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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	A Siemens 3 Tesla MRI scanner with a 12-channel head coil was used to collect functional and structural MRI data. PSG data was recorded using a TREA EEG Amplifier (Grass Technologies) with a total of 23 electrodes (17 cortical, 2 EOG ,3 EMG and 1 EKG channel). In the second in-lab PSG study COMET EEG amplifier was used (Grass Technologies) with a total of 27 electrodes (21 cortical, 2 EOG ,3 EMG and 1 EKG channel) Behavioral data was collected using PsychoPy v 1.83.
Data analysis	fMRI preprocessing and data analysis were performed using Statistical Parametric Mapping software implemented in Matlab (SPM12; Wellcome Department of Cognitive Neurology, London, UK). All EEG analyses were performed in MATLAB 8.6 (The MathWorks), including the add-in toolbox EEGLAB (http://sccn.ucsd.edu/eeglab/) Behavioral data was analyzed using SPSS (v25, IBM corp.) and JASP (v. 0.8.3 for bayesian analysis).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding authors upon request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined in line with our previous work on the effects of sleep loss in healthy adults(N of ~40 experimental sessions, two per participant).
Data exclusions	no participant was excluded from fMRI analysis. One participant was excluded from spectral EEG analysis due to a limited number of artifact-free NREM epochs (less than 40%). Micro-longitudinal participants (Online Study 1, N=293) were excluded if 1) they had not completed both nightly sleep surveys, 2) seep efficiency data exceeded 2.5 standard deviations from the mean or 3)they completed the same online survey more than once. Based on these quality control factors N=194 were eligible for further analysis. In Online Study 2 (N=187) ,participants were excluded if they failed to complete at least 3 daily surveys to allow for enough variability in assessing directionality effects. Final sample therefore included N=154 participants.
Replication	Experimental findings were confirmed using two additional two additional independent samples: a) 32 healthy participants took part in an in-lab overnight sleep study intended to confirm the association of NREM sleep to anxiety in an independent dataset and b) a subsample of the general population (N=154) took part in a second online micro-longitudinal study, now tracking their habitual sleep and subjective anxiety across a longer duration of four consecutive nights/days in order to replicate the original findings and confirm the directionality of the sleep-anxiety association.
Randomization	All Participants took part in both experimental sessions (sleep rested and sleep deprived), in a randomized order (10 subjects started with a sleep rested session and 8 with a sleep deprived session). Task versions were also randomized across sessions so both versions were presented equally in both experimental sessions. Within each version task stimuli were presented in randomized order. Survey questions in both Online Micro-longitudinal Studies were presented in random order.
Blinding	Experiments did not involve blinding because no neuroimaging or behavioral performance was predefined. Participants were kept blind to overall study objectives.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	In lab experimental fMRI study included Eighteen healthy adults, ages 18-24 years (mean: 20.2yrs, SD 1.5, 9 women) . Participants were well rested (average sleep duration>7 hr/night, validated using actigraphy prior to study participation). Participants were also free of sleep disorders, neurologic disorders, closed head injury, psychiatric disorders, history of drug abuse and current use of anti-depressant or hypnotic medication validated using a pre-screening questionnaire. In lab replication PSG study included 32 healthy adults, ages 18-24 years (mean: 20.47yr, SD1.8, 18 women) Online micro-longitudinal study 1 included 194 participants (mean age=37.03±11.3y, 54% women); Online micro-longitudinal study 2 included N=154 participants (mean age 36.78 yr, 45% women).
Recruitment	In-lab participants were recruited using local ads distributed across the campus in Berkeley as well as using social media groups relevant to Berkeley students. Online micro-longitudinal study participants (1 and 2) were recruited using Amazon Mechanical

Turk.

Ethics oversight: The study was approved by the local human studies committee of the university of California Berkeley, with all participants (in-lab and online) providing written informed consent.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Magnetic resonance imaging

Experimental design

Design type: task based block design

Design specifications: 28 videos were presented overall (14 in each experimental session, 2 runs per session). Each vidoe lasted lasted 32.3 s on average (SD 2.5 s). The different video trials (emotional, neutral) appeared in randomized order within each run with an inter-trial fixation jittered between 4-8s.

Behavioral performance measures: behavioral data (RT, button preses) was collected in order to verify attention to each video (a brief memory question about the content of the video at the end of each clip).

Acquisition

Imaging type(s): Functional and structural

Field strength: 3T

Sequence & imaging parameters: Blood oxygenation level-dependent contrast functional images were acquired with echo-planar T2*-weighted (EPI) imaging using a Siemens 3 Tesla MRI scanner with a 12-channel head coil. Each image volume consisted of 37 descending 3.5mm slices (96 x 96 matrix; TR = 2000 ms; TE = 22 ms; voxel size 3.5 x 3.5 x 3.2 mm, flip angle = 50, 0.3 mm interslice gap). One high-resolution, T1 weighted structural scan was acquired at the end of each session (256 x 256 matrix; TR=1900; TE = 2.52; flip angle = 9□; FOV 256 mm; 1 x 1 x 1 mm voxels).

Area of acquisition: whole-brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software: Preprocessing was carried out using SPM12 (Wellcome Department of Cognitive Neurology, London, UK).

Normalization: Data was normalized to MNI space using affine and non linear transformations as implemented in SPM12. In this process,deformation is estimated by deforming template data to match an individual's T1 scan (segmented to gray and white matter maps), a deformation which is then applied to the co-registered functional data.

Normalization template: SPM12's MNI normalized templates

Noise and artifact removal: To control for movement artifacts, six movement-related covariates (three rigid-body translations and three rotations determined from the realignment preprocessing step) were used as regressors in the design matrix. To further address the influence of motion on BOLD data, we calculated frame-wise displacement (FD) of head motion based on the motion parameters estimated during preprocessing using the ArtRepair toolbox. TRs including FD values larger than 1 were interpolated with the nearest artifact free TRs surrounding the motion.
To control for physiological noise 5 principal components of cerebrospinal fluid (CSF) and white matter signal were added as regressors to the design matrix, implemented through the CompCor pipeline. Extraction of white matter/CSF signal was derived using probabilistic maps segmented from the T1 weighted anatomical image of each participant using the segment function implanted in SPM12. Masks were then thresholded at a probability value of 0.99 for white matter and 0.95 for CSF, converted to functional resolution and eroded to eliminate isolated voxels.

Volume censoring: Subjects were excluded from further analysis if both movement regressors and FD values were larger than 2mm.

Statistical modeling & inference

Model type and settings: A general linear model (GLM) was specified for each participant to investigate the effects of interest. The resulting contrasts were then taken to a second level, random effects analysis to assess group-level effects, examined using a paired ttest (Sleep Rested < > Sleep Deprived).

Effect(s) tested: Contrasts were created at the first level focusing on Emotional vs. Neutral clips to target affective brain regions known to be sensitive to anxiety.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s): Regions of interest (ROIs) were independent, literature-defined, 5mm spheres centered around reported coordinates of a-priori brain regions known to be sensitive to anxiety (coordinates are listed in Table S2 of supplementary information)

Statistic type for inference
(See Eklund et al. 2016)

Condition differences in ROI activity were examined using a repeated measure ANOVA with the factors of sleep (SR\SD) and task condition (emotional\neutral clips) .

Correction

In the repeated measure ANOVA, post-hoc tests were computed using two-sided T-tests corrected for multiple comparisons using the Bonferroni correction.

Models & analysis

n/a	Involved in the study
☐	☒ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

To identify changes in mPFC-amygdala circuit functional connectivity as a function of sleep, a psychophysiological interaction (PPI) analysis was conducted separately for each session (sleep-deprived/ sleep-rested) using SPM12. Consistent with standard PPI practices, an individual design matrix for each participant included three regressors: 1) the BOLD signal time course from the mPFC seed region, 2) regressors coding the temporal ordering of task conditions (emotional and neutral videos), and 3) the PPI term, reflecting the product of the deconvolved time course in the mPFC with a vector representing the order of the psychological variables of interest