDI) and change in BDI (ΔBDI) were 4.2 (4.5) and 0.6 (2.3), respectively (Table 1). Mean ΔBDI of all participants was not significantly different from zero ($p = 0.09$). LMM analysis showed that weekly IS_{SW} was negatively associated with end-of-week BDI ($b = -0.16$, 95% CI: −0.24 to −0.08, $p = 0.0003$; Figure 2E and Table 2, Model 1a) and its change from baseline value (ΔBDI: $b = -0.11$, 95% CI: −0.17 to −0.05, $p = 0.0009$; Figure 2F and Table 2, Model 1a) independently of demographics, sleep duration, and physical activity. We then matched BDI scores with the subsequent week's IS_{SW}, but surprisingly found no significant relationship ($p > 0.05$). There were no significant differences in demographics, IS_{SW} or BDI (all $p > 0.05$) between the first 27 subjects and the final 15 subjects (same protocol, additional home BDI assessment on weeks 1 and 2).

Nocturnal heart rate variability moderates the association between sleep–wake regularity and mood

Mean weekly heart rate (HR) was significantly lower ($p < 0.0001$; Supplementary Figure S2A) and HF power of HRV was significantly higher during sleep compared with wake ($p < 0.001$; Supplementary Figure S2B) regardless of whether in the first two quartiles (Irregular Weeks) or the upper two quartiles (Regular Weeks). We did not find a significant relationship between IS_{SW} and HF during sleep or wake. However, there was a significant interaction effect for IS_{SW} and HF during sleep (IS_{SW}*HF sleep;

Table 1. Study participant characteristics by sleep–wake regularity (IS_{sw})

	Lower half of IS_{sw}	Upper half of IS_{sw}	All
Subjects	21	21	42
IS_{sw}	61.9 (12.1)	81.4 (4.8)	71.6 (13.4)
Demographics			
Age (years)	22.2 (2.8)	23.2 (2.6)	22.7 (2.7)
Female	52.4% (11)	52.4% (11)	52.4% (22)
Caucasian	47.6% (10)	38.1% (8)	42.9% (18)
Asian	38.1% (8)	52.4% (11)	45.2% (19)
Black/African American	14.3% (3)	9.5% (2)	11.9% (5)
Baseline vital signs			
HR (bpm)	69.5 (11.6)	70.2 (10.6)	69.8 (11.0)
BP (mmHg)	117/68 (13/10)	116/67 (10/8)	116/68 (12/9)
BMI (kg/m^2)	24.1 (3.1)	23.3 (3.9)	23.7 (3.5)
RR (breaths/min)	17.3 (3.1)	17.8 (2.4)	17.6 (2.8)
Sleep and activity measures			
Chronotype (MEQ score)—baseline	45.8 (7.0)	52.0 (7.3)	48.9 (7.7)
Sleep duration (hr/night)	7.8 (0.7)	7.6 (0.8)	7.7 (0.7)
Delayed bedtime (hr/week)	10.7 (8.5)	5.0 (6.4)	7.8 (8.0)
Social jetlag (min)	53.0 (99.4)	26.9 (51.3)	40.6 (80.4)
Physical activity (MET-min/week)†	8.0 (0.6)	7.8 (0.9)	7.9 (0.7)
Mood			
BDI—during study	6.2 (5.1)	2.1 (2.6)	4.2 (4.5)
ΔBDI—weekly change	1.4 (2.7)	−0.2 (1.6)	0.6 (2.3)
Hear rate variability (during study)			
Heart rate (sleep)	65.3 (10.1)	62.6 (6.6)	63.9 (8.5)
Heart rate (wake)	79.5 (7.7)	83.1 (7.9)	81.3 (7.9)
High-frequency power (sleep)†	2.91 (0.42)	3.12 (0.25)	3.02 (0.36)
High-frequency power (wake)†	2.63 (0.34)	2.60 (0.25)	2.62 (0.29)

Data for 42 participants. Mean (standard deviation) and percentages (number of subjects). Lower half (1st and 2nd quartiles), upper half (3rd and 4th quartiles) for mean participant IS_{sw} across the study.

IS_{sw} = interdaily stability of sleep–wake times; HR = heart rate; bpm = beats per minute; BP = blood pressure; mmHg = millimeters mercury; BMI = body mass index; RR = respiratory rate; MEQ = morning–eveningness questionnaire; min = minutes; MET-min = Metabolic Equivalent of Task x minutes product; BDI = Beck Depression Inventory; ΔBDI = change in BDI.

†log-transformed.

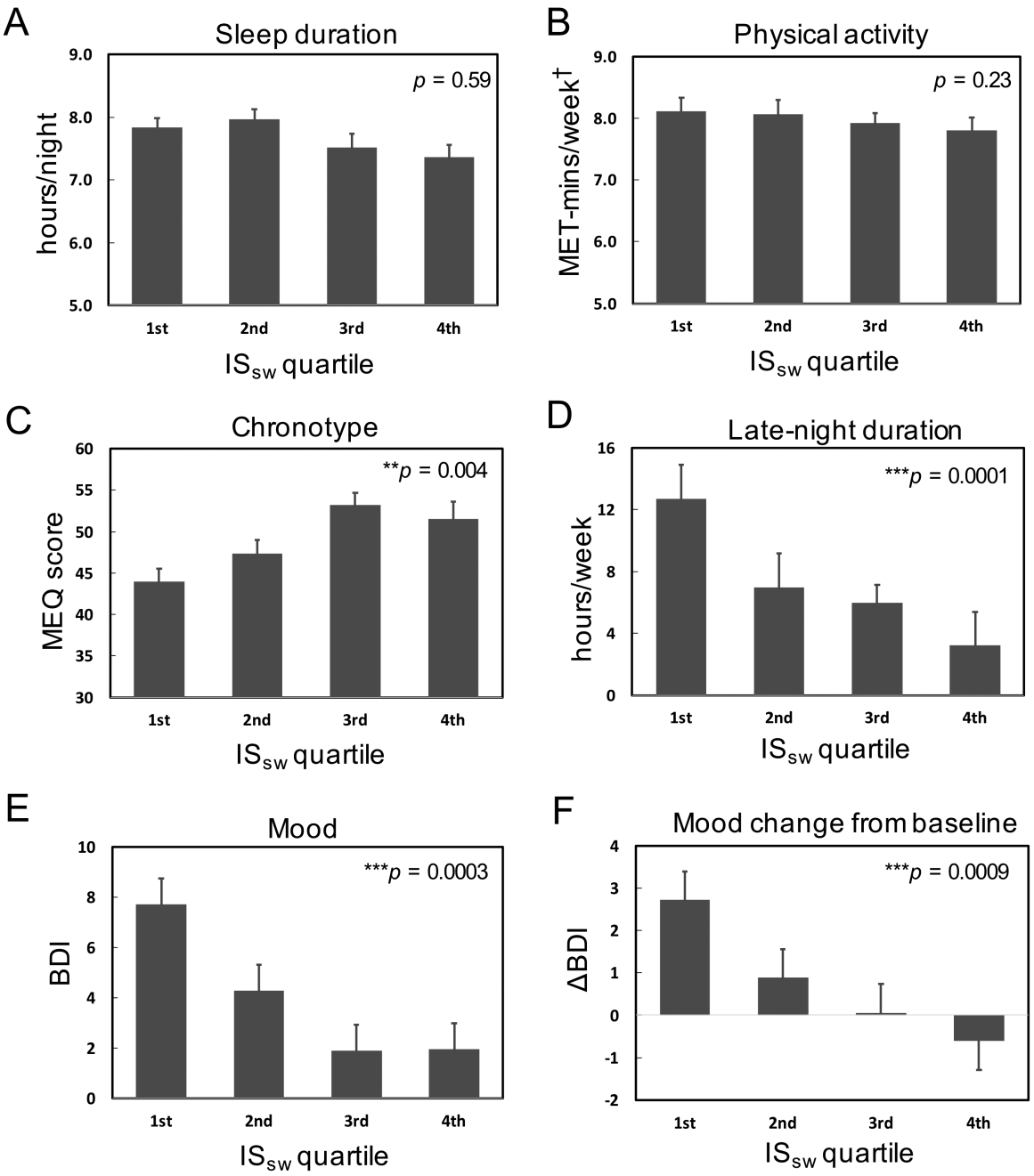

Figure 2. Effects of sleep–wake regularity on sleep, activity, and mood measures. Data (mean plus standard error bars) binned by weekly IS_{sw} quartiles for illustration purposes; *p* values represent results from mixed effects models, in which IS_{sw} was included as a continuous variable. (A) Weekly sleep duration. (B) Weekly physical activity assessed by Metabolic Equivalent of Task x minutes product (*MET-mins*). (C) Chronotype determined by morning–eveningness questionnaire (*MEQ*). (D) Weekly late-night duration. (E) Weekly mood assessed by Beck Depression Inventory (BDI). (F) Weekly BDI change from baseline (*ΔBDI*). Weekly sleep–wake regularity was estimated by interdaily stability of sleep–wake times (IS_{sw}). †log transformed. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Error bars indicate standard errors.

Table 2, Model 1c) on both BDI (*b* = −0.27, 95% CI: −0.45 to −0.09, *p* = 0.005) and ΔBDI (*b* = −0.21, 95% CI: −0.34 to −0.08, *p* = 0.003).

To further explore the interaction effect, we examined the differences in BDI/ΔBDI between "Irregular Weeks" (bottom half of IS_{SW} values) and "Regular Weeks" (top half of IS_{SW} values) by separating the cases with "HF-high" (top half of HF values) and the cases with "HF-low" (bottom half of HF values) during sleep. We found that after Irregular Weeks with HF-high during sleep, students reported significantly higher BDI (Figure 3A) and ΔBDI (Figure 3B) than Regular Weeks. Groups were equally sized and compared using post hoc Student's *t*-test for the IS_{SW}*HF sleep interaction term, adjusted for demographics, physical activity, mean sleep duration, and heart rate during sleep. Consistently, similar interaction effects on BDI and ΔBDI were observed for other HRV variables associated with vagal modulation (e.g. SDNN, RMSSD, and pNN50). The interaction effect on BDI (but not on ΔBDI) was borderline significant for LF (*p* = 0.046 for BDI and *p* = 0.24) but not for LF/HF (see Supplementary Table S1).

Table 2. Linear mixed effects models for associations between sleep–wake regularity (IS_{sw}), heart rate variability (HF), and mood (BDI)

		BDI			ΔBDI		
Model		*b*	95% CI	*P*	*b*	95% CI	*P*
1a	IS_{sw}	−0.16	(−0.24 to −0.08)	**0.0003*****	−0.11	(−0.17 to −0.05)	**0.0009*****
1b	IS_{sw}	−0.19	(−0.27 to −0.11)	**0.00002*****	−0.13	(−0.19 to −0.06)	**0.0002*****
	HF sleep	5.87	(1.77 to 9.98)	**0.006****	3.03	(0.16 to 5.91)	**0.039***
1c	IS_{sw}	−0.21	(−0.29 to −0.13)	**0.000002*****	−0.14	(−0.20 to −0.08)	**0.00001*****
	HF sleep†	6.46	(2.68 to 10.2)	**0.002****	3.08	(0.44 to 5.72)	**0.023***
	IS_{sw} * HF-sleep†	−0.27	(−0.45 to −0.09)	**0.005****	−0.21	(−0.34 to −0.08)	**0.003****

Linear mixed effects models showing the effects of weekly IS_{sw} (Model 1a), with HF sleep (Model 1b), and also with IS_{sw} *HF sleep (Model 1c) on weekly mood (BDI) and change in mood (ΔBDI). Adjusted for demographics, physical activity, mean sleep duration, and heart rate during sleep.
BDI = Beck Depression Inventory; ΔBDI = change in BDI; IS_{sw} = interdaily stability of sleep–wake times; HF sleep = high-frequency power during sleep.
Bold values indicate statistically significant ($p < 0.05$).
†log-transformed. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

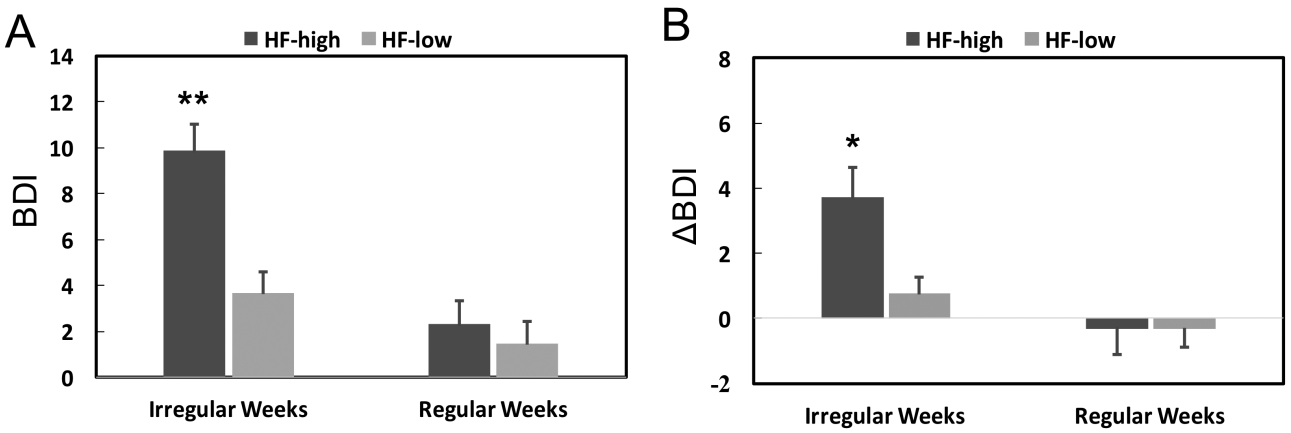

Figure 3. Interaction effect of sleep–wake regularity and high-frequency power component of heart rate variability during sleep on mood. (A) Weekly mood assessed by Beck Depression Inventory (BDI). (B) Weekly BDI change from baseline (*ΔBDI*). For illustration, data were divided into four categories base on HF (HF-high: 1st and 2nd quartiles; HF-low: 3rd and 4th quartiles) and weekly IS_{sw} (Irregular weeks: 1st and 2nd quartiles; regular weeks: 3rd and 4th quartiles). Comparison between groups using mixed effects models to account for repeated measures across weeks, and post hoc Student's *t*-test for the IS_{sw}*HF sleep interaction term, adjusted for demographics, physical activity, mean sleep duration, and heart rate during sleep. HF was log transformed. $^{*}p < 0.05$; $^{**}p < 0.01$ and represents difference from irregular weeks and HF high group. Error bars indicate standard errors.

Sleep–wake regularity and mood are associated with chronotype, delayed bedtime, but not social jetlag

We further explored whether participants with preference towards evening chronotype were more likely to suffer from lifestyle constraints leading to greater delayed bedtime, social jetlag, or lower IS_{sw}, and potentially, poorer mood. Mean MEQ score was 48.9 (7.7) suggestive of "intermediate types" on average. Lower IS_{sw} was associated with preference towards evening (lower MEQ; Figure 2C) and greater delayed bedtime (Figure 2D). Lower MEQ ($b = -0.23$, CI: −0.41 to −0.05, $p = 0.012$; Table 3, Model 2a) and delayed bedtime ($b = 0.16$, CI: 0.03 to 0.28, $p = 0.014$; Table 3, Model 2b) predicted higher BDI scores, but was not related to ΔBDI. However, neither remained significant once IS_{sw} was introduced into the models (Table 3, Model 2c). Mean social jetlag was 41 ± 52 (*SD*) min and surprisingly not associated with IS_{sw} ($r^2 = 0.01$, $p = 0.16$), MEQ ($r^2 = 0.01$, $p = 0.57$), or mood ($r^2 = 0.01$, $p = 0.96$). We did not find any differences in HF during sleep or wake related to chronotype, delayed bedtime, or social jetlag.

Discussion

To the best of our knowledge, this is the first study to examine the relationship between sleep–wake regularity (SWR), mood, and HRV. We measured the HF power component, a marker of vagal modulation, in healthy young college students free from sleep and mood disorders, and medications, over an extended period of time. We identified a strong temporal association for low weekly IS_{sw} preceding poor mood, and worsening mood, independent of age, sex, physical activity, and sleep duration in this cohort. There was a significant interaction effect between IS_{sw} and nocturnal HF on mood such that significantly worse mood and worsening mood was reported by students after irregular weeks with high HF during sleep. Irregular sleep–wake cycles were more likely in individuals of evening types and were associated with greater delayed bedtime, whereas social jetlag in these students was unrelated.

Directional effect of sleep–wake regularity on mood

The observed effect of sleep–wake irregularity (IS_{sw}) on mood is in keeping with recent studies linking regularity to happiness [38], wellbeing [11, 37], and even academic performance [2, 39]. A recent study also showed that irregular sleepers aged 50 and over-reported higher rates of depression symptoms [3]. Although studies have shown short-sleep duration to be associated with poor health outcomes [40] including depression [41], there was no relationship here between weekly IS_{sw} and mean sleep

Table 3. Linear mixed effects models for associations between sleep–wake regularity (IS_{sw}), chronotype (MEQ), delayed bedtime, and mood (BDI)

		BDI			ΔBDI		
Model		*b*	95% CI	*P*	*b*	95% CI	*P*
2a	Chronotype (MEQ)	−0.23	(−0.41 to −0.05)	**0.012***	−0.03	(−0.16 to 0.10)	0.62
2b	Delayed-bedtime	0.16	(0.03 to 0.28)	**0.014***	0.02	(−0.07 to 0.11)	0.68
2c	IS_{SW}	−0.10	(−0.18 to −0.01)	**0.022***	−0.08	(−0.18 to −0.01)	**0.018***
	Chronotype (MEQ)	−0.12	(−0.31 to 0.06)	0.18	0.02	(−0.12 to 0.16)	0.77
	Delayed bedtime	0.05	(−0.08 to 0.25)	0.43	0.05	(−0.08 to −0.19)	0.43

Linear mixed effects models showing the effects of chronotype (Model 2a), late-night duration (Model 2b) and both with IS_{sw} included (Model 2d), on mood (BDI), and change in mood (ΔBDI). Adjusted for demographics, physical activity, and mean sleep duration.
IS_{sw} = interdaily stability of sleep–wake times; HF sleep = high-frequency power during sleep; BDI = Beck Depression Inventory; ΔBDI = change in BDI.
*$p < 0.05$.

duration. Interestingly, we found that prior BDI did not predict the next week's IS_{SW}. Change in BDI ranged from −4 to 9 but was not significantly different from zero, implying stable group mood with some degree of intraparticipant variability during the 3 week study period, which is typical for a healthy cohort [42]. That IS_{SW} was able to predict intraparticipant changes in mood in our LMMs, however, lends further evidence that IS_{SW} may be tightly coupled to weekly individual changes in mood.

To put some context on these findings, moving a student from the lower quartile (mean IS_{SW} = 52) to the upper quartile (mean IS_{SW} = 88) for a given week would translate into a decrease of 7.5 points (95% CI: 4.7–10.4; using our fully adjusted model in Table 2, Model 2b) for BDI. Given that moving from "mild mood disturbance" (BDI 11–16) to "moderate depression" (BDI 21–30) may require as little as a 5-point change, this is potentially a clinically significant effect size. Moreover, although the BDI-II is a robust assessment of mood with a retest interval mean of 2 weeks (ranging from 1 week to 6 months in 82% of studies) [43], there is evidence that testing at 1 week intervals (as we did in this study) leads to an underestimation of true depression symptoms [42]. Thus, the measured BDI scores in our study are unlikely to be overestimations of mood severity.

Despite the strong association and effect size, we remain cautious regarding interpretation of these findings. It is likely that many confounders exist, including timing of meals, personal or academic stressors, and type of physical activity. In addition, some have argued that mood may cyclically worsen during the luteal phase of the menstrual cycle [44, 45]. Although this study was not set up to address this, it is reassuring that no sex differences were found, and the study duration (21 days) encompassed three-quarters of a typical 28 day cycle. Finally, the study was designed to incorporate a full week for SWR/mood assessment; it could well be that the association between irregularity and poor mood is stronger for a different time lag (e.g. less than 7 days). Future studies are warranted to clarify the temporal relationship between SWR and mood, before causality or potential interventions can be considered.

Heart rate variability during irregular weeks and low mood

Preserved HRV, a marker of ANS control, has been thought to reflect physiological wellbeing, a healthy degree of social engagement and flexible adjustment to environmental demands [46]. Surprisingly, we found that participants who experienced weeks with irregular sleep–wake cycles (low IS_{SW}) and nocturnally high vagal tone (indicated by HF) reported the highest BDI and increases in BDI. The interaction was independent of sleep duration and persisted after inclusion of chronotype and delayed bedtime. In addition, we replicated the same finding using other HRV markers of vagal modulation (e.g. SDNN, RMSSD, and pNN50; Supplementary Table S1).

Many possible mechanisms may underlie the effect of higher HF during sleep on the SWR-mood association. (1) One possibility is that those with low SWR and poor mood exhibited greater sleepiness or sleep propensity; this has been shown to increase cardiac vagal modulation in the first cycle of nonrapid eye movement (NREM) sleep [47]. A prospective study of 13 medical interns also showed increased HF during their first sleep episode after 33.5 hr shifts that corresponded to sleepiness scores [48]. (2) Activities close to sleep could have led to HRV changes during sleep. For example, moderate, habitual drinking of alcohol was suggestive of higher HF in healthy young adults of similar age (mean 20.5) to our participants [49], and higher SDNN in women with cardiovascular disease [50]. (3) The circadian rhythm of HRV and its relation to sleep stages has been noted by others [19, 20, 51, 52] such that the relative time spent in different sleep stages or shifted circadian phases may partially explain our nocturnal HF findings. It is possible that the higher nocturnal HF may be a reflection of stage-specific alterations in the intensity of cardiac vagal modulation secondary to irregular sleep–wake cycles and poor mood. The potential ramifications are unclear, but markers of vagal modulation such as HF during sleep have already been shown to play a role in human memory consolidation [53] and warrant follow-up studies using polysomnography.

We were able to demonstrate the well-documented increase in vagal modulation (lower HR and higher HF) during sleep versus wake, in keeping with prior studies [32, 51, 54]. However, it was surprising that only HRV during sleep (not during wakefulness) was a significant moderator and certainly needs replicating in future studies. Reassuringly, there was an appropriate representation of sleep (one third of the ECG data) compared with wake episodes, which was equally represented across weeks. We speculate that the observed HRV during sleep episodes was least likely to be affected by the unmeasured confounders mentioned above, and thus, closest to in-lab conditions.

Finally, it is worth noting that persistently high HRV may not always be protective. For instance, increased HRV during sleep was associated with cardiovascular events in diabetics [55] and predicted mortality in the elderly [56]. Persistent vagal rebound after repetitive psychological stressors is an adaptation noted in rodents [57] and humans [58] and has been linked to

abnormal mood repair [59], recurrent depression [60], and even mood-disordered suicidal ideation [61]. High-nocturnal vagal modulation during young adulthood, together with poor mood and irregular sleep–wake cycles, has unknown impacts on long-term cardiovascular health. There could feasibly be burnout of a protective vagal system, where it is unable to keep check on sympathetic surges associated with cardiac events in later adult life [57, 58, 62], a potential mechanism for the coexistence of mood disorders and cardiovascular diseases [63, 64].

The role of chronotype, delayed bedtime, and social jetlag

SWR and mood are likely affected by a mixture of inherent and external lifestyle-related factors. Prior studies have linked evening chronotype to depression [65–67]. However, the underlying mechanism is not clear. In this study, we confirmed that students with preference towards evening chronotype exhibited greater delayed bedtime and irregular sleep–wake times (lower IS_{sw}). Although chronotype and delayed bedtime were associated with poor mood, they were not related to change in mood. In addition, their associations with mood disappeared when adjusting for the effect of IS_{sw}. These results suggest that SWR likely explains a significant amount of the effects of chronotype and delayed bedtime on mood, but also has independent predictive value for change in mood.

Despite recent studies linking social jetlag and health outcomes [12], we did not find any links for social jetlag with mood or other sleep variables in this cohort. This is somewhat surprising given that IS_{sw}, to some extent, is supposed to incorporate social jetlag. One explanation is the small sample size and large variability, i.e. irregular students did report on average double the weekday–weekend shift, but also double the standard deviation (53 [99.4] vs 26.9 [51.3] min, Table 1). This is perhaps not surprising given that students are not yet constrained to a typical work week schedule and is in keeping with prior findings [68]. IS_{sw} likely better represents daily fluctuation in college students' lifestyle than social jetlag, since the weekday–weekend shift may not be pronounced in these individuals; thus, controlling for the weekday–weekend shift had no effect on our final model.

Overall, our findings point to a strong link between SWR and mood, which is significantly moderated by nocturnal HRV, as measured by HF. Irregular sleep–wake cycles has already been shown to disrupt circadian timing [2]; together with these findings, we postulate that SWR represents a common endpoint between evening chronotype and delayed bedtime, acting as a marker of habitual disruption to the circadian system.

Strengths, limitations, and future directions

Strengths of this study include good non-Caucasian representation, control for physical activity levels, unique extent of ECG recordings, and weekly study design for temporal assessment of regularity, mood, and autonomic signals. However, these findings should be interpreted with caution. This is a healthy cohort with subclinical BDI scores that limit generalizability. Mechanisms linking SWR and depressive disorder itself may differ and need to be further clarified. Although the study aimed to recruit healthy participants with normal BMI and vital signs, we were unable to definitively exclude disorders that are undiagnosed, nondisclosed, or typically not self-reported (e.g. sleep apnea or periodic limb movement disorder).

One potential difficulty in both the interpretation of the nocturnal HRV results and in SWR assessment lies in the fact that sleep–wake classification itself is known to be challenging regardless of methodology [69, 70]. Unfortunately, polysomnography is not feasible in field studies of this type and duration. Although we did review diaries with participants, alongside heart rate tracings, where available, it is conceivable that those who slept at irregular times (low SWR) and reported poor mood, either overestimated their sleep duration, or had unreported awakenings/activity at night, leading to an overestimation of nocturnal HRV, particularly since vagal modulation predominates before each transition from wake to sleep [71]. However, when we excluded 30 min either side of reported sleep and wake times, our results remained the same. Others have begun implementing nonlinear ECG and/or respiratory signals to detect sleep–wake states; this may best serve as an aid to sleep diaries since they remain inaccurate 1 in 5 times [72–74]. However, given our findings, this is certainly a direction for future analysis with ECG data, where available, to supplement sleep diaries, not only for state classification, but also for potential markers of sleep quality.

Taken together, further studies may be able to combine continuous cardiac and sleep monitoring with accurate recording of timing of activities, food, light exposure, and daily life stressors to delineate potential stage-specific vagal modulation differences in irregular sleepers with poor mood; this may represent an opportunity for identifying important factors affecting mood, performance, and lifelong productivity at an early stage in adulthood.

Supplementary Material

Supplementary material is available at *SLEEP* online.

Funding

This work was supported by National Institutes of Health (NIH) grants T32GM007592 (to L.G.), R01AG048108, and RF1AG059867 (to K.H.). P.L. was supported by the International Postdoctoral Exchange Fellowship (20150042) from the China Postdoctoral Council. F.A.J.L.S. was supported in part by NIH grants R01HL118601, R01DK099512, R01DK102696, R01DK105072, and R01HL140574.

Conflict of interest statement. F.A.J.L.S. has received lecture fees from Bayer HealthCare, Sentara HealthCare, Philips, Vanda Pharmaceuticals, and Pfizer Pharmaceuticals.

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