Received: 18 May 2024 | Revised: 17 June 2024 | Accepted: 15 July 2024

DOI: 10.1111/jsr.14298

RESEARCH ARTICLE

Habitual rapid eye movement sleep predicts changes in test-anxiety levels weeks in advance

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Funding information
National Science Foundation, Grant/Award Number: 2237419; Southwest Center for Occupational and Environmental Health, Grant/Award Number: 5T42OH008421

Summary

Previous research has linked rapid eye movement sleep to emotional processing, particularly stress. Lab studies indicate that rapid eye movement sleep deprivation and fragmentation heighten emotional reactivity and stress response. This relationship extends to natural settings, where poor-quality sleep among college students correlates with increased academic stress and lower academic performance. However, there is a lack of research into how specific sleep stages, like rapid eye movement, affect real-life stress development. This study investigated whether habitual rapid eye movement sleep in college students can predict the future development of real-life stress symptoms associated with final exams. Fifty-two participants (mean age = 19 years, 62% females) monitored their sleep for a week during the academic semester using a mobile electroencephalogram device, and then completed self-evaluations measuring test anxiety and other relevant factors. They completed the same evaluations again just prior to final exams. We found that rapid eye movement sleep was the most dominant factor predicting changes in participants' test anxiety. However, contrasting with our predictions, habitual rapid eye movement sleep was associated with an increase rather than decrease in anxiety. We discuss these results in terms of the rapid eye movement recalibration hypothesis, which suggests rapid eye movement sleep modulates activity in stress-encoding areas in the brain, leading to both decreased sensitivity and increased selectivity of stress responses.

KEYWORDS
emotional processing, rapid eye movement sleep, stress development, test anxiety

1 | INTRODUCTION

Decades of research highlight the importance of sleep in emotional processing. Sleep deprivation has been linked to irritability, heightened emotional reactivity to negative stimuli and decreased emotional regulation (Fairholme & Manber, 2015; Menz et al., 2013). Electroencephalographic (EEG) studies suggest one particular sleep stage, rapid eye movement (REM) sleep, may play a pivotal role; REM sleep suppression was shown to enhance emotional reactivity, whereas longer REM sleep was linked to weaker emotional reactivity and enhanced emotional stability (Groch et al., 2013; Lara-Carrasco et al., 2009; Rosales-Lagarde et al., 2012).

The effects of sleep, particularly REM sleep, have also been widely observed in patients with mental conditions such as post-traumatic stress disorder (PTSD). Patients with PTSD exhibit abnormal sleep architecture and disrupted REM sleep, which has been associated with sustained nightmares and increased symptom severity (Habukawa et al., 2007; Kobayashi et al., 2007). Recent research revealed a bidirectional relationship between sleep and stress, where pre-deployment sleep deficiencies correlate with increased risk of PTSD development in military soldiers (Gehrman et al., 2013; van Liempt et al., 2013). Conversely, REM sleep was shown to mitigate future stress responses and associated brain activation in PTSD models in both humans and animals (Feng et al., 2018; Lerner et al., 2017; Polta et al., 2013).

A far less studied aspect of REM sleep is its association with future stress responses occurring in everyday life scenarios, such as college settings. College is a transition period that involves new educational and social environments that can lead to increased stress from various sources, including academic stress (Beiter et al., 2015; Lee & Oh, 2017). Increased academic stress, especially during final examinations, has been associated with reduced immunity and decreased academic performance (Segerstrom & Miller, 2004). Furthermore, academic stress has been associated with poor sleep, where about 27% of college students are at risk of developing a sleep disorder (Lund et al., 2010). A potential result of academic stress is an increase in test anxiety, characterized by arousal, worry and negative thoughts related to exams (Khan et al., 2019). Unlike recent research on sleep and its effect on fearful memory encoding, most studies that assessed the effects of sleep on stress in everyday academic life have used self-rating measures of sleep or, at best, wrist actigraphy that can only differentiate reliably between sleep and wake (Schlarb et al., 2017). Therefore, these studies cannot determine if any particular sleep stage, such as REM, is involved in the effects.

Here, we aimed to understand if habitual sleeping patterns, particularly REM sleep, could predispose students to heightened test anxiety. Thereby, we collected objective sleep data from college students during either the fall or spring semester to determine if these could predict increased test-related stress during the final exams. To focus on the predictive nature of habitual sleep, we monitored sleep many weeks prior to exams. Based on previous studies on fear learning, we predicted that higher amounts of and less fragmented REM sleep will be associated with reduced test-related stress weeks to months later (Davidson & Pace-Schott, 2020; Feng et al., 2018; Lerner et al., 2017; Polta et al., 2013).

2 | MATERIALS AND METHODS

2.1 | Participants

Fifty-two students from the undergraduate program in Psychology at the University of Texas at San Antonio completed the study for academic credit. The number of participants was chosen based on power calculations using Gpower 3.1.9.2 to detect R^2 increase in linear multiple regression with one tested predictor and 30 control predictors, assuming a medium–large effect size of $f^2 = 0.2$ (based on a prior related study; Lerner et al., 2016), $\alpha = 0.05$ and power $(1 - \beta) = 0.8$, yielding a minimum sample size of 42. Potential participants completed an electronic questionnaire to assess eligibility based on the inclusion and exclusion criteria. Inclusion criteria were: (a) commitment to participate during the entire period of the study (one full semester); (b) reporting having regular sleep of at least 6 hr per night; (c) willingness to try keeping a regular, consistent sleep schedule during the period of baseline sleep monitoring; (d) keeping a typical alcohol and caffeine consumption during the period of baseline sleep monitoring. Exclusion criteria included: (a) pre-existing neurological or medical conditions that may affect sleeping patterns, or a history of mental disorders, drug abuse or trauma; (b) regular consumption of medications that influence sleep; (c) a diagnosis of any sleep disorder; (d) failure to complete good recordings of at least three good nights of sleep. Of the 52 participants, 47 had complete data while five others had partial behavioural data, having not completed the behavioural testing in either of the two timepoints (Table 1). Two additional participants who completed the protocol were dropped from the study, one for showing indications of potentially experiencing a trauma between the two timepoints of data collection (e.g. significant increase in suicidal thoughts and indicating severe life events happening during the relevant semester) and one for having an outlier score for the dependent variable (more than 4.5 SDs from the participants' average).

TABLE 1 Main demographic details and objective sleep measures.

Sample characteristics	
Gender	
M/F/NB	34.6%/61.5%/3.8%
Age	19 ± 1.2
Race	
White	44.2%
Black	15.4%
Asian	13.5%
Other	26.9%
Ethnicity	
Hispanic	48.1%
Non-hispanic	51.9%
Income	
< $10,000	30.8%
$10,000–$20,000	9.6%
$20,000–$35,000	7.7%
$35,000–$50,000	17.3%
$50,000–$100,000	11.5%
> $100,000	23.1%
Relationship	
Single	61.5%
In relationship	32.7%
Living together	5.8%
Days from 1st to 2nd assessment	51 ± 14.7
TST (hr)	6.2 ± 1.2
N1/N2 (hr/% of TST)	3.1 ± 0.7/50.2 ± 6.9%
N3 (hr/% of TST)	1.5 ± 0.3/24.4 ± 4.6%
REM (hr/% of TST)	1.6 ± 0.6/25.4 ± 6.3%

Note: $N = 52$. Numbers above represent either percentages of the total sample or mean ± standard deviation.
Abbreviations: F, females; M, males; NB, non-binary; REM, rapid eye movement; TST, total sleep time.

2.2 | Sleep monitoring measures

Multiple-night baseline sleep data were collected using a mobile EEG recorder (the Zmachine® Insight+ Model DT-200; General Sleep Corporation). This device consists of three self-applicable EEG sensors connected to a small pocket-size recorder that is charged daily and can hold over 7 days of data. The sensors are applied over each mastoid process (signal electrodes) and to the back of the neck (ground electrode). The recorder is placed in a pocket or on a nearby nightstand. The Zmachine was designed for easy self-monitoring of sleep at home, and automatically records and detects wake, light sleep (combined sleep stages N1 and N2), N3 and REM sleep in 30-s epochs. It has shown substantial agreement with polysomnography, achieving an overall Cohen's kappa of 0.72, indicating reliable sleep stage detection (Wang et al., 2015; Wood et al., 2023).

2.3 | Behavioural measures

We tested stress and related factors using an assessment battery delivered through an online survey platform, Qualtrics (Qualtrics, 2022). The use of Qualtrics ensured standardized data collection procedures and facilitated the electronic submission of responses. The main dependent variable was test anxiety, measured using the Test Anxiety Inventory (TAI; Spielberger, 1980).

The TAI is a well-validated tool that assesses test anxiety across 20 items on a four-point Likert-type scale. Beyond the total score across all items, specific items can be classified into dimensions: worry and emotionality, where worry refers to concerns about achievement and consequences of failure and emotionality refers to autonomic nervous system self-perceived arousal (Sud & Sharma, 1990). The TAI has an overall Cronbach's alpha of 0.893, while worry and emotionality dimensions have 0.743 and 0.899, respectively.

A variety of additional questionnaires quantifying factors that affect real-life stressors were also collected and served as control variables. These factors included measures of depression (Patient Health Questionnaire [PHQ-9]; Kroenke et al., 2001), suicidality (Depressive Symptom Inventory – Suicidality Subscale [DSI-SS]; Joiner Jr et al., 2002), resilience (Dispositional Resilience and Disability Scale [DRDI]; Moore et al., 2022), experience of stressful events (Life Events Checklist for DSM-5 [LEC-5]; Weathers et al., 2013), emotion regulation (Emotion Regulation Questionnaire [ERQ]; John & Gross, 2004), and subjective sleep quality: Epworth Sleepiness Scale (ESS; Johns, 1991), Insomnia Severity Index (ISI; Bastien et al., 2001) and Pittsburgh Sleep Quality Index (PSQI; Buysse et al., 1989). Table 2 presents the mean values for all participants' subjective measures. In addition, basic demographics (age, gender, race, ethnicity, education, income, relationship status and overall perceived sleeping difficulties) were also collected. Another included factor was the time, in days, from completion of the sleep measurement until the beginning of final exams. See additional descriptions of each questionnaire in the Supplementary Materials S1.

2.4 | Procedure

During the first half of the fall or spring semester at the university, participants were brought into the lab and were properly trained on

TABLE 2 Subjective assessment across time 1 and time 2.

	Time 1		Time 2		
Measure	Mean	SD	Mean	SD	*t*
Depression (PHQ-9)	6.48	5.81	5.90	5.04	1.247
Suicidality (DSI-SS)	0.18	0.75	0.18	0.66	0.000
Resilience (DRDI)	5.44	0.85	5.40	0.88	0.485
Cognitive reappraisal (ERQ-CR)	28.76	8.29	30.04	7.89	−0.949
Expressive suppression (ERQ-ES)	16.66	5.73	16.24	5.96	0.780
Subjective sleep quality (PSQI)	6.02	2.52	6.12	3.30	−0.620
Daytime sleepiness (ESS)	5.84	4.28	7.88	4.93	−2.792**
Insomnia symptoms (ISI)	6.42	4.69	6.76	5.41	−0.032
Stress exposure (LEC-5)	2.70	2.49	2.86	2.72	0.00
Test anxiety (TAI Total)	42.90	14.78	40.90	14.85	2.22*
Worry dimension (TAIw)	16.52	6.38	15.67	6.22	1.92
Emotionality dimension (TAIe)	17.08	6.09	16.69	6.38	0.97

Note: Means and SDs were calculated across subjects that had valid data for that timepoint. Pairwise *t*-tests were calculated for the 47 participants with data for both timepoints.
Abbreviations: DRDI, Dispositional Resilience and Disability Scale; DSI-SS, Depressive Symptom Inventory – Suicidality Subscale; ERQ-CR, cognitive reappraisal dimension of Emotion Regulation Questionnaire; ERQ-ES, expressive suppression of Emotion Regulation Questionnaire; ESS, Epworth Sleepiness Scale; ISI, Insomnia Severity Index; LEC-5, Life Events Checklist for DSM-5; PHQ-9, Patient Health Questionnaire; PSQI, Pittsburgh Sleep Quality Index; TAI, Test Anxiety Inventory; TAIe, emotionality dimension of TAI; TAIw, worry dimension of TAI.
*$p < 0.05$. **$p < 0.01$.

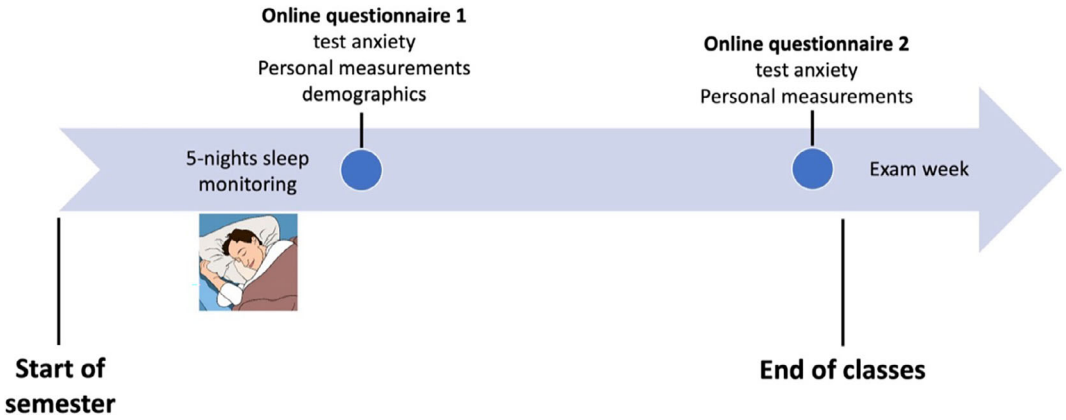

FIGURE 1 Experimental timeline.

operating the mobile-sleep monitoring system, including the preparation and placement of the EEG electrodes and usage of the recorder. Participants then took the system home and recorded their sleep for a minimum of five successive nights at their home environment during a week with no anticipated exceptional stress (e.g. relatively early in the semester after settling in but before any exams). In addition, participants maintained a sleep diary indicating, for each night, the time they went to bed, woke up, how long it took them to fall asleep and whether they were awakened in the middle of the night. Following the monitoring period, participants returned the sleep device and completed the assessment battery online to obtain baseline values of the relevant behavioural variables mentioned above, as well as a basic demographic form. Participants were measured again with the same online battery at the end of the semester just before exam week began, when academic stress was expected to peak (Figure 1).

2.5 | Data analysis

We conducted two main statistical analyses using SPSS Statistics 27.0 (IBM), a stepwise regression and a linear mixed-models analysis. We used both approaches because each method compensates for the other's limitations: while stepwise regression considers all factors, it usually selects only a few of them and does not include missing data; conversely, the mixed-models analysis requires pre-selecting factors based on a-priori assumptions but can handle missing data and ends up addressing all the selected factors.

The stepwise regression model included only the 47 participants with full sleep and behavioural data. The outcome variable was the difference in scores on the TAI between the two time periods, Finals and Baseline (positive values indicating increase in anxiety). The sleep factors evaluated by the model were chosen based on published data showing their potential impact on emotional regulation and stress (Talamini et al., 2013): duration of time spent in each sleep stage (N1/N2 sleep, N3 sleep, REM sleep); REM fragmentation (total number of arousals occurring during REM sleep per night; Vethe et al., 2022); REM latency (amount of time before first entry to REM sleep); and wake after sleep onset (WASO; the amount of time spent in wake after sleep onset). All sleep variables were calculated for each participant and each night based on the sleep staging data extracted from the monitoring devices and averaged over the entire monitoring period. Following our previous study (Lerner et al., 2016), only data for "acceptable" nights were counted for this averaging to prevent inclusion of nights where the mobile sleep monitoring device malfunctioned or was erroneously disconnected by the user. The selection process for "acceptable" nights was conducted by comparing the objective sleep data with participants' subjective sleep diaries to verify their fidelity. Only nights where reported and measured sleep onset and offset were within ~30 min of each other with no prolonged discrepancies in mid-night wake periods were counted. An average of 17.55% of nights across subjects were removed due to inconsistencies with the sleep diary. Nevertheless, all participants included in the study had at least three valid nights with no inconsistencies, adhering to the exclusion criteria stated above ($M = 4.69$, $SD = 1.11$ of valid nights).

In addition to the sleep predictors, to control for a variety of factors that could potentially influence the outcome variable, the model also considered the scores of the additional collected scales: depression, resilience, occurrence of stressful events, emotion regulation, and subjective sleep rating. For each of these factors, both their average over the two testing periods (reflecting the general level typical to the individual) and the difference between the two time periods (reflecting any change occurring between the testing periods, which could potentially be influenced by the increasing proximity to exam week) were included. The difference scores were calculated similarly to the TAI by subtracting scores at time 1 (baseline questionnaire) from those at time 2 (prior to finals). Finally, principle demographics data were also entered as potential predictors. A total of 31 sleep and control factors were included, and were subject to a stepwise selection procedure. Starting with a default intercept-only model, at each step, variables were selected based on p-values of the F-statistics, with $p < 0.05$ being the criterion to add a term to the model and $p > 0.10$ being the criterion to remove an included term from the model.

To examine potential associations between the predictors and a general tendency for test anxiety rather than an increase in test anxiety, a similar stepwise regression procedure was repeated with the average TAI scores across the two time periods serving as the outcome variable instead of their difference. When appropriate, several follow-up analyses were run based on the results of the two stepwise models, as detailed in the Results.

To supplement the stepwise regression, our second statistical procedure was a linear mixed-models analysis, using all 52 participants (with missing data for those who did not complete the full assessment battery at both time periods). Time was entered to the model as a within-subjects factor with two levels (baseline, exam week), and the TAI score in each level was the dependent variable. The average times spent in each sleep stage were also added as three independent factors. In addition, to choose control factors to include in the model, we computed the pairwise Pearson correlations between each potential control predictor (same as those in the stepwise procedure) and the dependent variable. Any predictor that had a significant correlation with either the average or difference of TAI was added as a factor to the mixed-models. Because some of these factors were highly correlative with each other, we omitted a few of them from the final model based on existing knowledge, to avoid multicollinearity issues. The model also included a random intercept for subjects and was run using a Maximum-Likelihood estimation method, with follow-up analyses when needed as detailed in the Results.

3 | RESULTS

We started by conducting a stepwise regression analysis to identify factors that predict an increase in test anxiety before exam week. The stepwise procedure included TAI difference as the outcome variable, and demographic data, scores on the subjective questionnaires (both the difference between and average across the two timepoints), the time between observations, and objective sleep measures (average hours spent in N1/N2 sleep, N3 and REM sleep, as well as REM fragmentation) as predictors. The stepwise procedure resulted in including hours of REM sleep as a single factor in the model in addition to the intercept ($F_{1,45} = 4.678$, $p = 0.036$, adjusted $R^2 = 0.074$), with a positive coefficient ($\beta = 3.944$), indicating that TAI scores increased from time 1 to time 2 as participants spent more time in REM sleep. In other words, consistent with our prediction, we found REM sleep predicts changes in test anxiety but, in contrast to our prediction, the correlation between the two was positive rather than negative (Figure 2a, Main).

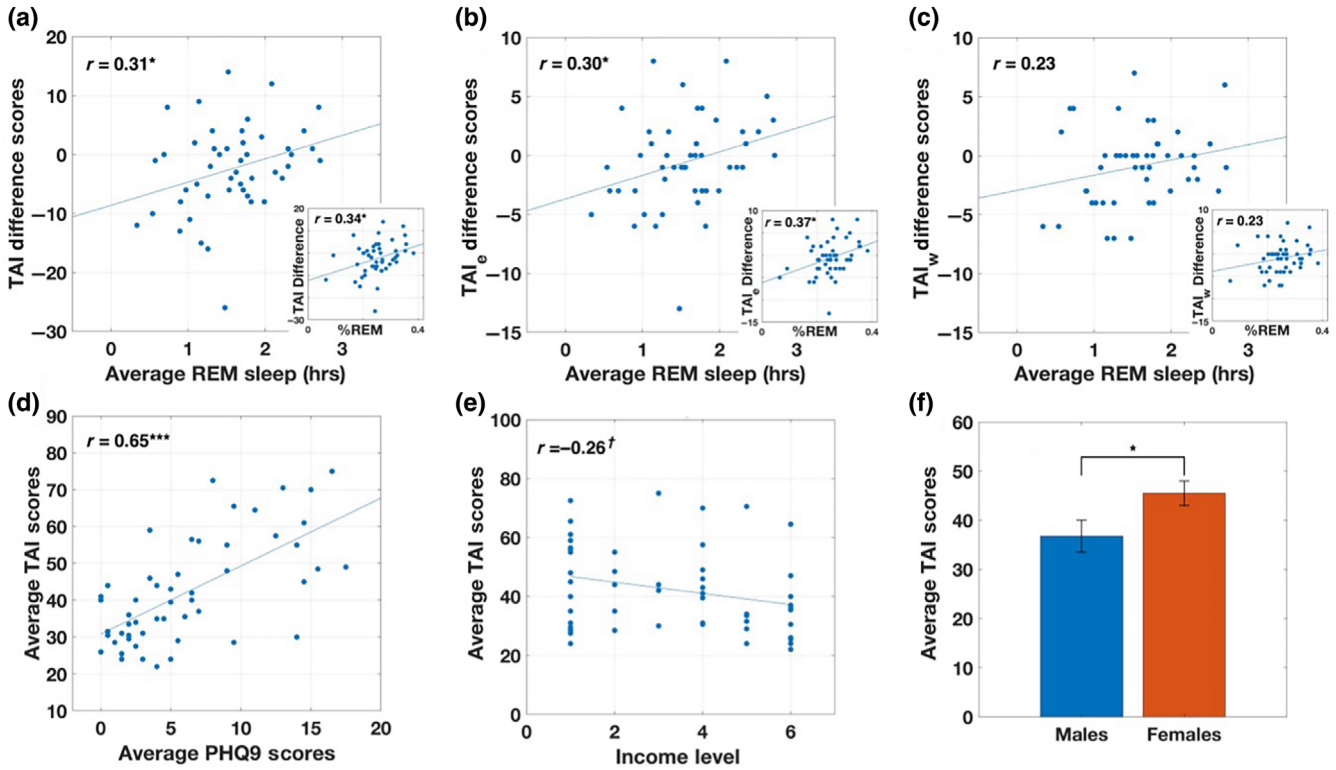

FIGURE 2 Predictors of test-anxiety inventory scores. (a) Difference in test anxiety from baseline to exam week as a function of average time spent in REM sleep (Main) and percent of time spent in REM sleep out of TST. (b) Same as (a), for the emotionality dimension of the test-anxiety scores. (c) Same as (a), for the worry dimension of the test-anxiety scores. (d) Average test anxiety as a function of average PHQ-9 scores. (e) Average test anxiety as a function of income level. (f) Average test-anxiety scores by gender. Two non-binary participants omitted from the graph. Bars representing standard errors of the mean. PHQ-9, Patient Health Questionnaire-9; REM, rapid eye movement; TAI, Test Anxiety Inventory; TAI_e, emotionality dimension of the TAI scores; TAI_w, worry dimension of the TAI scores; TST, total sleep time. *$p < 0.05$; ***$p < 0.001$; †$p < 0.06$; p-values represent significance level for the simple correlations.

To verify that the correlation with REM sleep was not a by-product of the total time spent in sleep (TST), we ran a follow-up stepwise regression analysis with REM sleep percentage and TST as the only sleep predictors, alongside the same demographic data and subjective scores. Confirming our first result, this stepwise procedure results in the inclusion of REM sleep percentage as a sole predictor (in addition to an intercept) of the difference in TAI from time 1 to time 2 ($F_{1,45} = 5.716$, $p = 0.021$, adjusted $R^2 = 0.093$), with REM sleep percentage having a positive relationship with TAI ($\beta = 0.336$; Figure 2a, inset).

To get more insight on the nature of the associations identified in the stepwise procedure, we calculated Pearson correlations between each of the two factors of TAI, worry and emotionality, and the time spent in REM sleep as well as the REM sleep percentage. We found that both hours of REM sleep ($r = 0.298$, $p = 0.042$) and REM sleep percentage ($r = 0.367$, $p = 0.011$) were significantly correlated with the emotionality dimension of TAI (Figure 2b). In contrast, neither hours of REM sleep nor REM sleep percentage were significantly correlated with the worry dimension (Figure 2c).

We then ran a separate stepwise regression analysis with the average TAI scores across both timepoints as the outcome variable. This analysis attempted to identify whether any sleep metrics can predict overall test anxiety (as reflected by the average TAI score) and not just the increase in test anxiety when approaching final exams. The same demographic data, subjective questionnaires and objective sleep measures were used as predictors. The stepwise procedure identified the difference in ISI and the average of PHQ-9 scores as significant predictors in addition to the intercept ($F_{2,44} = 19.494$, $p < 0.001$, adjusted $R^2 = 0.446$). The higher the average PHQ-9 scores and the greater the increase in ISI scores were, the higher the overall test anxiety ($\beta = 2.004$ and $\beta = 0.743$, respectively). No objective sleep measures were included in the model.

Finally, to identify which TAI dimensions contributed to the discovered associations with average TAI scores, we ran multiple regression models for the average score on the worry and emotionality dimensions of TAI. Predictors were the average score on PHQ-9 and the difference score of ISI, the two factors that were found to contribute to the average total TAI score in the stepwise procedure. For the emotionality dimension, the model was significant ($F_{2,44} = 15.705$, $p < 0.001$), driven by a positive relationship with the average PHQ-9 score ($t = 5.359$, $p < 0.001$), as well as a marginally significant positive relationship with the ISI difference score ($t = 1.883$, $p = 0.066$). Similarly, a multiple regression model with the average score on the worry dimension of TAI as the outcome variable was significant ($F_{2,44} = 19.221$, $p < 0.001$, adjusted $R^2 = 0.442$), driven by a positive relationship with both the average PHQ-9 scores ($t = 5.887$, $p < 0.001$) and the difference score of ISI ($t = 2.213$, $p = 0.032$). To sum up, participants exhibited higher average levels of anxiety on both the emotionality and worry dimensions as their average PHQ-9 scores were higher and there was a larger increase in ISI from time 1 to time 2.

Next, to complement the results of the stepwise procedure, we performed a linear mixed-models analysis using all 52 participants. TAI score was the outcome variable with Time as a within-subject factor with two levels (Baseline, Exam week). The average time spent in each sleep stage, our main variables of interest, were also included as factors. In addition, control factors were chosen to be potentially included in this model based on whether they had a Pearson correlation with the total TAI difference or average at a significance level below 0.05. Based on this criterion, we found no significant correlations between the control factors and difference in TAI; in contrast, gender, income, sleeping difficulties, and the average scores of PHQ-9, DRDI, PSQI, ESS, ISI, and the emotional suppression dimension of ERQ (ERQes) were all significantly correlated with the average scores of TAI and marked as potential factors to be included in the model.

Next, we examined the pairwise correlations between these factors (excluding Gender) to identify risks of multicollinearity (Table 3). We found that the correlation coefficient between PHQ-9 and DRDI, as well as the correlations of PHQ-9, PSQI and ISI among themselves, were all at a level higher than 0.65 (Table 1). According to Dormann et al. (2012), correlations of 0.5–0.7 are often chosen as thresholds to remove variable to avoid multicollinearity, with 0.7 being a recommended choice. In our case, all variables correlated at a level higher than 0.65, in addition to being close to the 0.7 threshold, had strong conceptual and empirical background supporting their association: ISI and PSQI are known to be highly correlated given that they measure

TABLE 3 Pairwise correlations between significant factors.

	Sleeping difficulties	Income	PHQ-9 AVG	DRDI AVG	ISI AVG	PSQI AVG	ESS AVG	ERQ-ES AVG
Sleeping difficulties	–	−0.131	0.519*	−0.384**	0.560**	0.518*	0.047	0.178
Income		–	−0.248	0.280	−0.069	0.018	−0.142	−0.075
PHQ-9 AVG			–	−0.663**	0.672**	0.653**	0.254	0.258
DRDI AVG				–	−0.334*	−0.364*	−0.049	−0.343*
ISI AVG					–	0.651**	0.305*	0.409*
PSQI AVG						–	0.027	0.230
ESS AVG							–	0.069

Abbreviations: AVG, average; DRDI, Dispositional Resilience and Disability Scale; ERQ-ES, expressive suppression of Emotion Regulation Questionnaire; ESS, Epworth Sleepiness Score; ISI, Insomnia Severity Index; PHQ-9, Patient Health Questionnaire; PSQI, Pittsburgh Sleep Quality Index.
*$p < 0.05$. **$p < 0.001$.

sleep in a similar way (Veqar & Hussain, 2020); PHQ-9 is known to have high correlations with both ISI and PSQI as it tests depression symptoms that include sleep disruption, and its high correlation with DRDI is not surprising because both measure mental health (Chang et al., 2021; Hughes et al., 2021; Schulte et al., 2021). Because our model already included other measures of sleep (sleeping difficulties as well as the objective measures of sleep), and because DRDI is a relatively new survey compared with the much more established PHQ-9, we decided to include PHQ-9 in the model and omit PSQI, DRDI and ISI (this choice did not have a substantial effect on our results; the eventual model yielded similar results when, instead of PHQ-9, it included PSQI + DRDI or ISI + DRDI).

The final factors included in the model were, therefore, Time, average time spent in N1/N2, N3 and REM sleep, Gender, Income, Sleeping Difficulties, and scores in the PHQ-9, ESS and ERQes questionnaires. Each of these factors, as well as their interaction with Time, was added to the model as a fixed effect. In addition, the model included a random intercept to account for individual variability.

Results of the mixed-models analysis indicated an estimated subject variability of 108.34 (confidence interval [CI] = [65.69178.67]) and a residual variability of 22.85 (CI = [14.37 36.31]). Estimates of fixed effects are displayed in Table 4. We found a significant interaction between hours spent in REM sleep and Time ($F_{1,36.52} = 4.497$, $p = 0.041$), with the parameter estimates indicating that more REM sleep predicted an increase in TAI scores from time 1 to time 2. In addition, there were significant main effects of Income ($F_{1,41.81} = 4.579$, $p = 0.038$) and PHQ-9 ($F_{1,66.06} = 5.391$, $p = 0.023$) on TAI scores, indicating that smaller income and higher PHQ-9 scores predicted overall higher test anxiety (Figure 2d,e). Finally, the main effect of Gender was marginally significant ($F_{2,42.06} = 3.189$, $p = 0.051$), mostly driven by estimated stronger anxiety for females compared with males (Figure 2f).

Following the significant interaction between hours in REM sleep and Time, we ran a follow-up mixed-models analysis to verify that this result was not a by-product of time spent in TST. This model contained the same control factors as the first, but instead of time in each sleep stage it included the average percent of time spent in REM out of TST. Estimates of fixed effects are displayed in Table S1. We found a highly significant interaction between REM sleep percentage and Time ($F_{1,38.74} = 6.852$, $p = 0.013$), indicating an increase in TAI between time 1 and time 2 as REM percent increases ($\beta = 0.485$) but no effect of TST or interaction of TST with Time (both $p > 0.29$). In other words, the effect of REM sleep was not a by-product of the TST.

TABLE 4 Estimates of fixed effects in linear mixed-model with TAI as the dependent variable.

					95% CI	
Parameter	**Estimate**	**Df**	***t***	***p***	**Lower bound**	**Upper bound**
Intercept	19.813	49.739	1.167	0.249	−14.299	53.925
Time (Time 2 versus Time 1)	0.014	36.788	−0.001	0.999	21.148	−21.176
Gender (NB versus M)	−2.834	53.057	0.313	0.755	−20.993	15.325
M (Time 2 versus 1)	−4.940	37.508	0.870	0.390	−16.438	6.558
Gender (NB versus F)	−12.268	52.416	1.353	0.182	−30.460	5.924
F (Time 2 versus Time 1)	−1.816	36.949	0.325	0.747	−13.122	9.490
Income	−1.491	55.803	−1.687	0.097	−3.262	0.280
Income (Time 2 versus Time 1)	0.554	39.469	−0.975	0.336	−0.595	1.703
Sleeping difficulties	1.955	58.072	0.638	0.526	−4.177	8.087
Sleeping dif. (Time 2 versus Time 1)	−1.496	36.700	0.754	0.456	−5.520	2.527
N1/N2_hr	2.529	55.301	1.017	0.313	−2.453	7.510
N1/N2 hr (Time 2 versus Time 1)	−2.174	36.954	1.374	0.178	−5.381	1.032
N3_hr	−5.748	53.413	−0.717	0.476	−21.814	10.318
N3 hr (Time 2 versus Time 1)	0.438	37.489	−0.087	0.931	−9.782	10.659
REM_hr	6.072	51.245	1.650	0.105	−1.314	13.457
REM hr (Time 2 versus Time 1)	4.783	36.528	−2.121	0.041	0.211	9.354
PHQ-9	0.561	58.415	1.904	0.062	−0.029	1.150
PHQ-9 (Time 2 versus Time 1)	0.100	41.992	−0.327	0.746	−0.517	0.717
ERQes	0.096	65.000	0.477	0.635	−0.305	0.496
ERQes (Time 2 versus Time 1)	0.124	38.918	−0.591	0.558	−0.301	0.549
ESS	−0.101	56.501	−0.429	0.669	−0.574	0.372
ESS (Time 2 versus Time 1)	−0.217	40.680	0.772	0.444	−0.783	0.350

Abbreviations: CI, confidence interval; Df, degrees of freedom; ERQes, emotional suppression dimension of Emotion Regulation Questionnaire; ESS, Epworth Sleepiness Scale; F, female; M, male; NB, non-binary; PHQ-9, Patient Health Questionnaire; REM, rapid eye movement.

Finally, we ran follow-up mixed-models analyses to explore which TAI dimension was influenced by the factors we found to be significant. One model was run for the emotionality dimension and the other for the worry dimension. Each model included Gender, Income, PHQ-9, and hours spent in REM sleep, as well as their interaction with Time. For the worry dimension, only the main effects of Income ($F_{1,43.53} = 6.838$, $p = 0.012$) and PHQ-9 ($F_{1,88.50} = 10.956$, $p = 0.001$) were once again significant, as well as the main effect of Time ($F_{1,39.26} = 5.098$, $p = 0.03$), indicating a decrease in overall test anxiety from time 1 to time 2 ($\beta = -3.832$; see Table S2 for estimates of fixed effects). In contrast, for the emotionality dimension, we found a significant main effect of REM sleep ($F_{1,45.40} = 4.427$, $p = 0.041$) and, once again, a significant interaction between REM sleep and Time ($F_{1,41.61} = 4.236$, $p = 0.046$), both with a positive correlation to the emotionality scores. The main effects of Time ($F_{1,42.12} = 4.192$, $p = 0.047$), Gender ($F_{2,45.12} = 3.74$, $p = 0.031$) and PHQ-9 ($F_{1,98.25} = 10.167$, $p = 0.002$) were also significant (see Table S3 for estimates of fixed effects). To exclude the possibility that REM sleep effects were driven by TST, we reran the model but substituted hours of REM sleep with the factors TST and percent of time spent in REM sleep out of TST. We found that the interaction of REM sleep percentage with Time remained highly significant ($F_{1,41.15} = 9.307$, $p = 0.004$). However, the main effect of REM sleep percentage and the main effect of TST and its interaction with time was not significant (all $p > 0.12$; see Table S4 for estimates of fixed effects).

4 | DISCUSSION

The current study aimed to investigate the role of habitual sleep, particularly REM sleep, in the future development of stress in everyday life. Monitoring students' sleep and test anxiety over the academic semester, we found REM sleep early in the semester predicts changes in test anxiety before finals; however, while confirming our general hypothesis, the direction of the effect was opposite to the one predicted, with more REM sleep being associated with increased, rather than decreased, test anxiety over the course of the semester.

Inconsistent findings regarding the direction of the REM sleep effect on emotional processing are common. For example, longer REM sleep has been associated with both decreased (Gujar et al., 2011) and increased (Wagner et al., 2002) negative emotional reactivity. Similarly contradictory findings across studies also characterize investigations of REM sleep and stress response following fear conditioning (Davidson & Pace-Schott, 2020). One potential explanation for these contradictions could derive from the REM recalibration hypothesis, which predicts REM sleep both decreases sensitivity to and increases selectivity of stress responses (Goldstein & Walker, 2014). Accordingly, Lerner et al. (2021) found that such heightened selectivity can increase stress reactions to few selective threats, specifically if the threats are highly predictive of an upcoming unpleasant experience relative to other cues. Thereby, it is possible the looming exam week was conceived by our participants as a highly probable source of stress, leading to REM sleep contributing to maintaining test anxiety rather than decreasing it as originally predicted. This explanation is also consistent with our finding that REM sleep was specifically associated with the emotionality dimension of test anxiety, which reflects self-perceived arousal of the autonomic nervous system (Sud & Sharma, 1990). Likewise, in our previous study, REM sleep was found to be positively associated with increased skin conductance response during fear recall, reflecting autonomic activity, but only when exposed to cues that were highly predictive of threat (Lerner et al., 2021; Sud & Sharma, 1990).

In contrast to the time spent in REM sleep, we did not find any associations between test anxiety and REM sleep fragmentation. REM fragmentation, a facet of REM sleep often considered to reflect its quality, has been previously linked to stress development, particularly in relation to PTSD. For example, REM fragmentation after trauma is associated with an elevated risk of PTSD development and, in mice, low continuity of baseline REM sleep episodes predicts PTSD-like hyperarousal symptoms after an electric shock (Grafe et al., 2024; Polta et al., 2013). The milder nature of test-related stress might explain our null result, as our REM fragmentation values were slightly below those of previous studies ([0.05–0.14] versus [0.01–0.2] in Vethe et al., 2022).

While the difference in anxiety levels between baseline and exams week showed an association with REM sleep, the average anxiety levels across both timepoints did not exhibit correlations to any of the objective sleep measures. In other words, we found physiological sleep traits that are predictive of changes in anxiety before a stressful period rather than of overall individual levels of anxiety. It is possible that REM sleep influences coping mechanisms that are active when needed the most, but has smaller effects on regular, day-to-day anxiety.

Apart from objective sleep factors, we examined various demographic and self-evaluated subjective factors to control for their potential influence on test anxiety, including academic year, demographics, stressors between baseline and exam week, and coping strategies. Most factors did not predict TAI scores, but some were linked to overall test anxiety. For example, higher test anxiety was found in females and low-income individuals, reflecting existing research on social expectations and financial hardship (King et al., 2024; Núñez-Peña et al., 2016). For example, low-income students might face increased financial stress, leading to full-time jobs, less social interaction and less time for exam preparation, which increases test anxiety (Adams et al., 2016; Martinez et al., 2009). Another possibility is that low income signals other factors, such as being a first-generation student, which itself reflects less familiarity with academic settings and potentially leads to higher test anxiety (Adams et al., 2016).

Additionally, we found positive correlations between average test anxiety and levels of depression, insomnia symptoms, and self-reported difficulties in sleeping, aligning with previous research (Blankstein et al., 1990; Jung et al., 2001). Moreover, higher average resilience (expressed as high DRDI scores) was associated with lower test anxiety, consistent with prior studies showing resilience mediating the connection between emotion regulation and test anxiety (Liu et al., 2021). Because REM has been previously linked to increased emotional regulation, it is possible that the effect of REM sleep on

test anxiety is the indirect result of its effect on resilience (Tempesta et al., 2018); however, our data showed no correlation between REM sleep and resilience, reducing the likelihood of such an explanation.

Notably, overall test-anxiety scores across subjects did not increase near exam week compared with baseline (and, in some of the analyses, showed a small but significant decrease). This contradicts the expectation that test anxiety would be elevated prior to finals week. However, the acclimation of students to college life and the testing environment after midterms might have contributed to adjusted stress levels. Furthermore, due to logistical reasons, some participants completed their first test-anxiety assessment closer to the middle of the semester when midterms take place, whereas the second test-anxiety assessment was collected for all participants during the days just prior to the final exams rather than during the final exam week itself when stress is expected to peak. The combination of these two constraints could have limited the strength of the stress manipulation. Either way, it points to an effect of REM sleep in maintaining stress rather than increasing it, with lower amounts of REM leading to decreased stress (Figure 2a).

5 | CONCLUSION

Overall, findings from the present study demonstrate that habitual sleep, particularly REM sleep, can predict increases in stress occurring in proximity to future life events, such as test anxiety before final examinations in university students. The REM recalibration hypothesis can serve as a framework to understand our results by assuming a selection process in which the association between stress and stimuli predicting it can be either strengthened or weakened by REM sleep, depending on the predictive value of the stimuli. This study opens avenues for further research into how habitual sleep architecture affects real-life stress and stress development, and what determines the direction of this association.

AUTHOR CONTRIBUTIONS

Emerson Larios: Conceptualization; methodology; writing – original draft; formal analysis; investigation; visualization. **Itamar Lerner:** Conceptualization; funding acquisition; methodology; formal analysis; supervision; writing – review and editing; visualization.

FUNDING INFORMATION

This work was partly supported by the National Science Foundation (NSF/Career 2237419) and the Southwest Center for Occupational and Environmental Health (SWCOEH NIH/NIOSH 5T42OH008421).

CONFLICT OF INTEREST STATEMENT

The authors have indicated no financial conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Larios, E., & Lerner, I. (2025). Habitual rapid eye movement sleep predicts changes in test-anxiety levels weeks in advance. *Journal of Sleep Research*, *34*(1), e14298. https://doi.org/10.1111/jsr.14298