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Baker, Amanda
Tashjian, Sarah
Goldenberg, Diane
et al.

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Original Article

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Sleep variability over a 2-week period is associated with restfulness and intrinsic limbic network connectivity in adolescents

Amanda E. Baker[1], Sarah M. Tashjian[1], Diane Goldenberg[1] and Adriana Galván[1,2,*]

[1]Department of Psychology, University of California, Los Angeles, 502 Portola Plaza, Los Angeles, CA 90095, USA and
[2]Department of Psychiatry and Biobehavioral Sciences, University of California, Los Angeles, 757 Westwood Plaza, Los Angeles, CA 90095, USA

*Corresponding author. Amanda Elina Baker, Department of Psychology, University of California, Los Angeles, 502 Portola Plaza, Los Angeles, CA 90095, USA. Email: amandaelina@ucla.edu.

Abstract

Study Objectives: Sleep duration and intraindividual variability in sleep duration undergo substantial changes in adolescence and impact brain and behavioral functioning. Although experimental work has linked acute sleep deprivation to heightened limbic responding and reduced regulatory control, there is limited understanding of how variability in sleep patterns might interact with sleep duration to influence adolescent functioning. This is important for optimal balancing of length and consistency of sleep. Here, we investigated how objective indices of sleep duration and variability relate to stress, restfulness, and intrinsic limbic network functioning in adolescents.

Methods: A sample of 101 adolescents ages 14–18 reported their stressors, after which they wore wrist actigraph watches to monitor their sleep and rated their restfulness every morning over a 2-week period. They also completed a resting-state fMRI scan.

Results: Adolescents reporting more stress experienced shorter sleep duration and greater sleep variability over the 2-week period. Longer nightly sleep duration was linked to feeling more rested the next morning, but this effect was reduced in adolescents with high cumulative sleep variability. Sleep variability showed both linear and quadratic effects on limbic connectivity: adolescents with high sleep variability exhibited more connectivity within the limbic network and less connectivity between the limbic and frontoparietal networks than their peers, effects which became stronger once variability exceeded an hour.

Conclusions: Results suggest that cumulative sleep variability is related to stress and limbic network connectivity and shows interactive effects with sleep duration, highlighting the importance of balancing length and consistency of sleep for optimal functioning in adolescence.

Key words: adolescence; sleep; fMRI; limbic network; actigraphy

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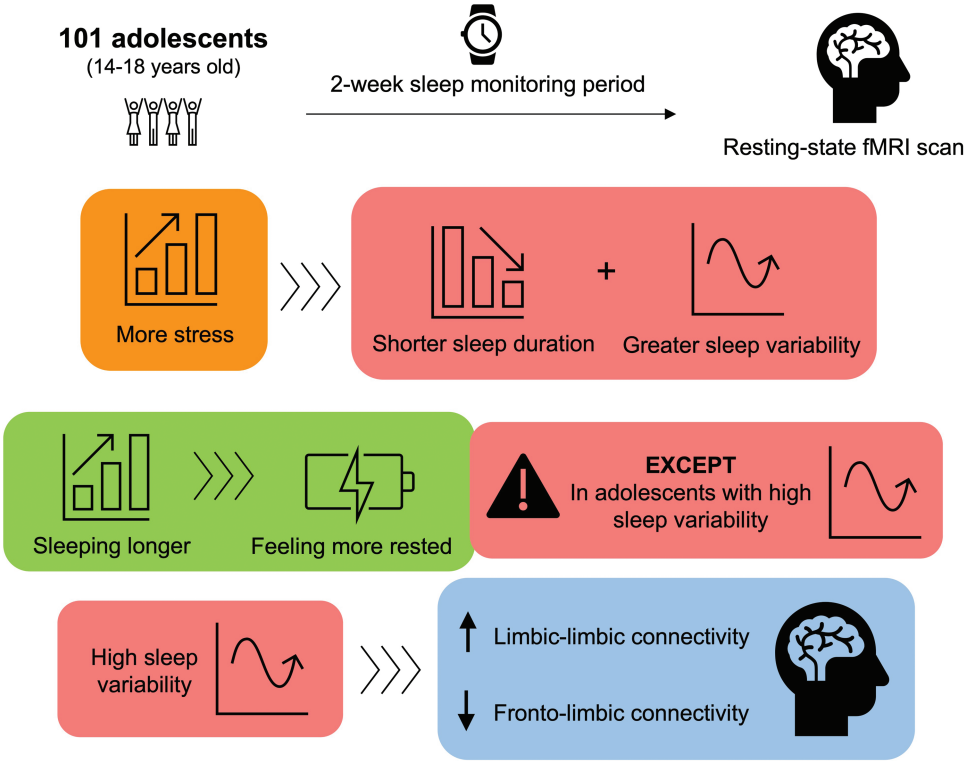
Graphical Abstract



Statement of Significance

Sleep patterns undergo significant changes in adolescence and are important for health and well-being. While experimental studies have linked acute sleep deprivation to functioning of the limbic system, a brain network anchored in the threat-sensitive amygdala that governs emotional responding, less work has examined how cumulative sleep patterns interact in their associations with limbic and socio-emotional functioning. Here, we explore how naturalistic differences in objective indices of sleep duration and variability relate to stress, restfulness, and intrinsic limbic network connectivity in 101 adolescents. This is important for recommending sleep length and consistency for optimal functioning in adolescence. Future work will be critical for understanding how these factors contribute to the development and maintenance of disorders such as anxiety.

Introduction

Sleep is an essential bodily function that is crucial for a healthy adolescent's brain and behavioral development. As with many behaviors, sleep undergoes developmental shifts in adolescence: newfound independence combined with delays in the circadian timing system and changes to the homeostatic sleep regulating system push bedtimes later and later [1]. Conversely, rise times on weekdays are generally determined by the school and therefore remain constant. The combination of these changes and suboptimal environmental influences can lead to declines in sleep duration in high school [2], with two-thirds of students in grades 9–12 reporting less than 7 hr of sleep on school nights [3]. This constitutes a major health problem, as sleep problems in adolescence are linked to negative outcomes such as emotional disturbances, academic failure, health problems, substance abuse, and driving crashes [2, 4–6].

To compensate for lost sleep during the week, adolescents often use "recovery" strategies such as extending sleep on weekends or free days. While additional sleep can be a much-needed relief for the sleep-deprived teen, this can introduce additional variability and once again shift the circadian phase delay [7], making it more difficult to return to regular sleep schedules during the week. Although extra sleep may seem innocuous or even beneficial, sleep variability may be as detrimental to adolescent health outcomes as chronically low sleep duration [8]. For example, variability in sleep duration has been associated with lower levels of subjective well-being [9] and predicts academic achievement and symptoms of anxiety and depression 1 year later, over and above sleep duration [10]. Higher levels of sleep variability predict greater perceived stress in college students [11], while greater sleep variability in adolescence has been associated with higher levels of C-reactive protein (CRP), a biomarker of inflammation that predicts chronic health problems [12]. A study combining daily diaries and diffusion tensor imaging (DTI) found that sleep duration variability, over and above sleep duration, was associated with lower white matter integrity a year later [13]. These findings suggest that sleep variability, over and above sleep duration, may be uniquely associated with adolescent stress and well-being both at physiological and neurobiological levels.

Research suggests that inadequate sleep in adolescence can exacerbate preexisting adolescent brain dynamics by heightening

the sensitivity of the limbic system, a brain network anchored in the amygdala that governs emotional responding, while undermining the ability of regulatory systems in the prefrontal cortex (PFC) to dampen subcortical responding [14]. Fronto-limbic connections undergo extensive development in adolescence and are especially sensitive to stress and sleep problems during this time, contributing to the high prevalence of adolescent-onset mood and anxiety disorders [15, 16]. These affective disorders that emerge in adolescence are often preceded by sleep difficulties and sleep-related emotional disturbances [17], underscoring the complex bidirectional relationships between sleep and adolescent socio-emotional development. Given the important role that limbic network functioning plays in adolescent socio-emotional development and its vulnerability to the effects of insufficient sleep, this brain system is a key target when assessing how sleep patterns correlate with adolescent brain function.

While experimental work has been crucial for highlighting the immediate impacts of insufficient sleep on limbic network function, this method lacks ecological validity and explains little about how naturalistic sleep patterns relate to adolescent brain and behavioral functioning. Some adolescents may pull the occasional "all-nighter," but even more experience the downstream effects of cumulative sleep patterns on brain and behavioral development [13, 14, 18]. Studies using daily diaries to measure sleep, on the other hand, provide important information regarding adolescent perceptions of their sleep patterns, but are susceptible to self-report bias and require memory of what occurred during sleep for later reporting. Therefore, studies that combine objective measures of sleep (e.g. actigraphy) with daily diaries to fill in missing information are promising approaches for accurately assessing cumulative adolescent sleep patterns.

There are numerous metrics of adolescent sleep (e.g. duration, variability, and quality), some of which may be more amenable to intervention than others. Different metrics of sleep may also show interactive effects on adolescent brain and behavioral functioning such that improvements in one area (e.g. achieving more consistent sleep overall) could have the potential to ameliorate some of the negative effects of insufficient sleep, leading to improvements in adolescent health and well-being. Although extensive research has shown that poor sleep is common in adolescents and relates to brain and behavioral functioning [13, 19], less work has explored how variability of sleep may interact with duration and ameliorate or exacerbate the effects of insufficient sleep on adolescent brain and behavioral functioning. This is an important next step for understanding how sleep consistency works alongside sleep duration in its associations with brain and behavioral functioning in adolescents.

In the present study, we combine objective sleep indices measured through actigraphy and subjective daily diary ratings with resting-state fMRI to examine the associations between naturalistic differences in sleep duration and variability over a 2-week period and resting-state limbic network functional connectivity in a group of 101 adolescents (14–18 years old). As stress, sleep problems, and well-being are often tightly linked in adolescence [20–24], participants reported on their perceived stress levels prior to sleep monitoring, as well as rating their restfulness every morning over the 2-week period. As research suggests that there may be an optimal amount of sleep for peak emotional and cognitive functioning [10, 25], we assessed both linear and quadratic effects of sleep duration and variability on brain and behavioral functioning. To understand how sleep duration and variability work together in adolescence, we also tested for interactive effects of these metrics on adolescent functioning.

Given previous work linking stressful demands, shorter sleep duration, and poor emotional well-being in adolescents [26], we predicted that higher levels of stressful demands would be linked to shorter sleep duration and greater sleep variability over the 2-week period which would in turn be associated with reductions in self-reported restfulness. As shorter self-reported sleep duration has been associated with reduced cortico-limbic connectivity [21], we predicted that shorter objectively measured sleep duration over the 2-week period would be associated with reduced connectivity between the limbic network and higher-order cortical areas, highlighting the importance of sleep duration for overall network communication. Given that sleep, variability has been uniquely associated with adolescent stress, well-being, and brain development over and above sleep duration [8, 12, 13], we predicted that sleep variability would be associated with stress and cortico-limbic connectivity over and above sleep duration. Finally, we predicted that sleep variability would show interactive effects with sleep duration on adolescent functioning such that adolescents achieving more consistent sleep over the 2-week period would be partially protected from the negative effects of insufficient sleep on adolescent well-being.

Methods

Participants

A total of 112 typically developing adolescents ages 14–18 years (M_{Age} = 16.51 years; 53F) were recruited to UCLA for this study. Of these, one participant was excluded from the fMRI scan due to a metal implant, one was excluded due to a self-reported attention-deficit hyperactivity disorder (ADHD) diagnosis, and one was excluded for taking psychotropic medications. Data from five participants were unusable due to excessive motion in the scanner (>1 mm mean relative motion and/or >2 mm mean absolute motion). Four participants were excluded due to missing sleep data. Data are presented for 101 participants (M_{Age} = 16.62 years; 52F). Forty-seven percent of our sample identified as Hispanic/Latino, 25% Caucasian, 15.6% African American, 7.3% Mixed Race, 4.2% Asian, and 1% Middle Eastern. All included participants were right-handed, free of metal, and reported no current medical or neurological disorders. Participants completed written consent and assent in accordance with UCLA's Institutional Review Board and were compensated for their participation.

Eligibility criteria

Participants were screened for eligibility based on the following guidelines: (1) ages 14–18 years, (2) right-handed, (3) free of metal or other contraindications to imaging, (4) no medical or psychiatric conditions contraindicating study participation (e.g. suicidality, head trauma, and pregnancy), (5) no current use of psychotropic medication, and (6) no history of claustrophobia.

Self-reported stressors

Prior to the sleep monitoring period, participants reported on recent stressors they had faced to assess between-person differences in stress levels. Specifically, participants answered eight items assessing whether they had felt stressed due to demands (e.g. "A lot of work at home") and five items assessing whether they had felt stressed due to arguments (e.g. "You got into an argument with your friend") over the last 2 weeks. Items from each scale were averaged together to calculate demand and argument stressor scores for each participant, as well as a cumulative stressor score. Participants in this sample reported an average of 1.9 demand-based stressors (SD = 1.11), 1.34 argument-based

stressors (SD = 0.80), and a cumulative stress score of 3.24 (SD = 1.58). Stressors were not significantly associated with age or sex.

Sleep

Objective sleep indices were generated using Micro Motionlogger Sleep Watch actigraphs by Ambulatory Monitoring, Incorporated (AMI). Actigraphy is a well-validated measure for use in adolescents [27–29] and a valid measure in field and workplace studies when compared to polysomnography [30]. Participants were instructed to wear the actigraph on their non-dominant wrist at night for 14 days. Body movement during nighttime sleep was monitored in 1-min epochs using zero crossing mode (ZCM). In ZCM, frequency of movement is measured by the number of times per epoch the signal voltage crosses zero. Participants were asked to push the event marker button when they turned off the lights to go to sleep and when they got out of bed in the morning. Participants were reminded to wear their watch via daily text messages, and reports of sleep and wake times were also collected via daily text messages to verify the accuracy of the actigraphy data. The in-bed period began at the time of the first event marker indicating when participants turned off the lights to go to sleep and ended at the time when the participant woke up in the morning. If event markers were not available for a particular night, adolescent-reported sleep and wake times were used in place. Significant discrepancies between adolescent-reported sleep and wake times and the actigraph record were reconciled by discussion among two trained coders using additional indices of sleep onset and offset (e.g. light monitoring and time stamps). Sleep onset time was recorded once there were at least three consecutive minutes of sleep, while sleep offset time was recorded as the last five or more consecutive minutes of sleep. Actigraph data were scored using the Sadeh algorithm [28] in the Action4 software package for the portion indicated as nighttime sleep (sleep onset to sleep offset).

The average number of days of actigraphy data for each participant was 13.25 with a range of 8–21 days. Participants with fewer than 8 days of actigraphy data were excluded ($n = 4$). Additionally, temperature and light data were missing for one participant, daily diary data were missing for three participants, and event markers were missing for one night for three participants. The amount of actigraphy data for each participant was not significantly associated with any brain or behavioral metrics of interest in the current sample, although older age was marginally associated with more days of data [$r(101) = 0.19$, $p = .054$] and more days of data was marginally associated with longer average sleep duration [$r(101) = 0.19$, $p = .057$].

Actigraphy data across the 14-day period was used to calculate two sleep indices of interest: sleep duration and sleep duration variability. Sleep duration was calculated by taking the mean of the number of minutes of sleep from the time participants fell asleep to the time they awoke the next morning. Sleep duration variability was calculated by taking the mean of the absolute differences between a participant's mean nightly sleep duration and each individual night's sleep duration over the 2-week period in accordance with previous studies that have examined associations between sleep variability and brain and behavioral functioning in adolescents [12, 18, 26].

Daily diary

Every morning upon waking up over the 2-week period, participants rated their restfulness by answering the question: "How do you feel?" on a scale from 1 to 7, with 1 being worst ("I'm so tired I want to go back to bed") and 7 being best ("I woke up feeling great"). Over the 2-week period, participants in this sample reported an average score of 4.66 (SD = 0.96). Average restfulness ratings were not significantly associated with age or sex. As three participants were missing daily diary data, the analyses involving restfulness ratings were conducted with the remaining 98 participants. Average restfulness ratings over the 2-week period were significantly associated with self-reported stressors [$r(98) = –0.30$, $p = .002$], driven primarily by the arguments subscale of stress [$r(98) = –0.43$, $p < .001$].

Stress and sleep

Associations between self-reported stressors and cumulative sleep indices over the 2-week period were assessed using a path model in MPlus version 8.2 [31]. Cumulative sleep duration and sleep variability metrices were allowed to covary and regressed on age, sex, weekend, demands, and arguments.

Multilevel model

Within-person associations between sleep and restfulness ratings over the 2-week period were assessed using a multilevel linear model in R with sleep metrics (duration, variability) and restfulness ratings nested within participants. A binary weekend variable (1 = weekend, 0 = weekday) was also included in the model to control for weekend effects on restfulness ratings. Age and sex were included in the model as between-person variables. Within-person duration and variability variables were created by centering nightly duration and variability around each participant's mean over the 2-week period. Between-person duration and variability variables were created by centering each participant's average duration and variability scores around the group mean. This resulted in a within-person measure indexing how sleep fluctuated around each participant's average as well as a between-person measure indicating how each participant's average sleep duration and variability compared to the rest of the group.

Resting-state fMRI paradigm

After the 2-week actigraphy monitoring period, participants completed a resting-state fMRI scan (duration: 5 min and 6 s) during which they were instructed to lie still and relax in the scanner with their eyes open and focused on a white fixation cross centered on a black screen. Due to scheduling considerations, only 46 of the 101 participants were able to complete the scan immediately following the sleep monitoring period, while the rest of the group returned for their scan 1–35 days later ($M = 5.91$ days, SD = 8.57 days). All neuroimaging analyses were therefore repeated using only the subset of participants who returned immediately after the monitoring period to assess whether results were primarily driven by cumulative sleep patterns or previous night's sleep. Participants were scanned at different times of day depending on participant availability; weekend scans typically occurred earlier in the day, while weekday scans occurred in the afternoon and evening [average scan time on weekday = 4:40 pm, average scan time on weekend = 12:00 pm, $t(99) = 8.79$, $p < .001$]. Time-of-scan effects were considered in analyses, but no significant associations were found.

fMRI data acquisition

Scanning was performed using a 32-channel head coil on a 3-Tesla Siemens Trio MRI machine at the UCLA Center for Cognitive Neuroscience. Prior to scanning, participants completed a mock scanner session to ensure they were prepared, were not claustrophobic, and could easily remain still in the machine. All

participants were screened for metal with a metal detector prior to entering the scanning suite. Image acquisition parameters were voxel size = 3.8 × 3.8 × 3.8 mm, slices = 33, slice thickness = 3.8 mm, repetition time = 2000 ms, echo time = 30 ms, flip angle = 90°C, interleaved slice geometry, field of view (FOV) = 240 mm, 150 vol. Preprocessing was conducted using FEAT (FMRI Expert Analysis Tool) version 6.00 within FSL (FMRIB Software Library; https://fsl.fmrib.ox.ac.uk/fsl/).

fMRI preprocessing

Preprocessing steps included discarding the first four volumes to ensure magnet stabilization, non-brain removal using FSL BET, spatial smoothing using a Gaussian kernel of FWHM 6 mm, intensity normalization, and rigid body motion correction using MCFLIRT. A T2*weighted, matched bandwidth (MBW), high-resolution, anatomical scan, and magnetization-prepared rapid-acquisition gradient echo (MPRAGE) scan were acquired for registration purposes (TR: 1900 ms; TE 2.26 ms; FOV: 250 mm; slice thickness: 1 mm; 176 slices). Each participant's functional data were registered to their MBW, then to the MPRAGE, and finally to Montreal Neurological Institute (MNI) stereotaxic space with 12°C of freedom using FSL's registration method via FLIRT. Participants did not exceed 1 mm mean relative motion or 2 mm mean absolute motion as determined using motion parameters generated by FSL. To remove potential confounds resulting from head motion, data were next denoised using Independent Component Analysis (ICA)-based Automatic Removal of Motion Artifacts (ICA-AROMA [32]). ICA-AROMA is a highly effective method for addressing head motion when compared to 18 other commonly employed denoising pipelines [33]. Data were then high-pass filtered (100-s cutoff), and white matter and cerebrospinal (CSF) masks for each participant were created using FSL's Automatic Segmentation Tool (FAST).

Group ICA

Group Independent Component Analysis (ICA) is a popular data-driven method for identifying common brain networks among different people. Unlike a typical seed-based analysis, ICA is model-free and multivariate, which switches the focus from evaluating the functional connectivity of single brain regions identified a priori (e.g. the amygdala) to evaluating brain connectivity of resting-state networks that simultaneously engage in oscillatory activity [34]. ICA also retains magnitude information, so is sensitive to changes in the spatial distribution of the network as well as the amplitude of the network activity and preserves individual differences in spatial patterns [35]. Resting-state networks identified using group ICA have been shown to exhibit high spatial consistency across subjects and closely resemble discrete functional networks in the brain [36]. Therefore, ICA is a promising method for (1) identifying a common "limbic network" across a group of participants and (2) assessing how naturalistic differences in sleep patterns relate to functioning of this network in adolescents.

To identify the set of resting-state networks common across subjects, FSL's MELODIC (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/MELODIC) was used to temporally concatenate the functional data from the 101 participants and conduct a multivariate group probabilistic ICA [36]. This method is useful in cases where one is looking for common spatial patterns without assuming that the temporal response is consistent across subjects, which is the case with data acquired without stimulation such as resting-state fMRI. The dimensionality (number of components) was left unspecified to allow the program to identify the networks without top-down modulation. MELODIC identified 22 spatial components explaining the variance in the group's resting-state data. The resulting 22 independent component maps were visually inspected to identify the subcortical limbic network based on previously reported studies [37, 38].

To investigate how naturalistic sleep patterns relate to functioning of the limbic network, subject-specific time courses and spatial maps corresponding to the group limbic network component were estimated using dual regression [34, 39]. For each subject, the group-average spatial map of the limbic network was regressed into their 4D space-time dataset, resulting in a subject-specific limbic network time series. This time series was then regressed into the same 4D dataset, resulting in a subject-specific spatial map of the limbic network for each subject.

Group-level analysis was performed using FSL's randomize (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise/UserGuide) with Threshold-Free Cluster Enhancement and 5000 permutations. Demeaned scores for sleep duration and sleep duration variability were included as covariates of interest. As previous work has found sex- and age-related differences in limbic resting-state connectivity [40], sex and age were also included in the model as covariates of no interest. Fifty-five out of 101 participants were scanned on a weekend (vs. a weekday); to minimize potential confounding day-of-the-week effects, a binary weekday/weekend variable was also included as a covariate of no interest. All sleep analyses were repeated using sleep metrics from weekdays only (Sunday–Thursday nights) to assess whether results were driven primarily by weekday or weekend sleep patterns. Thirty-three participants were scanned over winter or summer break; therefore, analyses were repeated controlling for whether the scan occurred over a free period, and all results remained consistent. Thresholded Z-statistic images were generated to visualize clusters ($Z > 3.1$, $p < .05$). Statistical maps were projected onto a standard MNI brain and visualized using MRIcron software, and brain regions were labeled using the Harvard-Oxford Subcortical and Cortical Structural Atlases (≥25% probability) in FSLView (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FslView).

Results

Sleep findings

Over the 2-week monitoring period, participants slept an average of 7.1 hr a night (425 min; *SD* = 50.59 min) and varied in nightly sleep duration by an average of 58.96 min (*SD* = 22.82 min; Supplementary Figure S1). When considering weeknights only, participants slept an average of 6.95 hr on weeknights (417 min; *SD* = 41.21 min) and varied in weeknight sleep duration by an average of 55 min (*SD* = 14.63 min; Supplementary Figure S2). The monitoring period included an average of 3.7 weekend nights for each participant. Participants slept approximately 33.6 min longer on weekends than on weekdays ($p < .001$), while variability increased by a factor of 14.63 min ($p < .001$). Average sleep duration and sleep variability across the 2-week period were not correlated [$r(101) = -0.17$, $p = .09$], although weekday duration was negatively correlated with weekday variability [$r(101) = -0.23$, $p = .019$].

Sleep and stress

Path model results demonstrated that more self-reported stressful demands prior to the sleep monitoring period were associated with shorter sleep duration and greater sleep duration variability over the 2-week period such that one additional demand was associated with a 10-min reduction to sleep duration and a 5-min

increase in sleep variability (Table 1, Figure 1). Results stayed consistent when testing a model with weekday sleep duration and variability only (Supplementary Table S1). Self-reported stressful arguments were not significantly associated with either sleep duration or variability. Age and sex were also associated with sleep duration such that older participants and participants who were male experienced significantly shorter sleep durations than younger and female participants.

Sleep and daily restfulness ratings

Multilevel model results revealed significant effects of nightly sleep duration on restfulness ratings such that achieving longer nightly sleep duration was associated with feeling more rested the next morning (b = 0.32, SE = 0.05, p < .001). Nightly differences in sleep variability did not show direct effects on restfulness ratings the next morning (b = −0.02, SE = 0.04, p = .69), although participants who experienced relatively high variability in sleep over the 2-week period rated their restfulness marginally lower overall (b = −0.17, SE = 0.10, p = .08). There was also an interaction between previous night's sleep duration and cumulative sleep variability on restfulness ratings such that participants who experienced relatively high cumulative sleep variability over the period did not show the positive effects of long sleep duration on restfulness ratings (b = −0.07, SE = 0.04, p = .03; Figure 2). These results suggest that additional sleep may be most beneficial for adolescent restfulness when sleep patterns are relatively consistent overall.

Table 1. Path model results demonstrate that self-reported stressful demands are associated with lower sleep duration and greater sleep duration variability when considering both weekday and weekend sleep

	Standardized Estimate (SE)	Unstandardized Estimate (SE)	*P*-value
Duration WITH Variability	−0.16 (0.10)	−160.20 (101.35)	.101
Duration ON			
Age	−0.24 (0.09)	−10.43 (4.22)	.011
Sex (1 = male)	−0.22 (0.09)	−21.68 (9.51)	.020
Weekend	0.12 (0.10)	11.94 (9.80)	.220
Demands	**−0.21 (0.10)**	**−9.69 (4.56)**	**.031**
Arguments	0.09 (0.10)	5.68 (6.19)	.358
Variability ON			
Age	−0.02 (0.10)	−0.36 (1.96)	.854
Sex (1 = male)	−0.15 (0.10)	−6.70 (4.40)	.124
Weekend	−0.17 (0.10)	−7.84 (4.53)	.080
Demands	**0.24 (0.10)**	**4.86 (2.11)**	**.019**
Arguments	0.05 (0.10)	1.39 (2.87)	.627

SE = standard error.

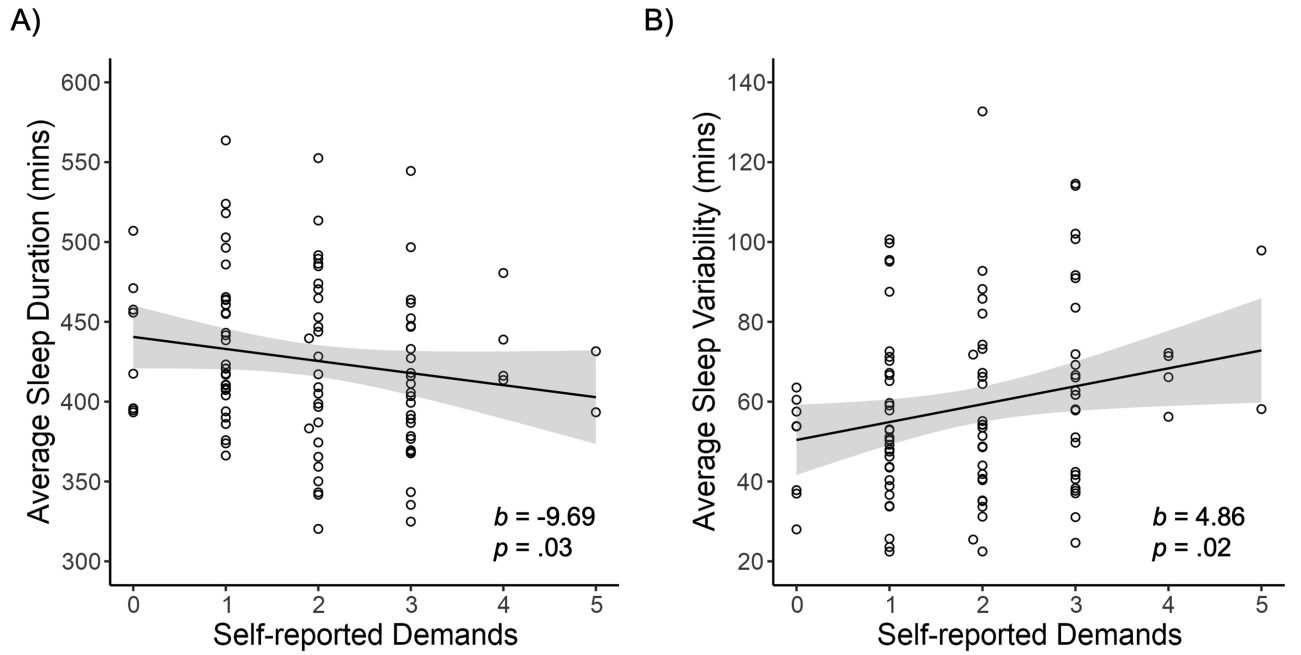


Figure 1. Self-reported stressful demands are associated with sleep duration and variability. (**A**) As the number of self-reported stressful demands increased by one demand, average sleep duration over the 2-week period decreased by 9.69 min. (**B**) As the number of self-reported stressful demands increased by one demand, average sleep variability increased by 4.86 minutes.

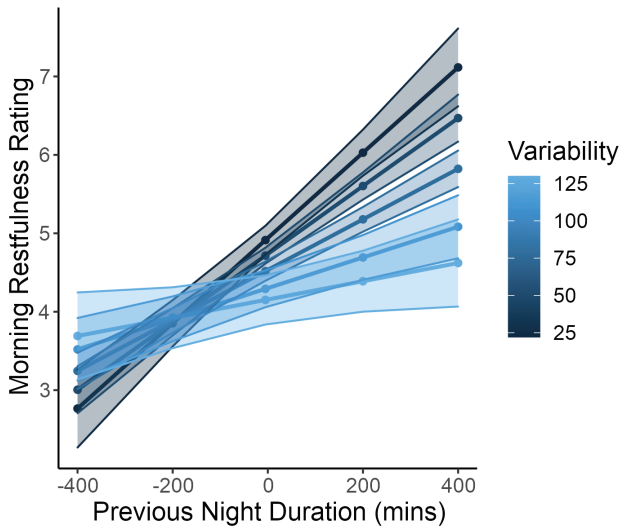


Figure 2. Within-person increases in nightly sleep duration are associated with feeling more rested the next morning except in adolescents with relatively high cumulative sleep variability. Within-person increases in minutes of nightly sleep duration were associated with higher restfulness ratings the next morning (b = 0.16, $p < .001$); however, between-person differences in cumulative sleep variability interacted with nightly sleep duration such that youth who experienced high cumulative sleep variability over the 2-week period showed reduced duration-related benefits to restfulness the next morning ($b = -0.07$, $p = .03$). *Note:* Previous night duration is mean-centered within participants and therefore indicates whether the participant experienced relatively more minutes or fewer minutes compared to their mean over the 2-week period.

Participants also rated their restfulness significantly higher on weekends than on weekdays ($b = 0.20$, $SE = 0.09$, $p = .03$).

Limbic network functioning

Main effects

Dual regression revealed a common intrinsic limbic network across the 101 participants that was anchored in the bilateral amygdala and extended into the hippocampus, subcallosal cortex, temporal pole, and ventromedial prefrontal cortex (vmPFC; Figure 3A). Activation of the limbic network during the resting state was associated with deactivation of frontoparietal network regions such as the dorsal prefrontal cortex, posterior parietal cortex, and dorsomedial thalamus (Figure 3B).

Linear effects of sleep variability.

Participants who experienced greater variability in sleep over the 2-week period showed heightened communication within the limbic network, especially in the hippocampus and subcallosal cortex (Figures 4A and B), while they showed more negative coupling between the limbic network and frontoparietal regions such as the middle frontal gyrus (MFG), frontal pole, and posterior parietal cortex (Figures 4C and D). There were no significant linear effects of sleep duration on limbic network functioning in the current sample of participants.

Quadratic effects of sleep variability.

Upon adding quadratic sleep metrics to the model, the whole-brain linear effects of sleep variability on limbic network functioning were no longer significant. However, sleep variability showed significant quadratic effects on limbic network

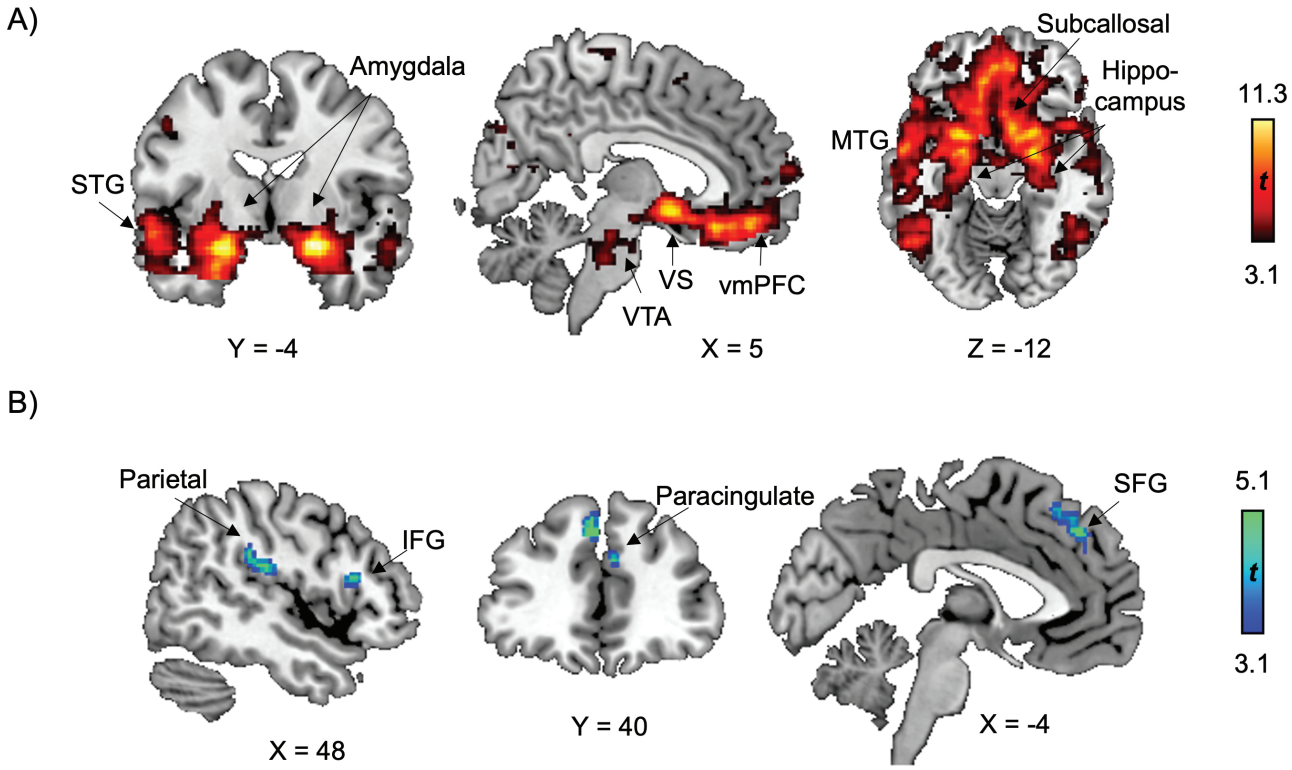


Figure 3. Group-level whole-brain limbic network connectivity at rest. (**A**) Dual regression revealed a common intrinsic limbic network across participants encompassing the bilateral amygdala, hippocampus, ventral tegmental area (VTA), ventral striatum (VS), ventromedial prefrontal cortex (vmPFC), middle temporal gyrus (MTG), and subcallosal cortex. (**B**) Activation of the limbic network at rest was associated with deactivation in regions of the frontoparietal network including the parietal operculum cortex, inferior frontal gyrus (IFG), paracingulate cortex, and superior frontal gyrus (SFG). $Z > 3.1$, $p < .05$.

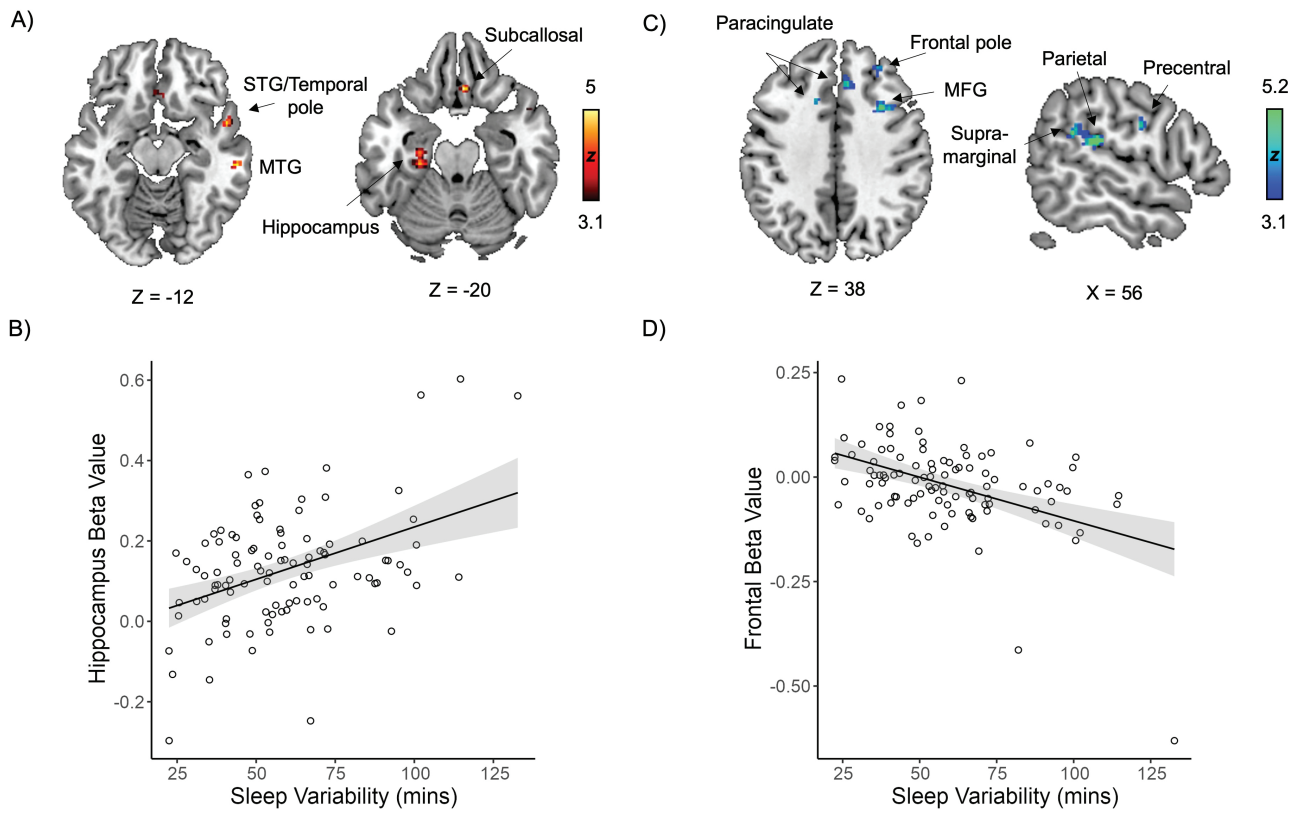


Figure 4. Linear effects of sleep variability on intrinsic limbic network connectivity. (**A**) Greater variability in sleep over the 2-week period was associated with heightened communication within the limbic network, especially in regions such as the superior temporal gyrus (STG), temporal pole, middle temporal gyrus (MTG), hippocampus, and subcallosal cortex. (**B**) Visual depiction of the association between sleep variability and limbic network connectivity with the hippocampus. As sleep variability increased, connectivity with the hippocampus increased. (**C**) Greater variability in sleep over the 2-week period was associated with reduced communication between the limbic network and regions of the frontoparietal network such as the paracingulate cortex, frontal pole, middle frontal gyrus (MFG), supramarginal gyrus, parietal cortex, and precentral gyrus. (**D**) Visual depiction of the association between sleep variability and limbic network connectivity with the frontal pole. As variability increased, fronto-limbic connectivity decreased. $Z > 3.1$, $p < .05$.

functioning—participants who varied in nightly sleep duration by over an hour showed the strongest associations with limbic network connectivity, while participants who experienced less than an hour of variability did show associations with limbic network connectivity (Figure 5). The quadratic effects were most robust in participants who experienced relatively low average sleep duration over the 2-week period, suggesting that while a small bit of variability may be worth the extra sleep time for sleep-deprived adolescents, it may begin to show associations with limbic network functioning once the average cumulative variability exceeds an hour. Sleep duration did not show significant quadratic effects on limbic network functioning in this sample.

Weekday sleep and limbic network functioning.

All analyses were repeated using sleep metrics from weekdays only (Sunday–Thursday nights). When considering only weekday sleep, sleep variability showed significant linear and quadratic negative associations with limbic network connectivity with the frontoparietal network (Supplementary Figure S3). Weekday sleep duration did not show significant associations with limbic network connectivity.

Previous night and day-of-scan effects.

Due to scheduling considerations, not all participants were able to complete their scan immediately upon finishing the 2-week monitoring period. However, 46 out of the 101 participants wore actigraphs the night before and rated their restfulness the morning of the scan. Therefore, in these 46 participants, we examined how previous night sleep metrics, cumulative sleep metrics over the 2-week period, and day-of-scan morning restfulness ratings related to limbic network functioning by including all variables in the same model along with age, sex, and weekend (1 = scanned on a weekend) as covariates of no interest. Previous night sleep metrics did not show significant associations with limbic network connectivity; however, cumulative sleep variability was again positively associated with within-limbic network communication (Supplementary Figure S4A–B), suggesting that the associations between sleep variability and limbic network functioning we found here were driven by cumulative sleep patterns rather than one night of rest. Furthermore, cumulative sleep duration was positively associated with limbic network connectivity with the left inferior frontal gyrus (IFG) pars opercularis (Supplementary Figure S4C–D), suggesting that longer average sleep duration over the 2-week period was linked to heightened limbic-cortical connectivity in this subset of participants. Interestingly, day-of-scan restfulness ratings were negatively associated with within-limbic network communication such that participants who felt more rested that morning evinced lower limbic network connectivity with the thalamus and hippocampus as compared to their peers who felt less rested that morning (Supplementary Figure S5).

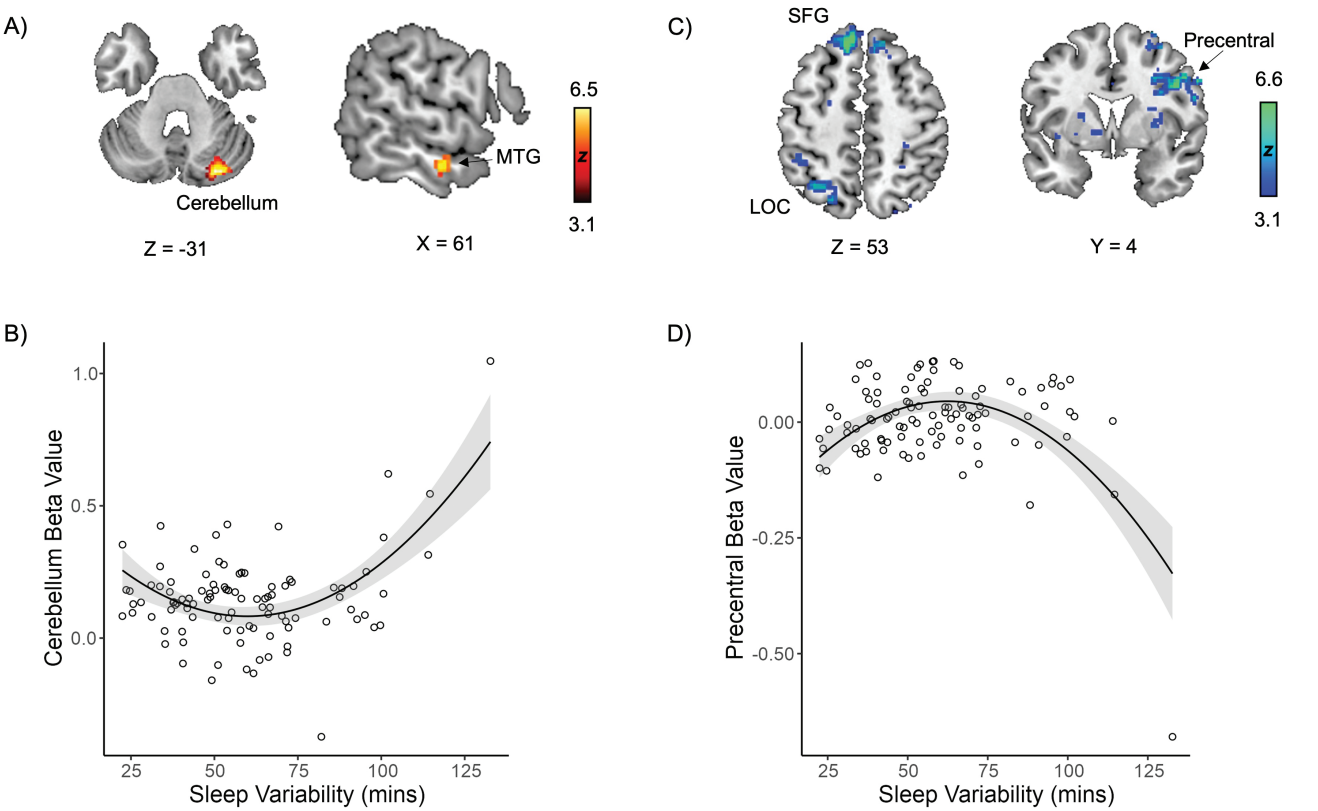


Figure 5. Quadratic effects of sleep variability on intrinsic limbic network connectivity. (**A**) Sleep variability showed a positive quadratic association with within-limbic network communication with the cerebellum and middle temporal gyrus (MTG). (**B**) Visual depiction of the association between sleep variability and limbic network connectivity with the cerebellum. Sleep variability showed the strongest positive associations with cerebellum connectivity once it exceeded about one hour. (**C**) Sleep variability showed a negative quadratic association with communication between the limbic network and the lateral occipital cortex (LOC), superior frontal gyrus (SFG), and precentral gyrus. (**D**) Visual depiction of the association between sleep variability and precentral connectivity. Sleep variability showed the strongest negative associations with precentral connectivity once it exceeded about 1 hr. $Z > 3.1$, $p < .05$.

Discussion

In this study, we investigated how objective measures of sleep duration and variability relate to stress, restfulness, and intrinsic limbic network functional connectivity in a sample of 101 adolescents. We found that adolescents who reported a high level of stressful demands (e.g. schoolwork or responsibilities at home) experienced shorter sleep duration and greater sleep variability over the 2-week period compared to their peers. Within-person fluctuations in nightly sleep duration were linked to how participants felt the next morning—when adolescents slept longer, they felt more rested the next day, although this effect was not as robust in adolescents who experienced high cumulative sleep variability over the 2-week period. Sleep variability, but not duration, showed linear, and quadratic associations with intrinsic limbic network connectivity such that greater variability was associated with heightened within-network limbic connectivity and reduced limbic connectivity with the frontoparietal network, and these associations were most pronounced once sleep variability exceeded an hour. Together, findings from this study suggest that although sleeping longer is beneficial for many adolescents, these benefits may be reduced if cumulative sleep patterns become too variable. Furthermore, as cumulative sleep variability, but not duration, showed tight associations with limbic network functioning, sleep consistency may be an especially important metric of focus when considering adolescent health and well-being.

Findings from experimental studies suggest that adolescents need about 9–9.25 hr of sleep a night for cognitive function and attention [41] and emotion regulation [25]. However, adolescents in this sample experienced less than 7 hr of sleep on weeknights and varied by an hour in nightly sleep duration. This juxtaposition of ideal and observed sleep patterns aligns with the patterns found in a recent meta-analysis of youth ages 12–18 years [42] and speaks to the conflict between bioregulatory mechanisms and psychosocial factors that contributes to widespread sleep deprivation in adolescence. When probing the factors contributing to these sleep patterns, we found that self-reported stressful demands at school and at home were related to both objective metrics of sleep in this sample of participants: more self-reported stressful demands at the start of the sleep monitoring period were associated with shorter average sleep duration and greater average sleep variability over the 2-week period. These findings align with previous research using daily diaries to measure sleep patterns that found stressful demands during the day were associated with shorter sleep duration that night [26], while extending this research by highlighting an equally strong association between stressful demands and sleep variability.

In addition to reporting on their stressors, adolescents in this study also rated how rested they felt every morning upon waking over the 2-week period. Using multilevel modeling of actigraphy-measured sleep indices and self-reported restfulness ratings, we found that longer nightly sleep duration was associated with a boost in restfulness ratings the next morning, highlighting the

importance of a good night's sleep for adolescents to feel properly rested. Nightly variability in sleep duration was not associated with restfulness ratings the next morning; however, having higher cumulative variability over the 2-week period was associated with lower restfulness ratings overall, suggesting that while one night of sleep may impact adolescent restfulness immediately, cumulative patterns of sleep variability (rather than acute nightly variability) may be important for downstream effects on adolescent restfulness. Interestingly, cumulative sleep variability patterns over the 2-week period interacted with nightly sleep duration in its associations with restfulness such that adolescents who experienced relatively high variability in sleep did not show the same benefits of longer nightly sleep duration on morning restfulness ratings. While previous research has highlighted the importance of both sleep duration and variability for optimal functioning in adolescents, this study extends these results by suggesting that once sleep becomes too variable, the benefits of extra hours of sleep are reduced. Therefore, instead of focusing on sleep duration or variability in isolation, it may be more fruitful to consider both metrics as important factors that require balancing to achieve the most restful and restorative sleep. Although this study only looked at variability in sleep duration, it is possible that consistency in other metrics (e.g. sleep and wake times) would also help adolescents reap the benefits of extra hours of sleep. Sleep variability may be an untapped resource—if small changes to schedules could improve consistency of sleep, and thereby improve how rested adolescents feel, perhaps this would be a fruitful avenue for sleep intervention, even among youth who cannot get the recommended dose of sleep.

In addition to probing how sleep duration and variability relate to how rested adolescents feel, we also investigated how cumulative indices of sleep duration and variability relate to the intrinsic functioning of the limbic network, a large-scale brain network important for emotional processing and responding. Sleep variability, but not duration, showed linear, and quadratic effects on limbic network functioning: adolescents who experienced greater variability in sleep over the 2-week period evinced more within-limbic network connectivity than their peers, especially in regions such as the hippocampus and subcallosal cortex. Limbic network connectivity with the cerebellum and middle temporal gyrus (MTG) showed quadratic associations with sleep variability such that the positive effects of variability on connectivity became strongest after variability exceeded one hour on average. Sleep variability also showed negative associations with connectivity between the limbic network and nodes of the frontoparietal network such as the middle frontal gyrus (MFG) and parietal cortex, and these effects again became strongest once cumulative variability exceeded an hour.

These results suggest that cumulative sleep variability may play an important role in the neural functioning of bottom-up emotion processing systems, as well as their communication with the rest of the brain. Interestingly, a study investigating adolescent narcolepsy, or chronic daytime sleepiness, found that adolescents with narcolepsy demonstrate decreased communication between the limbic system and regions of the superior frontal gyrus (SFG), putamen, and parietal cortex [43] compared to healthy controls. Here, we found similar associations between naturalistic differences in sleep variability and limbic network connectivity, suggesting that cumulative patterns of sleep variability in healthy adolescents might have similar effects on neural functioning as chronic sleepiness. Although nightly sleep duration showed significant associations with restfulness ratings the next morning, cumulative sleep duration patterns did not show significant associations with limbic network functioning. Once again, these results suggest that while sleeping enough hours is crucial for daily functioning, the cumulative consistency of sleep may be important for reaping the benefits of extra sleep, which is important to keep in mind while balancing extra sleep with alterations to regular sleep schedules.

Finally, almost half (46/101) of the participants in this study wore their wrist actigraph watches the night before the scan, as well as rating their restfulness the morning of the scan. Therefore, in this subset of adolescents, we were able to probe the competing influences of sleep duration and variability from the previous night, cumulative sleep duration and variability over the 2-week period, and day-of-scan morning restfulness ratings on limbic network connectivity. There were no significant associations between sleep metrics from the previous night and limbic network connectivity, but cumulative sleep variability once again showed significant positive associations with within-limbic network connectivity, suggesting that the findings presented here were driven by cumulative variability in sleep patterns rather than simply one night of rest. In this subset of participants, achieving higher cumulative duration over the 2-week period was associated with heightened limbic network connectivity with the left inferior frontal gyrus (IFG) opercularis, which aligns with previous work reporting low white matter integrity between the left IFG and the thalamus in patients with insomnia disorder [44]. Interestingly, day-of-scan morning restfulness ratings also showed significant associations with within-limbic network connectivity such that feeling more rested was associated with lower limbic network connectivity with the hippocampus and thalamus. Taken together, these findings suggest that adolescents experiencing more consistent sleep and adolescents who felt more rested on the day of the scan evinced lower within-network limbic connectivity than their peers, while adolescents who slept longer on average over the 2-week period evinced greater limbic-IFG connectivity than their peers.

Although a previous study combining actigraphy, daily diaries, and resting-state fMRI revealed associations between quality of sleep and intrinsic functioning of the default mode network (DMN) [19], this study did not consider variability alongside duration and quality and therefore did not speak to the role that cumulative sleep variability may play over time or its potential interactive effects with sleep duration. Although we utilized a portion of the same sample (n = 101 in this study; n = 45 in the Tashjian study), the current study uniquely examines the role of sleep variability in limbic network functioning whereas the Tashjian study focused on duration, number of and duration of nighttime awakenings, and sleep efficiency. The current study also employs a data-driven, model-free, and multivariate Independent Components Analysis (ICA) approach to identify a common limbic network across all participants. Networks identified with this approach have been shown to exhibit high spatial consistency across subjects and closely resemble discrete functional networks in the brain [36], adding to the implications of the current work.

This study should be interpreted in the context of several limitations. First, while this study uncovered important associations between sleep variability and limbic network functioning, we cannot make causal claims regarding the effects of sleep on functional connectivity, as sleep metrics were observed rather than manipulated directly or examined longitudinally. Although this type of naturalistic sleep study has the benefit of ecological validity, experimental sleep research is important for determining the causal nature of events. The resting-state fMRI sequence

used in this study was also relatively short (just over 5 min) and therefore may not have captured the full range of networks in the adolescent brain. However, as the limbic network was determined using a data-driven group ICA approach in this study, it suggests that the scan was at least long enough to capture limbic network functioning in most of the participants. Although resting-state fMRI can provide valuable insight into intrinsic functional brain network dynamics, it cannot be mapped directly to behavior and therefore runs the risk of reverse inference. As this study did not collect data regarding participants' psychological functioning, we cannot speak to how sleep patterns and neural functioning relate to adolescent mental health. Future research utilizing experimental and longitudinal designs, multimodal imaging, and thorough clinical measures will be needed to fully map the relation between sleep patterns and adolescent brain and behavioral functioning. This study also did not consider daytime napping, which could play an important role in the tradeoff between hours and consistency of sleep. Future work will be needed to determine whether consistent sleep paired with daytime naps would show similar associations with emotional and neural functioning in adolescents.

Despite these limitations, this study makes an important contribution to the literature regarding sleep in adolescence by demonstrating associations between objectively measured naturalistic sleep patterns, self-reported stressors and restfulness, and intrinsic limbic network functioning in a large sample of adolescents. Notably, this study highlights the interacting roles of sleep duration and variability on adolescent self-reported restfulness and suggests that extra sleep should be balanced with consistency of sleep for optimal functioning in adolescence.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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Conflict of interest statement

None declared.

Data availability statement

Data from this study are available upon request.

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